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CONTENT

CUPRINS

THE VARIATION OF THE IONIC CALCIUM ADSORPTION COEFFICIENT ON A RESIN WITH SULPHONIC GROUPS

VARIAȚIA COEFICIENTULUI DE ADSORBȚIE A CALCIULUI IONIC PE CATIONIT CU GRUPĂRI SULFONICE

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Abstract. *Determination of the adsorption coefficient is essential for all retention processes of ionic species on surfaces, to determine the process efficiency and influence of the various factors on the adsorption. Calcium ions are present in significant quantities in many natural waters, causing high values of hardness, implicitly restricting the possibilities of using these waters. Decreasing the content of ionic calcium from aqueous solutions can be done by retaining them on cation exchangers, so in the present paper we chose a sulphonic resin - Dowex 50 - to study the calcium adsorption from aqueous solutions of different concentrations. We determined both the values of the adsorption coefficient over time for each concentration in the dynamic process as well as the correlation between the amount of ion in the initial solution and that retained on the surface of the adsorbent, depending on the amount of resin used.*
Key words: ionic calcium, adsorption, softening, water

Rezumat. *Determinarea coeficientului de adsorbție este esențială în cazul tuturor proceselor de reținere a unor specii ionice pe suprafețe, pentru a stabili eficacitatea procesului şi influența diferiților factori asupra adsorbției. Ionii de calciu sunt prezenți în cantități însemnate în multe ape naturale, determinând valori mari ale durității, implicit restricționând posibilitățile de utilizare ale acestor ape. Scăderea conținutului în calciu ionic din soluții apoase se poate face prin reținere pe schimbători de cationi, de aceea am ales în prezenta lucrare o rășină cu grupări sulfonice, Dowex 50 pentru studiul adsorbției calciului din soluții apoase de diferite concentrații. S-au determinat atât valorile coeficientului de adsorbție în timp, pentru fiecare concentrație, în procedeu dinamic cât şi corelația dintre cantitatea de ion aflat în soluția inițială şi cea reținută pe suprafața adsorbantului, în funcție de cantitatea de rășină folosită.*
Cuvinte cheie: calciu ionic, adsorbție, dedurizare, apă

INTRODUCTION

Ion exchange materials are insoluble substances containing loosely held ions and are able to exchange those with other ions, as calcium or magnesium, in solutions coming in contact with them (Bandrabur *et al*, 2012).

Strong acid cation (SAC) resins proved to be an option for water softening process for the domestic or food industry specific applications (Zagorodni, 2007).

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A group of Chinese researchers synthesized a Ca-selective zeolite characterized by SEM, XRD and FT-IR and used it as adsorbent for calcium in an ion exchange and hydroxyl complex adsorption mechanism. It indicated that the zeolite has potential application value in zero discharge of seawater desalination (Qin *et al*, 2010).

MATERIAL AND METHOD

The chosen adsorbent material was a strong acid changing resin with sulphonic groups, Dowex 50, added in three different doses – 0.5; 1.0 and 1.5 g per 50 ml sample solution. The tested ionic calcium solutions contained calcium chloride prepared in situ from calcium carbonate and hydrochloric acid, with the following initial concentrations: 1.672 mg Ca^{2+}/mL; 2.376 mg Ca^{2+}/mL; 3.28 mg Ca^{2+}/mL. We adsorption was conducted in dynamic conditions, under continuous magnetic stirring took samples of 1 mL solution at specific intervals, considering that the resin is rapidly adsorbing calcium and the surface tends to be quickly covered.

We calculated the decrease in calcium levels for the analysed solutions and the adsorption coefficients variation in time and after that, traced the correlation between these coefficients and the initial calcium concentration for all three resin quantities added as adsorbent in the process.

RESULTS AND DISCUSSIONS

The considered adsorbent retained calcium ions from the aqueous solutions, tending to equilibrium in a very short time, therefore we tested and calculated the adsorption coefficient at 0.5, 1, 2 and 5 minutes after we added the adsorbent in the solution. Table 1 and figure 1 present the variation of the adsorption coefficient in time for the initial concentration of 1.672 mg Ca/mL depending on the amount of added adsorbent.

Table 1

Variation of the adsorption coefficient in time for the initial concentration of 1.672 mg Ca/mL

Time (min)	C (mg Ca/mL)	mg adsorbed Ca	γ(mg ads Ca /g Dowex)
0.5 g Dowex			
0	1.672	0	0
0.5	1.436	0.236	0.450897975
1	1.26	0.412	0.787160871
2	1.044	0.628	1.199847153
5	0.984	0.688	1.314482232
1.0 g Dowex			
0	1.672	0	0
0.5	1.332	0.34	0.315340382
1	0.924	0.748	0.693748841
2	0.708	0.964	0.89408273
5	0.512	1.16	1.075867186
1.5 g Dowex			
0	1.672	0	0
0.5	1.084	0.588	0.383186706
1	0.928	0.744	0.484848485
2	0.456	1.216	0.792440534
5	0.288	1.384	0.90192245

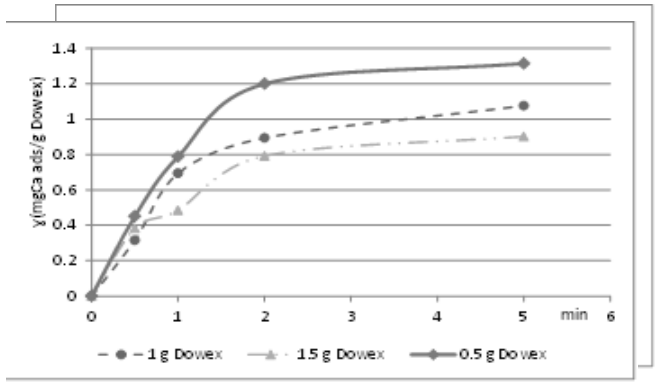

Fig. 1 Variation of adsorption coefficient at 1.672 mg Ca/mL in time depending on the amount of adsorbent

Table 2 and figure 2 present the variation of the adsorption coefficient in time for the initial concentration of 2.376 mg Ca/mL depending on the amount of added adsorbent.

Table 2

Variation of the adsorption coefficient in time for the initial concentration of 2.376 mg Ca/mL

Time (min)	C (mg Ca/mL)	mg adsorbed Ca	γ(mg ads Ca /g Dowex)
0.5 g Dowex			
0	2.376	0	0
0.5	2.152	0.224	0.43956044
1	1.984	0.392	0.769230769
2	1.732	0.644	1.263736264
5	1.632	0.744	1.459968603
1.0 g Dowex			
0	2.376	0	0
0.5	1.952	0.424	0.420176395
1	1.756	0.62	0.614408879
2	1.412	0.964	0.955306709
5	1.236	1.14	1.129719552
1.5 g Dowex			
0	2.376	0	0
0.5	1.812	0.564	0.375424349
1	1.496	0.88	0.585768488
2	1.068	1.308	0.87066498
5	0.816	1.56	1.038407775

Fig. 2 Variation of adsorption coefficient at 2.376 mg Ca/mL in time depending on the amount of adsorbent

Table 3 and figure 3 present the variation of the adsorption coefficient in time for the initial concentration of 3.28 mg Ca/mL depending on the amount of added adsorbent.

Table 3

Variation of the adsorption coefficient in time for the initial concentration of 3.28 mg Ca/mL

Time (min)	C (mg Ca/mL)	mg adsorbed Ca	γ (mg ads Ca /g Dowex)
0.5 g Dowex			
0	3.28	0	0
0.5	2.804	0.476	0.911877395
1	2.628	0.652	1.249042146
2	2.536	0.744	1.425287356
5	2.488	0.792	1.517241379
1.0 g Dowex			
0	3.28	0	0
0.5	2.72	0.56	0.543161979
1	2.4	0.88	0.853540252
2	2.136	1.144	1.109602328
5	2.1	1.18	1.144519884
1.5 g Dowex			
0	3.28	0	0
0.5	2.48	0.8	0.523457436
1	2.24	1.04	0.680494667
2	1.78	1.5	0.981482693
5	1.588	1.692	1.107112478

For each quantity of adsorbent we determined the correlation between the adsorption coefficients and the initial concentrations (fig 4, fig. 5, fig. 6).

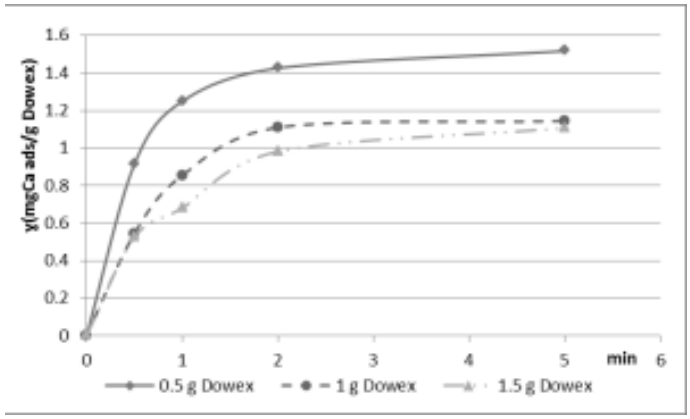

Fig. 3 Variation of adsorption coefficient at 3.28 mg Ca/mL in time depending on the amount of adsorbent

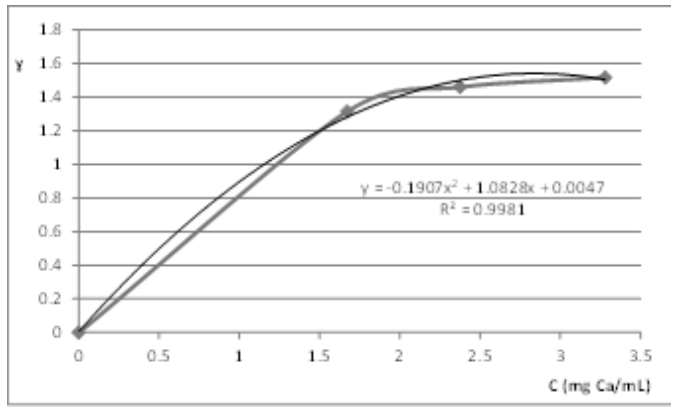

Fig. 4 Correlation adsorption coefficient - calcium concentration for 0.5 g Dowex

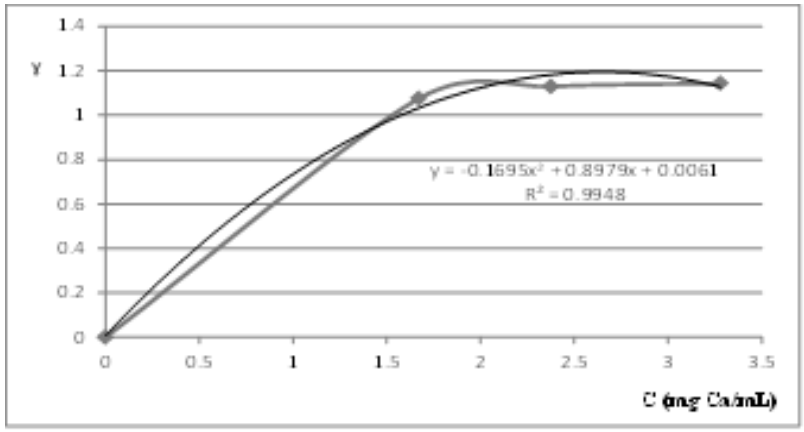

Fig. 5 Correlation adsorption coefficient - calcium concentration for 1.0 g Dowex

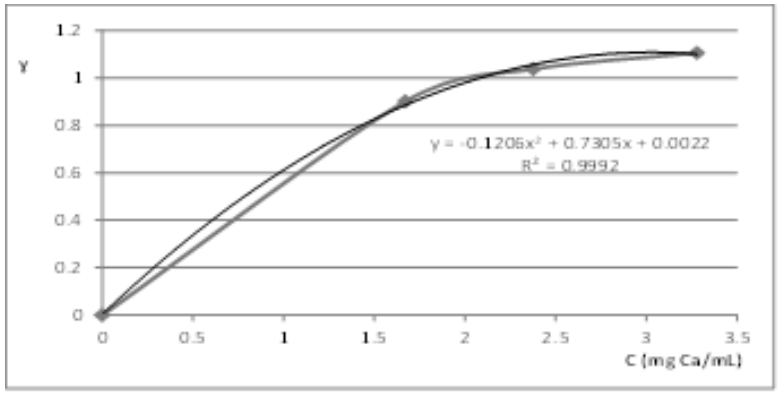

Fig. 6 Correlation adsorption coefficient - calcium concentration for 1.5 g Dowex

CONCLUSIONS

1. Dowex 50 is very efficient as adsorbent material for calcium ions from aqueous solutions, including natural water with an elevated hardness degree;

2. The adsorption procedure, in dynamic conditions, proved to be very simple and quick;

3. The sorption equilibrium installs in a short time after mixing the adsorbent in the tested solution;

4. Considering we tested solutions with an equivalent of 200 or more German degrees of hardness (1 Gd=10mg CaO/L), using small quantities of adsorbent for natural water would soften them quickly, being easily removed afterwards by simple filtration.

REFERENCES

1. Bandrabur B., Tataru-Fărmuş R.E., Lazăr L., Gutt Gh., 2012 - *Application of a strong acid resin as ion exchange material for water softening – equilibrium and thermodynamic analysis*, Scientific Study & Research, Chemistry & Chemical Engineering, Biotechnology, Food Industry, ISSN 1582-540X, 13 (4), pp. 361 – 370;

2. Qin C., Wang R., Ma W., 2010 - *Characteristics of calcium adsorption by Ca-Selectivity zeolite in fixed-pH and in a range of pH*, Chemical Engineering Journal, 156(3), 540–545;

3. Zagorodni, A.-A., 2007 - Ion Exchange Materials: Properties and Applications, Elsevier, ISBN 978-008-044-5526.

LIGNIN DERIVATIVES MODIFIED BY HYDROXYMETHYLATION AND EPOXYDATION REACTIONS

DERIVAŢI LIGNINICI MODIFICAŢI PRIN REACŢIILE DE HIFROXIMETILARE ŞI EPOXIDARE

UNGUREANU Elena[1], JITĂREANU Doina [1], TROFIN Alina[1], UNGUREANU O.C. [2], POPA V.I. [3]
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Abstract. *Lignin derivative (the commercial product - Protobind 2000) offered by the Granit Recherche Developement S.A. company, Lausanne-Schwitzerland was synthesized from annual plants. The present study's aim was to modify commercial lignins by the reaction of hydroxymethylation (produced in alkaline medium) and epoxydation (reaction with epichlorohydrin was performed in basic catalysis, aiming at increase the functionality) and to characterize the lignin derivatives chemical, spectral (^{1}H NMR) and thermogravimetric analysis (TG). Studies have revealed some functional changes related to the difference in reactivity and reaction conditions.*
Key words: Protobind 2000, lignin, hydroxymethylation, epoxydation, spectral and thermogravimetric analysis.

Rezumat. *Lignina derivativă (produsul comercial Protobind 2000) oferită de firma Granit Recherche Developement S.A. Lausanne-Elveţia a fost sintetizată din plante anuale. Scopul prezentului studiu este a de a modifica ligninele comerciale prin reacţia de hidroximetilare (produsă în mediul alcalin) şi epoxidare (reacţie cu epiclorhidrina în cataliza bazică ce creşte funcţionalitatea) şi de a caracteriza derivaţii ligninici prin analize chimice, spectrale (^{1}H RMN) şi termogravimetrice (TG). Studiile au relevat unele modificări funcţionale legate de diferenţa de reactivitate şi condiţiile de reacţie.*
Cuvinte cheie: Protobind 2000, lignină, hidroximetilare, epoxidare, analize termogravimetrice şi spectrale.

INTRODUCTION

Lignin is a macromolecular compound, much more active than cellulose or other natural polymers, due to functional groups contained in its macromolecule, constituting the main aromatic component of vegetal tissues, representing 20-40 % of the higher plants' mass located in the cellular wall and in intercellular spaces (Benar *et al,* 1999; Ungureanu, 2011).

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It is known that lignin has a very complex structure, which varies depending on the plant species, separation method and modification reactions that may induce particular characteristics. Regarding functional groups lignin presents at least three base functional groups in its structure: methoxylic, hydroxylic (alcoholic and phenolic) and the lateral propanic chain. Alongside these functional groups, in lesser amounts, there can be found carbonylic groups (approximate 1 group of CO at 5 C9 units), most of the times, fixated at the lateral chain. In some cases, the presence of carboxylic groups into the lignin can be noticed under the form of phenol carboxylic acids or of some small quantities of lactonic groups (Ungureanu *et al,* 2009, 2016).

Lignin modification through hydroxymethylation and epoxydation offers possibilities of developing its functionality and this allows the extension of the application area for the synthesized derivatives.

Taking into account all these aspects, the objectives of this study are the modification of some lignin's from annual plants through a reaction of hydroxymethylation produced in an alkaline medium in the presence of formic aldehyde and of epoxydation effected out in an alkaline medium in the presence of epichlorhydrin and the characterization of lignin derivatives from a chemical, spectral (^{1}H NMR) and thermogravimetric methods.

MATERIAL AND METHOD

The following materials have been used:

- Protobind 2000 (Pb2000), commercial lignin offered by Granit Recherché Dévelopement Switzerland, with the following chemical characteristics presented in table 1.

Table1

The characteristics Protobind 2000

Characteristics	Protobind 2000
Solide, %	95
Ash, %	1.3
pH (10 % dispersion)	4.80
Densitatea, g/mL	~ 0.6
Aromatic OH, mmole/g	1.6-1.8
COOH, mmole/g	2.1-2.3
T softening, °C	~ 130
Solubility in furfuryl alcohol, %	41
Solubility in aqueous alkali, %	95

- Formic aldehyde (37 %);
- Dimetil sulfoxid (DMSO);
- NaOH solution 0.1 N;
- Epichlorhydrin.

Work procedure:

The hydroxymethylation reaction

The method used in the hydroxymethylation of the three lignin products was performed in a basic medium, in the presence of formic aldehyde (37 %), according to the technical literature *(*Ungureanu, 2011).

Determination of total hydroxyl groups

The total OH groups content was determined by chemical method with acetic anhidryde in pyridine medium and from FT-IR spectral analysis.The Ar-OH group's content was determined by a UV-VIS method.

The epoxidation reaction

The epoxydation method achieved in a basic medium in the presence of epichlorhydrin through which the three types of lignin studied have been modified has been effected out according to the technical literature *(*Ungureanu, 2011).

Epoxydation index

Determination of the epoxy group was effected out by HCl addition on the epoxy group and titration of the acid excess with NaOH solution 0.1 N.

Proton nuclear magnetic resonance spectroscopy (^{1}H NMR)

Nuclear magnetic resonance (NMR) offers the richest and most complete information on the structure of organic compounds. For this purpose it was used a Bruker Avance DRX 400 MHz spectrometer.

Process: For investigation was necessary lignin acetylation and derivatives for a better dissolution in DMSO-d6. To obtain a "good" spectrum it is required to have concentrations of about 0.2 mmol/mL. Spectra processing was performed with a specialized program from SpectraManager series.

Thermogravimetry

The thermal analysis was performed using the METTLER TOLEDO derivatograph in N_2 atmosphere with a flow of 20mL/min and a heating rate of 15ºC/min, in the temperature range 25-800 °C and sample mass of 4 ÷ 6 mg.

RESULTS AND DISCUSSIONS

During the reaction of hydroxymethylation performed for lignin, the reaction conditions have been varied (50°C temperature, 90°C respectively, reaction duration of three hours and pH 10.5, pH 12 respectively) in order to obtain highly functional products.

The content of functional groups was determined according to the methods presented by different research groups. The other methods applied for chemical characterization were: the determination of carboxylic groups and of the metoxyl groups, the determination aromatic hydroxyl groups, the calculation of the fenolic groups/aliphatic groups' ratio, as well as, the determination siringyl/guaiacyl unit's ratio (S/G).

The information obtained has allowed the determination from this point of view of the optimal reaction conditions, namely: 90°C temperature, pH 10.5 and the reaction duration of three hours (tab. 2).

Table 2

The content of functional groups of modified and unmodified lignins

Sample	T, ^{O}C	pH	OH total groups	Ar-OH groups	OCH_3 groups	Ak/Ar ratio	C=O groups	S/G ratio
Pb 2000	-	-	1.11	0.89	1.05	1.17	0.89	0.83
	90	12.0	1.23	0.98	1.15	1.27	0.95	0.96
	90	*10.5*	*1.15*	*0.98*	*1.13*	*1.20*	*0.91*	*0.96*
	50	10.5	1.14	0.98	1.12	1.22	0.95	0.96
	50	12	1.16	0.99	1.14	1.21	0.94	0.96

The lignin obtained in optimal conditions there was characterized from the point of view spectral and thermogravimetric. As a consequence of the thermal analyses, it can be noticed that the modified product has a higher degradation temperature in the third stage, compared to the unmodified sample (tab. 3).

Table 3

Characteristics of the thermal degradation process of the lignin derivatives

Samples	Degradation stage	T_i (°C)	T_{max} (°C)	T_f (°C)	Mass losses (%)
Pb2000	I	205	234	326	22.78
	II	326	381	496	39.23
	III	56	79	115	5.06
Pb2000H	I	216	248	328	12.90
	II	329	3374	580	36.37
	III	205	234	326	22.78

(Ti - initial temperature at which the degradation starts; Tmax – temperature corresponding to the maximum rate of degradation, Tf –final temperature şi W – mass losses %).

The characterization of the lignin has been achieved by monitoring the influence of temperature (50°C and 70°C respectively), the mass ratio between the lignin (L) and NaOH (L:NaOH = 1:3 and 1:6) and the reaction duration (3, 5 and 7 hours respectively). It can be noticed from table 4 that the best results can be obtained when the reaction is achieved at 70°C, for a L:NaOH=1:3 ratio and a three-hour reaction duration, appreciated as being ***optimal reaction*** conditions. The reaction yield was included in the 50-90 % interval, related to the mass of the reactants and it differs according to the type of sublayer and the purification degree after washing the derivatives. It can also be noticed that along with the temperature increase and the reaction duration, from 3 to 7 hours, appear a decrease of the epoxydation number (tab. 4) (Ungureanu *et al,* 2015).

Table 4

Characteristics of the modified lignin's by epoxydation

Sample	T, ^{0}C	L:NaOH (w/w)	t, h	CE, %		η, %	U, %	Ash %	Const. f.liq, %
				f.sol.	*f.liq.*				
Pb2000E	70	1:6	3	1.20	0.21	50	5.5	8.12	16.40
	70	*1:3*	*3*	1.70	0.64	*64*	*5.1*	*2.1*	*18.20*
	50	1:3	3	1.85	0.36	60	7.2	7.85	16.25
	70	1:3	5	1.50	0.32	69	6.78	6.22	12.32
	70	1:3	7	1.63	0.40	52	6.27	5.63	15.30

For characterization by ^{1}H-NMR spectroscopy the lignin was subjected to acetylation to aid dissolution in DMSO-d6. In figures 1 and 2 are shown the ^{1}H-NMR spectra for Pb1000 lignin unmodified (Pb2000N) and hydroxymethylation

(Pb2000H), and the results were interpreted using literature data. The spectrum recorded for the two lignins weak signals in the aromatic domain at 8.64 ppm and the methoxyl groups.

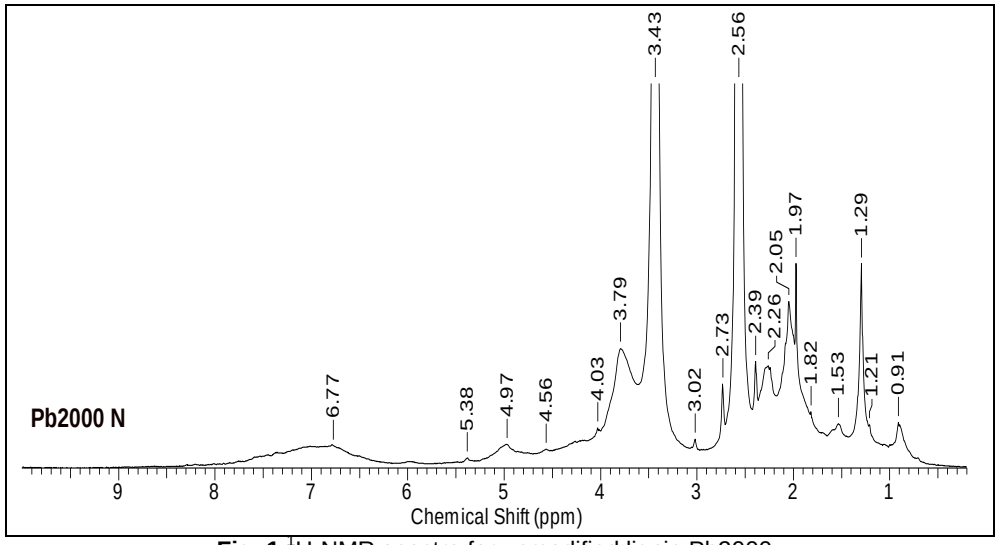

Fig. 1 ^{1}H-NMR spectra for unmodified lignin Pb2000

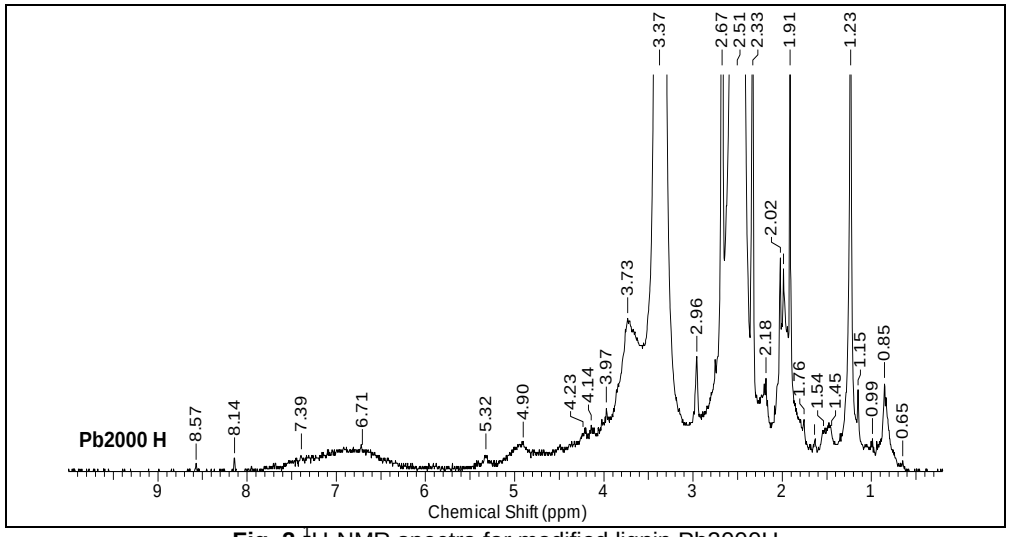

Fig. 2 ^{1}H-NMR spectra for modified lignin Pb2000H

Signals from 9.08-7 ppm confirms the presence of epoxy groups in lignin structure. Also stands out the signals of methoxyl and acetyl groups, more intense in the spectra of epoxidised lignin (fig. 3).

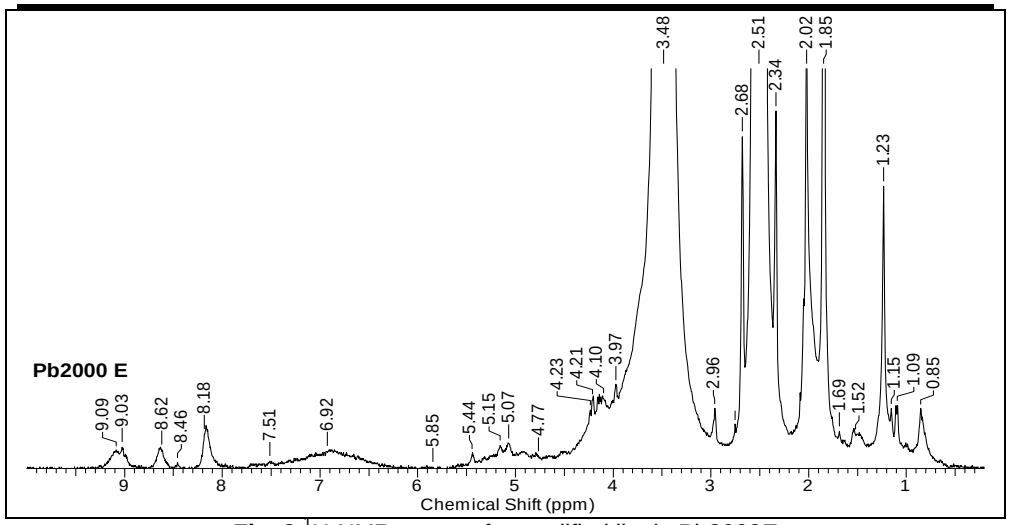

Fig. 3 ^{1}H-NMR spectra for modified lignin Pb2000E

CONCLUSIONS

1. The ^{1}H-NMR spectroscopy shows the change of functionality for lignin as a result of hydroxymethylation and epoxydation reaction.

2. The thermogravimetric analyses have proved that thermal degradation occurs in two and three stages respectively, according to the type and the degree of modification of the products tested but the hydroxymethylated/epoxydated derivatives have a higher thermostability, compared to the unmodified lignin.

REFERENCES

1. **Ariton Adina-Mirela, Creangă T., Trincă Lucia Carmen, Borş S.I., Ungureanu Elena, Măluţan Th., Popa V.I., 2015** - *Valorization of lignin modified by hydroxymethylation to ensure birch veneer bioprotection,* Cell. Chem. and Technol., 49 (9-10), p. 765-774.
2. **Benar P., Gonplves A. R., 1999** - *Principal component analysis of the hydroxymetylation of sugarcane lignin: A time-depending study by FTIR*, J. Wood Chem. Technol., 19 (1-2), p.15 1-165.
3. **Ungureanu Elena, Jităreanu Carmen Doina, Trofin Alina, Popa V.I., Sandu Tatiana, 2015** - *Chemical and spectral characterizations for some lignin products*, Lucrări Ştiinţifice, Seria Horticultură, nr.58, vol.1, p.23-28.
4. **Ungureanu Elena, 2011** - *Lignina, polimer natural aromatic cu ridicat potenţial de valorificare*, Ed. PIM, Iaşi, p. 112.
5. **Ungureanu Elena, Ungureanu O., Căpraru Adina-Mirela, Popa V.I., 2009** - *The chemical modification and the characterization the straw lignin,* Cell. Chem. and Technol., 43 (7-8), p. 263-269.
6. **Ungureanu Elena, Trofin Alina, Ariton Adina-Mirela, Jităreanu Carmen Doina, Ungureanu Ovidiu, Gîlcă Valerica, Bors S.I., Popa V.I., 2016 -** *Applications of epoxidated lignins for bioprotection of lignocellulosic materials***,** Cell. Chem. and Technol., 50 (1), p. 77-85.

INFLUENCE OF PHOSPHORUS FERTILIZATION AND *BRADYRHIZOBIUM JAPONICUM* INOCULATION OF SOYBEAN ON ACTIVITIES OF ACID PHOSPHATASES IN ROOTS AND RHIZOSPHERE SOIL UNDER PHOSPHORUS AND WATER LIMITED CONDITIONS

INFLUENŢA FERTILIZĂRII CU FOSFOR ŞI INOCULĂRII CU *BRADYRHIZOBIUM JAPONICUM* ASUPRA ACTIVITĂŢII FOSFATAZEI ACIDE ÎN RĂDĂCINI ŞI SOLUL RIZOSFERIC LA SOIA ÎN CONDIŢII LIMITATE DE FOSFOR ŞI UMIDITATE

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Abstract. *A pot experiment was conducted to examine the impact of Bradyrhizobium japonicum rhizobacteria along with P fertilizer on the activity of acid phosphatases (APase) in roots and rhizosphere soil of soybean under phosphorus and water limited conditions. Non-inoculated and inoculated soybean plants (cv Horboveanca) were supplied with three levels of P: 0 mg P/kg soil, (P0, insufficiency P), 20 mg P/kg (P20, medium) and 100 mg P/kg (P100, sufficient). At the flowering stage a set of plants was subjected to moderate drought stress for 12 days. Experimental results have shown that the root enzyme activity was much higher in the treatment without fertilization compared to those fertilized with phosphorus irrespective of soil moisture level. Inoculated plants with B. japonicum exhibited greater acid phosphatases activity in roots than non-inoculated plants. The same trend was observed in soil acid phosphatase activity under well-watered and water deficit conditions.*
Key words: acid phosphatase, *B. japonicum*, moisture, phosphorus, soybean

Rezumat. *S-a organizat un studiu în vase de vegetaţie pentru a evalua influenţa bacteriilor Bradyrhizobium japonicum aplicate separat sau în combinare cu fosforul asupra activităţii fosfatazei acide în rădăcini şi solul rizosferic la plantele de soia, cultivate în condiţii limitate de fosfor şi umiditate a solului. Plantele (cv Horboveanca) neinoculate şi celea inoculate le-au fost administrate diferite doze de fosfor: 0 mg P/kg sol, (P0, nefertilizat), 20 mg P/kg (P20, mediu) şi 100 mg P/kg (P100, suficient aprovizionat). Plantele au fost supuse deficitului de apă la faza de înflorire pentru 12 zile. Rezultatele experimentale au demonstrat că activitatea enzimei a fost mai înaltă în variantele fără fertilizarea chimică comparativ cu cea depistată la fertilizare indiferent de regimul de umiditate a solului. Aplicarea B. japonicum a majorat activitatea enzimei în rădăcini faţă de plantele neinoculate. Aceiaşi tendinţă s-a observat şi la nivel de activitate în solul rizosferic.*
Cuvinte cheie: *B. japonicum*, fosfataza acidă, fosfor, umiditate, soia

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INTRODUCTION

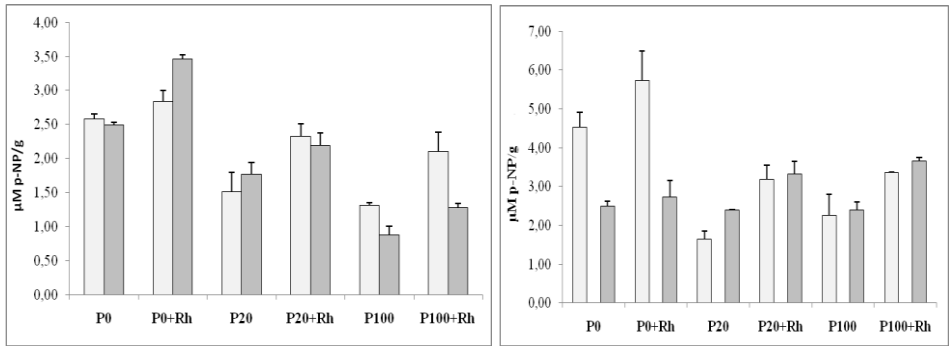
Nowadays the farmers are largely cash limited and mineral fertilizers being costly, restricts their capacity to purchase fertilizers and hence it is needed to develop sustainable agricultural production especially under scarce water and nutrient environments. The low yields of legumes are partly due to infertility caused by carbonated soils which have low nutrient contents, particularly of available phosphates (Andries, 2007), insufficient water supply and compatible *Rhizobium* for adequate N2 fixation. The microorganisms application as biofertilizers is considered a promising alternative that supports an effective approach for improving plant nutrition, decreasing agricultural costs, maximizing crop yield by providing them with available nutrients, particularly P (Lugtenberg and Kamilova, 2009). Almost half of the microorganisms present in soil or on plant roots possess the ability to mineralize organic phosphorus through the action of phosphatases (Tarafdar and Classen, 1988). These enzymes catalyze the cleavage of mineral P from organic phosphate esters, in acidic and alkaline soils that are poor in P (Nannipieri *et al*, 2011), thus making P more available in these soils. Rhizobium inoculation appears to increase P use efficiency in field grown soybean plants and in faba bean under controlled conditions (Boudanga *et al*, 2015). The aim of this study was to asses root acid phosphatase, rhizosphere acid and alkaline phosphatase activities of soybean in relation to application of *B. japonicum* and P fertilizer under water limited conditions.

MATERIAL AND METHOD

To accomplish the study's objectives a pot experiment was conducted under controlled soil moisture conditions. The soil was represented by chernoziom carbonated with low available phosphates. The experiment was laid out in a randomized complete block design, with four replicates for each treatment. Phosphorus (P) was administered to soil before the sowing. Soybean seeds (*cv Horboveanca)* were inoculated with suspension of rhizobacteria *Bradyrhizobium japonicum* (Rh) before sowing. The seeds of soybeans were grown in experimental pots (10L capacity) filled with non-sterilized soil: sand mixture (3:1). Water status was monitored by weighing the pots and water was supplied to maintain soil moisture at 70% of water holding capacity (WHC) before exposing the plants to water stress. The soil moisture content was reduced through natural evapotranspiration and then the water stress was maintained at 35% of WHC for 12 days. Plant morphological and physiological parameters were determined at the end of drought. The acid and alkaline phosphatase activity in soil was performed as described by Tabatabai and Bremner (1969). The root and nodules acid phosphatase activity was analyzed by procedure described by Kaous S. *et al*, (Kaous *et al*, 2009). Soil pH in distilled water (1:2.5 v/v soil:water) was determined in rhizosphere soil. Subsamples of soil were air-dried and used for measuring soil available P. The content of available soil phosphates was determined using the Murphy and Riley's method (Murphy and Riley, 1962). Statistical analyses were carried out using a STATISTICA 7 software program.

RESULTS AND DISCUSSIONS

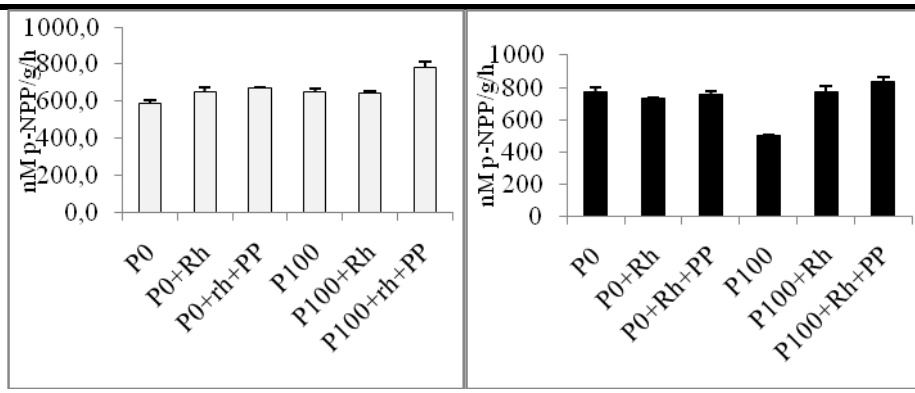
The use of microorganisms provides better plant nutrition and promotes plant growth. It is well documented that leguminous species, particularly soybean (*Glycine max* L) have a higher demand in phosphorus nutrition. Nitrogen-fixing microorganisms besides their ability to assimilate nitrogen from atmosphere have a contribution for promoting the growth and nutrition of crops.



A B

Fig. 1 Effect of P fertilizer and *B. japonicum* inoculation on the root (A) and nodules (B) acid phosphatase activity of soybean under well water (grey bar) and water stress (dark bar). Bars represent the means with SE.

Experimental results showed that treatment with such kind of rhizobacteria affected the activity of acid phosphatases in roots and rhizosphere soil. It was observed that under water deficit conditions the phosphatase activity was enhanced in roots of inoculated plants grown under P deficiency in comparison to uninoculated plants (fig. 1A). The same trend was revealed in treatments with combined application of rhizobacteria and P in well-watered and drought stressed plants. Hence, it was found that soybean with application of *B. japonicum* had higher root phosphatase activity than their uninoculated counterparts. As a consequence, more soil available phosphorus could be released with an increase in rhizobacteria mediated acid phosphatase resulting in partial alleviation of drought stress as well as P deficiency (Stancheva *et al,* 2008). According to investigations of Araujo A. (2008) bean plants subjected to P-deficiency increased the activities of phosphatases and phytases in nodules. This response constitutes an adaptative mechanism for N2-fixing legumes to tolerate P deficiency by improving the utilization of the P within nodules. Similarly, in our study the utilization of rhizobacteria had beneficial impact on the activity of acid phosphatase of nodules in relation to water supply (fig. 1B). The P insufficiency treatment (P0) caused an increase in nodule phosphatase activity under inoculation when compared to the P sufficient supply. In addition, it was established that the enzyme activity increased in treatment with *Bradyrhizobium japonicum* application together with phosphorus fertilizer.

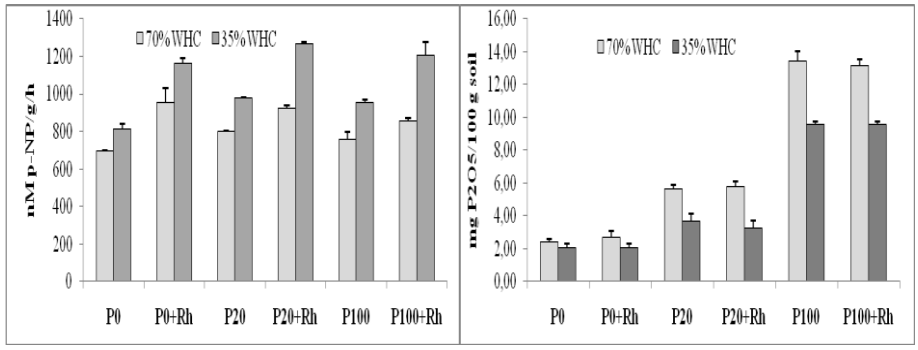


A B

Fig. 2 Effect of P fertilizer and *B. japonicum* inoculation on the soil acid phosphatase activity under normal water (A) and water deficit (B). Bars represent the means with SE.

The P fertilization alone decreased this parameter irrespective of soil moisture level compared to treatment without fertilization. We agree with the assumption that the increase of the acid phosphatases activities in nodules and roots may constitute a biological option to improve P assimilation and to attenuate partial P deficiency (Boudanga *et al*, 2015).

Soil enzyme activities in the rhizosphere soils of legumes serve many fundamental biochemical roles. The experimental data showed significant differences in acid and alkaline phosphatases activities in soil with the application of *B. japonicum* bacteria and P (Fig. 2 and 3). In general, increases in acid and alkaline phosphatase activities of soil were recorded with the inoculation of *Bradyrhizobium japonicum.* This may be attributed to increased plant roots growth (Lugtenberg and Kamilova, 2009), which in turn stimulated the proliferation of soil microorganisms in the rhizosphere. Their influence was observed in both soil water regimes well-watered and water deficit. Likewise, the impact of rhizobacteria was evident in the pots with P fertilization, but the increases were lower compared to the unfertilized treatment. We suppose that the roots of *Glycine max* L. supplied with P in doses 20 and 100 mg per kg of soil secreted less phosphatases compared to the treatment without fertilization. In general, drought contributed to the increase of the enzyme activity of soil. Probably, water deficit diminished the content of available phosphates in the soil. The application of phosphorus in low dose (20 mg P/kg soil) alone or in combination with *B. japonicum* stimulated the alkaline phosphatase activity of rhizosphere soil in comparison to unfertilized and non-inoculated treatments (fig. 3B). The use of this kind of rhizobacteria increased the alkaline phosphatase activity in soil by 28% in pots of normal soil water regime and by 38% in the rhizosphere soil of plants subjected to water deficit. Under utilization of rhizobacteria together with P fertilizer the increases were less and was 11.8% under optimal water regime and 24.4% under moderate drought conditions. The administration of a higher dose of P (100 mg/kg) did not change this parameter comparing with a low dose.



A B

Fig. 3 The soil alkaline phosphatase activity (A) and the phosphates content (B) in the rhizosphere soil of soybean inoculated with *B. japonicum* and P fertilization grown under sufficient moisture (grey bar) *versus* deficit moisture (black bar). Data are means ± SE.

In agricultural soils the solubilization of inorganic phosphate is closely associated with the activity of soil microorganisms including rhizobia (Tarafdar and Claassen, 1988). The content of available phosphates in the rhizosphere soil was significantly higher in P fertilized treatments than in the treatment without fertilization (fig. 3B). The use of *B. japonicum* had no significant impact on the content of available phosphates in soil. Only a moderate increase (by 10%) of mobile phosphates in rhizosphere soil was observed in treatment with rhizobacteria administration under insufficient P supply.

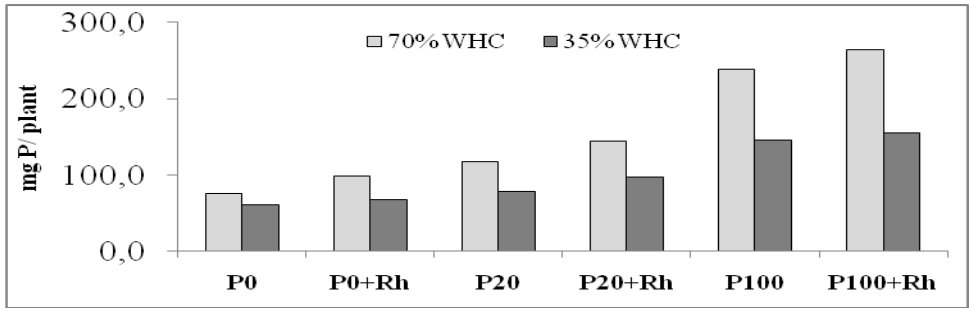


Fig. 4 The influence of rhizobacteria application and P fertilization on phosphorus uptake of soybean in relation to soil moisture conditions.

This increase was associated with a small decrease of pH in the rhizosphere soil. In this study, the values of soil pH were registered in relation to rhizobacteria application and P fertilization (data are not shown). There was only a trend of decreasing soil pH as the result of the application of rhizobacteria under limited water condition. An insignificant increase of this parameter was observed in treatment with rhizobacteria in combination with a low dose of P. The estimtion of P uptake revealed that the supplemental nutrition affected this parameter in

both water soil regimes (Fig. 4). But its effectivness was more pronounced on plants that were not limited with water. The rhizobacteria application increased P acquisition by plants especialy grown in soil with P deficiency. Thus, the increase of phosphatases activities under application of rhizobacteria would have a beneficial influence on growth and tolerance of legumes to abiotic factors.

CONCLUSIONS

1. The inoculation of soybean plants with *Bradyrhizobium japonicum* grown on low phosphorus fertility increased root and soil acid phosphatases activity and P uptake of plants in comparison with the uninoculated treatment. The enzymes activities were lowest under P fertilization without rhizobacteria administration.

2. The application of *B. japonicum* in P-deficit soil alone or together with a low dose of phosphorus fertilizer improved the drought tolerance of plants through modulation of the root acid and alkaline phosphatases as well as soil rhizosphere, thus facilitating phosphorus acquisition.

REFERENCES

1. **Andrieş S., 2007** - *Optimizarea regimurilor nutritive ale solului şi productivitatea plantelor de cultură.* Chişinău, 384p.
2. **Araújo A.P., Plassard C., Drevon J.J., 2008** - *Phosphatase and phytase activities in nodules of common bean genotypes at different levels of phosphorus supply.* Plant Soil, 312, 129-38.
3. **Boudanga L. Farissi M., Bouizgaren A., Ghoulam** C., **2015** - *Growth and nodulation in faba bean-rhizobia symbiosis under different soil phosphorus levels: acid phosphatase and phosphorus deficiency tolerance.* J. Mater. Env. Sci. 6, 997-1003.
4. **Kaous S., Dabes A., Slatni T., Labidi N., Drevon J.J., Abdelly C., 2009** - *Root proliferation, proton efflux, and acid phosphatase activity in common bean under phosphorus shortage.* J. Plant Biol. 5, 395-402.
5. **Lugtenberg B., Kamilova F., 2009** - *Plant-growth-promoting rhizobacteria.* Annu. Rev. Microbiol. 63, 541-556.
6. **Murphy J., Riley J.P., 1962 -** *A modified single solution method for the determination of phosphate in natural waters.* Anal. Chim. Acta. 27, 31-36.
7. **Nannipieri, P., Giagnoni, L., Landi, L., Renella G., 2011** - *Role of phosphatase enzymes in soil. Phosphorus in action, (Bunemann A. et al.,),* 215-243, Berlin.
8. **Stancheva M., Geneva E., Djonova N., Kaloyanova M., Sichanova M., Boychinova G., 2008** - *Response of slfalfa (Medicago sativa L.) growth at low accessible phosphorus source to the inoculation with mycorrhizal fungi and nitrogen fixing bacteria.* GEN Appl. Plant Physiol., 34 (3-4), 319-326.
9. **Tabatabai M.A., Bremner J.M., 1969** - *Use of p-nitro phenyl phosphate for assay of soil phosphatase activity.* Soil Biol Biochem; 1:301-307.
10. **Tarafdar J.C., Claassen N., 1988** - *Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms.* Biol. and Fertility of Soils, 5, (4), 308-312.

EFFECT OF DISTILLED BEVERAGES ON THE ANTIOXIDANT STATUS AND ON THE STABILITY OF POLYPHENOLIC COMPOSITION AFTER THE GASTROINTESTINAL DIGESTION, *IN VITRO* STUDY

INFLUENȚA DISTILATELOR ALCOOLICE ASUPRA STATUSULUI ANTIOXIDANT ŞI A STABILITĂȚII COMPOZIȚIEI POLIFENOLICE ÎN URMA DIGESTIEI GASTROINTESTINALE, STUDIU *IN VITRO*

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Abstract. *The in vitro effect of digestion on the quantity of phenols and on the antioxidant status after consuming three types of alcoholic beverages (bilberry brandy from commerce, cherry brandy from craft production and a topinambur distillate supplemented with natural extracts) was determined. There were identified a high stability of the total polyphenolic content and a relatively constant value of ABTS scavenging activity and of cupric reducing antioxidant capacity. This study also indicated a high stability related to the action of the pancreatin and of bile salts in the case of the artisanal sample. If the effect of alcohol intake was eliminated, the results have shown a reduced stability of the beverages containing bilberry and sour cherries, but also a reduced action of the distillate supplemented with spices – Sample 3.*
Key words: polyphenols, *in vitro* gastrointestinal digestion, antioxidant activity

Rezumat. *Efectul digestiei in vitro asupra cantității de fenoli şi a statusului antioxidant în urma consumului a trei tipuri de băuturi alcoolice (afinată din comerț, vişinată din producție artizanală şi un distilat din topinambur suplimentat cu extracte naturale) a fost determinat. S-a observat o stabilitate ridicată la nivel gastric a conținutului polifenolic total şi o valoare relativ constantă a inhibării radicalului ABTS şi a capacității antioxidante de reducere a cuprului. În acest studiu, a rezultat o stabilitate ridicată şi la acțiunea pancreatinei şi a sărurilor biliare a probei obținute artizanal. Dacă se elimină efectul ingestiei de alcool, rezultatele au demonstrat o stabilitate redusă a băuturilor ce conțin afin şi vişine, dar şi o acțiune mai redusă a distilatului suplimentat cu mirodenii – Proba 3.*
Cuvinte cheie: polifenoli, digestie gastrointestinală *in vitro*, activitate antioxidantă

INTRODUCTION

Fruits are the fermentable substrate most used to make natural distillates. In rural areas, from these, apples, plums and grapes are the most common sources of

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sugars, omnipresent in every peasant household. To improve the taste and to increase the palatability, the distillate obtained is supplemented with various natural juices from: cherries, sour cherries, blueberries etc. Beside the first two, the main juices are prepared from berries (Bermudez-Soto *et al.,* 2007). Excluding the ethanol and/ or sugar content, the consumptions of these beverages may be a beneficial one due to the content of active biological compounds in natural juices.

Among the bioactive compounds present, we distinguish mainly those which are part of the flavonoids category (for example, anthocyanins), which are found in high amount in *Vaccinium myrtillus* fruits. These compounds, identified in most of the fruits, are responsible for the antioxidant status which they express, which is significantly important against pathologies determined by the oxidative stress. Beside these, a strong antimicrobial effect can be added (Benzie *et al.,* 2011).Therefore, the action of gastrointestinal digestion on the antioxidant status and on total polyphenol stability was determined, following the consumption of two natural beverages (distillate of fruits supplemented with cherries syrup and distillate of topinambur supplemented with cumin, cinnamon and ginger). The antioxidant status was determined by the inhibition of two free radical species and of cupric reducing antioxidant capacity. *In vitro* simulation tests aimed at the pH and pepsin at gastric level, as well as that of bile salts and pancreatin for the small intestine.

MATERIAL AND METHOD

Samples. The following samples were used: bilberry brandy from commerce – Sample 1 (Angelli …), cherry brandy from craft production – Sample 2 (provided from Claudiu Raicu master student) and a topinambur distillate supplemented with natural extracts – Sample 3 (provided from SC Hypericum Impex SRL) – Sample 3.

ABTS scavenging activity. ABTS radical cations are produced by reacting ABTS (7 mM) and potassium persulfate (2.45 mM) on incubating the mixture at room temperature in darkness for 16 h. The solution thus obtained was further diluted with phosphate buffered saline to give an absorbance of 1.000. 50 µL sample was added to 950 µL of the ABTS working solution to give a final volume of 1 mL. The absorbance was recorded immediately at 734 nm with the Helios λ spectrophotometer. The percentage of inhibition was calculated with the following equation: % inhibition = [(Absorbance of control –Absorbance of test sample)/Absorbance control] x100 (Vamanu, 2013).

Reducing power. Cupric ion reducing power was evaluated by the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent, measuring the absorbance at 450 nm with a Helios spectrophotometer (Thermo Fisher Scientific, Inc., USA) (Pop *et al.,* 2016).

Total phenolic content. The reaction mixture was made from 0.5mL of the sample, 2.5 mL of Folin-Ciocalteu reagent in a dilution ratio of 1:10, and a saturated solution of sodium carbonate (75 g/L, 2 mL on average) was added 4 min later. The reaction mixture's absorbance was identified at 760 nm after a 2h incubation period, at room temperature. The reference standard engaged was Gallic acid, with the outcome provided as mg GAE (Gallic acid equivalent) of the extract (Vamanu, 2017).

***In vitro* simulation.** It was realized after the technology presented in the following patent request (Vamanu *et al*., 2011) by using sterile Duran screw thread tubes, GL with silicone seal for samples obtaining. The samples were taken with a seringe, 2 mL volume.

RESULTS AND DISCUSSIONS

The effect of gastric digestion

The antioxidant status and the total phenol level determined following the gastric transit is presented in table 1. Considering also the action of the lysozyme in the simulated saliva solution, it was determined that the total phenol content has increased a bit, mainly after one hour of action of the digestion determined by the pepsin. Results have been confirmed by the product Sample 1 (alcoholic beverage from commerce). Sample 2 was an exception, as it had an increase of the total phenols quantity which exceeded 50%. The proteolytic action of the pepsin combined with the low pH leads to an additional release of phenol compounds from the molecules resulted from the fruits juice added to the final formula.

The antioxidant status was determined after the consumption of Sample 2, which has maintained its ABTS scavenging activity. *In vitro* tests of evaluation of the antioxidant status for Sample 1 have decreased in time. For Sample 3, stability was noticed regarding the power of reduction, at the gastric transit, which might be interpreted as a constant presence of reductones.

Table 1

Total phenolic content and antioxidant status after *in vitro* digestion

Level of digestion		Total phenolic content (µg/mL gallic acid)			ABTS scavenging activity (%)			CUPRAC (450 nm)		
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Gastric level	0 h	18.25	22.25	13.85	75.47	94.91	29.21	1.14	1.31	0.69
	1 h	21.10	26.25	18.60	68.36	93.00	45.62	1.09	1.00	0.72
	2 h	20.00	30.00	20.00	53.00	72.75	50.00	1.05	2.10	0.67
Small intestine level	3 h	10.00	20.00	11.00	15.80	54.35	11.42	0.72	1.82	0.43
	4 h	10.00	18.76	9.69	14.68	53.87	10.67	0.68	1.74	0.45
	5 h	9.58	21.57	3.68	14.77	54.00	9.40	0.60	1.76	0.45
	6 h	7.65	19.67	4.78	15.04	54.24	8.75	0.51	1.67	0.48
	7 h	7.00	19.87	3.00	15.12	54.06	8.25	0.52	1.70	0.47

The effect of digestion in the small intestine

Following the digestion suffered by the pancreatic juice and the bile salts (tab. 1) it has resulted that Sample 2 was the most stable from the point of view of

the phenol composition. The differences were of maximum 6%. In exchange, the rest of the samples have manifested a low resistance after minimum two hours of digestion at this level. Sample 3 has showed a loss of over 60% of the total phenols quantity. This compared to the product traded – Sample 1, approximately 3 times higher. Results have demonstrated a low stability at this level, compared to the gastric digestion. An exception was represented by Sample 2 which showed a mix of stable phenol compounds under these conditions. Results obtained have demonstrated the presence of some similar phenol compounds in Sample 1 and Sample 3, which do not resist to the pH change, to enzymes and bile salts of the human digestive tube.

The antioxidant activity determined after the pancreatic digestion was high only for Sample 2, being of over 50%. For the other two samples, the ABTS scavenging activity did not exceed an average of 15%. These findings were directly correlated with the polyphenol level of table 1. The degradation suffered by auto-oxidation by these compounds during the digestion at neutral pH is a known aspect and corresponds with the presence of some derivates of the catechin.

Results of the value of the reduction power for Sample 3 might demonstrate an increase in time of the pH value due to the entire composition. The low pH value determines, normally, a chemical instability of the compound, which is compensated by the composition in bioactive compounds of the products (Lee *et al.*, 2003).

The same trend was also determined for CUPRAC, the maximum loss being calculated for Sample 1 (approximately 50%). The differences between the three samples are due to the conditions of products and to the fruit species from which they have been obtained, which directly affect the composition and stability of the bioactive compounds. Some of the compounds of Sample 1, which can be preserve the stability of the product in time, are added to all these.

According to some previous studies, following the gastric digestion, the derivates of the caffeic and cholorogenic acids are not affected by the pepsin attach and by that of the low pH. These compounds are found in the composition of extracts from species of plants used in making these drinks (Mechikova et al., 2010). Results obtained confirm the stability in various food products of the polyphenol acids. In addition, the slight increase of the total quantity (tab. 1) of polyphenols can be interpreted as a degradation of some compounds with higher molecular weight binding these molecules. Retention of these compounds is also confirmed by studies which showed absorption of the anthocyanins at gastric level (Bermudez-Soto *et al.,* 2007).

According to some previous studies there are important differences, of approximately 50%, between the quantities of polyphenols which shall be available and which can be absorbed, by studies *in vitro* in a static simulation system. For this research, a protocol similar to that of the in vitro testing of some microbial strains was used, but normally, there are changes in protocol. In

exchange, no other enzymes were added, for the digestion in the small intestine, as there is data in the literature that support the stability of various phenol compounds directly. If there was a direct consumption of fruits, the enzyme composition is important for the bioavailability of these compounds (for example, catechin) (Hur *et al*., 2011).

An important aspect is the estimation of results *in vivo*, as well as the comparison with other results obtained in other models of simulation *in vitro*. Most of the times, a compromise will be made between the constructive type (static or dynamic) of the simulator *in vitro* and the physiological requirements which need to be complied with. These types of results are valuable and fully accepted in case of extreme pathological conditions, such as gastric hypo or hyperacidity and/or pancreatic insufficiency (Guerra *et al*., 2012).

CONCLUSIONS

The results thus obtained demonstrate that the three types of beverages determine, after consumption, an important contribution of active biological compounds. Within reasonable limits, the beverages can be consumed without problems for the health, with the purpose to act as functional products. The beverage obtained from sour cherries presented the highest antioxidant status after the gastrointestinal digestion (expressed as ABTS scavenging activity and reduction power). The other two samples have shown a low stability, mainly after the action of the pancreatin and bile salts.

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REFERENCES

1. **Benzie I.F.F., Wachtel-Galor S., 2011** - *Herbal Medicine: Biomolecular and Clinical Aspects.* 2nd edition. CRC Press.
2. **Bermudez-Soto M.J., Tomas-Barberan F.A., Garcia-Conesa M.T., 2007 -** *Stability of polyphenols in chokeberry (Aronia melanocarpa) subjected to in vitro gastric and pancreatic digestion*. Food Chemistry, 102, p. 865–874.
3. **Guerra A., Etienne-Mesmin L., Livrelli V., Denis S., Blanquet-Diot S., Alric M., 2012** - *Relevance and challenges in modeling human gastric and small intestinal digestion*. Trends in Biotechnology, 30, p. 591-600.
4. **Hur S.J., Lim B.O., Decker E.A., McClements D.J., 2011** - *In vitro human digestion models for food applications*. *Food Chemistry*, 125, p. 1–12.
5. **Lee J.B., Ahn J., Lee J., Kwak H.S., 2003 -** *The microencapsulated ascorbic acid release* in vitro *and its effect on iron bioavailability*. Archives of Pharmacology Research, 26, p. 874-879.
6. **López de Lacey A.M., Giménez B., Pérez-Santín E., Faulks R., Mandalari G., López-Caballero M.E., Montero P., 2012** - *Bioaccessibility of green tea polyphenols incorporated into an edible agar film during simulated human digestion*. Food Research International, 48, p. 462–469.

7. Mechikova G.Y., Kuzmich A.S., Ponomarenko L.P., Kalinovsky A.I., Stepanova T.A., Fedorov S.N., Stonik V.A., 2010 - *Cancer-preventive activities of secondary metabolites from leaves of the bilberry Vaccinium smallii A. Gray*. Phytotherapy Research, 24, 1730-2.

8. Pop O.V., Vamanu A., Pop A.E., Vamanu E., Nita S., 2016 - *Evaluation of antioxidant and cytotoxic activity of alcoholic beverages from topinambour by in vitro and ex vivo tests.* Revista de Chimie (Bucharest), 67, p. 1301 – 1305.

9. Vamanu E., Vamanu A., Niţă S., Pelinescu D., Rusu N., 2012 – *Procedeu de testare a viabilităţii bacteriilor lactice la tranzitul tractului gastrointestinal uman*. Cerere brevet 01175/17.11.2011.

10. Vamanu E., 2013 - *In vitro antiradical activity of Betula verrucosa leaves*. Revista de Chimie (Bucharest), 64, p. 254 – 259.

11. Vamanu E., 2017 - *Antioxidant components and antioxidant activities of hydroglyceroalcoholic extract from fresh mushroom mycelium*. Revista de Chimie (Bucharest), 68, p. 534 – 540.

THE EFFECT OF ZINC ON GERMINATION AND SEEDLINGS GROWTH OF *ERUCA SATIVA* Mill.

EFECTUL ZINCULUI ASUPRA GERMINAȚIEI ŞI CREŞTERII PLANTULELOR LA *ERUCA SATIVA* Mill.

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Abstract. *The effect of zinc on seed germination and growth in the first ontogenetic stages in the species Eruca sativa was investigated. Zinc was used as sulphate solutions, in five different concentrations: 50 mg/L, 100 mg/L; 150 mg/L, 200 mg/L; 250 mg/L. We analyzed the following indicators: the percentage of germinated seeds; the length of root, the length of the hypocotyl, the tolerance index, the seedling vigor index, water content and dry matter content of the seedlings. The following effects were found: the insignificant modifications of the germination percentage; the significant delay of the growth in length of the root and of the hypocotyl; the decrease of the tolerance index, of the seedling vigor index and of the water content.*
Key words: zinc, germination, tolerance index

Rezumat. *S-a investigat efectul zincului asupra germinației semințelor şi creşterii în primele stadii ontogenetice la Eruca sativa Mill. Zincul s-a folosit sub formă de sulfat, în 5 concentrații diferite: 50 mg/L, 100 mg/L; 150 mg/L, 200 mg/L; 250 mg/L. S-au luat în studiu următori indicatori: procentajul de germinație, lungimea rădăcinii, lungimea hipocotilului, indicele de toleranță, indicele de vigoare a plantulelor conținutul de apă şi de substanță uscată din plantule. S-au constatat următoarele efecte: modificări nesemnificative statistic ale procentajului de germinație; reducerea semnificativă a lungimii rădăcinii şi a lungimii hipocotilului; scăderea indicelui de toleranță, a indicelui de vigoare a plantulelor şi a conținutului de apă.*
Cuvinte cheie: zinc, germinație, indice de toleranță

INTRODUCERE

Zinc is a microelement involved in the metabolism of some groups of substances (glucides, lipids, protein, auxins, etc.), in germination, growth, photosynthesis, etc. (Davidescu *et al*., 1988; Rout and Das, 2003; Tsonev and Lidon 2012), but in high concentrations it becomes toxic (Rout and Das, 2003). Symptoms of toxicity were visible in concentrations of zinc from leaf, higher than 300 mg/kg dry material or less than 100 mg/kg dry material (Chaney, 1993 and Marschner, 1995 quoted by Broadley *et al.*, 2007 and Malecka *et al*., 2012). As symptoms of zinc toxicity are quoted: delay/inhibition of plant growth, reduction of chlorophyll synthesis, reducing the rate of the photosynthesis, chlorosis, etc (Rout and Das,

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2003; Krusdar *et al.*, 2004; Broadley *et al*., 2007). The occurence of the toxicity phenomena was reported on land affected by human activities: depositing of the industrial and domestic waste; excessive application of fertilizers and pesticides that contain zinc, etc. (Broadley *et al*., 2007; Vasiliev *et al.,* 2011). Romanian legislation provides that the maximum permissible limit for the zinc content from soil is 100 mg/kg; the alert threshold is 300 mg/kg for the land with sensible use and 700 mg/kg for the land with less sensible use (Ordin nr. 756/1997 pentru aprobarea Reglementării privind evaluarea poluării mediului).

Eruca sativa Mill. (Brassicaceae family) with the updated name *Eruca vesicaria* (L) Cav. ssp. sativa (Mill.) Thell. is an annual herbaceous species, 20-60cm high, of Mediterranean origin (Săvulescu, 1958; Sârbu *et al.,* 2013). In Romania, it is spread sporadically in the field area and in the hills (Sârbu *et al*., 2013). The young leaves are used in alimentation. It is considered to be a good source of compounds with important nutritional value. A series of studies have underlined the chemical composition of the leaves (protein, glucides, mineral salts, vitamins, fatty acids, etc) (Bukhsh *et al.,* 2007; Nurzyńska-Wierdak, 2015) and seeds (Bukhsh *et al.,* 2007) and the potential medical properties (to prevent cancer, gastro-protective, antioxidant, etc.) (Michael, 2011; Saleh *et al.*, 2016). *Eruca sativa* has the ability to tolerate high metals (zinc, copper, cadmium, lead) in the environment of growth (Saleh, 2001; Zhi *et al*., 2015) and to accumulate some heavy metals (lead, cadmium) (Saleh, 2001).

This paper has as purpose the investigation of the effect of zinc on seed germination and growth of seedlings in the species *Eruca sativa* Mill.

MATERIAL AND METHODS

The biological material, was represented by seeds of *Eruca sativa* Mill. purchased from a shop specialized in seed marketing. The seeds were disinfected for 5 minutes with 1 % sodium hypochlorite solutions and afterward washed several times with distilled water. The seeds were placed to germinate in Petri dishes, on a filter paper humidified with distilled water (a control variant) and zinc sulphate solutions (treatment variants). The concentration of zinc from solutions were: 50mg/L (V1), 100mg/L (V2); 150 mg/L (V3), 200 mg/L (V4), 250 mg/L) (V5). Plates were kept at laboratory condition: at room temperature(20^0C - 24^0C), a photoperiod corresponding to the month of May, 2017. The total duration of the experiment was of 7 day after mounting. For each variant we used 4 replications, each replication with 15 seeds Germinated seeds were counted each 24 hours. After 7 days the root length and hypocotyl length was measured at a number of 40 seedlings for each experimental variants; the water and dry matter content of seedlings was determined by the gravimetric method (Boldor et. al., 1983). The following indicators were analyzed: the percentage of germinated seeds (at 1- 4 day, 7 days); the length of the root, the length of the hypocotyls and the seedlings; the tolerance index; the seedling vigor index, the water and dry matter content of seedlings. The tolerance index of heavy metals (TI) was calculated by the formula described by Iqbal and Rahmati, (1992) (Ahmad *et al*., 2012). The seedling vigor index was calculated by the formula described by Moradi *et al*., 2008. Also, the ratio of root length and hypocotyl length was calculated. The results presented in figure and tables are expressed as mean value ± standard error

(for the germination percentage n = 4; for the morphological indicators n = 40). The data obtained from the germination percentage, and the morphological indicators were statistically interpreted. The unifactorial Anova test followed by the Tukey test (α = 0.05) was used in order to test the differences between means (Zamfirescu and Zamfirescu, 2008).

RESULTS AND DISCUSSIONS

During the first 4 days after assembling the experiment, the *percentage of germination* presented average values smaller in the variants of treatment than in the control. The lowest average values were registered in the variant of treatment V5; in this case, the difference from the control was reduced gradually from the first day until the fourth day. After seven days, the *percentage of germination* registered a slight increase in value comparing with the control in the variants V1-V4 and with a decrease in value, respectively, by 10.35% in the variant V5; these modifications are insignificant statistically ($p>0.05$) (Figure 1). The results obtained suggest the fact that the germination of the seeds of *Eruca sativa* is tolerant to zinc in the concentrations used, fact signalled by other authors as well, but for other concentrations of zinc (Ozdener and Kutbay, 2009; Zhi *et al.*, 2015).

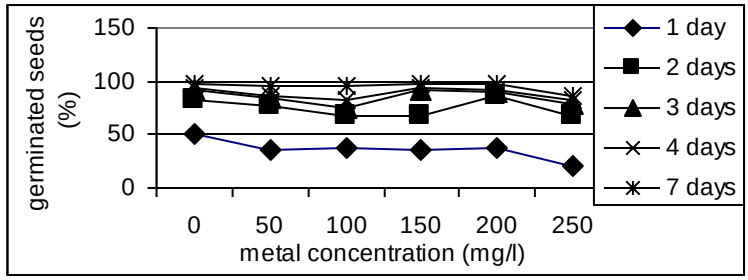

Fig. 1 The dynamics of germination at *Eruca sativa*

The length of the root (LR), *the length of the hypocotyl* (LH) and *the length of the seedling* (LS) registered a decrease in value comparing with the control in all the variants of treatment (by 15.58 % - 86.91 % comparing with the control in the case of the root; by 11.42 % - 62.35 % in the case of the hypocotyl; by 14.05 % - 76.93 % in the case of the seedling). From the statistic point of view, the reduction was significant ($p\leq0.05$) in the variants V2-V5 in the case of the root and in all the variants in the case of the hypocotyl and seedling (tab. 1). The unfavourable effect was very acute in concentrations of 200mg/L and 250mg/L zinc in the environment of growth. The proportion between the length of the root and the length of the hypocotyl (LR/LH) reduced in value gradually with the increase of the concentration of the metal, the reduction being significant in the variants V3-V5 (tab. 1). The morphological parameters of the seedlings exposed to the treatment with zinc varied inversely proportional with the concentration of the metal.

Table 1

The analyzed indicators

The indicator	The variants					
	Control	V1 (50mg/l)	V2 (100mg/l)	V3 (150mg/l)	V4 (200mg/l)	V5 (250mg/l)
LR (mm)	34.9±1.61	29.37±1.4	26.25±1.58*	17.02±1.75*	8.6±1.09*	4.57±0.72*
LH (mm)	23.82±0.73	21.1±0.62*	18.05±0.81*	15.32±0.63*	13.32±0.65*	8.97±0.47*
LS(mm)	58.72±1.66	50.47±1.6*	44.3±1.8*	32.22±2.09*	21.95±1.58*	13.55±1.12*
LR/LH	1.53	1.43	1.57	1.06*	0.60*	0.47*
IT (%)	-	84.15	75.21	48.76	24.64	13.09
ISV	567.39	478.8	420.8	317.6	215.34	116.99
W (g%)	90.5	89.76	87.18	87.4	86.24	82
DS (g%)	9.5	10.24	12.82	12.60	13.76	19.80

Note: * indicates significant differences (Tukey test, $p<0.05$)

The reduction of the process of growth in length for the root and the hypocotyls in the case of the treatment with zinc was also reported by other authors: Ozdener and Kudbay (2009), Al-Quariny (2010) at *Eruca sativa*; Çavuşoğlu *et al*., (2009) at *Phaseolus vulgaris;* Sharma *et al.,* (2010) at *Cicer arietinum;* Ashagre *et al.,* (2013) at *Lycopersicon esculentum*. The results obtained have underlined the fact that the process of growth for the seedlings (especially the growth in length of the root) comparing with the process of germination was more sensitive to the treatment with zinc in the concentrations used. Similar observations have been reported also by other authors: Ozdener and Kudbay (2009), at *Eruca sativa;* Li *et al.,* (2005), at *Arabidopsis thaliana* for zinc and other heavy metals. The effect noticed on the process of incipient growth of the seedlings of *Eruca sativa* would be probably due to the metabolic and physiological disorders caused by the ions of zinc in excess in the solutions used for treatment. The ions of zinc in excess induce the appearance of the oxidative stress (Jain *et al.,* 2010), the chromosome aberrations (Liu *et al.,* 1996; Jain *et al*., 2010); they inhibit the cell division and elongation (Khudsar *et al.,* 2004), the growth of the root (Liu *et al.,* 1996), the growth of the plants; they determine the browning of the root (Vassilev *et al.*, 2011).

Index of tolerance (IT) presented a gradual decrease in value, with the increase of the metal concentration. Values of IT less than 50 % were registered in concentrations between 150mg/L – 250mg/L (tab. 1). *Index of seedling vigour* (ISV) registered a decrease in value gradually with the increase of the zinc concentration in the solutions used for the treatment (tab. 1). For concentrations between 150mg/L - 250mg/L, the rate of reduction comparing with the control for the ISP was between 44.05 % - 79.33%. The decrease of the index of tolerance and the index of vigour with the increase in the zinc concentration was reported by Ashagre *et al*. (2013) in the species *Lycopersicon esculentum*.

The water content (W) in the seedlings presented a decrease in value in the variants of treatment comparing with the control (tab. 1). Similar effects were reported also by Singh *et al.* (2007) in the seedlings of *Triticum aestivum* in the

case of the treatment with concentrations of 5-100 mg/L copper. Water is essential for germination and growth; the results obtained suggest a possible disorder of the processes of absorption and use of water in the germination stage and post germination. *The content of dry substance* (DS) presented reversed amplitudes comparing to those described in relation with the water content (tab. 1).

CONCLUSIONS

1. *Eruca sativa* has the ability to germinate and grow in the first ontogenetic stages, in the presence of some moderate and high concentrations of zinc; the level of tolerance to zinc was higher in the phase of germination.

2. The process of growth during the first ontogenetic stages was affected proportionally with the increase in the metal concentration.

REFERENCES

1. **Al –Qurainy F., Alameri A. A., Khan S., 2010 -** *RADD profile for the assessment of genotoxicity on a medicinal plant, Eruca sativa*. Journal of Medicinal Plant Research, 4 (7), p. 579-586.
2. **Ahmad I., Akhtar M., J., Zahir Z. A., Jamil A., 2012** - *Effect of cadmium on seed germination and seedling growth of four wheat (Triticum aestivum L.) cultivars*. *Pak. J. Bot*., 44 (5)p. 1569-1574.
3. **Ashagre H., Almaw D., Feyisa T., 2013** - *Effect of copper and zinc on seed germination, phytotoxicity, tolerance and seedling vigor of tomato (Lycopersicon esculentum L. cultivar Roma VF).* International Journal of Agricultural Science Research, 2 (11), p. 312-317.
4. **Boldor O., Raianu O., Trifu M., 1983** – *Fiziologia plantelor (lucrări practice).* Editura Didactică şi Pedagogică, Bucureşti, p. 5-8.
5. **Broadley M. R., White P. J., Hammond J. P., Zelko I., Lux A**., **2007**- *Zinc in plants.* New Phytologist, 173, p. 677-702.
6. **Bukhsh E., Malik S. A., Ahmad S. S., 2007**- *Estimation of nutritional value and trace elements content of Carthamus oxyacantha, Eruca sativa and Plantago ovata*. Pak. J. Bot., 39(4), p. 1181-1187.
7. **Çavuşoğlu K., Yalçin E., Ergene A., 2009** - *The citotoxic effects in zinc and cadmium metal ions on root tip cells of Phaseolus vulgaris L. (Fabaceae).* SDU Journalo Science (E-Journal), 4 (1), p. 1-11.
8. **Davidescu D., Davidescu Velicica, Lăcătuşu, L**., **1988** - *Microelementele în agricultură.* Editura Acad. R. S. R., Bucureşti, p. 75-80.
9. **Jain R., Srivastava S., Solomon S., Shrivastava A.K., Chandra A., 2010** - *Impact of excess zinc on growth parameters, cell division, nutrient accumulation, photosynthetic pigments and oxidative stress of sugarcane (Saccharum spp.).* Acta Physiol. Plant., 32, p. 979-986.
10. **Li W., Khan M. A., Yamaguchi S., Kamya Y., 2005** - *Effects of heavy metals on seed germination and early seedling growth of Arabidopsis thaliana*. Plant Growth Regulation 46, p. 45-50.
11. **Liu D., Jiang W., Wang C., 1996** - *Effects of Zn^{2+} on root growth, cell division and nucleoli of Allium cepa L.* Journal of Environmental Science, 8(1), p. 21-27.
12. **Khudsar T., Mahmooduzzafar Iqbal M., Sairam E. K., 2004** - *Zinc induced changes in morpho-physiological and biochemical parameters in Artemisia annua*. Biologia plantarum, 48 (2), p. 255 - 260.

13. Malecka A., Piechalak A., Mesinger A., Hanć A., Barałkiewicz D., Tomaszewska B., 2012 - *Antioxidative defense system in Pisum sativum roots exposed to heavy metals (Pb, Cu, Cd, Zn).* Pol. J. Environ. Stud., 21 (6), p. 1721-1730.

14. Michael H. N., Shafik R. Z., Rasmy G. E., 2011 - Studies on the chemical constituents of fresh leaf of *Eruca sativa* extract and its biological activity as anticancer agent in vitro. Journal of Medicinal Plants Research Vol. 5(7), p. 1184 -1191.

15. Moradi D., Sharifzadeh P.F., Janmohammadi M., 2008 - *Influence of priming techniques on seed germination behavior of maize inbred lines (Zea mays L.).* ARPN J. Agric. Biol. Sci., 3, p. 22-25.

16. Nurzyńska-Wierdak R., **2015** - *Nutritional and energetic value of Eruca sativa Mill. leaves*. Acta Sci. Pol. Hortorum Cultus, 14(4), p. 191-199.

17. Nurzyńska-Wierdak R., **2015** - *Protein nutritional value of rocket leaves and possibilities of its modification during plant growth*. Turkish Journal of Agriculture and Forestry, 39, p. 1- 6.

18. Ozdener Y., Kutbay H. G., 2009 – *Toxicity of copper, cadmium, nickel, lead and zinc on seed germination and seedling growth in Eruca sativa*. Fresenius Environmental Bulletin, 18 (1), p. 26-31.

19. Rout G. J., Das P., 2003 - *Effect of metal toxicity on plant growth and metabolism*, I. Zinc. Agronomie, 23, p. 3-11.

20. Saleh A. A. H., 2001- *Effects of Cd and Pb on growth, certain antioxidant enzymes activity, protein profile and accumulation of Cd, Pb and Fe on Raphanus sativus and Eruca sativa seedlings*. Proceeding of the First International Conference (Egyptean British Biological Society, EBB Soc). Egyptean Journal of Biology, 3, p. 131-139.

21. Saleh M. M., Qader S. W., Thaker A. A., **2016** - *Gastroprotective activity of Eruca sativa leaf extract of ethanol - induced gastric mucosal injury in Rattus norvegicus*. Jordan Journal of Biological Science, 9 (1): 47-52.

22. Sârbu I., Stefan N. Oprea A., 2013 **-** *Plante vasculare din România. Determinator ilustrat de teren*. Ed. Victor B Victor, Bucuresti, p.466.

23. Săvulescu T., **(Ed.) 1958** – *Flora R.P. R*. vol.VI, Editura Academiei R.S.R, Bucureș ti, p.168.

24. Sharma S., Sharma P., Datta S. P., Gupta P., 2010 - *Morphological and biochemical response of Cicer arietinum L. var. pusa-256 towards an excess of zinc concentration*, Life Science Journal, 7 (1), p. 95 - 98.

25. Singh D., Nath K., Sharma Y. K., 2007 - *Response of wheat seed germination and seedling growth under copper stress*. J. Environ. Biol., 28 (2), p. 409 - 414.

26.Tsonev T., Lidon F.J.C., 2012 - *Zinc in plants* - An overview. Emir J. Food Agric., 24 (4), p. 322 - 333.

27. Vassilev A., Nikolova A., Koleva L., Lidon F., **2011 -** *Effects of excess zinc on growth and photosynthetic performance of young bean plants.* Journal of Phytology, 3 (6), p. 58-62.

28. Zamfirescu Ş., Zamfirescu Oana, 2008 - *Elemente de statistică aplicate în ecologie.* Editura Univ. „Al. I. Cuza", Iaşi, p. 108-116.

29. Zhi Y., Deng Z., Luo M., Ding W., Hu Y., Deng J., Li Y., Zhao Y., Zhang X., Wu W., Huang B., 2015 - *Influence of heavy metals on seed germination and early seedling growth in Eruca sativa Mill*. American Journal of Plant Science, 6, p. 582-590.

30. *****,** Ordin nr. 756/1997 pentru aprobarea Reglementării privind evaluarea poluării mediului.

THE EFFECT OF MULCH AND DENSITY ON THE RHUBARB YIELD

EFECTUL MULCIULUI ŞI DENSITĂŢII ASUPRA PRODUCŢIEI DE REVENT

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Abstract. *The aim of the present work has been to study the influence of technological factors (density and methods of mulching), on the total yield of rhubarb, in the case of Victoria and Glaskin's perpetual cultivars and the local population "De Moldova". Applying differential cultivation technology, the rhubarb yield varies according to the mulching system and crop density. The highest total production was obtained at straw mulching and density of 13.330 plants·ha*$^{-1}$*. Statistically assured yields were also obtained at the same density but without mulching. The total yield varied within wide limits according to the two technological factors, ranging from 26.37 t·ha*$^{-1}$ *to 43.72 t·ha*$^{-1}$*.*
Key words: cultivar, local population, yield, density

Rezumat. *Scopul lucrării de faţă a fost acela de a studia influenţa unor factori tehnologici (densitate şi metode de mulcire), asupra producţiei totale de revent, in cazul cultivarelor Victoria, Glaskin's perpetual şi populaţia locală „De Moldova". Prin aplicarea diferenţiată a tehnologiei de cultivare, producţia de revent variază în funcţie de sistemul de mulcire şi de densitatea culturii la înfiinţare. Cea mai ridicată producţie totală s-a înregistrat în cazul în care mulcirea s-a realizat cu paie, iar plantarea s-a făcut la o densitate de 13.330 plante/ha. Producţii, de asemenea, asigurate statistic au mai fost obţinute şi în cazul acelorași densităţi, dar în situaţia nemulcirii. Producţia totală a variat în limite foarte largi în funcţie de cei doi factori tehnologici, variind de la 26,37 t/ha la 43,72 t/ha.*
Cuvinte cheie: cultivar, populaţie locală, producţie, densitate

INTRODUCTION

The rhubarb (*Rheum rhabarbarum* L.) is a less known and spread crop in Romania. It is a perennial vegetable species, adapted to cold temperate climate (Ciofu *et al*., 2004; Indrea *et al.*, 2007).

Rhubarb originated in the Himalayas, where its root was an important medicine believed to purge the body of ill humors (Stan *et al.*, 2003).

In our country rhubarb is more cultivated in the western part of the country and it is used for compote, jam and other deserts (Treptow H, 1985).

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In recent years, rhubarb products have been spread by the supermarkets on all over the country. So, it is a good opportunity for farmers to cultivate this species in other parts of the country as well, not only in the traditional ones.

For this reason, our research was focused on evaluating the possibilities to cultivate rhubarb in the environmental conditions of the Eastern part of Romania.

To achieve this goal, our objective was to study the influence of the planting distances and mulching methods on the crop and, mainly, on the yield (Stoleru, 2013).

The distance between plants in the row and between rows is a technological factor influencing crop density, which is the number of plants per unit area. This technological factor can be determined directly from the feeding soil surface, light regime etc. (Loughton, 1969).

Mulching is a technique through which the surface between cultivated plants is covered with a thin layer of different materials, a process which clearly shows a number of features highlighted over time through experience and practice: preventing the barnyard grass and weeds emergence, keeping moisture in the soil and allowing faster soil warming, improving air system and soil porosity, keeping clean the edible parts in contact with soil, favorably influencing production, precocity and quality (Bakker *et al.*, 1985).

MATERIAL AND METHOD

Experiment site. To achieve the goal and objectives of this research work, an experimental design was done at "V. Adamachi" Experimental Station of the Agronomic University, using root cuttings of the Victoria cultivar (fig. 1), Glaskin's perpetual cultivar (fig. 2) and the local Population "De Moldova" (fig. 3). The harvested area of the experimental plots covered the 12 plants.

Fig. 1 Rhubarb – Victoria (original)

Fig. 2 Rhubarb – Glaskin's perpetual (original)

Fig. 3 Rhubarb – Local population „De Moldova"

Considering the importance of studying factors in the growing technology, their ability to change, and taking into account the possibilities of organizing experience, a hierarchy of factors was established, as follows:

1. A factor – density, with two graduations: 13.330 plants·ha^{-1} and 10.000 plants·ha^{-1};

2. B factor – mulching system, with four graduations: mulching with straw, mulching with black polyethylene film of 15 µ, mulching with black polyethylene film of 30 µ and unmulched.

Collection and processing of the experimental data. The experimental data collection was carried out by observations and weight measurements, according to the experimental technique used in experiments. During 2016, a total of eight harvestings were made: 16.04, 29.04, 11.05, 26.05, 8.06, 23.06 and 8.07.

The experimental variants were compared with the experimental mean, using the reported percentages and differences. The influence of the experimental factors was assessed using ANOVA. The significance of differences was assessed on the basis of LSD (least significant difference) for three degrees of confidence (95%, 99%, 99.9%).

RESULTS AND DISCUSSION

Applying differential cultivation technology, the rhubarb production varies according to the mulching system and crop density.

Regarding the influence of the density x mulch combination, the total yield of rhubarb crop ranged from 26.37 t·ha^{-1}, in the case of 10.000 plants·ha^{-1} density and mulching with black polyethylene film of 15 µ (a_2m_2), to 43.72 t·ha^{-1}, for 13.300 plants·ha^{-1} density and mulching with straw (a_1m_1), compared with the experimental average of 35.47 t·ha^{-1} (table 1).

The best yields were obtained for the 13.330 plants·ha^{-1} density and mulching with straw (a_1m_1), respectively unmulching (a_1m_4), with an average yield of 43.72 t·ha^{-1}, respectively of 42.43 t·ha^{-1}. The lowest yields were obtained in the case of 10.000 plants·ha^{-1} density and mulching with black polyethylene film of 15 µ (a_2m_2), respectively with black polyethylene film of 30 µ (a_2m_3), with an experimental average of 26.37 t·ha^{-1}, respectively of 28.03 t·ha^{-1}.

Table 1

Total yield of rhubarb crop

Variants	Total yield (t/ha)	% to the average	Difference to average (t/ha)	Significance of differences
a_1m_1	43,72	123,26	8,25	***
a_1m_2	35,24	99,35	-0,23	ns
a_1m_3	32,70	92,19	-2,77	00
a_1m_4	42,43	119,62	6,96	***
a_2m_1	38,87	109,59	3,40	**
a_2m_2	26,37	74,34	-9,10	000
a_2m_3	28,03	79,02	-7,44	000
a_2m_4	36,40	102,62	0,93	ns
x□ (Average)	35,47	100,00	0,00	-

LSD 5% = 0,97 t.ha; LSD 1% = 1,74 t.ha; LSD 0,1% = 3,94 t.ha
a_1 –13.330 plants.ha; a_2 –10.000 plants.ha;
m_1 – mulching with straw; m_2 – mulching with black polyethylene film of 15 µ, m_3 - mulching with black polyethylene film of 30 µ, m_4 - unmulched

CONCLUSIONS

1. Regarding the influence of the density and mulch methods on the rhubarb total yield during 2016, it ranged from 26.37 t·ha^{-1} for 10.000 plants·ha^{-1} density and mulching with black polyethylene film of 15 µ, to 43.72 t·ha^{-1} for variant mulching with straw, planted at 13.330 plants·ha^{-1} density.

2. The best yields were obtained for the 13.330 plants·ha^{-1} density and mulching with straw, respectively unmulching, with an average yield of 43.72 t·ha^{-1}, respectively of 42.43 t·ha^{-1}.

REFFERENCES

1. **Bakker J. J., Schroen G., Roelands C., 1985** – *With rhubarb cultivars there are large differences in yield and stem colour*. Groenten en Fruit, 41, 15, pp 62-63, 65.
2. **Ciofu Ruxandra, Stan N., Popescu V., Chilom P., Apahidean S., Horgos A., Berar V., Lauer K. F., Atanasiu N., 2004** – *Tratat de Legumicultura*. Editura Ceres Bucuresti.
3. **Indrea D., Apahidean S., Apahidean Maria, Maniutiu D., Sima Rodica, 2007** – *Cultura Legumelor*. Editura Ceres Bucuresti.
4. **Loughton A.; 1969** – *Rhubarb forcing*. Publications. Department of Agriculture, Ontario, 346, pp 15.
5. **Stan N., Munteanu N., Stan T., 2003** – *Legumicultura*, Vol III. Editura " Ion Ionescu de la Brad " Iasi.
6. **Stoleru V., 2013** - *Managementul sistemelor legumicole ecologice*. Editura "Ion Ionescu de la Brad" Iasi.
7. **Treptow H., 1985** – *Rhubarb (Rheum species) and its uses*. Flauss. Obst., 52, 8, pp. 419-422.

THE INFLUENCE OF THE CULTIVAR ON THE MAIN BIOCHEMICAL INDICATORS ON PEA SEEDS

INFLUENŢA CULTIVARULUI ASUPRA PRINCIPALILOR INDICI BIOCHIMICI ÎN SEMINŢELE DE MAZĂRE

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Abstract: *The main biochemical quality indices of seeds for the cultivated plants are influenced by a series of factors, such as: gentic, technological, abiotic or biotic factors. The storage period, the germination capacity and seed vigor are in direct correclation with the seeds' biochemical indices, which vary depending on the cultivar. The present work presents a study regarding the influence of the cultivar and storage period on the main biochemical indices of the garden pea seeds. The ash content varied in the case of pea seeds between 1.4% for the Skinado cultivar and 2.4% for the Television cultivar. The crude protein varied in the case of the cultivar selection under study between 20.20% for the Television cultivar and 27.40% for the Skinado cultivar, and the total lipids varied between 5.10% (Ambrosia cultivar) and 6.50% (Ran 1 round-seed and Kelvedon Wonder cultivars). The reducing sugars varied between quite large limits, from 10.20% in the case of the Television cultivar, up to 18.30% in the case of the Ran 1 wrinkled-seed cultivar.*
Key words: *Pisum sativum*, moisture, dry matter, proteins, sugars, lipids

Rezumat: *Principalii indici biochimici de calitate ai semințelor la plantele cultivate sunt influențați de o serie de factori, precum: factori genetici, factori tehnologici, abiotici sau biotici. Durata de păstrare, capacitatea de germinație şi vigoarea semințelor sunt în corelație directă cu indicatorii biochimici ai semințelor, care sunt variabili în funcție de cultivar.*
Lucrarea de fata prezintă un studiu cu privire la influența cultivarului şi a duratei de păstrare asupra principalilor indici biochimici ai semințelor la mazărea de grădină. Conținutul de cenuşă a variat la mazăre între 1,4% la Skinado până la 2,4% la cultivarul Television. Proteina brută a variat în cazul sortimentului studiat între 20,20% la Television până la 27,40% la cultivarul Skinadoiar lipidele totale au variat între 5,10% (Ambrosia) şi 6,50% (Ran 1 bob neted şi Kelvedon Wonder). Glucide reducătoare au variat în limite destul de largi, de la 10,20% în cazul cultivarului Television, până la 18,30% în cazul soiului Ran 1 bob zbârcit.
Cuvinte cheie: *Pisum sativum*, umiditate, substanță uscată, proteine, glucide, lipide

INTRODUCTION

It is well known that the main physical and biological quality indices of seeds are directly and clearly determined by the cultivation technology and,

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especially, by the harvesting and conditioning work and operations (Ciofu *et al.,* 2004).

These quality indices of seeds determined upon reception for storage are the phenotypic expression of each cultivar, an expression that sums up the influence of the genotype and the environment (the environmental conditions).

The seeds' physical and biological characteristics, expressed by moisture, germination, physical purity, health state, etc., are to a great extent the expression of the metabolic processes that have as a substrate the chemical and biochemical composition of seeds (Butnariu and Butu, 2014).

The chemical composition of pea seeds varies a lot depending on the maturation state of the seeds, the crop, as well as on the nutrition-related conditions (Dogaru *et al.,* 2006; Marin and Burada, 2007). The dry matter contained in the seeds varies a lot between 11,6 and 33,1%, depending on the cultivar. The proteins may vary between 2.48 and 9.12%, and the sugars content may rise from 8.1% to 20,1% out of the dry matter content (Enăchescu, 1984). The total nitrogen content varies within reduced limits in the case of pea seeds (15.08-15.80 g/100 g protein), depending on the crop (Atanasiu and Atanasiu, 2000). The total aminoacid quantities in the pea grains, from the same variety, have highlighted the fact that that the crop localization and the soil type determines a great variability of the chemical composition (Păcurar *et al.*, 2007).

Generally, in the case of the wrinkled-seed varieties, the dry matter content is more reduced, compared with the round-seed varieties. The sugars and reducing sugars content is greater in the case of the wrinkled-seed varieties, compared with the round-seed varieties, but from a qualitative perspective, the wrinkled-seed varieties are far superior (4.82% proteic matter in round-seed varieties and 2.74% proteic matter in wrinkled-seed varieties). The reducing sugars content depending on the crop varies from 2.41%, in round-seed varieties, up to 4.28%, in wrinkled-seed varieties.

Under these circumstances, the aim of the research was to establish to what extent the cultivar determines the quality of the seeds' metabolic substrate, namely their chemical and biochemical composition.

The present experiment offers solutions regarding the way in which the chemical and biochemical composition influences the seeds' quality, specifically their germination, as a main factor on which the seeds depend for their reception for storage, circulation and use in establishing new crops.

MATERIAL AND METHOD

The pea seeds from the 2015 harvest, from six cultivars, were used as a biological material: the Ambrosia, Television, Ran 1- non-wrinkle grain, Skinado, Ran 1- wrinkle-grain and Kelvedon Wonder cultivars.

The moisture determination was done according to AOAC 1999. Approximately 5 g from a sample in ampoules containing a known dry mass, previously numbered. After weighing them, they are introduced in the drying oven, previously set at a 105ºC

temperature. After the drying time is up, the ampoules are taken out of the drying oven and the mass is recorded.

This process is repeated until a constant mass is reached. For the majority of products, they are considered to have reached a constant mass when the difference between two successive weighings does not surpass 0.005 g.

The mineral substances (ash) determination was done according to AOAC 942.05 (Thiex *et al*., 2012).

The ash content was determined after the samples were calcinated in the calciner, at a 525 ± 25ºC temperature, until reaching a constant weight.

The total fibers content determination was done according to AOAC 973.18.

The method employs cationic detergents in order to remove the carbohydrates, the proteins uncomplexed by Maillard reactions and fats, leaving a fibrous residue made for the most part from cellulose and lignin or from insoluble protein complexes. After weighing the reflux tubes, the sample used for analysis is homogenized and then some quantities between 0.9 and 1.1 g are added in the tubes.

The weighed and noted tubes are introduced in the device fuelled with sulphuric acid and the cationic detergent, where the washing process begins. After this process ends, the reflux tubes are dried over night (100ºC).

The crude protein determination was done according to AOAC 955.04, the Kjeldahl method of determinig the total nitrogen (Butnariu and Butu, 2014).

The total lipids determination was done according to AOAC 963.15, the Soxhlet method of determining the total fats.

The reducing sugars determination is done by preparing the carbohydrate extract starting from a part of the quantity from the material to be analyzed, so that the sample would contain a certain amount of carbohydrates, which in turn would lead to obtaining a final extract with a sugar content below 1% (Stan *et al.,* 2003). The samples were treated with acetic acid (the precipitation with lead acetate is done in a weak acetic solution, because this way, the precipitate formed retains the carbohydrates). The excess of lead acetate is precipitated in turn with a saturated solution of sodium sulphate and it is filtered again. The solution is made up to volume with distilled water, in a volumetric flask of 250 ml capacity. In the case in which the materials used are dried fruits and vegetables, the necessary quantity needed to work with is around 5 – 10 g dried plant material.

RESULTS AND DISCUSSION

The results obtained show the fact that the cultivar factor may have a certain influence on the seeds' chemical and biochemical composition, which practically translates into the characteristics that determine the germination, as main characteristics depending on which the seed vigor and the quality that would ensure the production of an appropriate crop are assessed.

The cultivar, as a main production factor for any agricultural crop, proves its influence starting from the seed stage (table 1).

The seed moisture varied within the cultivar selection from 9.7% (in the case of the Television cultivar) to 13.4% (in the case of the Skinado cultivar), with an experimental avarage of 10.8%.

The moisture level for most cultivars falls within the limit of the experimental avarage, except for the Skinado cultivar, for which the moisture was

of 13.4%. This value also has a positive correlation with a higher moisture retention capacity in the case of this specific cultivar.

Table 1

The chemical composition of pea seeds regarding moisture, ash (mineral salts) and fibers

No.	Cultivar	Moisture (%)	Ash (%)	Total fibers (%)	Observations
1	**Ambrosia**	10,1	2,2	7,2	-
2	**Television**	9,7	2,4	5,8	Pea-weevil samples
3	**Ran 1 wrinkle-grain**	10,2	1,8	8,7	-
4	**Skinado**	13,4	1,4	7,6	-
5	**Ran 1 non-wrinkle grain**	10,8	1,6	7,4	-
6	**Kelvedon Wonder**	10,6	1,6	7,0	-
7	**x□ (Average)**	10,8	1,8	7,25	-

The ash content varied within the cultivar selection between 1.4% for the Skinado cultivar, and 2.4%, for the Television cultivar, with an experimental avarage of 1.8%. In the case of the Television cultivar, the greater ash content was also determined by the fact that the seeds were damaged by *Bruchus pisorum*.

In the case of the wrinkled-seed variety (Ran 1), the ash content was greater then the one found in the Ran 1 round-seed variety, with a growth of 0.2%.

The fibers content varied within wide limits, from 5.8% in the case of the Television cultivar, up to 8,7% in the case of the Ran 1 wrinkled-seed variety, with an average within the cultivar selection of 7.25%.

The crude protein, percentually determined, varied in the case of the cultivar selection under study between 20.20% in the case of the Television cultivar, up to 27.40% in the case of the Skinado cultivar, with an average recorded at the level of the entire experiment of 23,10%. For the majority of cultivars, the crude protein content varied between 22% and 23%, which is also shown in the literature on the matter (Enăchesu, 1984; Păcurar *et al.,* 2007; Butnariu and Butu, 2014).

A greater protein content indicates the fact that the variety is more stable for industrialization and it is positively correlated with a more reduced starch content, which makes it better for preservation.

In the case of the Ran 1 cultivars, the total protein varied within small limits, from 23.20% to 23.70% (table 2).

The total lipids varied between 5.10% (Ambrosia) and 6.50% (Ran 1 round-seed and Kelvedon Wonder), with an experimental average of 5,95%. Higher values of the total lipids content were also obtained in the case of the Skinado cultivar (6.40%).

The reducing sugars content varied within wide limits, from 10.20% in the case of the Television cultivar, up to 18.30% in the case of the Ran 1 wrinkled-seed cultivar, with an experimental average of 14.60%.

Values that were close to the experimental average were obtained in the case of the Skinado and Ran 1 round-seed varieties. Values that exceeded the experimental average were obtained in the case of the Kelvedon Wonder cultivar.

The starch content varied within wide limits, from 30.65% (Skinado) up to 48.30% (Television), with an experimental average of 38.29%. Values that were close to the experimental average were obtained in the case of the Ran 1 round-seed (36.70%) and Kelvedon Wonder varieties (36.59%). The high values of the starch content are correlated with low values of the lipids and proteins content.

Table 2

The chemical composition of pea seeds regarding the crude proteins, total lipids and reducing sugars

No.	Cultivar	Crude proteins (%)	Total lipids (%)	Reducing sugars (%)	Starch (%)
1	**Ambrosia**	21,80	5,10	12,20	43,80
2	**Television**	20,20	5,80	10,20	48,30
3	**Ran 1 wrinkled-grain**	23,70	5,40	18,30	33,70
4	**Skinado**	27,40	6,40	14,50	30,65
5	**Ran 1 non-wrinkled grain**	23,20	6,50	15,40	36,70
6	**Kelvedon Wonder**	22,31	6,50	17,00	36,59
7	**x□ (Average)**	23,10	5,95	14,60	38,29

CONCLUSIONS

In all cases, the value of the seeds' moisture level is under the maximum limit accepted by the standard, which should be under 14%, and this fact shows that the pea seeds were kept under optimal conditions.

In the majority of the cases, the average fibes content falls within the average limit. Lower levels of the fibes content are due especially to the degree of pea-weevil infestation.

The data presented highlight the fact that the Kelvedon Wonder cultivar is more equilibrated from a biochemical perspective, which made this variety to be highly appreciated by consumers, for over five decades.

The reducing sugars content varied within wide limits, from 10.20% in the case of the Television cultivar, up to 18.30% in the case of the Ran 1 wrinkled-seed variety, and the starch content varied within very wide limits, from 30.65% (Skinado) up to 48.30% (Television).

REFFERENCES

1. **Atanasiu C., Atanasiu N., 2000** - *O monografie a mazărei*. Editura Vertus Bucureşti.
2. **AOAC, 1999 -** *Official methods of analysis, 16th edn*. Association of Official Analytical Chemists, Washington.
3. **Butnariu M., Butu A., 2014** - *Chemical composition of Vegetables and their products*. Handbook of Food Chemistry, Springer, pp. 1-49.
4. **Ciofu R., Stan N., Popescu V., Chilon P., Apahidean S., Horgoş A., Berar B., Lauer F.K., Atanasiu R**., **2004** - *Tratat de legumicultură*, Editura Ceres, Bucureşti.
5. **Dogaru S., Ionete S., Criste E.A., 2006** - *Depozitarea semințelor de consum. Ghid de bune practici pentru siguranța alimentelor*. Editura Uranus Bucureşti.
6. **Enăchescu Georgeta, 1994** - *Tratat de biochimie vegetală. Compoziția chimică a principalilor plante de cultură. Legumele*. Editura Academiei Române, pp. 301-325.
7. **Marin Ş., Burada C., 2007** - *Controlul calității semințelor şi construcții pentru depozitarea semințelor*. Editura Universitaria Craiova.
8. **Păcurar I**. **et al., 2007** - *Producerea semințelor de cereale, leguminoase pentru boabe şi plante tehnice*. Editura Phoenix Braşov, pp. 133-147.
9. **Stan N., Munteanu N., Stan T., 2003** - *Legumicultură, vol. III*. Editura "Ion Ionescu de la Brad" Iaşi, pp. 127-141.
10. **Thiex N., Novotny L., Crawford A., 2012** - *Determination of ash in animal feed: AOAC official method 942.05 revisited*. J AOAC Int. Vol. 95(5), pp. 1392-7.

THE INFLUENCE OF THE STORAGE PERIOD OF PEA SEEDS ON THEIR GERMINATION CAPACITY

INFLUENȚA DURATEI DE PĂSTRARE A SEMINȚELOR DE MAZĂRE ASUPRA CAPACITĂȚII DE GERMINARE

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Abstract: *The present paper presents a study regarding the influence of the storage period on the germination capacity of garden peas seeds. The germination capacity of the garden peas seeds was done according to the Romanian standard SR 1634/1999, during a period of three years, between 2012-2014. The number of germs resulted naturally has constantly declined starting from 2012 up until 2014, in all cultivars, but the biggest decline was recorded in the Skinado (11.80 %) and Television (12,32 %) cultivars. The final value of the total germination in the case of the Television cultivar was of 76.5%, being below the acceptable limit for germination, that is of 80%.*
Key words*: Pisum sativum,* storage conditions, total germination

Rezumat: *Lucrarea de față prezintă un studiu cu privire la influența duratei de păstrare asupra capacității de germinare a semințelor la mazărea de grădină. Determinarea capacității de germinare a semințelor de mazăre s-a efectuat în conformitate cu SR 1634/1999, pe parcursul a trei ani, în perioada 2012-2014. Numărul germenilor dezvoltați normal s-a redus constant din anul 2012 până în 2014, la toate cultivarele, însă, cea mai mare scădere s-a înregistrat la cultivarele Skinado (11,80 %) şi Television (12,32 %). Valoarea finală a germinației totale în cazul cultivarului Television a fost de 76,5 %, fiind sub limita acceptabilă pentru germinare, respectiv 80%.*
Cuvinte cheie: *Pisum sativum,* condiții de păstrare, germinație totală

INTRODUCTION

In the past years, the expression "quality seeds" has been increasingly used, and it is a relative concept, defined, on the one hand, by the tougher requirements of the final seed users, and on the other hand by the evolution of the legislation and standards in the field, which establish the minimum requirements to be met, higher and more restrictive with each passing year, a concept which supposes that the seed should derive from some efficiend varieties and hybrids whose identity should be defined by: authenticity and varietal purity, specific morphological characteristics, as well as physical and physiological characteristics that would attest an appropriate cultural value.

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In order to identify the species and varieties, as well as to employ the correct approach to the conditioning, packaging and processing processes, there are some important characteristics that influence the seeds' behaviour in the mentioned processes, related to *shape and size*, *colour and sheen*, *chemical composition*, which may influence the storage conditions, through the different affinity that these have on the external factors that produce the deterioration of the quality, as well as related to the ratio between the carbohydrates, proteins, lipids, water and mineral salts (Butnariu and Butu, 2014).

Beside the biological value of seeds, that attests their belonging to a variety with proven productivity and quality characteristics, a great importance is placed on the seeds' physical characteristics, represented by: the percentage content of pure seed from that particular species compared to the total mass of the analyzed sample (*physical purity*), the ratio of seeds belonging to other species present in the basic seed, respectively, of seeds of other crop species and weed seeds, represented either in a numerical or gravimetric way (*botanical composition*), the seed moisture content, that should not surpass a certain critical level, depending on the species (*moisture*), as well as the physiological characteristics that define the quality of the seed, standardised through specific norms: the *germination faculty*, which shows the number of pure seeds, expressed in percentages, capable of producing normal germs in the laboratory, in the period and in the temperature, moisture and light conditions established as being optimal for each species, and which can be improved to a lesser extent than the physical purity and decides if the seed lot keeps or loses the value of seed fit for sowing; the *germination energy*, which expresses the germination speed of seeds in optimal conditions in which the germination faculty is determined, being for a long time considered as a valuable indicator, being correlated with a better power germination, with a faster and more uniform plant emergence, with a better resistance of plants to the unfavourable conditions from the first period of growth; the *vigor*, which is considered to be the sum of the characteristics that determine the seeds' level of activity and performance during the germination and emergence of the germs in the field, the seeds that emerge well in the field conditions, not always optimal, being the seeds with high vigor, and the seeds that have a weak emergence have a reduced vigor; the *viability* expresses the percentage of viable germs specific to a certain lot, allowing an orientation towards the germination faculty and it is determined when a quick orientation is needed regarding a seed lot or when the seeds are in a germinantion stand-by, and the *phytosanitary condition*, when thinking about the contamination of seeds with diseases or pest infestation, is very important in the characterization of the seeds' quality, the presence of quarantine organisms being banned, and the attack of harmful organisms being limited, in order to ensure a proper emergence and growth of the seeds, during the first stages of development, being recommended and necessary to treat the seeds in order to improve their resistance to the attack of harmful organisms.

The seeds' germination is a succession of biological phenomenons that happen at a cellular level and that ultimately determine the transformation of the embryo into a germ, on the basis of some chemical and physical modifications.

There are many situations in which, under normal environmental conditions and at the same germination percentage, the growth vigor of young seedlings varies a lot.

The main aim of the present paper was to highlight the germination capacity of six pea cultivars in two years of experiments.

MATERIALS AND METHODS

The process of determining the germination takes into account at least two working standards, the one for sampling and the one for determining the germination, coupled with the determination of the seeds' vigor and the standard for determining the seeds' viability.

Out of the total mass of pure seeds well-homogenized, 400 seeds are randomly counted – 8 repetitions of 50 seeds each, placed at a sufficient distance in order to ensure the necessary space needed for the germs' growth and nutrition, as well as for the protection of the seeds that are not contaminated by diseases. In the case in which the seeds are strongly infected, it is necessary for the paper substrate to be changed, at an intermediate count.

As a method of germination, the germination between-paper (BP) was used.

The seeds are placed to germinate between strips of paper, rolled and uniformly distributed (fig. 1). The placement of the seeds on the paper is done manually because the pea seeds are big enough to allow the proper development of the germs. The repetitions of placing the seeds in between stripes of paper are rolled and put into plastic bags, in order to maintain a constant level of moisture, and then they are placed in the germinator (Sanyo MLR), in a horizontal position.

Fig. 1. Germination pea seeds stage

Taking into account that the germination analysis is applied to a great number of species, in order to render uniform the methods used, the standard SR 1634/1999 establishes the requirements of each species in relation to the environmental factors, which are mandatory to be respected in any official accredited or authorized laboratory.

For the pea, the optimal germination temperature is around 20^{o}C and the readings for recording the values are done 5 days and 10 days respectively after the seeds are put in the germinator.

The seeds from the six cultivars have been analyzed in dynamic between 2012-2014.

After determining the physical purity, which was between 99.6 and 99.9%, the seeds were prepared in order to determine the germination, for the seeds obtained in 2011.

According to standard SR 1634/1999, the minimal germination for the pea seeds must be of 80%.

RESULTS AND DISCUSSION

The germ considered to be normal has the following normal essential structures:

- the radicular system is intact;
- the main root is intact or with slight defects, discolored or with necrotic stains, scars of cracks or fissures, superficial tears and cracks.

The germs that have a defective main root are classified as normal if they have enough well-developed secondary roots.

The data regarding the influence of the storage period on the germination of the pea seeds are presented in table 1. The data are presented for each cultivar and year of storage, as follows: the normally developed germs, the abnormal germs, the dead seeds and the value of the germination energy.

It is important to mention the fact that after germination, there have been no hard seeds or fresh seeds non - germinated.

In 2012, the seeds' germination has varied between 87.3%, in the case of the Television cultivar, and 98.5% in the case of the Kelvedon wonder variety, in 2013, the germination has varied between 81.5 % (Television cultivar) and 97.3% (Kelvedon wonder variety) and in 2014 the germination has varied between 76.5% (Televison cultivar) and 91.3 % in the Ambrosia cultivar.

In the case of the Ambrosia cultivar, we can say that the number of germs normally developed has decreased from 391 (2012) to 365 (2014), the germination percentage being reduced with 6.65 % during three years of storage.

In the case of the Television cultivar, we can say that the number of germs normally developed has decreased from 349 (2012) to 306 (2014), the germination percentage being reduced with 12.32 % during three years of storage.

In the case of the Ran -1 smooth seed cultivar, we can say that the number of germs normally developed has decreased from 384 (2012) to 343 (2014), the germination percentage being reduced with 10.68 % during three years of storage.

In the case of the Skinado cultivar, we can say that the number of germs normally developed has decreased from 373 (2012) to 329 (2014), the germination percentage being reduced with 11.80 % during three years of storage.

In the case of the Ran -1 wrinkled seed cultivar, we can say that the number of germs normally developed has decreased from 364 (2012) to 338 (2014), the germination percentage being reduced with 7.14 % during three years of storage.

In the case of the Kelvedon cultivar, we can say that the number of germs normally developed has decreased from 394 (2012) to 364 (2014), the germination percentage being reduced with 7.61 % during three years of storage.

The highest number of abnormal germs was recorded in the Televison cultivar, 32 (2012), which then grew up to 66 (2014).

From the data presented in figure 2, we can say that after three years of storage, the highest germination value was recorded in the Ambrosia cultivar (91.3) and Kelvedon wonder cultivar (91%), and the lowest value was recorded in the Television cultivar (76.5%).

Table 1

Results regarding the germination and plant emergence on paper substrate (2012-2014)

No. crt.	Cultivar	Values of germination indices*											
		2012				2013				2014			
		Normally developed germs (no.)	Abnormal germs (no.)	Dead seeds (no.)	Germination value (%)	Normally developed germs (no.)	Abnormal germs (no.)	Dead seeds (no.)	Germination value (%)	Normally developed germs (no.)	Abnormal germs (no.)	Dead seeds (no.)	Germination value (%)
1	Ambrosia	391	8	1	97.8	387	10	3	96.8	365	25	10	91.3
2	Television	349	32	19	87.3	326	41	33	81.5	306	66	28	76.5
3	Ran 1-bn	384	12	4	96.0	362	28	10	90.5	343	36	21	85.8
4	Skinado	373	23	4	93.3	354	33	13	88.5	329	51	20	82.3
5	Ran 1-bz	364	28	8	91.0	353	29	18	88.3	338	41	21	84.5
6	Kelvedon wonder	394	4	2	98.5	389	7	4	97.3	364	27	9	91.0

*with no hard or fresh ungerminated seeds

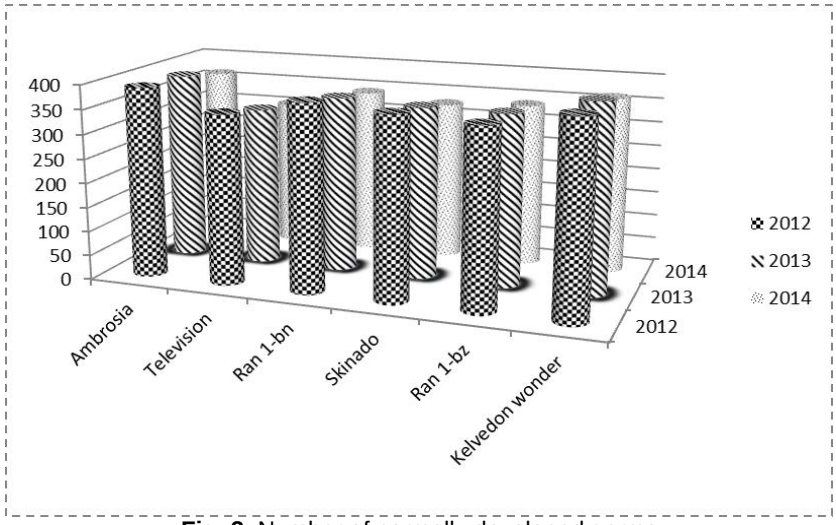

Fig. 2. Number of normally developed germs

CONCLUSIONS

The results presented in Table 1 highlight a direct correlation that exists between the pea cultivar and the seeds' germination percentage, which suggests that the storage process influences directly this process of germination.

The highest reduction of the germination percentage in the Television cultivar and the highest number of abnormal germs may be explained by the fact that in the seeds of this cultivar there has been identified the *Bruchus pisorum* pest, which results in the fact that after three years of storage, the seeds from this cultivar are unfit for sowing the crop.

REFERENCES

1. Atanasiu C., Atanasiu N., 2000 – *O monografie a mazării*. Editura Vertus, Bucureşti.
2. Butnariu M., Butu A., 2014 – *Chemical composition of Vegetables and their products*. *Handbook of Food Chemistry*, Springer, 1-49.
3. Ciofu R., Stan N., Popescu V., Chilon P., Apahidean S., Horgoş A., Berar B., Lauer F.K., Atanasiu R., 2004 – *Tratat de legumicultură*, Editura Ceres, Bucureşti.
4. Dogaru S., Ionete S., Criste E.A., 2006 – *Depozitarea semințelor de consum. Ghid de bune practici pentru siguranța alimentelor*. Editura Uranus, Bucureşti.
5. Marin Ş., Burada C., 2007 – *Controlul calității semințelor şi construcții pentru depozitarea semințelor*. Editura Universitaria, Craiova.
6. Muntean L.S. et al., 2003 – *Fitotehnie*. Editura Didactică şi Pedagogică, Bucureşti.
7. Munteanu N., 2000 – *Ameliorarea plantelor ornamentale*. Editura „Ion Ionescu de la Brad", Iaşi.
8. Mureşan T., Pană N.P., Cseresnyes Z., 1986 – *Producerea şi controlul calității semințelor agricole*. Editura Ceres, Bucureşti.
9. Stan N., Munteanu N., Stan T., 2003 – *Legumicultură, vol. III*. Editura "Ion Ionescu de la Brad", Iaşi.

MĂGURA - NEW VARIETY OF VINE FOR RED WINES CREATED AT S.C.D.V.V. ODOBEŞTI

MĂGURA - SOI NOU PENTRU OBȚINEREA VINURILOR ROŞII DE CALITATE CREAT LA S.C.D.V.V. ODOBEŞTI

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Abstract. *The studying the long-term of vine germplasm, results in choosing the most valuable genitors and perform a variety of intra- and interspecific hybridisation with obtaining valuable new genotypes, tolerant to pests and diseases and resistance to stressors. Of the many elite hybrid obtained at S.C.D.V.V. Odobeşti, in 2014 was approved the elite hybrid 18-46 under the name 'Măgura'- variety for quality red wines obtained by hybridization sexual of variety Babească neagră with the hybrid combination (Merlot x Alicante Bouschet). The new creation is characterized by middle-sized grapes (175g), small to medium berry (3.3 g), colored in black-blue, rich in anthocyanins in both the skin and core. The average grape production is 4.5 kg/vine or 16.6 tons/ha. It has a good resistance to the main cryptogamic diseases. The grapes reach maturity in the epoch IV. The wines obtained are extractive, intensely colored, tinctorial.*
Key words: variety, hybridization, elite hybrid, biological resistance

Rezumat. *Studierea pe termen lung a germoplasmei viticole are ca rezultat alegerea celor mai valoroşi genitori şi efectuarea unei game largi de hibridări intra şi interspecifice cu obținerea de genotipuri noi valoroase, cu toleranță la boli şi rezistențe la factorii de stres. Dintre numeroasele elite hibride obținute la S.C.D.V.V. Odobeşti, în anul 2014 a fost omologată elita hibridă 18-46 sub denumirea 'Măgura' - soi pentru vinuri roşii de calitate, obținut prin hibridarea sexuată a soiului Băbească neagră cu combinația hibridă (Merlot x Alicante Bouschet). Noua creație se caracterizează prin struguri de mărime mijlocie (175 g), boabe mici spre mijlocii (3,3 g), colorate în negru - albăstrui, bogate în antociani, atât în pieliță cât şi în miez. Producția medie de struguri este de 4,5 kg/butuc, respectiv 16,6 tone/ha. Are rezistență bună la principalele boli criptogamice. Strugurii ajung la maturitate în epoca a IV-a. Vinurile obținute sunt extractive, intens colorate, tinctoriale.*
Cuvinte cheie: soi, hibridare sexuată, elită hibridă, rezistență biologică

INTRODUCTION

The value of the varieties for obtaining the red wines is show both to level and the constant of grape production, its quality, defined by the ability of accumulation of sugars and anthocyanins in beans, as well as their tolerance to diseases and stress conditions. Research conducted in the last four decades in

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Romania, led to valuable vine, with high tolerance to disease, resistant to drought and frost (Moldovan *et al.*, 1994, Oprea *et al.*, 2007 Calistru and Damian 1999, Culcea *et al.*, 2004).

The valorization of valuable hybrid combinations with genetic resistance, obtained over time in scientific research in the area of improving the vine, is one of the major objectives of Research and Development Station for Viticulture and Oenology Odobeşti. In this contex it has created a new variety called ”Măgura”, which completes the varietal range of the varieties for obtain red wines quality, adapted to climatic conditions in vineyards in southeastern of Moldova.

MATERIAL AND METHOD

The study was carried out between 2012 - 2013, on a plantation aged 25 years, planting on soil type levigated,, located in the biological field SCDVV Odobeşti. For comparison, as a witness was used Băbească neagră variety, that represents the parents maternal and is similar in direction of production and epidermis' color.

The varieties Măgura and Băbească neagră was grafted on Kobber 5 BB rootstock, the training system with trunk of hub to the ground, with Dr. Guyot cutting system. Fruit load was 44 eye/hub, distributed on the canes with 9 eye and the spur with 2 eye. Distance of planting by 2.2 m x 1.2 m is returning 3788 but/ha. Were studied the ampelographic main characters, were made measurements and determinations on elements of fertility and productivity, the amount and quality of grape production, disease resistance, physico-chemical characteristics of the wine. The main climatic conditions of during the study period and multiannual values are presented in table 1.

Table 1

The main climatic conditions of study period (Odobeşti, 2012-2013)

Climatic Indicator	Multiannual (1946 -2011)	Crop year		Average 2012 - 2013
		2011 -2012	2012 -2013	
Annual				
The average temp., ºC	10.5	11.8	11.2	11,.
Temp max. abs. ºC	39.4	39.2	36.8	38.0
Temp min. abs., ºC	-22.8	-22.4	-17.3	-19.9
Amount degrees usuful temp.(Σºtu) ºC	1604.0	1961.4	1879.2	1920.2
The amount heatstroke hours	2113.9	2585.1	2318.9	2452.0
Precipitation amount, mm	617.3	382.4	800.8	591.6
On the vegetation period				
The average temp., ºC	16.9	19.7	18.4	19.0
Temp max. abs. ºC	39.4	39.2	36.8	38.0
Temp min. abs., ºC	-8.2	-0.3	-7.5	-5.8
Amount degrees usuful temp.(Σºtu) ºC	1581.2	1938.0	1865.0	1901.5
The amount heatstroke hours	1629.7	1949.8	1842.9	1896.4
Precipitation amount, mm	431.2	327.2	561.8	444.5

RESULTS AND DISCUSSIONS

Research period was characterized by availability heliothermic large, but with very low rainfall in 2012 and very high in 2013, unevenly distributed during the growing season. Amount degrees useful temperature was 1920.2 compared to multiannual value of 1604.0 and the amount of annual rainfall was 591.6 mm, of which 444.5 mm during the growing season compared to multi annual value (617.3 mm), of which 431.2 mm during the growing season.

On the background of this weather conditions, Măgura variety has started its vegetation through disbudding between 19th - 23th of April, without significant differences in comparison with the witness. The flowering phenophase located between 23th–25th of May, and the veraision recorded between 25th - 30th of July. Grapes' ripenning was realized between 25th of August - 5th of September, with approximately two weeks earlier that the witness variety (tab. 2).

In regards as the phenological spectrum cover in Odobeşti ecosystem conditions, the new variety Măgura completed their vegetation period after 190 days. Is the variety with the half-erlier maturation, grapes reach to full maturity in the first decade of September (epoch IV -a).

Table 2

Phenological spectrum in the conditions of Odobeşti vineyard

Variety	Disbudding	Flowering	Veraison	Physiological maturity	Fall leaves
Măgura	19-23.04	22-25.05	25-30.07	25.08 -05.09	31.10-10.11
Băbească neagră (Mt.)	20-25.04	23-27.05	05 - 08.08	18.09- 18.09	04.11-15.11

Fertility and productivity of the new variety appreciated by the percentage of fertile shoots, fertility coefficients (absolute and relative) and productivity indices (absolute and relative) shows the superiority of the new creation for the elements analyzed in comparison with to the witness. The primary buds' viability had values close to witness (tab. 3).

Table 3

Viability and the fertility/ productivity elements of the Măgura variety

(Odobeşti, average data 2012 – 2013)

Variety	Viability (%)	Fertile shoots (%)	Fertility coefficients		Productivity indices	
			Relative	Absolute	Relative	Absolute
Măgura	89.12	72.0	1.04	1.44	197.6	274.4
Băbească neagră (Mt.)	91.30	63.3	0.77	1.21	168.7	266.3

The Măgura variety show a potential fertility of 72.0%, higher than the control (63.3%). The same hierarchy exists and in the case the absolute fertility coefficient (1.44 to Măgura variety, respectively 1.21 to Băbească neagră) and the absolute productivity index (274.4 – Măgura, respectively 266.3 - Băbească neagră.

The main characters ampelographic of Măgura variety (fig. 1 and fig. 2), according 2nd edition of the OIV Descriptor list for grape varieties and Vitis species, 2009.

- Young shoot
  - Opening of the shoot tip: half open;
  - Intensity of anthocyanin coloration: low;
  - Density of prostrate hairs on the shoot tip: low;
- Young leaf
  - Upper side color of blade: green areas anthocyanins;
- Shoot:
  - Attitude (before tying); semi-erect
  - Color of the dorsal side of internodes: green with red;
  - Color of the ventral side of internodes: green with red;
  - Length of tendrils: medium/long;
- Mature leaf
  - Size of blade: small;
  - Shape of blade: pentagonal;
  - Number of lobes: five;
  - Shape of teeth: both sides straight;
  - Degree of opening/overlapping of petiole sinus: overlapped;
  - Depth of upper lateral sinuses: deep;
  - Density of erect hairs on main veins on lower side of blade: medium;
  - length of petiole compared to length of middle vein: slightly shorter;

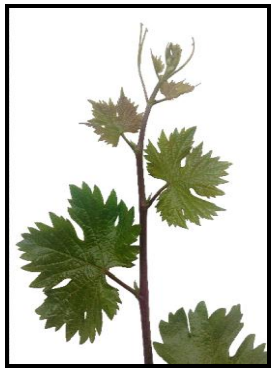

Fig. 1 Young shoot

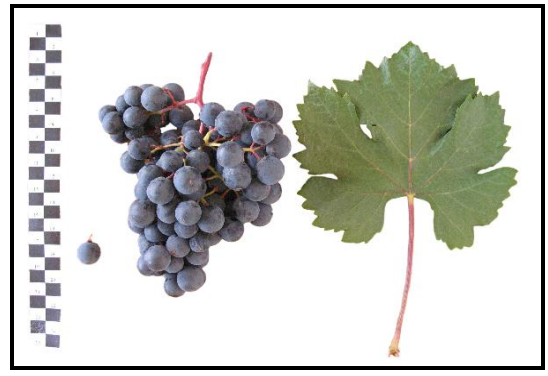

Fig. 2 Măgura variety (adult leaf, grape)

- Bunch - length (peduncle excluded): medium;
  - width: medium;
  - Density: middle;
  - Peduncle length: short;
- Berry - Length: short;
  - Width: narrow
  - Shape: globose;

- color of skin: blue black;
- thickness of skin: thick;
- intensity of flesh anthocyanin coloration: very strong;
- firmness of flesh: slightly firm;

Studying the technological characteristics of grape production was completed knowledge elements for the new grape variety (tab. 4).

Table 4

The technological properties of the Măgura variety
(Odobeşti, average data 2012 – 2013)

Variety	No. bunch/ vine	Average weight grapes (g)	Average weight 100 berry (g)	Production grape		Sugars g/l	Total acidity g/lH_2SO_4
				kg./vine	t/ha		
Măgura	24.0	190.0	185.0	4.5	16.6	212.0	4.2
Băbească neagră (Mt.)	19.1	220.0	215.0	4.2	15.9	208.0	5.3

Though the average weight of bunch at Măgura variety is smaller (190 g) than the reference variety (220 g), the higher number of grapes per vine to determined a higher production (4.5 kg/vine) compared to a reference variety - Băbească neagră (4.2 kg/vine). As regards the quality of grape production, sugar content in the juice at the variety Măgura was 212.0 g/L (with 4 g higher than the control) and total acidity of 4.2 g/L H_2SO_4 (lower 1.1 g/L H_2SO_4 than the control).

In the climatic conditions of the years 2012 - 2013, the Magura variety manifested high resistance to the main cryptogamic diseases compared to the control – Băbească neagră (tab. 5).

Table 5

The behavior at the main diseases of the vine
(OIV descriptor list for grape varieties and *Vitis* species, 2nd edition – 2009)

Variety	Downy mildew (*Plasmopara viticola*)		Powdery mildew (*Uncinula necator*)		Black rot (*Botrytis cinerea*)	
	Leaf OIV 452	Grape OIV 453	Leaf OIV 455	Grape OIV 456	Leaf OIV 458	Grape OIV 459
Măgura	7 - 9	7 - 9	7 - 9	7 - 9	7	5- 7
Băbească neagră (Mt.)	5 - 7	5	5	5	7	5

Main characteristics of the wines produced are shown in table 6.

Table 6

Physico-chemical characteristics of wines (average data 2012 – 2013)

Variety	Alcohol vol. %	Total acidity g/l H_2SO_4	Dry extract unreducible g/l	Anthocyanins mg/l
Măgura	13.38	4.83	22.24	442.8
Băbească neagră (Mt.)	13.25	5.31	18.49	358.7

The wine made from the variety Măgura had an alcoholic strength of 13.38% vol., upper the witness (Băbească neagră) with 0.13% vol. Total acidity was 4.83 g/L H_2SO_4, less with 0.48 g/L H_2SO_4 compared to the control (5.31 g/L H_2SO_4). Also anthocyanin content in wine (442.8 mg/L) is superior than the witness (358.7 mg/L). Higher values were obtained and for unreducible dry extract (3.75 mg/L greater than the witness).

The values of the technological indices resulted from the mechanical analysis of the grapes, complete the properties of the Măgura variety (tab. 7).

Table 7

Mechanical composition of grapes for Măgura variety (average data 2012 – 2013)

Elements determined		Măgura	Băbească neagră (Mt.)
1 kg grapes:	berry, g	959.5	960.5
	bunch, g	40.5	39.5
	no. berry normally developed	530	445
	must, g	796	780
	volume of must,cm_3	657	635
100 berry:	average weight, g	185	215
	skin weight, g	21	32.5
	core weight, g	157.8	175
	seeds weight, g	6.2	7.5
	number of seeds	165	167
	weight of 100 seeds, g	3.7	3.3
Technological indices:	berry index	53	45
	structure of the grape index	23.69	24.31
	composition of berry index	7.51	5.38

CONCLUSIONS

1. Variety Măgura stems from a hybridization of Băbească neagră variety with the hybrid combination (Merlot x Alicante Bouschet). During the study period it maintained qualities of distinctness, uniformity and stability.

2. In comparison with the control (Băbească neagră), the elite has shown potential for high productivity and constant throughout the study period.

3. The elite has proved superior than the witness in terms of grape production / vine and grape sugar content;

4. Being a variety with genetic tolerance is recommended introduction into the vineyards suitable for green technologies.

REFERENCES

1. Calistru Gh., Damian Doina, 1999 – *Golia, soi nou de viță de vie cu rezistență sporită la ger.* Lucr. st. seria Horticultura, vol nr. 42, USAMV Iaşi.

2. Culcea Valeria, Preda Daniela, Marian I., 2004 – *Rosina, soi rezistent pentru vinuri albe.* Analele ICDVV, vol. XVII, Bucureşti, pp.61-65.

3. Oprea St., Moldovan S.D., 2007 – *Ameliorarea viței de vie în România.* Editura Poliam, Cluj Napoca.

4. *, 2009** – The 2nd edition of the OIV Descriptor list for grape varieties and Vitis species

AGROBIOLOGICAL AND TECHNOLOGICAL CHARACTERISATION OF SOME CLONAL ELITES FOR WINE GRAPES OBTAINED WITHIN S.C.D.V.V. IAŞI

CARACTERIZAREA AGROBIOLOGICĂ ŞI TEHNOLOGICĂ A UNOR ELITE CLONALE PENTRU STRUGURI DE VIN OBŢINUTE ÎN CADRUL S.C.D.V.V. IAŞI

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Abstract. *Autochthonous and cosmopolitan grapevines varieties represent a valuable source of germoplasm, which is particularly important for the breeding of the currently cultivated genotypes.Thestructural improvement of the national viticulturalassortment is supported by scientific research in the field of vine breeding, whose mission was and is to renew, diversify and increase the biological value of the vine assortment by creating new qualitative and productive genotypes with superior resistance to disease and stress factors, through both genetic engineering and clonal selection. The present paper contains the results obtained at the Research Development Station for Viticulture and Winemaking Iasi, referring to the agrobiological and technological characteristics of clonal elites selected from the populations of the varieties: Sauvignon blanc, Pinot gris and Cabernet Sauvignon. The clonal elites obtained, through the cultural and qualitative features for which they were selected, complementarily contribute to achievement of high quality grape productions.*
Key words: clonal elites, wine grapes, breeding schemes *Vitis vinifera* L.

Rezumat. *Soiurile de viță de vie autohtone şi cosmopolite reprezintă o sursă valoroasă de germoplasmă, deosebit de importantă pentru ameliorarea materialului genetic cultivat în prezent. Îmbunătățirea structurală a sortimentelor viticole naționale este sprijinită de cercetarea ştiințifică din domeniul ameliorării viței de vie, a cărei misiune a fost şi este, înnoirea, diversificarea şi creşterea valorii biologice a sortimentelor viticole prin crearea de noi genotipuri mai valoroase calitativ şi productiv, cu rezistență mai bună la boli şi factori de stres, atât prin inginerie genetică, cât şi prin selecție clonală. Prezenta lucrare cuprinde rezultatele obținute la Stațiunea de Cercetare Dezvoltare pentru Viticultură şi Vinificație Iaşi, cu referire la caracteristicile agrobiologice şi tehnologice ale unor elite clonale, selectate din populațiile soiurilor: Sauvignon blanc, Pinot gris şi Cabernet Sauvignon. Elitele clonale obținute, prin aptitudinile culturale şi calitative pentru care au fost selectate, contribuie în mod complementar la obținerea unor producții de calitate superioară.*
Cuvinte cheie: elite clonale, struguri pentru vin, scheme de ameliorare, *Vitis vinifera* L.

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INTRODUCTION

Maintaining and improving the biological potential of valuable vinevarieties by using the clonal selection as a fast and efficient means of quantitative and qualitative increase of grape production, is a major concern of scientific research in the field of vine breeding (Damian *et al*., 2006, 2010).

In this regard, by applying the clonal selection, at the Research Development Station for Viticulture and Winemaking (S.C.D.V.V.) Iasi, were obtained two clonal elites for white wines and one for red wine.The obtained clonal elites are well adapted to the ecological conditions specific to the vineyards from north-eastern Moldova, with high yield, superior qualities characteristics and genetic stability compared to population of the variety.

Presentwork refers to clonal elites Sauvignon blanc 12.9.5, Pinot gris 5.7.5 and Cabernet Sauvignon 16.6.9, obtainedat S.C.D.V.V. Iasi, and studied during several years of vegetation.

MATERIAL AND METHOD

The studies were carried out in experimental plantations located on cambic-chernozemsoil, practicing planting distances of 2.1/1.2m, semi-hightraining system, semi-protectedduring winter. Field technologies applied were those recommended by viticulturalagrotechnicsfor this area, specific to wine grape varieties. To highlight the agrobiological and technological characteristics, in the period 2012-2016, the researchwas focused on observations and determinations on the phenological spectrum, fertility and productivity of clonal elites, the quantity and quality of grape production, and also theirbehaviouron frost and cryptogamic diseases, in direct relation with the environmental factors.

RESULTS AND DISCUSSIONS

From the climatic point of view, a general analysis of the reference period (2012-2016) highlights the presence of moderate temperatures in the years 2013 and 2016, with temperatures below -14 °C (tab. 1). In 2012, 2014 and 2015, were recordedthe lowest temperatures, of -26.7 °C, -20.6 °C and -21.0 °C respectively, producing bud losses corrected by compensation pruning.

The sum of global temperatures oscillated during the years of study between 3755 °C in 2014 and 4222 °C in 2016. In 2014, thesum of active and useful temperatures was 1500.3 °C, and respectively 3460.3 °C, favouring the normal deployment of physical processes and grape maturation. The summers were warm, sometimes torrid, with a maximum of 40.1 °C in 2012 and 37.0 °C in 2016 respectively.Regarding the hydric regime, the year 2015 proved to be a dry year, accumulating during the vegetation period only 247.3 mm. The amount of rainfall accumulated in the other years of study was over 535.6 mm, providing the necessary conditions for obtaining large and high quality productions.

Table 1

Climatic characteristics of the years of study in the Copou Iaşi area

Year	2012	2013	2014	2015	2016
Average temperature(°C)	10.4	10.3	10.2	11.5	11.0
Absolute minimum in air (°C)	-26.7	-14.3	-20.6	-21.0	-8.4
Absolute maximum in air (°C)	40.1	33.7	34.2	37.0	35.5
Sum of active temperatures(°C)	4023.5	3550.0	3460.3	3615.4	3576.8
Sum of global temperatures(°C)	3838.1	3788.3	3755.0	4222.0	4019.0
Sum of useful temperatures(°C)	1963.5	1530.0	1500.3	1785.4	1686.8
Real insolation (hours)	4181.5	3999.7	3985.1	4169.6	4102.8
Rainfall (mm)	535.6	656.7	618.0	311.1	646.8

On the background of the specific climatic conditions of each year, the bud break ofstudied clonal elites occurred between 18th and 30thof April, being noted the early bud break of the clone Sauvignonblanc 12.9.5 (18.04.), followed by Pinot gris 5.7.5 (22.04.) and Cabernet Sauvignon 16.6.9 (25.04) (tab. 2).

Table 2

The main agrobiological characteristics of the clonal elites studied

Analysed elements	Sauvignon blanc 12.9.5	Pinot gris 5.7.5	Cabernet Sauvignon 16.6.9
Bud break	18 IV - 25 IV	22 IV - 27 IV	25 IV - 30 IV
Flowering	29 V - 08 VI	28 V - 07 VI	28 V - 07 VI
Veraison	29 VII - 13 VIII	3 VIII -06 VIII	31 VII - 13 VIII
Grape maturation	9 IX - 29 IX	9 IX - 30 IX	18 IX - 10 X
Duration of vegetation period	166- 206	166- 206	164 - 210
Fertile shoots (%)	70 - 81	70 - 81	82 - 85
Absolute fertility coefficient	1.18 - 1.56	1.18 -1.56	1.56 -1.90
Relative fertility coefficient	0.91 -1.28	0.91 -1.28	1.32 -1.56
Absolute productivity index	109 - 113	109 - 113	124 - 187
Relative productivity index	64 - 105	64 - 105	120 - 156
Growth vigour	average	average	average
Frost resistance (% viable buds)	90 - 93	90 - 93	85 - 92
Resistance to downy mildew (OIV notes)	9	8 - 9	7 - 9
Resistance to powdery mildew (OIV notes)	9	8 - 9	7 - 9
Resistance togrey mould (OIV notes)	7 - 8	7 - 8	6 - 8

Blooming occurred between 28.05 and 08.06, being conditioned by a useful thermal balance, with values ranging from 256.0 °C to 276.0 °C, while grape veraison took place between 29.07 and 13.08. Grape maturity of consumption coincided with the harvest date and took place in September. The first grapes that reached technological maturity were those of the clonal elites for white wines (Sauvignon blanc 12.9.5 and Pinot gris 5.7.5).

Regarding the elements defining the fertility of the studied clonal elites,

was found that the percentage of fertile shoots was high, ranging between 70 and 85%, particularly pointing out the clonal elite Cabernet Sauvignon 16.6.9.

Under the terms of an almost equal bud load attributed by pruning, the average number of inflorescences varied according to the hereditary specificity of the elites. The values of the absolute fertility coefficient were supraunitaryat all three elitesstudied, while the relative fertility coefficient rangedbetween 0.91 and 1.56. Elite Cabernet Sauvignon 16.6.9 showed the highest values of the absolute (> 124) and relative (> 120) productivity indices.

By comparing the results inrespect to the fructification capacity of the studied clones, it can be concluded that they have reached the known biological potential of population of the variety, with small differences between the repetitions of the same genotype, demonstrating that they have acquired genetic stability and have a good adaptability to the conditions of the ecosystem in which they were studied.

Applying a number of seven anticryptogamic treatments, the studied clonal elites showed a good resistance to the attack of the main grapevine diseases, specific to the *V. vinifera* varieties, appreciated with grades from 7 to 9 in the O.I.V. scale.

During the observation period (five years), the production and quality of grapes was influenced by environmental factors, thus, the yieldon vine stock ranged between 2.8 (Cabernet Sauvignon 16.6.9) and 5.4 kg (Pinot gris 5.7.5). Yield per hectare ranged between 13 and 20 t/ha forelite Pinot gris5.7.5 and between 10 and 15 t/ha for the other two studied elites (tab. 3).

Table 3

The main technological characteristics of the clonal elites studied

Analysed elements	Sauvignon blanc 12.9.5	Pinot gris 5.7.5	Cabernet Sauvignon 16.6.9
Average grape weight (g)	68 - 75	80 - 100	95 - 120
Weight of 100 berries (g)	177 - 186	136 - 151	120 - 129
Sugars in must (g/L)	185 - 230	205 - 230	185 - 216
Acidity of must (g/L H_2SO_4)	5.1 -5.9	4.2 - 4.6	3.9 - 4.3
Glucoacidimetric index	36 - 39	49 - 50	47 - 50
Colour of berry skin	yellow - green	red - grey	black - azure
Pulp consistency	succulent	succulent	succulent
Effective yield on vinestock (kg)	3.4 - 4.0	3.6 - 5.4	2.8 - 3.4
Calculated yield on ha (t/ha)	12.5 - 15.1	13.6 - 20.0	10.6 - 12.5

The size of the grapes, as mean weight, was superior to the control (population of the variety), with aweight of 100 berriesof over 120 g for all three elites.

The quality of the harvest, measured as the meanweight of a grape, the weight and volume of 100 berries, the sugar content and total acidity of the must, reflects both the genetic character of the variety and the influence of the climatic factors on these elements.

The studied genotypes showed high sugar concentrations, ranging between 185 g/L to 230 g/L at the technological maturity. Pinot gris 5.7.5 clonal elite was noted for its high potential ofsugars accumulation, which exceeded220 g/L at grape harvesting. The acidity of the must was situated within normal limits, being inverselycorrelated with the sugar concentration of grapes.The highest total acidity was recorded in clonal elite Sauvignon blanc 12.9.5 (5.1-5.9 g/L H_2SO_4).

Regarding the polyphenolic potential of the studied genotypes, the total polyphenolic index ranged from 8 to 25, the maximum value being obtained for the Cabernet Sauvignon 16.6.9 clonal elite (tab. 4).

Table 4

The polyphenolic potential of grapes at harvest

Clonal elites	DO 280	Polyphenolic index	Anthocyanins (mg/L)	Total anthocyanin potential (mg/kg)
Sauvignon blanc 12.9.5	0.028	8	0	0
Pinot gris 5.7.5	0.056	17	15	45
Cabernet Sauvignon 16.6.9	0.084	25	32	96

Clonal elite Cabernet Sauvignon 16.6.9, showed a total anthocyanin potential of 96 mg/kg, in accordance with its hereditary character and the climatic conditions of the harvest year.

CONCLUSIONS

1. The climatic conditions recorded during the study period allowed to the clonal elites obtained within S.C.D.V.V.Iaşi to complete the annual biological cycle and to ensure productions of superior quality comparing to the population of the variety.

2. Pinot gris 5.7.5 clonal elite was highlighted by constant quantitative and qualitative productions and a high potential for sugar accumulation (225 g/L) in the context of a balanced acidity, being suitable for obtaining quality white wines.

3. Clonal elite Sauvignon blanc12.9.5 ensures the obtaining of quality white wines, with typicity specific to the variety, accumulating over 200 g/L sugars. It is characterised by constant quantitative and qualitative productions, showing good resistance to low temperatures.

4. Clonal elite Cabernet Sauvignon 16.6.9 is a valuable creation that is distinguishedby an average yield of 4.8 kg/stock, providing anincreasein production of over26% compared to the variety. Cabernet Sauvignon 16.6.9 ensure the obtaining of high quality, extractive and balanced red wines, with a total acidity of 4.0 g/L of H_2SO_4.

5. The obtained clonal elites are well adaptedto the ecological conditions specific to vineyards from the north-eastern Moldova, with higher fertility and productivity, superior quality characteristics and genetic stability compared tothe population of the variety.

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REFERENCES

1. Damian Doina, Calistru G., Savin C., Vasile Ancuța, Zaldea Gabi, 2006 - *Agrobiological and technological value of newcultivars and clones of grape vine created in S.C.D.V.V.Iaşi.* LucrăriŞtiințifice – Seria Horticultură, USAMV Iaşi, vol. 49, p. 499-504.

2. Damian Doina, Savin C., Vasile Ancuța, Zaldea Gabi, 2010 - *The agrobiological and technological value of the vine clones obtained in the Research Development Station for Viticulture and Wine production of Iaşi.* Lucrări Ştiințifice – Seria Horticultură, USAMV Iaşi, vol. 53, p. 383-388.

3. Damian Doina, Vasile Ancuța, Zaldea Gabi, SavinC., 2010 - *Contributions to the knowledge regarding agrobiological and technologicalcharacteristics of genetic resources existent at the Research Development Station for Viticulture and Wine production of Iaşi.* Lucrări Ştiințifice – Seria Horticultură, USAMV Iaşi, vol. 53, p. 389-394**.**

SEPARATION AND CHARACTERISATION OF THE MAIN PROANTHOCYANIDIN FRACTIONS OF GRAPE SEEDS

SEPARAREA ŞI CARACTERIZAREA PRINCIPALELOR FRACŢIUNI DE PROANTOCIANIDINE DIN SEMINŢELE DE STRUGURI

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Abstract. *Grape seeds, as waste products of the winemaking industry, contain large amounts of monomers, oligomers and more highly polymerised proanthocyanidins (PA), being a good source of phytochemicals for the production of antioxidative dietary supplements. PA from defatted grape seeds were extracted by precipitation with diethyl ether from the crude alcoholic extract and fractionated into monomers (FI), oligomers (FII) and polymers (FIII) of flavan-3-ols by their separation on C18 Sep-Pak cartridges. FIII was the predominant class of proanthocyanidins (82.22%), while monomeric PA has only 5.71% of total. The ratio PA (by vanillin assay) / tannins (Bate-Smith assay) indicates the highest degree of polymerisation (DP) in FIII fraction (1.28). Thin layer chromatography (TLC) confirmed the presence of monomers in FI, the DP increasing significantly for the next two fractions. Oligomeric and polymeric PA showed the highest antioxidant activity (% scavenged DPPH), but the synergic antioxidant effect of PA classes was also observed.*
Key words: antioxidant activity, C18 cartridges, degree of polymerisation, grape seeds, tannins

Rezumat. *Semințele de struguri, ca deşeuri ale industriei de vinificație, conțin cantități mari de proantocianidine (PA) monomerice, oligomerice şi cu grad mare de polimerizare, fiind o sursă importantă de compuşi pentru producerea de suplimente alimentare cu rol antioxidant. PA din semințele de struguri delipidate, au fost extrase prin precipitare cu eter dietilic din extractul alcoolic crud şi fracționate în monomeri (FI), oligomeri (FII) şi polimeri (FIII) de unități flavan-3-ol prin separarea pe cartuşe de tip C18 Sep-Pak. FIII a constituit clasa predominantă de PA (82.22%), în timp ce formele monomerice au reprezentat doar 5.71% din totalul PA. Cromatografia în strat subțire (TLC) a confirmat prezența monomerilor în FI, gradul de polimerizare (GP) crescând semnificativ pentru fracțiunile FII şi FIII. Raportul catechine / taninuri a indicat cel mai ridicat GP pentru fracțiunea FIII (1.28). Formele oligomerice şi polimerice au prezentat cea importantă activitate antioxidantă, fiind remarcat efectul sinergic al claselor de PA.*
Cuvinte cheie: activitate antioxidantă, cartuşe C18, grad de polimerizare, semințe de struguri, taninuri

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INTRODUCTION

Discovered by Masquelier J. in 1947, in pine bark and grape seeds, proanthocyanidins (PA) or condensed tannins are phenolic compounds consisting of chains of flavan-3-ol units of varying degree of polymerization (DP). PA can occur as polymers of up to 50 units without being susceptible to be cleaved by hydrolysis (Sun *et al*., 1998). In nature, PA serve in the defensive mechanisms of plants against pathogens and predators (Amil-Ruiz *et al*., 2011).

As waste products of the wine industry, grape seeds are concentrated sources of monomeric phenolic copounds such as (+)-catechin (0.16 mg/mL), (-)-epicatechin (0.66 mg/mL), and dimeric (0.329 mg/g dry weight (d.w.)), trimeric (0.384 mg/g d.w.) and polymeric (0.905 mg/g d.w.) PA (Khanal *et al*., 2009). In grape seeds, PA represents the major phenolic fraction being characterised by a lower degree of polymerization than those of berry skin (Iriti and Faoro, 2010).

Tannins are potent antioxidants (Reed, 1995). Specific studies have shown that the antioxidant power of PA is 20 times higher than vitamin E and 50 times higher than vitamin C (Shi *et al*., 2003). Extensive research suggested that PA help to protect the body from sun damage, improve flexibility in joints and arteries, improve blood circulation and have anti-carcinogenic activity (Nandakumar *et al*., 2008). However, all of these properties largely depend on their chemical structure and degree of polymerization (Sun *et al*, 1998).

Different qualitative (thin layer chromatography) and quantitative (normal phase HPLC, chromatographic columns and resins) techniques were proposed to separate PA according to their DP, but having as main shortcomings that they are very difficult to use for routine analysis. Taking into account the different physico-chemical and health promoting properties of PA classes, their separation and specific use is of great interest in food, pharmaceutical and cosmetic fields.

In present work, was used an improved simplified method for the separation and quantification of PA from grape seeds, based on their DP, using preconditioned neutral C18-max Sep-Pak cartridges, silica-based bonded phase, with strong hydrophobicity. C18 cartridges are characterised by lower cost, higher accuracy and faster protocol, similar to reversed-phase HPLC columns.

MATERIAL AND METHOD

Grape seeds of autochthonous wine cultivar Fetească neagră were separated from skins, dried and grinded. Grinded seeds were defatted with hexane 99% (24 h), phenolic compounds being extracted with 96% ethanol (72 h) and concentrated by vacuum evaporation (Heidolph rotavapor) at 35 °C. PA were subsequently extracted by precipitation with diethyl ether 99.5%, and dried at 30 °C. Dried brute precipitate of PA was then fractionated in compliance with their DP into monomers (FI), oligomers (FII) and polymers (FIII) of flavan-3-ols by separation on C18-max Sep-Pak cartridges, 500 mg sorbent / 6 mL volume (Waters Corporation, USA). Aqueous solution of PA (100 mg) was passed through the C18 cartridge, previously activated with ethanol 96% and distilled water. Elution was carried out with 10 mL of distilled water to eliminate phenolic acids and eventually sugars or proteins. After the cartridge was

dried, elutions were carried out first with 25 mL of ethyl acetate to elute catechins and oligomeric PA, resulting the fraction (F) I + II, and then with 20 mL of ethanol to elute the polymeric PA (FIII). For the separation of monomers from oligomeric PA, FI+II was evaporated to dryness, dissolved in distilled water, and then redeposited onto the same preconditioned cartridge. Separation was realised by sequential elution with 20 mL of diethyl ether (FI) and then with 20 mL of ethanol (FII). Each PA fraction was evaporated in glass capsules and analysed gravimetrically (Shimadzu ATX 220).

PA purity was calculated as the sum of the three fractions. For each fraction was determined the total catechin content by vanillin-HCl assay according to Caceres-Mella A. *et al.* (2013) and tannin concentration (Procyanidolic Index) using the methodology proposed by Bate-Smith E. (1981).

Commercial TLC silica gel 60 F254, 20×20 cm aluminium sheets (Merck, Germany) were used to control the DP of PA in each fraction obtained from C18 Sep-Pak cartridges. The chromatography was carried out using an ascending elution with toluene/acetone/acetic acid (3:3:1, v/v/v), according to the method proposed by Sun B. *et al.* (1998). 10% (w/v) vanillin in concentrated HCl was used for peak detection.

Antioxidant activity of PA fractions (1 mg/mL) was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. Stock solution: 4.0 mg DPPH in 100 mL 96% ethanol. Sample (25 µL) / DPPH (975 µL) solution was mixed, measured at λ 517 nm (T_0), incubated at 37°C for 30 min and measured at λ 517 nm (T_{30}), using a UV-mini spectrophotometer (Shimadzu, Japan) and 10 mm path length Helma® glass cells. Inhibition (%) = [(Absorbance T_0 − Absorbance T_{30})/Absorbance T_0] × 100.

RESULTS AND DISCUSSIONS

After PA precipitation from the crude alcoholic extracts, grape seeds PA (100 mg) were separated into fractions on C18 Sep-Pak cartridges. After gravimetric determinations, the monomeric PA (FI) represents only 5.20 mg/100 mg d.w., oligomeric PA (FII) 11.00 mg/100 mg d.w. and polymeric PA (FIII) 74.90 mg/100 mg d.w. (tab. 1). Total amount of 91.15 mg, resulting from the cumulative of fractions, indicates the degree of purity of the PA brut precipitate.

Table 1

Total proanthocyanidin content of individual fractions by gravimetry

Fraction (F)	Type of PA	PA (mg/100 mg)	% of total PA
I	monomers	5.20	5.71
II	oligomers	11.05	12.07
III	polymers	74.90	82.22
Total	-	91.15	100.00

These results are slightly higher than those presented by US Food and Drug Administration for grape seed extracts, respectively up to 78.00% for polymeric flavan-3-ols and 5.50% for monomeric flavan-3-ols (FDA, 2003), but more closely to those reported by Monagas *et al.* (2003), which showed that the polymeric fraction in grape seeds represents 75 to 81% of total flavan-3-ols content.

Lower values for vanillin assay are explained through the fact that vanillin reacts only with free flavan-3-ol fraction or with the terminal units of PA (Price *et. al*, 1978). The total content of tannins (Procyanidolic Index or Bate-Smith assay)

varied between 8.51 and 33.17 g CE/L (tab. 2). Close values for FIII and brut precipitate (Bp), which include all the three fractions, confirmed the high percentage of polymeric PA forms in the initially obtained precipitate.

Table 2

Total PA (as catechins) of individual fractions

Fraction (F)	OD 500 nm (Vanillin assay)	OD 550 nm (Bate-Smith assay)	PA (catechins) (Vanillin assay) (g CE/L)	PA (tannins) (Bate-Smith assay) (g CE/L)	Cat/Tan (OD)
I	1.068	0.440	6.91	8.51	2.43
II	1.810	0.844	11.69	16.31	2.14
III	2.191	1.716	14.14	33.17	1.28
Bp	2.121	1.792	13.69	34.64	1.18

Note: Bp – initial brute precipitate (includes all three PA fractions); OD – optical density; CE – catechin echivalent; Cat/Tan – catechins/tannins ratio.

The ratio between the optical density of vanillin-coloured combinations and anthocyanidins formed by heating in acidic medium was used as the indicator of tannin DP. This ratio should be lower as the DP is higher (Goldstein and Swain, 1963). Thus, Cat/Tan ratio was the lowest in FIII and brute precipitate (Bp), confirming the presence of PA with higher DP in these samples (see tab. 2).

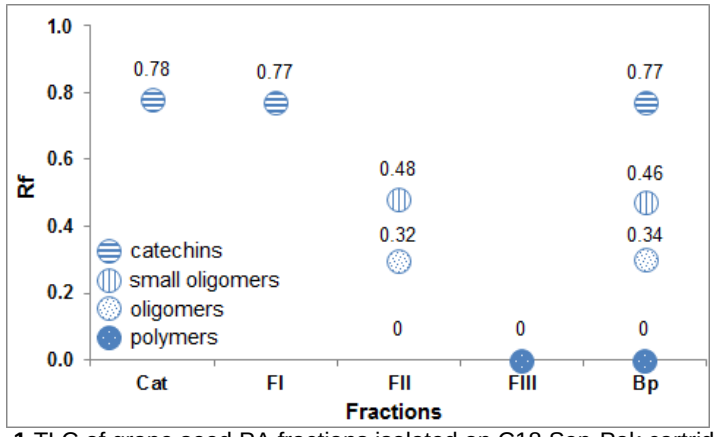

Fig. 1 TLC of grape seed PA fractions isolated on C18 Sep-Pak cartridges
Note: Cat - catechins; FI, FII, FIII - PA fractions; Bp - brut precipitate.

PA composition of each fraction was verified by TLC, using pure catechin solutions as standard. Individual compounds appeared as spots separated vertically (fig. 1). For each spot was calculated the retention factor (Rf), as ratio between the distance migrated over the total distance covered by the solvent (18 cm). The Rf values were compared with catechin standard and data reported by Sun *et al*. (1998). The migration of compounds on plates was according to their DP, confirming the presence of catechin units (monomers) in FI (similar migration to pure catechins) and of low molecular weight PA (oligomers) in FII.

FIII did not contain monomers or oligomers of PA, being composed by highly polymerised PA (no migration). TLC technique give only a qualitative answer on PA separation, further investigations (like HPLC technique) being necessary.

Grape seed extracts are known as powerful antioxidants that protect the body from premature aging, disease and decay (Shi *et al*., 2003).

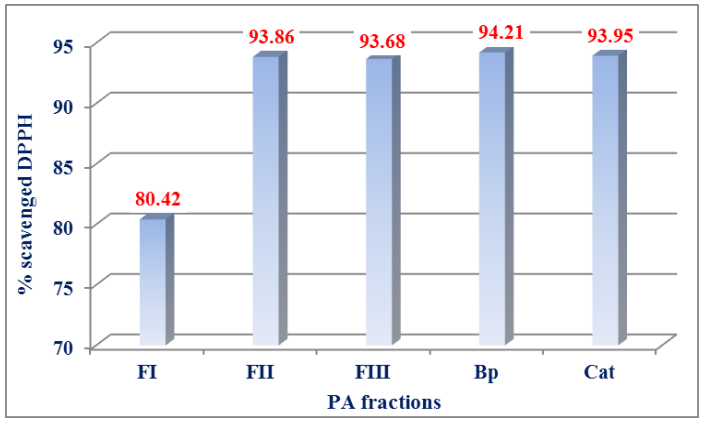

Fig. 2 Antioxidant activity of grape seed PA fractions isolated on C18 cartridges
Note: Bp - brut precipitate; Cat - catechin

The antioxidant activity of PA fractions (1 mg/mL) was high, FII being the most reactive, followed very closely by the polymeric fraction (FIII) (fig. 2). The highest antioxidant activity (94.21%) was that of the crude PA precipitate (Bp), highlighting the synergistic effect of PA classes. The results are consistent with those presented by Yilmaz and Toledo (2004), oligomeric and polymeric PA accounting for most of the superior antioxidant capacity of grape seeds.

CONCLUSIONS

1. Defatted grape seeds of wine cultivar Fetească neagră contained large amounts of proanthocyanidins, mainly in the polymeric form (>82%).

2. Oligomeric and polymeric PA showed the highest antioxidant activity, but the synergic antioxidant effect of PA classes was observed.

3. The use of C18 type cartridges to separate the PA fractions based on their DP is an efficient, accurate, fast and relatively inexpensive method for tannins preliminary analysis.

4. Considering the new possibilities of transforming these non-hydrolysable tannins into water-soluble compounds by controlled oxidation, grape seeds became a good source of phytochemicals, providing excellent protection against oxidative stress.

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REFERENCES

1. **Amil-Ruiz F., Blanco-Portales R., Munoz-Blanco J., Caballero J.L., 2011** - The strawberry plant defense mechanism: a molecular review. Plant Cell Physiol., 52 (11), p. 1873–903.
2. **Bate-Smith E., 1981** - *Astringent tannins of the leaves of Geranium species*. Phytochemistry, 20, p. 211–216.
3. **Caceres-Mella A., Pena-Neira A., Narvaez-Bastias J., Jara-Campos C., Lopez-Solis R., Canals J.M., 2013** - *Comparison of analytical methods for measuring proanthocyanidins in wines and their relationship with perceived astringency*. Int. J. Food Sci. Tech., 48, p. 2588–2594.
4. **Da Silva R.J.M., Rigaud J., Cheynier V., Cheminat A., Moutounet M., 1991** - *Procyanidin dimers and trimers from grape seeds*. Phytochemistry, 4, p. 1259-1264.
5. **FDA, 2003** - *Grape seed extract (GSE) notification*. US Food and Drug Administration, Silver Spring, Maryland, USA. Available online at: https://www.fda.gov/downloads /Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm267216.pdf.
6. **Goldstein J.L., Swain T., 1963** - *Methods for determining the degree of polymerization of flavans.* Nature, 198, p. 587-588.
7. **Iriti M., Faoro F., 2010** - *Bioactive chemicals and health benefits of grapevine products.* In Bioactive foods in promoting health: Fruits and Vegetables (Watson R.R. and Preedy V.R. eds.). Chapter 38, p. 581–620. Elsevier, Academic Press, London, UK.
8. **Khanal R. C., Howard L. R., Prior R. L., 2009** - *Procyanidin content of grape seed and pomace, and total anthocyanin content of grape pomace as affected by extrusion processing*. J. Food Sci., 74(6), p. H174-H182.
9. **Monagas M., Gómez-Cordovés C., Bartolomé B., Laureano O., Da Silva R.J.M., 2003** - *Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from Vitis vinifera L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon.* J. Agric. Food Chem., 51, p. 6475-6481.
10. **Nandakumar V., Singh T., Katiyar S.K., 2008** - *Multi-targeted prevention and therapy of cancer by proanthocyanidins.* Cancer Lett., 269(2), p. 378–387.
11. **Price M.L., Van Scoyoc S. , Butler L. G., 1978** - *A critical evaluation of the vanillin reaction as an assay for tannin in Sorghum grain*. J. Agric. Food Chem., 26 (5), p. 1214–1218.
12. **Reed J.D., 1995** - *Nutritional toxicology of tannins and related polyphenols in forage legumes.* J. Animal Sci., 73, p. 1516–1528.
13. **Saito M., Hosoyama H., Ariga T., Kataoka S., Yamaji N., 1998** - *Antiulcer activity of grape seed extract and procyanidins*. J. Agric. Food Chem., 46 (4), p. 1460 –1464.
14. **Shi J., Yu J., Pohorly J.E., Kakuda Y., 2003** - *Polyphenolics in grape seeds-biochemistry and functionality*. J. Med Food. Winter, 6(4), p. 291-299.
15. **Sun B., Conceição L., Da Silva R.J.M., Spranger I., 1998** - *Separation of grape and wine proanthocyanidins according to their degree of polymerization*. J. Agric. Food Chem., 46, p. 1390-1396.
16. **Yilmaz Y., Toledo R.T., 2004** - *Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid*. J. Agric. Food Chem., 52 (2), p. 255-260.

STUDIES ON THE PHENOLIC CONTENT OF RED SENESCENT GRAPEVINE LEAVES - A SUSTAINABLE SOURCE OF BIOACTIVE COMPOUNDS

STUDII PRIVIND CONŢINUTUL FENOLIC AL FRUNZELOR ROŞII SENESCENTE DE VIŢĂ DE VIE – SURSĂ SUSTENABILĂ DE COMPUŞI CU ROL BIOLOGIC ACTIV

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Abstract. *In order to identify new sustainable sources of bioactive compounds for food, pharmaceutical and cosmetic industries, anthocyanin, proanthocyanidin and total phenolic contents of red senescent leaves of 47 grapevine cultivars and chromatic parameters of their alcoholic extracts were evaluated. Leaves were picked between two to eight weeks after grape harvest, the remaining chlorophyll and carotenoid amounts being also quantified. Red grapevine leaves showed high antioxidant activity (% scavenged DPPH), proportionally correlated with the concentration of anthocyanins and total phenolic compounds. Chlorophyll and carotenoid content of red leaves was low, without exceeding 1.56 mg/g and 0.92 mg/g dry weight, respectively. High amounts of antioxidant anthocyanins, proanthocyanidins and total phenolic compounds, as well as the chromatic diversity of the obtained extracts, justify the use of senescent red grapevine leaves as raw material in the production of valuable dietary supplements.*
Key words: antioxidant activity, anthocyanins, chromatic parameters, grapevine leaves, proanthocyanidins

Rezumat. *În vederea identificării unor surse noi şi sustenabile de compuşi cu rol biologic activ pentru industriile alimentară, farmaceutică şi cosmetică, a fost evaluat conţinutul de antociani, proantocianidine şi compuşi fenolici totali din frunzele roşii senescente provenind de la 47 de soiuri de viţă de vie, precum şi caracteristicile cromatice ale extractelor alcoolice obţinute. Frunzele au fost colectate după două până la opt săptămâni de la recoltarea strugurilor, cantităţile remanente de clorofilă şi carotenoizi fiind de asemenea cuantificate. Extractele de frunze au prezentat o activitate antioxidantă ridicată (% DPPH), direct corelată cu prezenţa antocianilor şi compuşilor fenolici totali. Conţinutul de clorofile şi carotenoizi a fost redus, ajungând până la 1,56 mg/g şi respectiv, 0,92 mg/g masă uscată. Cantităţile mari de compuşi fenolici cu activitate antioxidantă ridicată, precum şi diversitatea cromatică a extractelor obţinute, justifică utilizarea frunzelor roşii senescente de viţă de vie ca materie primă în obţinerea alimentelor funcţionale.*
Cuvinte cheie: activitate antioxidantă, antociani, frunze roşii, parametri cromatici, proantocianidine

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INTRODUCTION

Red colouring of autumn leaves is a phenomenon that affects the normally green leaves, occurring mostly at grapevine cultivars with dark coloured grapes, varying depending on several factors such as: chlorophyll concentration (higher chlorophyll concentrations give leaves a purple to brown appearance), pH (basic pH in vacuoles results in blue coloration, whereas more acidic pH results in red), type of anthocyanins, co-pigmentation, glycosylation or the presence of metal ions. Anthocyanin synthesis in senescent leaves is thought to reduce light stress and provide protection against oxidation (Steyn *et al*., 2002). Also, red leaves may appear as a result of nutrient deficiency, viral or phytoplasma infection during the growing season, the symptoms being easily recognised.

Research conducted in recent years reported a high number of therapeutic properties of polyphenol rich extracts of red vine leaves. *In vitro* studies have shown vasorelaxing, antibacterial and anti-inflammatory activity, and particular antioxidant properties of grapevine foliar polyphenols (Katalinić *et al*., 2009).

The objective of this research was to assess new and sustainable sources of compounds with sanogenous potential, for use in food, pharmaceutical and cosmetic industries. Since phenolic compounds are considered responsible for various health benefits of red vine leaves, their content needs to be evaluated.

MATERIAL AND METHOD

The research has been carried out on red leaves of 47 grapevine cultivars for wine (31) and table grapes (16), growing in the Ampelographic Collection of the University of Agricultural Sciences and Veterinary Medicine Iaşi, N-E of Romania (27°53' E and 47°09' N). Red vine leaves were picked by hand at they phenolic maturity, between two to eight weeks after the grape harvest (depending on cultivar) as previously reported by Schneider *et al*. (2008). Phenolic compounds were extracted from grinded air-dried leaves (10 - 12 % moisture), with 0.1% HCl (v/v) in 96% ethanol (1:30, w/v), 20 h at 20 °C, in darkness. Chlorophylls and carotenoids were extracted with pure acetone (1:20, w/v), according to CIS (2011).

Total phenolic compounds (OIV, 2012), monomeric anthocyanins (Lee *et al*. 2005), total proanthocyanidins (Caceres-Mella *et al*., 2013), photosynthetic pigments (CIS, 2011) and the colour components (Glories, 1984), were determined by an UV-vis Shimadzu 1700 Pharmaspec spectrophotometer. Antioxidant activity of leaf extracts was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, at λ = 517 nm. Stock solution: 2.36 mg DPPH in 100 mL ethanol. The used formula was: Inhibition (%) = [(Absorbance T_0 − Absorbance T_{30})/Absorbance T_0] × 100.

RESULTS AND DISCUSSIONS

Analysis of the chromatic parameters can give information about the quality characteristics of a product and may be associated later with consumer acceptance. Chlorophyll (Chl) and carotenoid content of red leaves was low, specific for the final phenophase of annual cycle of grapevine vegetation. The percentage of red in the formation of extract colour was high (due to anthocyanins), along with yellow nuances (due to carotenoids). Red leaves of Fetească neagră cv. showed the highest colour intensity (62.08) (tab. 1).

Table 1

Photosynthetic pigments and phenolic compounds content of red leaf extracts and their colour components

No.	Cultivar	Photosynthetic pigments (mg/g)			TPC (g GAE/ 100 g)	A_{280}	PA (mg Cat / 100 g)	ANT (mg CE/ 100 g)	Colour components				
		Chl a	Chl b	Car					%Y	%R	%B	CI	H
0	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Alicante bouschet	0.28	0.19	0.28	4.27	75.95	70.91	355.31	60.31	35.26	4.43	11.74	1.71
2	Alidor	0.35	0.15	0.05	1.52	31.75	50.79	82.92	67.00	27.64	5.36	7.09	2.42
3	Alvarna	0.11	0.10	0.15	3.22	73.85	62.28	59.25	78.54	17.14	4.32	6.71	4.58
4	Andre	0.66	0.43	0.92	5.34	96.10	80.91	279.29	33.24	63.97	2.79	40.88	0.52
5	Aramon	0.26	0.20	0.05	2.78	64.15	57.69	68.97	79.92	15.19	4.89	7.57	5.26
6	Babească neagră	0.29	0.17	0.25	3.77	44.75	62.62	122.74	50.38	45.58	4.04	10.64	1.11
7	Balada	0.91	0.45	0.22	4.63	97.95	84.71	110.63	47.46	48.50	4.05	22.99	0.98
8	Bastard de Magaraci	0.57	0.31	0.73	5.60	106.05	76.18	354.72	32.53	65.00	2.47	39.26	0.50
9	Batută neagră	0.38	0.28	0.45	6.89	103.70	88.37	290.54	41.79	55.82	2.39	25.10	0.75
10	Black rose	0.34	0.21	0.28	5.51	63.40	77.11	298.06	51.66	44.37	3.97	13.86	1.16
11	Blauerzweigelt	0.44	0.02	0.10	5.41	74.25	72.28	213.33	39.97	56.74	3.30	23.67	0.70
12	Burgund mare	0.75	0.32	0.57	4.14	66.35	69.18	205.40	41.43	55.48	3.09	28.46	0.75
13	Cabernet Sauvignon	0.86	0.39	0.39	4.30	48.65	65.72	184.52	47.74	48.29	3.97	23.67	0.99
14	Cardinal	0.29	0.17	0.04	2.47	31.35	35.04	50.51	77.46	17.55	4.99	6.21	4.41
15	Cinsaut	0.95	0.45	0.45	5.29	76.95	75.38	203.31	48.55	47.51	3.93	26.94	1.02
16	Coarnă neagră	0.97	0.42	0.07	2.45	33.20	49.43	93.10	85.53	8.76	5.72	13.82	9.77
17	Codană	0.44	0.19	0.10	3.60	50.85	58.25	91.84	66.11	29.47	4.42	9.50	2.24
18	Dimiat	0.44	0.23	0.04	1.57	30.75	33.89	55.52	72.38	21.95	5.67	8.29	3.30
19	Dodrelabi	0.94	0.49	0.16	3.31	58.65	57.91	225.02	64.59	29.53	5.87	17.37	2.19
20	Durif	0.64	0.30	0.14	2.73	44.65	44.57	89.34	63.74	30.59	5.67	12.52	2.08
21	Fetească neagră	0.77	0.48	0.42	7.21	131.85	88.45	408.75	34.28	63.18	2.55	62.08	0.54
22	Gamay beaujolais	0.13	0.14	0.54	5.99	77.65	74.37	415.21	63.89	32.50	3.60	11.66	1.97
23	Gelu	0.25	0.12	0.11	1.61	25.00	7.09	120.65	69.05	26.39	4.56	5.04	2.62
24	Kismis negru	1.07	0.42	0.42	6.33	130.60	82.16	198.99	48.91	18.73	32.36	35.88	2.61

Table 1 – continuation

0	1	2	3	4	5	6	7	8	9	10	11	12	13
25	Merlot	0.90	0.44	0.23	4.89	90.15	56.94	163.23	74.03	19.35	6.62	16.02	3.83
26	Michele Palieri	0.41	0.29	0.51	6.09	129.55	80.33	189.41	57.34	37.79	4.87	16.62	1.52
27	Milcov	1.04	0.45	0.49	5.78	101.05	70.09	172.83	50.22	45.69	4.10	27.82	1.10
28	Mioriţa	0.44	0.25	0.25	2.75	55.45	41.37	42.16	69.31	23.58	7.11	8.44	2.94
29	Moldova	0.19	0.51	0.81	7.23	127.60	83.43	298.85	40.37	56.22	3.41	37.28	0.72
30	Muscat de Hamburg	0.69	0.34	0.58	2.06	26.00	38.25	149.46	76.87	16.61	6.51	9.21	4.63
31	Muscat timpuriu de Bucureşti	1.06	0.50	0.45	4.77	70.75	63.10	163.23	48.01	48.21	3.78	34.39	1.00
32	Napoca	0.76	0.43	0.29	2.49	49.75	29.21	129.83	51.57	43.64	4.79	18.79	1.18
33	Negru de Căuşani	0.36	0.34	0.66	6.59	118.45	74.81	233.73	51.29	44.51	4.21	21.39	1.15
34	Negru de Dragăşani	0.99	0.43	0.29	2.10	51.05	39.52	153.21	55.23	40.24	4.53	21.62	1.37
35	Negru vârtos	0.74	0.33	0.23	4.08	48.65	66.18	125.66	54.97	40.44	4.60	17.41	1.36
36	Novac	0.77	0.39	0.52	6.54	98.15	84.59	282.63	48.34	47.38	4.28	23.81	1.02
37	Oporto	1.03	0.46	0.27	3.48	67.45	49.06	95.18	61.74	33.08	5.18	23.55	1.87
38	Pinot noir	0.09	0.09	0.29	1.87	35.95	33.90	162.81	31.24	67.05	1.71	12.29	0.47
39	Princess	1.10	0.28	0.68	4.70	96.10	66.32	198.30	50.97	44.78	4.25	35.04	1.14
40	Purpuriu	0.35	0.11	0.22	3.07	71.80	57.79	172.00	75.58	18.58	5.84	5.65	4.07
41	Roz de Miniş	0.65	0.31	0.15	5.08	90.80	60.55	133.49	75.02	18.91	6.07	11.21	3.97
42	Someşan	0.20	0.08	0.03	2.37	36.25	50.10	200.40	68.78	26.59	4.63	4.10	2.59
43	Splendid	0.44	0.41	0.92	6.92	126.60	74.23	301.57	20.86	76.55	2.59	43.25	0.27
44	Sultanina	0.64	0.28	0.15	3.12	34.40	55.38	59.70	59.43	35.82	4.75	13.26	1.66
45	Transilvania	0.36	0.14	0.04	1.97	25.10	9.74	129.83	69.78	24.70	5.53	5.79	2.83
46	Trollinger	0.31	0.14	0.12	1.91	21.95	15.38	101.11	74.07	19.06	6.88	5.09	3.89
47	Vulpea	0.31	0.25	0.75	4.15	56.70	59.40	327.73	29.00	68.89	2.12	32.14	0.42

Note: Chl a - chlorophyll a; Chl b - chlorophyll b; Car - carotenoids (xanthophylls and carotenoids); TPC (g GAE/ 100 g) - total phenolic content (g Gallic acid equivalent/ 100 g dry weight); A_{280} - absorbance at 280 nm (estimation of total phenols); PA (mg Cat / 100 g) - proanthocyanidins (g catechin equivalent / 100 g); ANT (mg CE / 100 g) - monomeric anthocyanins (mg cyanidin-3-glucoside equivalent/100 g); %Y, %R, %B - percentages of yellow, red and blue in extract colour; CI - colour intensity (sum of absorbance at 620 nm, 520 nm and 420 nm); H - hue (ratio of optical densities at 420 nm and 520 nm).

A high concentration of anthocyanins was found in leaves of "teinturier" cultivars Gamay beaujolais and Alicante bouschet, and of Romanian cultivars Fetească neagră and Vulpea (327 - 415 mg CE/100 g), while total phenolic content of red leaves varied from 1.52 to 7.23 g GAE/100 g. Data are consistent with those reported by Schneider *et al*. (2008) and Ignat *et al.* (2016).

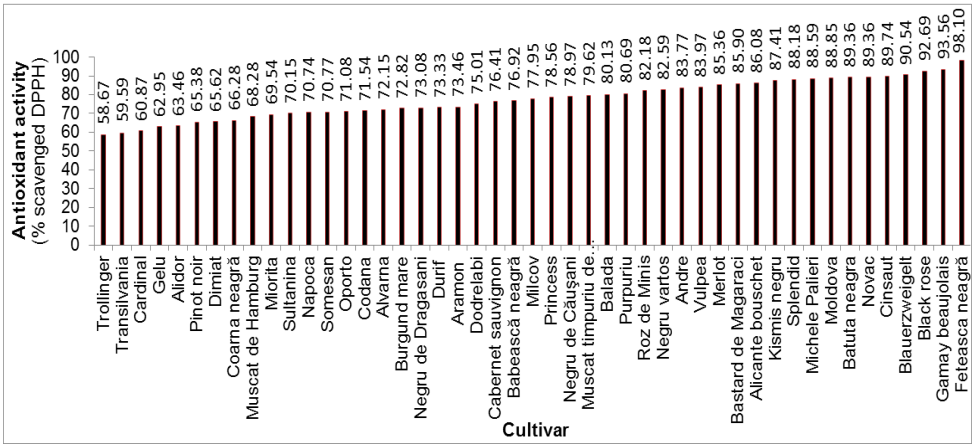

Fig. 1 Antioxidant activity of red senescent grapevine leaves

Antioxidant activity of leaf extracts was high, between 58.67 and 98.10 % scavenged DPPH (fig. 1), correlated with the abundance of anthocyanins (r = 0.7709), proanthocyanidins (r = 0.8621) and total phenolic content (r = 0.8915).

Table 2

The correlation of experimental data

Parameters	**Chl a**	**Chl b**	**Car**	**AA**	**ANT**	**PA**	**TPC**
Chl b	0.7607	-					
Car	0.1436	0.4574	-				
AA	0.1281	0.3245	0.5529	-			
ANT	-0.0724	0.2002	0.6756	**0.7709**	-		
PA	0.1929	0.3808	0.5510	**0.8621**	0.6038	-	
TPC	0.1682	0.4324	0.6606	**0.8915**	**0.7253**	**0.8703**	-
A_{280}	0.2002	0.4697	0.6438	**0.7916**	0.6083	**0.8062**	**0.9069**

Note: Chl a - chlorophyll a; Chl b - chlorophyll b; Car - carotenoids; AA - antioxidant activity; ANT - monomeric anthocyanins; PA - proanthocyanidins; TPC - total phenolic content; A_{280} - absorbance at 280 nm.

A strong correlation was found between anthocyanin and total phenolic content (r = 0.7253) and between proanthocyanidins and total phenolic content (r = 0.8703) of senescent leaves (tab. 2). As shown in our previous studies (Filimon *et al.*, 2015), neither in this cases, no relationship was found between anthocyanin and photosynthetic pigment content of red leaves.

CONCLUSIONS

1. Red senescent grapevine leaves contained high amounts of phenolic compounds, including anthocyanins and proanthocyanidins, which greatly contributed to the strong antioxidant activity of the leaf extracts.

2. Chlorophyll and carotenoid content of red leaves was low, but exerting a distinct influence on the composition of extracts colour.

3. Red senescent grapevine leaves represent a sustainable source of phenolic compounds for food, pharmaceutical and cosmetic industries, further research being necessary in order to identify the main compounds responsible for the related health benefits and to evaluate their real economic potential.

REFERENCES

1. **Caceres-Mella A., Pena-Neira A., Narvaez-Bastias J., Jara-Campos C., Lopez-Solis R., Canals J.M., 2013** - *Comparison of analytical methods for measuring proanthocyanidins in wines and their relationship with perceived astringency*. Int. J. Food Sci. Tech., 48, p. 2588–2594.
2. **Filimon V.R., Rotaru Liliana, Filimon Roxana, Niculaua M., 2015** - *Anthocyanin content and composition of red senescent leaves from Vitis vinifera L. table grape cultivars.* Lucrări Ştiințifice - UASM Chişinău, 42, no. 2, p. 82-92.
3. **Glories Y., 1984** - *La couleur des vins rouges II*. Connaiss. Vigne Vin, 18, p. 253–271.
4. **Ignat Gabriela, Colibaba Cintia, Costuleanu C.L., Balan A., Rotaru Liliana, Sandu I.C. A., 2016** - *Studies regarding the use of senescent viticultural material as colorants in the plastics industry.* Materiale Plastice, 53, no. 3, p. 367−369.
5. **Katalinić V., Generali I., Skroza D., Ljubenkov I., Teskera A., Konta I., Boban M., 2009** - *Insight in the phenolic composition and antioxidative properties of Vitis vinifera leaves extracts*. Croat J. Food Sci. Technol., 1, no. 2, p. 7-15.
6. **Lee J., Durst R.W., Wrolstad R.E., 2005** - *Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study*. J. AOAC Intl., 88, p. 1269-1278.
7. **Schneider E., Von der Heydt H., Esperester A. 2008** - *Evaluation of polyphenol composition in red leaves from different varieties of Vitis vinifera.* Planta Med., 74, p. 565-572.
8. **Steyn W.J., Wand S.J.E., Holcroft D.M., Jacobs G., 2002** - *Anthocyanins in vegetative tissues: a proposed unified function in photoprotection*. New Phytologist, 155, p. 349-361.
9. *****, CIS, 2011** - *Spectranomics Protocol: Chlorophylls and Carotenoids*. Carnegie Institution for Science (CIS), Stanford, USA, available online at: https://drive.google.com/file/d/0B58dyv8L3FpMdGw0QWtiZElHQzQ/view.
10. *****, O.I.V., 2012** - *Compendium of international methods of wine and must analysis*. Vol. I. International Organisation of Vine and Wine (OIV), Paris. Available online at: http://www.oiv.int/oiv/info/enplubicationoiv.

STUDIES REGARDING POLLEN VIABILITY AND GERMINATION CAPACITY OF SOME *VITIS VINIFERA* L. VARIETIES

STUDII PRIVIND VIABILITATEA ŞI CAPACITATEA DE GERMINARE A POLENULUI UNOR SOIURI *VITIS VINIFERA L.*

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Abstract. *Pollen quality is an important indicator in the estimation of grape production, being analysed based on its germination capacity. Thus, viability and germination potential of pollen from six Vitis vinifera L. varieties, grown in the climatic area of the Iasi vineyard, were analysed. To perform the determinations, were used comparatively three methods for observing the viable cells, by treating them with tetrazolium chloride (TTC), Lugol solution (IKI) and methylene blue (AM) solution. The germinating potential was analysed in vitro, the culture being performed on agar medium with added sucrose (0 to 20%). The viable cells were more clearly highlighted using the AM method, but the TTC method was more accurate in indicating the percentage of pollen viability. The highest pollen germination rate was observed in the 15% added sucrose variant.*
Key words: *Vitis vinifera* L., pollen viability, germination capacity

Rezumat. *Calitatea polenului este un indicator important în estimarea producției de struguri, fiind analizată pe baza capacității de germinare a acestuia. Astfel, au fost analizate viabilitatea şi potențialul germinativ al polenului provenit de la şase soiuri de viță de vie (Vitis vinifera L.) cultivate în arealul climatic al podgoriei Iaşi. Pentru efectuarea determinărilorau fost utilizate comparativ trei metode de evidențiere a celulelor viabile, prin tratarea acestora cuclorură de tetrazolium (TTC), soluție Lugol (IKI) şi soluție de albastru de metilen (AM). Potențialul germinativ a fost analizat in vitro, cultura fiind realizată pe mediu de agar cu adaos de sucroză (0 - 20%). Celulele viabile au fost cel mai clar evidențiate cu ajutorul metodei cu AM, metoda cu TTC fiind însă mai precisă în indicarea procentului de viabilitate a polenului analizat. Cea mai ridicată rată de germinare a polenului a fost observată la varianta cu adaos de 15% sucroză.*
Cuvinte cheie: *Vitis vinifera L.*, viabilitatea polenului, capacitate germinativă

INTRODUCTION

The quality of pollen is mainly represented by itsviability and germination capacity, being an essential characteristic that parental plants must accomplish to

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be used in vine-breeding experiments. In the breeding programs, only the descendants with normal hermaphrodite flowers, which allow the vine growing in monovarietalplots, are retained for promotion in production (Oprea and Moldovan, 2007). In the same time, pollen germination capacity is indicated by the potential of the pollen tubedevelopment and its vigor, as essential characteristics for optimal fecundation (Davarynejad *et. al*., 2008).

In vitro tests to determinate the viability and germination capacity of the pollen, indicate the percentage of viable cells, the pollen germination rate and the length of the pollen tube.

Present study aimedto analyse the pollen quality of some *Vitis vinifera* L. varieties for wine and table grapes by testing the viability of the pollen and its germination capacity. Comparative methods to highlight the pollen viability were also tested.

MATERIALAND METHOD

Determinations were performed on six *Vitis vinifera* L. varieties for wine (Chardonnay, Merlot and Cinsaut) and table grapes (Bicane, Muscat de Hamburg and Victoria), growing in the Ampelographic collection of the Research Development Station for Viticulture and Winemaking Iasi, in the years 2015 and 2016.For each variety, 20 inflorescences from 10 normally developed and healthy stocks were harvested randomly, before corollas opening.Inflorescences were placed in dry parchment bags and transported inthe laboratory.The corollas were removed, the anthersbeing isolated and stored overnight at room temperature (25 °C) for a better collection of pollen grains through brushing and sieving.

Pollen viability was estimated using three comparative methods.The tetrazolium chloride method (TTC 1%) is based on the reaction of reduction through respiration of 2,3,5-triphenyl-tetrazolium chloride to red triphenylformazan. The red and pink cells werecounted as viable, microscopic observations being performedaftertwo hours from thecontact of pollen with the solution (Sulusoglu and Cavasoglu, 2014).

When using Lugol's aqueous solution (IKI), viable cells were identified by changing their colour in brown and black, while using methylene blue solution (AM), viable cells remain unstained.Counting viable pollen cells was performed microscopically, after10 minutes fromstaining (Firmage and Dafni, 2001).

To determine the pollen germination capacity (microscopic examination of surfaces with about 100 pollen grains), was used a germination substrate consisting of: 1% agar in distilled water, boric acid (5 mg/L)and sucrose in concentrations of 5, 10, 15 and 20% (pH 6.5; in dark at 30 °C). Pollen wasconsidered germinated when the pollen tube exceeded the length of the pollen grain.

The results are presented as the meanvalues of two years determinations. Analysis ofvariance (ANOVA - Microsoft Excel) was used to investigate the differences between tested methods. P values lower than 0.05 ($p<0.05$) were considered statistically significant. For data dispersion analysis was calculated the coefficient of variation (± / average%).

RESULTSAND DISCUSSIONS

The highest percentage of viable pollen was observed when TTC reagent was used, the values ranging between 82.23 ± 1.31% (Muscat de Hamburg) and 88.34 ± 1.10% (Victoria), with a mean value of 84.87% (tab. 1).

The experimental results indicated statistically significant differences between the methods for testing the pollen viability at all studied varieties. Regardless of the test method used, Muscat of Hamburg showed the lowest percentage of fertile pollen. Also, the lowest pollen viability was obtained when the IKI solutionwas used.

Table 1

Pollen viability of *Vitis vinifera* L. analysed varieties (%)

Genotype/ Method	TTC	IKI	AM	Signification (p<0.05)
Muscatde Hamburg	82.23±1.31	59.79±2.34	75.49±0.89	***
Victoria	88.34±1.10	74.11±1.74	81.28±4.64	***
Bicane	86.44±1.27	71.18±1.50	82.44±3.15	***
Chardonnay	83.50±1.02	61.66±1.73	80.26±1.50	***
Merlot	85.51±1.82	70.34±0.78	84.33±1.57	***
Cinsaut	83.23±1.26	65.71±1.03	77.60±1.78	***
Mean	84.87±2.30	67.13±5.68	80.23±3.23	***
CV%	2.71	8.46	4.03	-

Note:TTC - tetrazolium chloride; IKI -Lugol solution; AM -methyl blue; ± - standarddeviation(between the two years of study); CV% - coefficient of variability.*** - very significant differences (between tested methods).

It was noticed the high variability between varieties of the results obtained with the IKI method, the coefficient of variability (CV%) exceeding 8%.

The concentration of sucrose in the germination media had a significant influence on pollen germination ($p < 0.05$), the germination rate exceeding 80% only for the media with 15% sucrose (tab. 2).

Table2

Pollen germination rates of *Vitis vinifera* L. analysed varieties

Genotypes	Sucrose concentration				Significance (p < 0.05)
	5%	10%	15%	20%	
Muscat de Hamburg	59.35±1.27	61.27±1.33	82.73±2.13	77.88±0.77	***
Victoria	61.44±2.52	69.54±1.25	88.83±0.25	72.38±1.63	***
Bicane	55.37±1.51	62.22±1.42	81.33±1.12	68.33±1.11	***
Chardonnay	61.21±1.71	65.56±1.89	83.42±1.70	70.33±2.70	***
Merlot	62.16±2.90	64.11±1.75	88.05±0.23	74.74±1.05	***
Cinsaut	59.04±3.05	63.39±1.34	89.63±1.29	68.12±1.11	***
Average	59.76±2.48	64.35±2.94	85.66±3.57	71.96±3.83	***
CV%	4.14	4.58	4.17	5.33	-

Note: ± - standard deviation (between the two years of study); CV% - coefficient of variability.*** - very significant differences($p < 0,05$)(between the germination rate on media with different concentrations of sucrose).

The results obtained are in accordance with the data presented by Sabir (2015), regarding the germination rateofpollen at *Vitis vinifera* L. varieties.

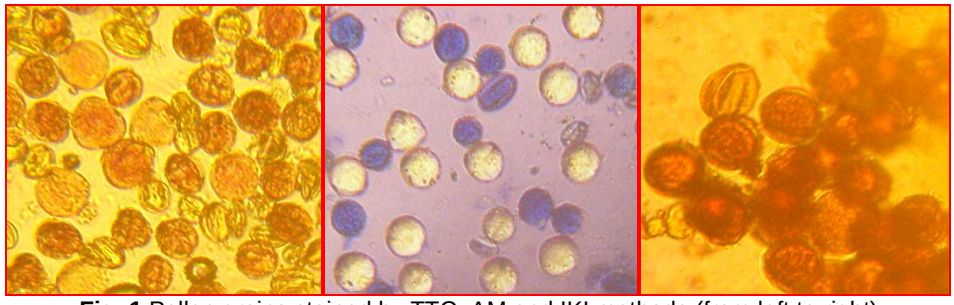

Fig. 1 Pollen grains stained by TTC, AM and IKI methods (from left to right)

The method that differentiated best the viable and non-viable cells was the AM test, followed by the TTC and the staining of the cells with IKI (fig. 1).

CONCLUSIONS

1. For all *Vitis vinifera* L. analysed varieties, the highest percentages of viable pollen were recorded when the TTC method was used, Muscat de Hamburg variety showing the lowest percentage of viable pollen regardless of the tested method.

2. The use of a medium with 15% sucrose led to higher ratesof pollen germination, up to 80% for all analysedvarieties, above the limit of 30% considered necessary for grapevine hybridization.

3. The method that highlighted best the viable cells was the AM method, the TTC method being considered the most accurate in indicating the percentage of viable pollen, in comparison to the germination rate.

REFERENCES

1. **DavarynejadG.H., SzaboZ., NyekiJ., SzaboT., 2008** - *Phenologicalstages, pollen production level, pollen viability and in vitro germination capability of some sour cherry cultivars.* Asian Journal of Plant Sciences, 7, p. 672-676.
2. **Firmage D., Dafni A., 2001** - *Field tests for pollen viability; a comparative approach*. Acta Hort. 561, ISHS 2001, p. 87-94.
3. **Oprea Ş., Moldovan S.D., 2007** – *Ameliorarea viței de vie în România*. Edit. Poliam, Cluj-Napoca.
4. **Sabir A., 2015** - *Improvement of the pollen quality and germination levels in grapes (Vitis vinifera L.) by leaf pulverizations with nanosize calcite and seaweed extract (Ascophyllium nodosum).* J. Anim. Plant Sci., 25(6), p. 1599-1605.
5. **Sulusoglu M, Cavusoglu A., 2014** - *In vitro pollen viability and pollen germination in cherry laurel (Prunus aurocerasus L.)*. The Scientific World Journal, p. 1-8.

VALUATION OF VEGETAL RESIDUE OF GRAPE SEEDS RESULTING FROM THE EXTRACTIVE PROCESSES OF PHENOLIC COMPOUNDS

VALORIFICAREA REZIDUULUI VEGETAL DE SEMINȚE DE STRUGURI REZULTAT DIN PROCESELE EXTRACTIVE ALE COMPUŞILOR FENOLICI

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Abstract. *The stepwise extraction process of phenolic compounds from grape seeds ultimately leads to the production of a residue rich in protein, cellulose and substances without nitrogen which can be biodegraded in nature by microorganisms from the soil. The purpose of this research was to verify whether the vegetable residue of grape seeds, resulting from the extraction of polymerized proanthocyanidins can be used as an organic fertilizer in the soil. The soil biodegradation process was assessed by microbiological analysis and analysis of current and potential dehydrogenase activity. The results obtained showed that the vegetal residue led to the increase of the number of soil microorganisms involved in the nitrogen circuit and carbon, as a result of the triggering of the biodegradation process as well as its non-polluting effect supported by current and potential dehydrogenase activity determined in dynamics over a year in experimental plots.*
Key words: organic fertilizer, microbiological, dehydrogenase activity

Rezumat. *Procesul de extracţie etapizată a compuşilor fenolici din seminţele de struguri conduce în final la obţinerea unui reziduu vegetal bogat în proteină, celuloză şi substanţe neazotate, ce pot fi biodegradate în natură, de către microrganismele din sol. Scopul cercetărilor efectuate a fost de a verifica dacă reziduul vegetal de seminţe de struguri rezultat după extracţia proantocianidinelor condensate polimerice poate fi utilizat ca îngrăşământ organic în sol. Procesul de biodegradare a reziduului administrat în sol a fost apreciat prin analize microbiologice şi analiza activităţii dehidrogenazice actuale şi potenţiale. Rezultatele obţinute au evidenţiat activitatea favorizantă a reziduului vegetal asupra creşteri numărului de microorganisme din sol implicate în circuitul azotului şi carbonului, ca urmare a declanşării procesului de biodegradare, precum şi efectul nepoluant al acestuia susţinut de activitatea dehidrogenazică actuală şi potenţială determinată în dinamică pe parcursul unui an în parcelele experimentale*
Cuvinte cheie: îngrăşământ organic, activitate microbiologică, enzimatică

INTRODUCTION

Maintaining and increasing soil fertility and biological activity is an important objective in organic viticulture (Bernaz *et. al*, 1999). The solution of this

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problem can be achieved by using conventional (organic and mineral fertilizers) or unconventional products provided that they are applied at well-defined doses in order not to induce soil imbalances in natural microflora (Ulea *et al*, 2008).

In the technology of exploiting the bioactive components in the grape waste, elaborated by Viticulture and Oenology Research and Development Station in Iasi (SCDVV Iasi), the gradual extraction process of phenolic compounds from grape seeds ultimately lead to a residue rich in vegetable protein (9.610 wt%), cellulose (51.720 wt%) and neazotate substance (34.80 wt%) or carbohydrates, which are the substances necessary for the activity of soil microorganisms.

The purpose of these researches was to verify the fertilizer quality of the plant residue resulting from the extraction of polymer condensed proanthocyanidins. The process of biodegradation in the soil of the residue given was assessed by the determination of pH, microbiological analysis and analysis of current and potential dehydrogenase activity.

MATERIAL AND METHOD

The experience was placed in a vineyard plantation belonging to SCDVV Iasi with plan land, chernozem cambic soil and south-western exposure. The amount of vegetable residue administered the test plots was calculated based on the results obtained in preliminary tests. Thus, in the plot V1 administered an amount of 1.5 kg/m^2 of vegetal residue and in plot V2 3.0 kg/m^2.The vegetal waste was applied by spreading on the vineyards, at the surface of the soil and incorporating it into the ground with the large digging up to the depth of 18-20 cm.

Determination of ecophysiological groups of microorganisms was carried out according to the method authors Pochon S. and Tardeux J. (1954) cited by Dunca S. (2007). The ecophysiological groups were assessed by the size order of the soil suspension dilution at which the microorganisms developed, and their number was expressed in logarithm based on 2/g soil.

Dehydrogenase enzyme activity current and potential were determined according to the method Casida (Drăgan - Bularda, 2000).

RESULTS AND DISCUSSIONS

Microorganisms in the soil a remarkable capacity to adapt the biodegradation processes of natural organic compounds. Most of the soil microorganisms prefer a pH close to neutrality. This aspect was monitored during the fertilizer quality assay of the plant residue.

The data on the variation of pH values in the soil in the control plot and the two plots in the experimental variants V1 and V2 are presented in table 1.

Table 1

The evolution of the soil pH values in the time March to September, 2016

Variantion	Months						
	March	April	May	June	July	August	September
Control plot (M)	6.50	6.41	6.50	5.90	5.90	6.11	6.68
Plot V1 1.5 kg/m^2	6.61	6.26	6.35	5.91	6.02	6.13	6.71
Plot V2 3.0 kg/m^2	6.50	6.21	6.20	5.85	6.04	6.19	6.72

From analysis of pH variation data was found, between March and June 2016 a moderate decrease of the values in the soil in the control plot (from 6.5 to 5.9) and the plot V1 (from 6.61 to 5.91) and more pronounced in the soil in the plot V2 (from 6.5 to 5.85).

The decrease in the pH values of the product was due to the precipitation of this time. In the plot V2 the decrease was also accentuated due to the higher quantity of vegetal residuals. Starting of month July observed a progressive increase in the soil pH values V1 and V2 plots, values higher than those recorded in the control plot soil.

Considering the chemical composition of the vegetable residue, namely rich in proteins, cellulose and carbohydrates, microbiological analyzes in this study focused on the determination of the eco-physiological groups of microorganisms involved in the nitrogen and carbon circuit.

In the context of climatic factors in the spring of 2016 (the average temperature higher than the normal level of soil moisture to 69%), determinations made before administration of the vegetable residue in the soil for the evaluation of potential microorganisms in ecophysiological groups, showed that they were well represented (tab. 2).

Table 2

The number of ecophysiological microorganisms (logarithm in base 2)

Plots	Total number of microorganisms								
	Ammo-niacal microflora	nitrification microflora		Denitrifying microflora	Nitrogen fixation microflora		Proteolytic microflora	Cellulosylium microflora	
		nitric bacteria	nitroas bacteria		aerobic	anaerobic		aerobic	anaerobic
MARCH 2016									
M	63.044	9.551	9.551	22.839	8.644	15.458	16.811	23.304	18.610
V1	63.043	8.814	6.845	21.254	7.966	12.873	15.988	22.666	17.932
V 2	59.932	9.343	9.892	22.632	8.814	14.610	15.458	21.966	19.310
JUNE 2016									
M	60.380	15.986	7.644	15.458	15.456	8.229	28.746	15.458	11.773
V1	74.082	20.510	8.814	14.873	17.922	10.966	36.467	16.536	11.966
V 2	75.389	20.133	9.892	13.214	17.923	12.316	36.467	21.245	12.136
SEPTEMBER 2016									
M	48.677	13.873	1.100	0.900	0.900	0.900	12.873	0.900	0.900
V1	47.507	17.195	1.100	0.900	0.900	0.900	12.666	0.900	0.900
V 2	52.529	17.610	1.150	0.900	0.900	0.900	12.873	0.900	0.900
MARCH 2017									
M	59.996	7.644	7.229	18.780	7.956	7.644	13.214	11.966	14.288
V1	59.865	11.288	11.288	18.417	7.956	8.451	16.205	12.001	17.932
V 2	63.317	12.883	11.966	21.932	8.451	11.288	16.747	16.536	19.858

In order of the number of microorganisms determined in the nitrogen circuit, the most abundant were the ammonifier, denitrifying, proteolytic, anaerobic nitrogen fixators, nitrite bacteria, nitratbacteria and aerobic nitrogen fixators. In order of the number of microorganisms involved in the carbon chain, the most numerous were aerobic cellulosic bacteria, followed by anaerobic

cellulosic.

The results obtained three months after soil vegetal application (June 2016) revealed a numerical increase of the microorganisms in experimental plots V1 and V2 compared to their numerical representation in the control soil, a positive aspect supporting the quality of organic fertilizer natural vegetable residue.

The analysis of September results shows that the ecophysiological groups of microorganisms were affected by the evolution of climatic factors during the summer months (absence of precipitation, accentuation of the water deficit in the first layer of soil 0-20 cm from 47% in June to 77% and 86% in July and August). The number of ammonifying microorganisms in the M and V 1 plots was lower compared to the one determined in June. Also, the number of nitrate bacteria increased by 19% in V1 and 21% in V2.

Microorganisms in eco-physiological groups: nitritbacteria, denitrifying, aerobic and anaerobic nitrogen fixators, as well as aerobic and anaerobic celluloses were identified in the first soil dilution but were not identified as a number by the Mc Cray method in logarithm on base 2.

One year after the administration of the vegetal residues into the soil (March 2017), the number of ammonifying microorganisms in the soil was almost equal to the control plot V1 and higher by 5.2% in the soil in the plot V2. The number of nitrate bacteria and nitrite bacteria increased in value in the soil in plots V1 and V2 compared to the number determined in plot M. The number of denitrifying microorganisms determined was almost to the soil in the plots M and V1, and by 14% higher in the plot V2. The number of aerobic nitrogen fixation microorganisms / g of soil was equal in the plots M and V1 and higher by 5.8% in the experimental plot V2, and the number of anaerobic nitrogen fixation microorganisms / g of soil increased by 9% in experimental plot V1 and by 32% in experimental plot V2.

Compared to the number of microorganisms determined by the plot M, V1 and V2 in the plots, the number of the proteolytic microorganisms grown in plots V1 and V2 with 18% to 21%. The number of aerobic cellulosic microorganisms determined in this group was almost equal to the number determined in the control plots and V1 and higher by 27% in the experimental plot V2. Regarding the number of anaerobic cellulosic microorganisms, compared to the control plot their number was higher in plot V1 by 20% and in the experimental plot V2 by 28%.

The results of microbiological analyzes are supported by current and potential dehydrogenase enzymatic analyzes performed monthly. The enzyme activity in the soil is one of the first parameters that change under the influence of fertilization products, representing an early indicator of soil quality changes (Lee *et al.,* 2002; Garcia-Ruiz *et al.,* 2008).

The results of dehydrogenase enzymatic assays are presented graphically in figures 1 and 2.

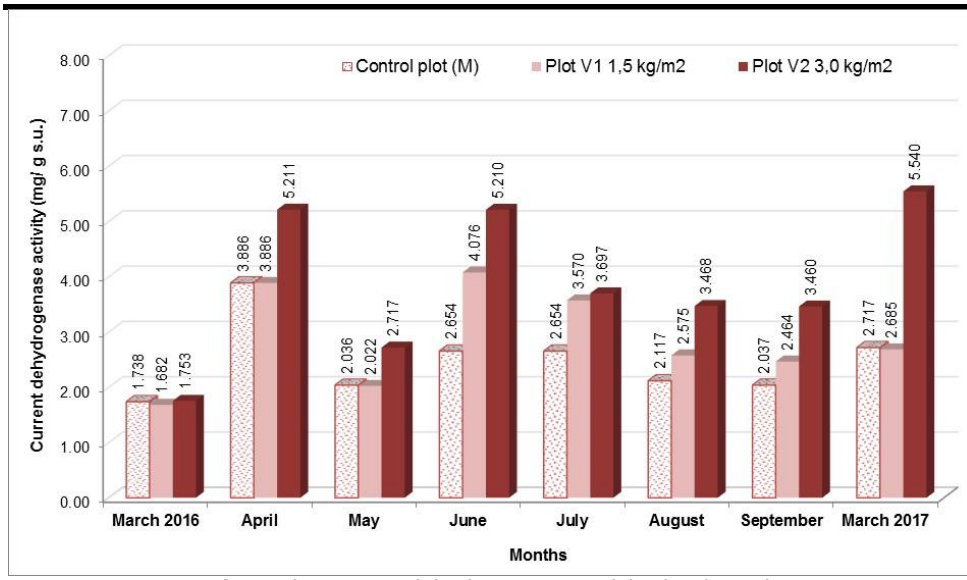

Fig. 1 The current dehydrogenase activity (mg/g s.u.)

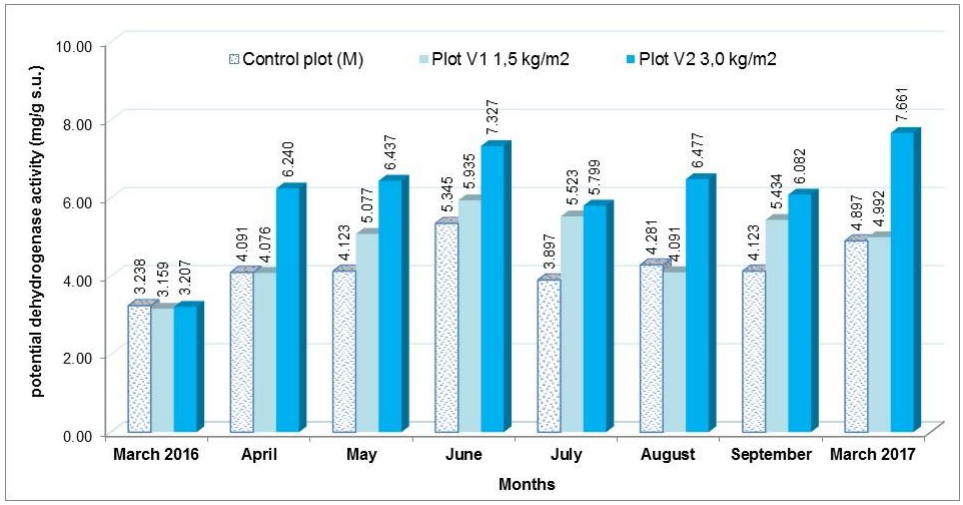

Fig. 2 The potential dehydrogenase activity (mg/g s.u.)

Determination of enzymatic activity, made in dynamics during the experiment, reveals that before application of the vegetal residue to the soil, the current and potential dehydrogenase activity values were close in the first case, between 1.682 – 1.753 mg formazan/g s.u. and 3.159 – 3.238 mg formazan/g s.u.

After administration of the residues in the soil, the values of dehydrogenase activity in the soil increased the plots V1 and V2, appearance which was maintained between April and September 2016.

One year after the administration of the vegetal residue into the soil, the actual and potential dehydrogenase activity values in plot V2 were higher

compared to the mean values determined in parcels M and V1 by 50% for current dehydrogenase activity and by 35% for potential dehydrogenase activity.

CONCLUSIONS

1. The test vegetal residue has favored the development of microorganism populations, an effect mainly observed in the soil in experimental plot V2 where the highest amount of vegetal residue (3.0 kg / m2) was administered, which led to a larger number of ecophysiological microorganisms compared to the soil in the plot of V1.

2. Dehydrogenase activity has been shown to be a sensitive indicator of global microbiological activity. Higher values of current and potential dehydrogenic activity in V1 and V2 plots support the non-polluting effect of the test vegetal residue, which did not inhibit the development of soil eco-physiological groups involved in the nitrogen and carbon circuit.

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REFERENCES

1. Bernaz Ghe., Dejeu L., 1999 - Fertilizarea viilor şi întreținerea solului în concepție ecologică. Editura Ceres

2. Drăgan-Bularda M., Samuel A.D., 2006 - *Microbiologie generală*, Ed. Universității din Oradea, Oradea.

3. Dunca S., Ailiesei O., Nimițan E., Ştefan M., 2007 - *Microbiologie aplicată*. Casa Editorială Demiurg, Iaşi.

4. Garcia C., Roldan A., Costa F., 1997 - *Potential use of dehydrogenase activity as an index of microbial activity in degraded soils.* Commun. Soil Sci. Plant., 12: 123-134.

5. Lee I.S., Kim O.K., Chang Y.Y., Bae B., Kim H. H., Baek K.H., 2002 - *Heavy metal concentration and enzyme activities in soil from contaminated Korean shooting range*, Journal of Bioscience and Bioengineering, 94: 400-411.

6. Ulea E., Lipşa F., Irimia Nicoleta, Bălan Gabriela Mihaela, 2008 - *Cercetări privind influența fertilizării şi amestecului de Onobrychis viciifolia scop. şi Bromus inermis leiss. asupra microflorei solului*. Lucrări Ştiințifice, vol. 51, seria Agronomie, pp. 132 - 139

ANTIMICROBIAL ACTIVITY OF AN ACTIVE BIOLOGICAL BIOPRODUCT OBTAINED FROM GRAPE SEEDS

ACTIVITATEA ANTIMICROBIANĂ A UNUI BIOPRODUS BIOLOGIC ACTIV OBȚINUT DIN SEMINȚELE DE STRUGURI

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Abstract. *At the Research Station for Viticulture and Enology Iasi, a polymeric condensed proanthocyanidins was obtained from Fetească neagră grape seeds, through a phase preparation, which under determined conditions with hydrogen peroxide, led to the production of a water-soluble bio product with antibacterial, antifungal and antioxidant properties. Evaluation of the antimicrobial activity of the bio product was performed by determining minimum inhibitory concentration (MIC) and minimal bactericidal concentration (CMB) against Staphylococcus aureus and Escherichia coli. From the analysis of the obtained data it was found that the bio product reacted differently from the tested species, being more active against Staphylococcus aureus (G +). The determined MIC was 1.5 mg/mL and the CMB 2.0 mg / mL. In the case of the Escherichia coli (G-) test, the MIC and CMB values were equal but increased to 3.0 mg/mL.*
Key words: seed, proanthocyanidins, inhibitoriy, bactericidal

Rezumat. *La Staţiunea de Cercetare Dezvoltare pentru Viticultură şi Vinificaţie Iaşi, din seminţele de struguri din soiul Fetească neagră, prin extracţii etapizate s-a obţinut un preparat de proantocianidine condensate (PA) polimerice, care tratat cu peroxid de hidrogen, în condiţii determinate, a condus la obţinerea unui bioprodus solubil în apă, cu proprietăţi antibacteriene, antifungice şi antioxidante. Evaluarea activităţii antimicrobiene a bioprodusului s-a efectuat prin determinarea concentraţie minime inhibitorie (CMI) şi a concentraţiei minime bactericide (CMB) faţă de speciile Staphilococcus aureus şi Escherichia coli. Din analiza datelor obţinute s-a constat că bioprodusul a reacţionat diferit faţă de speciile testate, fiind mai activ faţă de Staphilococcus aureus (G+). Valoarea CMI determinată a fost de 1,5 mg/mL, iar valoarea CMB de 2,0 mg/mL. În cazul testului efectuat faţă de specia (G-) Escherichia coli, valorile CMI şi CMB au fost egale, dar au crescut la 3,0 mg/mL.*
Cuvinte cheie: seminţe, proantocianidine, inhibitoriu, bactericid

INTRODUCTION

In the treatment of infectious diseases, the availability of products with antibacterial activity is insufficient, so numerous researches have focused on the investigation of natural products as sources of bioactive molecules (Valgas *et al,*

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2007). This research has grown, as it has been found to increase the resistance of pathogenic bacteria to antibiotics. Among the natural compounds extracted from plants, polyphenols have been shown to have antibacterial functional properties.

Grape seed is the richest source of phenolic extractable compounds whose antibacterial activity has been studied using either total polyphenolic extracts or subclasses of phenolic compounds, namely phenolic acids, quinones, flavanes, flavonols and tannins. The evaluation of antimicrobial activity of phenolic compounds extracted from various natural sources: grape marc, grape seeds, white and red wines has been the subject of numerous studies.

Rodriguez - Vaquero *et al*. (2007) and Papadopoulou *et al.* (2005) have determined the antibacterial activity of polyphenolic extracts obtained from white and red wine, establish the inhibition of the development of the species *Staphylococcus aureus* and *Escherichia coli*. Similar results were obtained by Radovanović *et al.* (2009), the diameter of the inhibition zone of the species *Staphylococcus aureus and Escherichia coli* being 16 - 22 mm and 12 - 20 mm respectively. Jayaprakaska *et al* (2003) determined the antibacterial activity on crude polyphenolic extracts obtained from grape seed extracted into acetone-water-acetic acid (90: 9.5: 0.5) and methanol-water- acetic acid (90: 95: 5) using different species of Gram-positive and Gram-negative bacteria. The minimum inhibitory activity (MIC) against *Staphylococcus aureus* was 1000 ppm / mL, and for *Escherichia coli* at 1250 ppm / mL.

Baydar *et al*. 2004, studied the antimicrobial activity by the diffusion method against 13 Gram positive and Gram negative bacteria using polyphenolic extracts obtained from grape seeds and found that Gram positive bacteria, *Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis* were inhibited by concentrations small amounts of phenolic compounds compared to Gram negative species *Escherichia coli* and *Pseudomonas aeruginosa*.

At the Viticulture and Oenology Research and Development Station in Iaşi, from the grape seed of Fetească neagră variety, by stepwise extractions a preparation of polymeric condensed proanthocyanidins (PA) was prepared, which was treated with hydrogen peroxide, under certain conditions, resulted in a water-soluble bioproduct with antibacterial, antifungal and antioxidant properties. The evaluation of the antimicrobial activity of the bioproduct was performed by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against *Staphylococcus aureus* and *Escherichia coli.*

MATERIAL AND METHOD

The bioproduct was physically and chemically characterized by determining the solubility in distilled water, the amount of total polyphenols - CFT, mg GAE / mg product (method Singleton and Rossi, 1965), polyphenolic index - IP (spectrophotometric at 280 nm) and antioxidant activity - AA (%) / 100 µg bioproduct (Method Brand-Williams *et al*., 1994, with modification of Miliauskas *et.al*.,2004). Determination of the antimicrobial activity of the active bioproduct was performed

qualitatively in the preliminary test by diffusion method on agarified medium with stainless steel cylinders and quantitatively by the dilution method on the liquid culture medium of the test microorganisms.

REZULTS AND DISCUSSIONS

The bioproduct obtained under optimal fractionation conditions is in the form of yellowish brown crusts, is completely soluble in distilled water, has an amount of polyphenolic compounds total of 0.625 mg GAE / mg and an antioxidant activity of 91.37% at concentration of 100 µg / mL (table1).

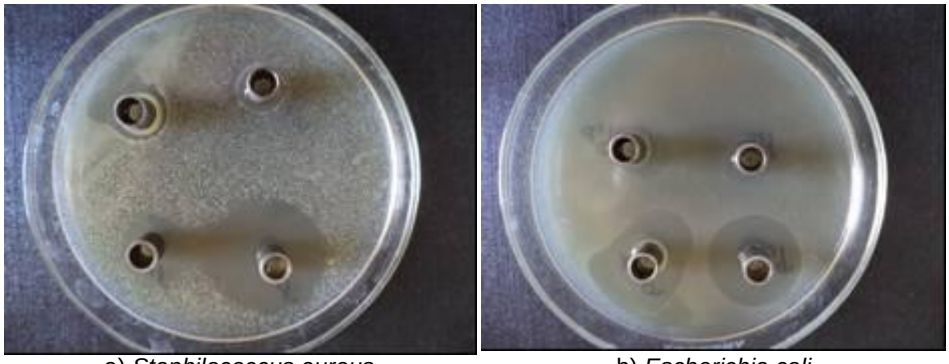
Table 1

Physico-chemical characteristics of the active bioproduct

Activ bioproduct	color		Solubility in distilled water	CFT, mg GAE/mg product	IP 280 nm	A. antioxidant % /100 µg	
	before fractionation	after fractionation				30 min	60 min
	brown crusts	yellow - brown powder	completely	0.625	18.4	91.37	92.44

Determination of antimicrobial activity in the preliminary test.

In the preliminary test carried out, the diffusometric method found that the bioproduct had antibacterial activity against both species of tested microorganisms. In the case of the species *Staphylococcus aureus* antibacterial activity not expressed at a concentration of 0.5 mg/mL, but was shown as a concentration of 1 mg/mL. The diameter of the inhibition zone of the test microorganism increased with the increase of the bioproduct concentration so that at the concentration of 2 mg / mL the largest diameter was 26 mm (fig. 1).



a) *Staphilococcus aureus* b) *Escherichia coli*

Fig. 1 Antimicrobial activity of the bioproduct against species *Staphilococcus aureus (a) and Escherichia coli (b)*

In the case of *Escherichia coli*, the antimicrobial activity was evidently starting with the bioproduct concentration of 1.5 mg/mL and 2 mg/mL, the diameters of the inhibition zones of the tested microorganism being close to 18 mm and 21 mm, respectively.

Also, from the data obtained in the preliminary test, the *Staphylococcus aureus* Gram positive species is more sensitive to the complex of phenolic compounds in the obtained bioproduct, compared to the Gram negative species *Escherichia coli.*

Determination of antimicrobial activity by dilution method on liquid medium

The preliminary test revealed that the bioproduct obtained has antibacterial activity starting from the concentration of 1 mg/mL and 1.5 mg/mL, respectively, relative to the species of microorganisms studied. Thus, in the experiment to evaluate the antimicrobial activity on the liquid medium, 7 concentrations/mL were used, conditions which ensure direct contact of the test microorganism cells with the phenolic compound complex from the bioproduct obtained by the physico-chemical treatment of the proanthocyanidinic preparation. The antimicrobial activity of the bioproduct was assessed by the MIC and MBC values. Table 2 and figures 2 and 3 show the results obtained in the determination of antimicrobial activity by the quantitative method on the liquid medium.

Table 2

Antibacterial activity MIC and MBC of the bioproduct obtained against *Staphylococcus aureus* and *Escherichia coli*

Microorganism test	Bioproduct mg/mL						
	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Staphilococcus aureus	+[x]	+[xx]	-[xxx]	-	-	-	-
Escherichia coli	+	+	+	+	-	-	-

[x] development of microorganisms test
[xx] inhibition of visible development of test microorganisms
[xxx] 99% inhibition of test microorganism development

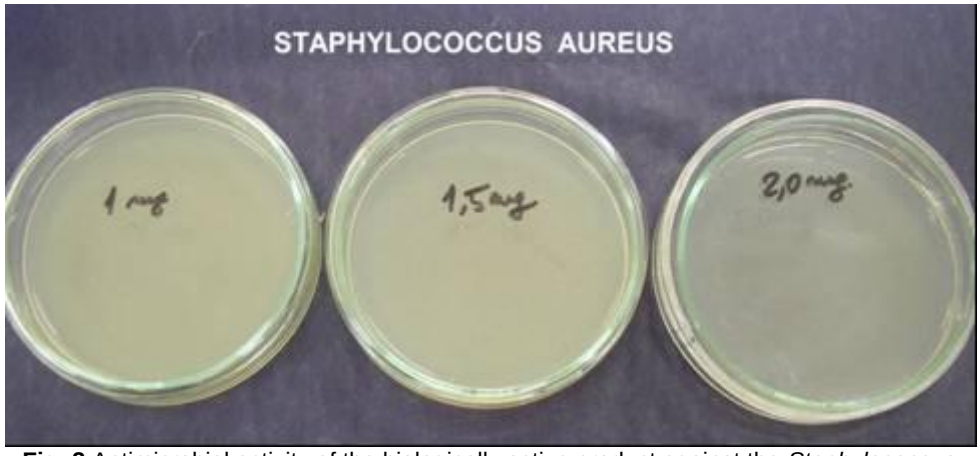


Fig. 2 Antimicrobial activity of the biologically active product against the *Staphylococcus aureus* test microorganism

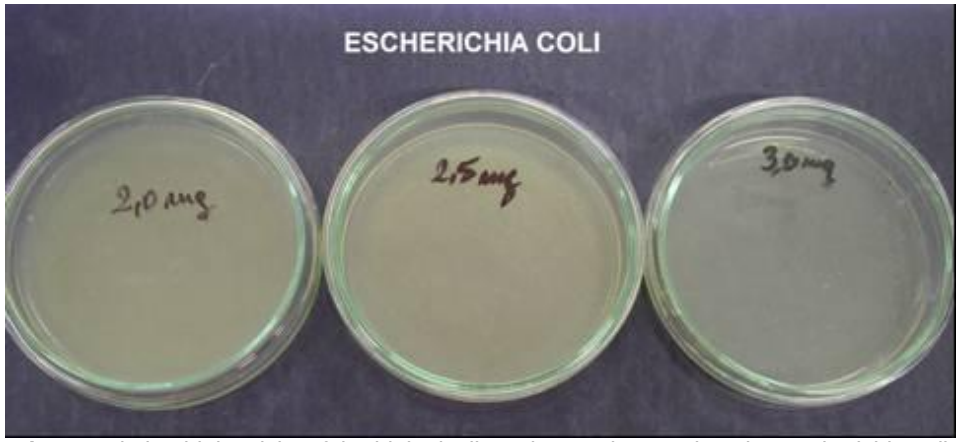


Fig. 3 Antimicrobial activity of the biologically active product against the *Escherichia coli* test microorganism

From the analysis of the data presented in table 2 and figures 2 - 3, the following aspects were found:

- ✓ the minimum inhibitory concentration (MIC) of the bioproduct for the species *Staphylococcus aureus* was 1.5 mg/mL and 3.0 mg/mL for the species *Escherichia coli*;
- ✓ the minimal bactericidal concentration (MBC) of the bioproduct for the species *Staphylococcus aureus* was 2.0 mg/mL and 3.0 mg/mL for the species *Escherichia coli*;
- ✓ In the case of *Escherichia coli*, the antibiotic activity of the CMI and CMB of the bioproduct was at the same concentration of 3.0 mg/mL.

The bioproduct show a more pronounced antibacterial reactivity to gram positive and temperate Gram negative species, as other authors have found when testing total polyphenolic extracts or phenolic compounds isolated from polyphenolic extracts from grape seeds.

The CMB / CMI ratio mark the bactericidal effect to values less than 4 and the bacteriostatic effect to values greater than 4 (O'neil *et al.*, 2004). In the case of the bioproduct tested, the antibacterial activity evaluated according to the CMB / CMI ratio (1.0 and 1.33) defines in particular the bactericidal potential of the test bacterial species.

Regarding the mechanism of action of phenolic compounds on microorganisms Xia *et al.* (2010), suggests that polyphenols by conjugating to the cell wall proteins of microorganisms and especially to key enzymes may be the main way to inhibit the development of microorganisms. Other authors (Cowan *et al,* 1999) have shown that the hydrophobic partial nature of phenolic compounds is responsible for antimicrobial activity. Also, the accumulation and attachment of phenolic compounds to the cytoplasmic membrane may eventually result in the death of microbial cells.

CONCLUSIONS

1. The bioproduct reacted differently to the test species, being more active against *Staphilococcus aureu*s (G+). The determined minimum inhibitory concentration (MIC) was 1.5 mg / mL and the minimal bactericidal concentration (MBC) of 2.0 mg/mL. In the case of the *Escherichia coli* (G-) test, the MIC and MBC values were equal but increased to 3.0 mg/mL.

2. Demonstration of antimicrobial activity against Gram positive and Gram negative microorganisms of phenolic compounds from the obtained bioproduct, is a way to capitalize on it chemically modified proanthocyanidins for solubilization in water, also representing a potential source with a wide utility spectrum in human and animal infectious therapy.

***Acknowledgments**: This work was carried out by the Partnership in priority areas - PN II, developed with the support of MEN -UEFISCDI, project no. 183/2014 (PN-II-PT-PCCA-2013-4-0333) "Technology of capitalization of the bioactive elements from the grape seed waste with usefulness in the food and pharmaceutical industry, plant and environmental protection (Acronym: PROVITIS)"*

REFERENCES

1. Baydar N.G., Özkan G., Sağdiç O., 2004 - *Total Phenolic Contents and Antibacterial Activities of Grape (Vitis vinifera L.)*. Extracts Food Control, vol. 15, pp: 335 - 339.

2. Cowan M.M., 1999 - *Plant product as antimicrobial agents*. Clinical Microbiology Reviews. vol. 12, no. 4, p. 564-582.

3. Jayaprakasha G. K., Selvi T., Sakariah K. K., 2003 - *Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extracts*. Food Research International, 36, pp: 117-122.

4. Miliauskas G, Yenkutonis PR, Van beek TA, 2004 - *Screening of radical scavenging activity of some medicinal and aromatic plants extracts*. Food Chem. vol. 85, pp:231–237.

5. O'Neill A. J., Chopra, I., 2004 - *Preclinical evaluation of novel antibacterial agents by microbiological and molecular techniques.* Expert Opin. Invest. Drug. 13, 1045–1063.

6. Papadopoulou C, Soulti K, Roussis IG., 2005 - *Potential antimicrobial activity of red and white wine phenolic extracts against strains of S. aureus, E.coli and C.albicans.* Food Technol Biotechnol; 43: 41 – 46.

7. Radovanović A, Radovanović B, Jovančičević B., 2009 - *Free radical scavenging and antibacterial activities of southern Serbian red wines*. Food Chem;117: 326 - 333.

8. Rodríguez-Vaquero, M. J., Alberto, M. R., & Manca de Nadra, M. C., 2007 - *Antibacterial effect of phenolic compounds from different wines*. Food Control,18, 93–101.

9. Singleton V.L., Rossi J.A., 1965 - *Colorimetry of total phenolics with phosphomolybdic – phosphotungstic acid reagents*. Am. J. Enol. Vitic., vol. 16, 144-158.

10. Valgas C., D'Souza S., Elza F., Smania Jr. A., 2007 - *Screening methods to determine antibacterial activity of natural products*. Brazilian J. Microbiol. 38, pp: 369-380.

11. Xia E.Q., Deng G.F., Guo, Y.J., Li H.B., 2010 - *Biological Activities of Polyphenols from Grapes*. International Journal of Molecular Sciences, vol. 11, pp: 622-646.

TREND OF THE EVOLUTION OF DAILY PRECIPITATION IN THE CONDITION OF PROBABLE CLIMATIC CHANGES IN THE DEALUL BUJORULUI VINEYARD

TENDINȚA EVOLUȚIEI PRECIPITAȚIILOR ÎN PODGORIA DEALUL BUJORULUI ÎN CONDIȚII DE SCHIMBĂRI CLIMATICE PROBABILE

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Abstract. *The evolution of the precipitations in the Dealul Bujorului vineyard was studied during 1980-2016. The periods 1980-2006 and 2007-2016 were compared. We analyzed the frequency of torrential rains, rainfalls, rainfall surplus / rainfall both during the vegetation period and during the winter. All these observations and determinations will allow the assessment of the consequences of extreme precipitation under the current climate change conditions. From the data analyzed was observed a tendency to accentuate the rainfall events during certain periods (increased frequency of extreme rains, torrential rain followed by long periods with rainfall, increasing the frequency of non-worthwhile rains).*
Key words: climate risk, vine, precipitations, rainfall deficit

Rezumat. *S-a studiat evoluția precipitațiilor în podgoria Dealul Bujorului în perioada 1980-2016. S-a luat comparativ perioadele 1980-2006 şi 2007-2016. S-a analizat frecvența ploilor torențiale, a ploilor valorificabile, a excedentului / deficitului de precipitații atât pe perioada de vegetație cât şi pe perioada de iarnă. Toate aceste observații şi determinări vor permite evaluarea consecințelor precipitațiilor extreme in condițiile actuale de modificare a climei. Din datele analizate s-a observat o tendință de accentuare a evenimentelor pluviale pe anumite perioade (creşterea frecvenței ploilor extreme, ploi torențiale urmate de perioade lungi cu deficit pluviometric, creşterea frecvenței ploilor nevalorificabile).*
Cuvinte cheie: risc climatic, viță de vie, precipitații, ploi torențiale

INTRODUCTION

Today we are assisting the global climate change due to global warming caused by the greenhouse effect. Climate change has been manifested more and more often in recent decades by increasing air temperature, the considerable reduction in atmospheric precipitation (rainfall and snowfall) and extreme weather phenomena (Enache *et al*, 2007). Analysis of climatic data over long periods has shown a climate change trend. Simulations with complex global climate models indicated that the phenomenon is determined by both natural and anthropogenic factors (IPCC, 2007). According to the researches, it is concluded

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that the air temperature may rise by 0.1°C/decade in the coming decades. Also, in the 2007 ICPP report it was mentioned that an essential feature of the temporary variability of precipitation amounts is the between decades component that makes it difficult to separate the long-term climate signal. The indicators regarding intense rainfall generally showed an increasing trend in winter and a downward trend in summer. In spring and autumn, clues have seen an increasing trend, but not as in winter (Yeshewatesfa Hundecha, Andras B"Ardossy, 2005).

Based on these considerations, the paper aims to present the trend of the precipitation evolution in the Dealul Bujorului vineyard analyzing a series of data from the 1980-2006 periods taking as 2007-2016.

MATERIAL AND METHOD

The research was carried out within the Research and Development Station for Viticultural and Winemaking Bujoru. Data on daily, monthly, and annual average precipitation were processed and analyzed. In order to evaluate the precipitation evolution trend, the data was collected from the Tg. Bujor weather station (AGROEXPERT system). Were analyzed the evolution of monthly and annual rainfall, during the winter and vegetation period, the precipitation rate greater than 10 mm, greater than 5 mm and less than 5 mm, the frequency of torrential rains relative to the total number of higher rainfalls 5 mm (useful rain). A comparative analysis was carried out between 1980-2006 and the reference period 2007-2016.

RESULTS AND DISCUSSIONS

In the figure 1 shows the annual precipitation over two time periods: 1980-2006 and 2007-2016**.** Annual precipitations were compared with the mobile media for 5 years and multi-annual average. The annual rainfall and precipitation during the vegetation period were distinct. The moving average for 5-year, indicates a declining rainfall trend and a growth trend over the period 2007-2016. Between 1980 and 2016, out of the total of 27 years, 10 years are below the multiannual average and 11 years below the multiannual average of the vegetation period. From these 11 years, 5 years have been with precipitation less than 250 mm (250 mm are necessary rainfall for vine cultivation during the vegetation period). Of the 10 years of the reference period 2007-2016, 3 years are below the multiannual average and 7 years below the multiannual average of the vegetation period of which 4 years with precipitation less than 250 mm. We observe that rainfall deficit is particularly high during the vegetation period. From the point of view of the monthly precipitation evolution, the number of rains smaller than 5 mm predominates in all months (fig. 2). In June, July and August the share of rain above 5 mm increased during the reference period 2007-2016 compared to the period 1980-2006 and the share of rain above 10 mm decreased. The monthly average of precipitation in the reference period 2007-2016 shows a downward trend in April, June, July and August and growth in the other months of the year (fig.3). The evolution of the monthly precipitation average reveals negative

deviations during the reference period 2007-2016 in April, June, July and August, with a maximum in August of 10,1mm and positive deviations in the other months (tab.1). The sum of the monthly precipitation from 2007-2016 is increasing compared to 1980-2006, with an average annual deviation of 73,1mm. In the reference period 2007-2016, the average positive deviation was registered in the winter and May. In the rest of the month the deviation was negative.

The rainfall recorded in the 37 years analyzed was differentiated as follows: rains below 5 mm, useful rains greater than 5 mm and more than 10 mm. Much of the precipitation had a torrential character and was not fully exploited by the soil. In the reference period 2007-2016 the torrential rains frequency is higher in June, July and August compared to the period 1980-2006. If during 1980-2006 the maximum torrential rainfall was recorded in June, in the reference period the maximum was 34,4% in July, followed by 24% in August. In April, May and September the torrential rains frequency is higher in the period 1980-2006 compared to the period 2007-2016 (fig.4).

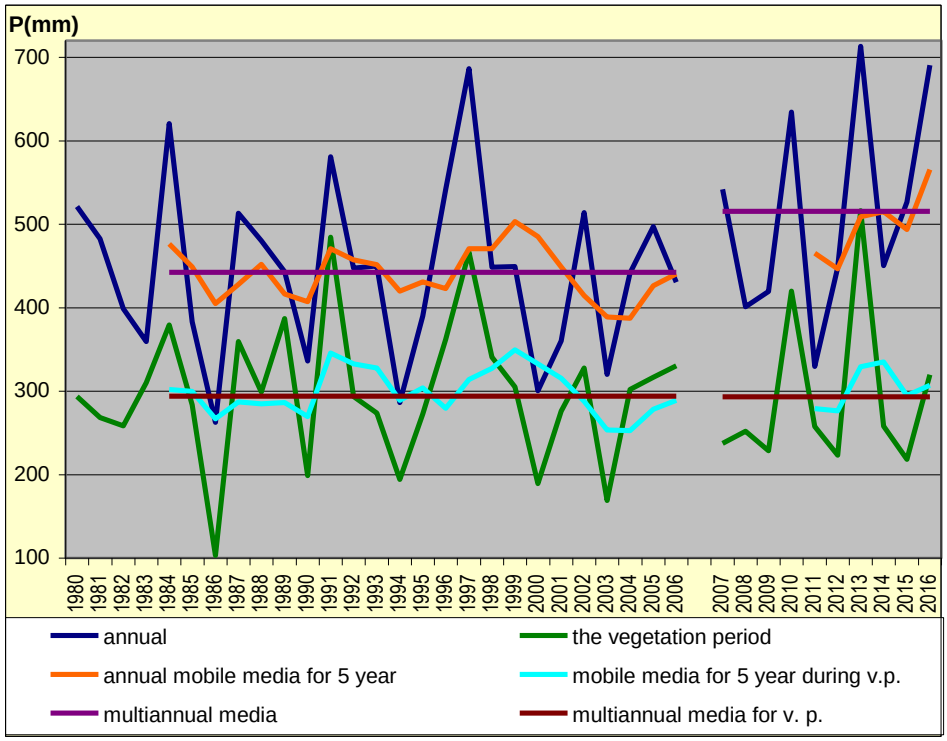

Fig. 1 Distribution of annual average precipitation between 1980-2006 and 2007-2016 period

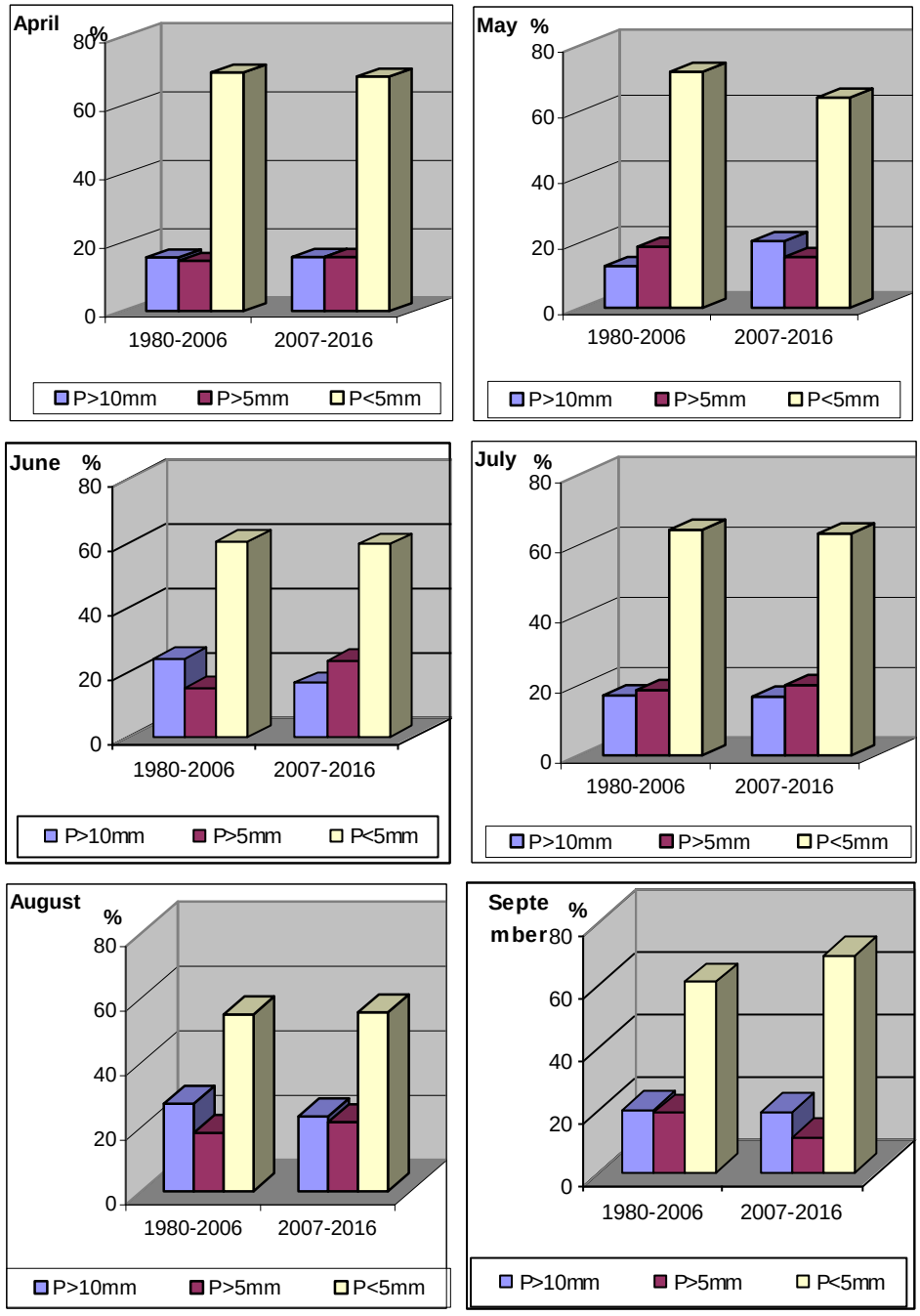

Fig. 2 Monthly precipitation rate P> 10mm, P> 5mm and P <5mm in the 1980-2006 interval and the 2007-2016 reference period

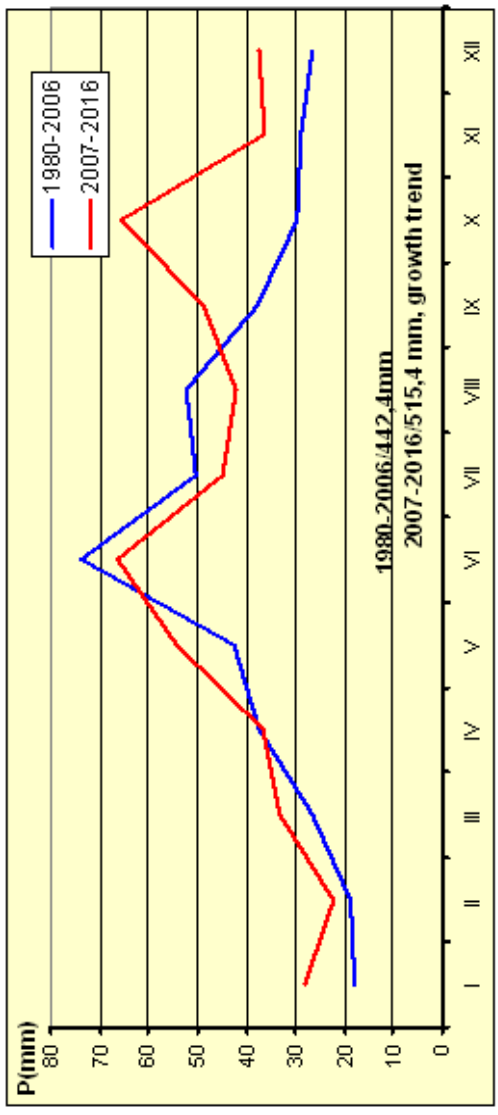

Fig. 3 Evolution of the average monthly rainfall in the Dealul Bujorului vineyard in the 1980-2006 periods compared to the 2007-2016 interval

The average of the monthly precipitation in the Dealul Bujorului vineyard in the period 1980-2006 as compared to the period 2007-2016

Table 1

Period	Monthly precipitation (mm)												Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2007-2016	28.1	22.3	33.2	36.7	54.2	66.5	44.8	42.1	48.6	65.5	36.1	37.3	515.4
1980-2006	17.9	18.6	26.7	37.4	42.7	73.7	50.2	52.2	37.8	29.5	28.8	26.7	442.4
deviation	10.2	3.7	6.5	-0.7	11.5	-7.2	-5.4	-10.1	10.8	36.0	7.4	10.6	73.1

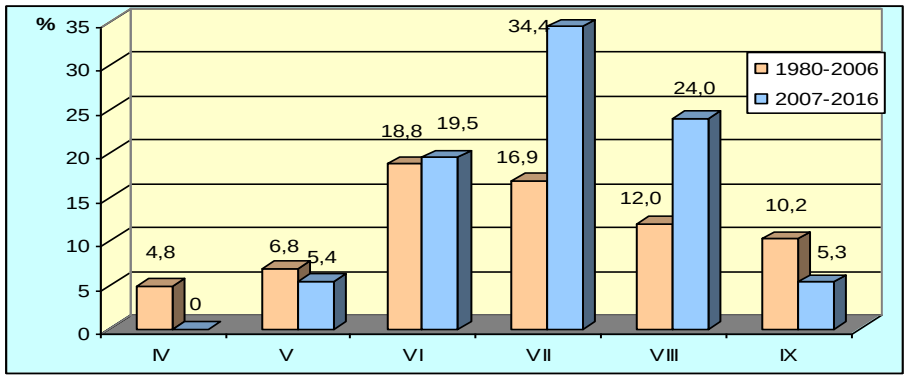

Fig. 4 Frequency of torrential rains relative to total rainfall greater than 5 mm

CONCLUSIONS

Comparing the 1980-2006 period with the reference period 2007-2016, it was found that:

1. The rainfall deficit has been particularly accentuated during the vegetation period of the reference range.

2. The share of useful rainfall (> 5 mm) in June, July and August increased during the reference period 2007-2016 compared to the 1980-2006 interval and the share of rain above 10 mm decreased.

3. The sum of the monthly precipitation from 2007-2016 is increasing compared to 1980-2006, with a positive average annual deviation of 73,1mm.

4. The monthly average rainfall for the reference 2007-2016 periods shows a downward trend in April, June, July and August and growth in the other months of the year compared to the 1980-2006 period.

5. Between 1980 and 2006, the maximum torrential rainfall was recorded in June and in the reference period the maximum moved to July (34.4%), followed by August (24%).

REFERENCES

1. Enache Viorica, 2007 - *Researches Regarding the Tendency in Some climatic factors evolusion from Moldavia south hilly region with reference ti viticultural plantation*, Lucrări ştiinţifice, Anul L, vol. 1 (50), Seria Horticultura, Editura Ion Ionescu de la Brad, p.639-642

2. Yeshewatesfa Hundecha, Andras B"Ardossy, 2005 - *Trend in dauly precipiations and temperature extremes across western Germanyin the second half of the 20th century*, INTERNATIONAL JOURNAL OF CLIMATOLOGY Int. J. Climatol. 25: 1189–1202 (2005) Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/joc.1182

3.*, IPCC, 2007** - *Climate change, 2007, The physical science basis. Contributionof working Grup I tothe fourthassessment raport of the IPCC*, Cambridge UP

EVALUATION OF FERTILITY OF NEW SEEDLESS VARIETIES INCLUDED IN GRAPEVINE ASSORTMENT OF THE REPUBLIC OF MOLDOVA

EVALUAREA FERTILITĂȚII SOIURILOR NOI APIRENE INCLUSE ÎN SORTIMENTUL VITICOL AL REPUBLICII MOLDOVA

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Abstract*. In the paper are presented the results of study of fertility of the new seedless varieties included in the grapevine assortment of the Republic of Moldova - Apiren roz timpuriu, Apiren alb, Apiren roz, Apiren Basarabean and Apiren negru de Grozeşti. The correlation between the main fertility elements (number of buds left after pruning, number of growing buds, total number of shoots, including fertile, number of formed grapes) was appreciated. In all cases a direct, very close correlation has been attested, especially for the varieties Apiren roz timpuriu and Apiren negru de Grozeşti. It is noted the advanced potential of all varieties for issuing shoots from multiannual wood, inclusive of fertile shoots.*
Key words*:* grapevine, assortment, seedless varieties, fertility

Rezumat. *În lucrare sunt prezentate rezultatele studiului fertilității soiurilor noi apirene incluse în sortimentul viticol al Republicii Moldova – Apiren roz timpuriu, Apiren alb, Apiren roz, Apiren Basarabean şi Apiren negru de Grozeşti. A fost apreciată corelația dintre principalele elemente de fertilitate (numărul de ochi lăsați la tăiere, numărul de ochi porniți, numărul total de lăstari, inclusiv fertirli, numărul de struguri formați). În toate cazurile s-a atestat o corelație directă, foarte strânsă, în special pentru soiurile Apiren negru de Grozeşti şi Apiren roz timpuriu. Pentru aceste soiuri se remarcă şi potențialul de emitere a lăstarilor din lemnul multiannual, inclusiv şi a lăstarilor fertili.*
Cuvinte cheie*:* vița de vie, sortiment, soiuri apirene, fertilitate

INTRODUCTION

According to the F.A.O.-O.I.V. study (2016), world table grape production holds a major share of global grape production (around 46%). For table grapes, in particular, the following consumer preferences are mentioned: mid-sized seedless grapes with uniformly colored, crispy berries and thin skin and harmonious taste. In the same context genetic-ameliorative research activities on the creation of new varieties of table grapes are also being developed: seedlessness, adaptability to

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environmental conditions, transportability (Careno, 2014, Ritchel *et al*., 2015, Xu *et al*., 2015; FAO-OIV, 2016; Ahmed *et al*., 2017).

The indigenous producers' orientation to this category of varieties requires the presence of a seedless assortment, adapted to the climatic conditions of our geographical area, located at the northern border of industrial viticulture. Cultivation in the Carpathian-Danubian-Pontic area of traditional seedless varieties of Oriental origin does not always provide sustained productions (Constantinescu and Indreaş, 1976): setting of elements of fructification for the next year begins late, often under restrictive weather conditions, is not completely realized; for some varieties predominate the luxuriant growth and poor fruiting (varieties from Kishmish group); agri-food and production characteristics do not meet market requirements.

The development of seedless assortment, adapted to the pedo-climatic conditions of the Republic of Moldova, is the result of grapevine breeding program, initiated at the beginning of the 80s of the last century (Juraveli and Savin, 1972), which confirmed the hypothesis of the possibility of combining in a single genotype the characteristics of resistance to winter conditions and downy mildew and grape quality (Savin, 1970). The first results were the creation of varieties Moldova, Pamiati Negrulea, etc., which were subsequently included in the standard assortment in republic and abroad. The hypothesis formulated was further reconfirmed and developed by creating, for the first time in the Euro-Asian area, of seedless varieties possessing biological resistance to low temperatures and pathogens, ensuring annual production, and grapes possessing a wide range of uses (Savin, 2012). The new varieties Apiren roz timpuriu, Apiren alb, Apiren roz, Apiren Basarabean and Apiren negru de Grozeşti have been patented, homologated and included in the list of varieties admitted to use in the Republic of Moldova (Catalogul de soiuri, 2017). The culture of these seedless varieties, relatively new for the viticulture of the republic, inclusive in the context of increasing climatic variability in recent decades, requires a wider assessment in space and time to establish the degree of correlation between the main elements of fertility.

MATERIAL AND METHOD

The observations were made during the years 2006-2016 within the experimental plots of the Institute's Genofond. The general climatic characteristic of sectors (located in the south of Chisinau, 46°58'39.65 "N and 28°46'21.68" E, 201 m altitude) corresponds to the conditions of the Codru wine region of the Republic of Moldova and the weather parameters represent the average values for the republic. Years of observation include varied weather conditions, especially with regard to rainfall, extreme temperatures during the year: the years 2006, 2010, 2012, 2014 were characterized by severe winter temperatures with an absolute minimum of -26...-30°C; during the summer period the maximum was above + 35 ° C, and in 2007-2010, 2012, 2014-2016 - over +38 ... + 42 ° C, being exceeded by 3-15 times the norm of

consecutive days with high temperatures; severe droughts or rainfall deficit, especially during the summer, were registered in 2007, 2011, 2012, 2015 (www.meteo.md).

In study were included new seedless varieties, created in the Republic of Moldova, approved for industrial cultivation: Apiren roz timpuriu, Apiren alb, Apiren roz, Apiren Basarabean (in the process of homologation) and Apiren negru de Grozeşti. The planting scheme is 3.0x1.25 m, training system is the double cordon on the high trunk (60 cm). They were determined: the total number of buds left on cutting, the number of buds that started the vegetation, the number of shoots grown, including the number of fertile shoots, the number of grapes

Processing of experimental data, the graphical presentation was performed with the STATISTICA 7.0 software package.

RESULTS AND DISCUSSIONS

In order to establish the correlation between the total number of buds left on cutting and the number of buds started to grow, between the total number of shoots and the number of fertile shoots and between the total number of buds and the number of grapes, the respective linear correlation coefficients were calculated and obtained the regression equations (tab. 1). In all cases was attested a direct, significant correlation (at significance level α=0,05). A stronger correlation is found between the total number of buds and the number of buds started to grow, the total number of shoots and the number of fertile shoots (linear correlation coefficient r=0,65÷0,90).

Table 1

Correlation between fruiting elements for new seedless varieties homologated in the Republic of Moldova

Variety	Total number of buds x Number of buds started to grow	Total number of shoots x Number of fertile shoots	Total number of buds x Number of grapes
Apiren roz timpuriu	y = 4.59 + 0.73 x r = 0.90	y = 0.76 + 0.67 x r = 0.84	y = 3.52 + 0.71 x r = 0.68
Apiren alb	y = 7.03 + 0.53 x r = 0.82	y = 1.04 + 0.38 x r = 0.67	y = 5.22 + 0.20 x r = 0.42
Apiren roz	y = 12.98 + 0.28 x r = 0.36	y = 0.81 + 0.47 x r = 0.65	y = 9.42 + 0.10 x r = 0.15*
Apiren Basarabean	y = 2.23 + 0.78 x r = 0.88	y = -6.69 + 0.83 x r = 0.82	y = 2.65 + 0.68 x r = 0.50
Apiren negru de Grozeşti	y = 14.72 + 0.51 x r = 0.78	y = -3.59 + 0.79 x r = 0.86	y = 17.27 + 0.43 x r = 0.47

* - insignificant correlation coefficient

The strong correlation between the total number of buds left on cutting and the number of buds starting to grow (fig.1) denotes the genetic resistance of studied varieties at severe temperatures, generally to wintering conditions. A weaker correlation between these parameters was found for the variety Apiren roz (r=0,36), the coefficient, however, was statistically significant.

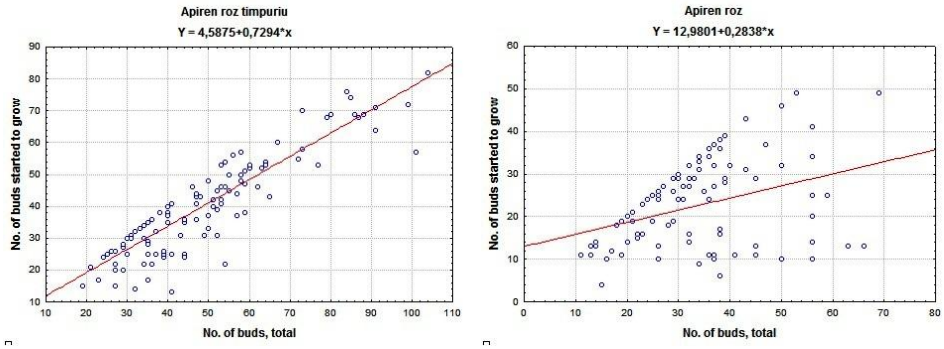

Fig. 1 Regression between total number of buds and buds started to grow

The high correlation coefficients (r=0.65÷0.84) between the total number of shoots and the number of fertile shoots (fig. 2) denotes a significant fertility potential of the newly created varieties, constituting a distinctive characteristic of them, in compared to some old *V. vinifera* seedless varieties.

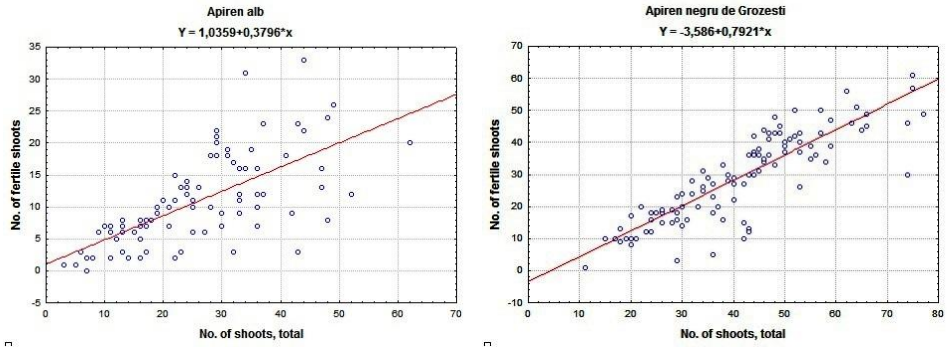

Fig. 2 Regression between total number of shoots and number of fertile shoots

The average percentage of fertile shoots ranges from 58.2 for Apiren roz to 71.8 for the Apiren Basarabean variety (tab. 2). Grape varieties with large and very large grapes (Apiren alb and Apiren roz) form on average one grape on each fertile shoot (absolute fertility coefficient, AFC=1.08÷1.10), and varieties with small-medium grape - on average 1.3 grapes per shoot (AFC=1.28÷1.30). Varieties Apiren Basarabean and Apiren negru de Grozeşti frequently have shoots with 2 grapes (20-35% of shoots). For the relative fertility coefficients (RFC) the same trend is observed - for Apiren alb and Apiren pink RFC=0.47÷0.65 - lower values compared to small-medium grape varieties with RFC=0.90÷0.94. In general, all varieties provide a favorable balance between the vegetative growth and fructification.

Table 2

Indixes of fertility and productivity for new seedless varieties (average for 2006-2016)

Variety name	Fertile shoots, %	AFC	RFC	Average weight of bunch, g	API*	RPI**
Apiren roz timpuriu	69.2±1.3	1.28±0.02	0.90±0.03	151.3	193.7	136.2
Apiren alb	43.4±1.8	1.08±0.01	0.47±0.02	310.3	335.1	145.8
Apiren roz	58.2±1.8	1.10±0.01	0.65±0.02	314.1	345.5	204.2
Apiren Basarabean	71.8±1.4	1.30±0.02	0.95±0.03	251.1	326.4	238.5
Apiren negru de Grozeşti	71.5±1.4	1.29±0.01	0.94±0.02	238.5	307.7	224.2

* API - absolute productivity index (API=AFC* weight of bunch)

* RPI – relative productivity index (RPI=RFC* weight of bunch)

The correlation coefficients between the total number of buds and the number of grapes (fig. 3), relatively smaller - r=0.42÷0.68 (in the case of Apiren roz r=0.16, statistically insignificant), indicates the need for some additional studies on the distribution of fertile buds along the shoot, in order to detect the optimal cutting length and total number of buds left on cutting.

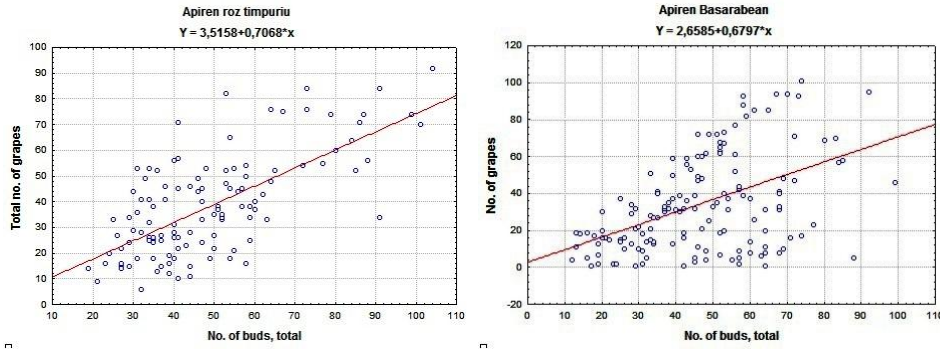

Fig. 3 Regression between total number of buds and total number of grapes

The studied varieties have an increased potential of development of shoots from dormant buds (on average 4-8 shoots/plant) and therefore the possibility of restoring of plant's architectonics after eventual climatic accidents. The variety Apiren Basarabean is characterized by the highest number of shoots developed from dormant buds (on average up to 12 shoots per vine) and Apiren negru de Grozeşti - by the high percentage of fertile shoots in this category (up to 20% of the issued shoots are fertile).

CONCLUSIONS

1. The presence, for the new seedless varieties included in standard grapevine assortment in Republic of Moldova, of a direct, very strong correlation between the total number of buds left to be cut and the number of buds started in grow (r=0.78÷0.90), the total number of shoots and the number of fertile shoots r=0.65÷0.86), and a reasonable correlation between the total number of buds and the number of grapes (r=0.42÷0.68) indicates a significant fertility potential of these varieties in our climatic conditions, ensuring also a balance between the vegetative growth and fructification.

2. The studied varieties have an increased potential of development of shoots from the dormant buds (on average 4-8 shoots/plant), including fertile shoots, thus having the possibility of restoring the plant architectonics after eventual climatic accidents.

REFERENCES

1. **Ahmed S., Roberto S.R., Colombo R.C. et al., 2017** – *Performance of the seedless grape 'BRS Isis' grown in subtropical area*. BIO Web Conferences 9, 01030 (2017), 40th World Congress of Vine and Wine. DOI: 10.1051/bioconf/20170901030
2. **Carreno J., Tomel M., Carreno I., 2014** – *New table grape varieties obtained in the breeding program by ITUM-IMIDIA in Spain*. 7th International Table Grape Symposium, Mildura Victoria, Australia, 12-14 November, 2014. ATGA, HAL, p. 37-38.
3. **Constantinescu Ghe., Indreaş A., 1976** - *Ampelologia soiurilor apirene*. Bucureşti: Editura Academiei RPR, 339 p.
4. **Juraveli M.S., Savin G.A., 1972** – *Vzaimosviazi morozoustoicivosti, mildiu-ustoicivosti i kacestva vinograda*. Sadovodstvo, vinogradarstvo i vinodelie Moldavii, № 12, s. 16-18.
5. **Ritschel P.S., Girardi C.L., Zanus M.C. et al., 2015** – *Novel Brazilian grape cultivars*. Acta Hort. (ISHS) 1082:157-163 http://www.actahort.org/books/1082/1082_21.htm
6. **Savin G.A., 1970** - *Nasledovanie morozoustoicivosti i mildiuustoicivosti v F1 ot skreşcivania sortov V.vinifera L. so slojnâmi mejvidovâmi ghibridami*: avtoref. diss. na soisk. uc. stepeni kand. s./h. nauk. K. 20 s.
7. **Savin Gh., 2012** - *Ameliorarea sortimentului viticol al Republicii Moldova*. Ch. : S.n., (Tipogr. AŞM). 260 p.
8. **Xu H.Y., Zhang G.J., Yan A.L. et al., 2015** – *Table grape breeding at the Beijing Institute of Forestry and Pomology.* Acta Hort. (ISHS) 1082:43-46 http://www.actahort.org/books/1082/1082_3.htm
9. *****, 2016** – *Table and dried grapes. FAO-OIV focus 2016. FAO* and OIV, 64 p. http://www.oiv.int/public/medias/4911/fao-oiv-grapes-report-flyer.pdf
10. *****, 2017** – *Catalogul soiurilor de plante al Republicii Moldova*. Ediție oficială. Editura „Lumina", Chişinău, 127 p.

THE INFLUENCE OF TRACE ELEMENTS AND PGPR ON GROWTH AND PHOTOSYNTHETIC ACTIVITY OF GRAPE SEEDLINGS

INFLUENŢA MICROELEMENTELOR ŞI PGPR ASUPRA CREŞTERII ŞI ACTIVITĂŢII FOTOSINTETICE A BUTAŞILOR VIŢEI-DE-VIE

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Abstract. *The possibility of joint application of a suspension or metabolites of plant growth promoting rhizobacteria (PGPR) and a complex of trace elements Microcom-VA for improving the growth and development of grape seedlings was established. Analysis of the experimental data obtained in controlled and field conditions showed that foliar fertilization of plants by a half dose of Microcom-VA together with bacterial products (suspensions of two- three strains of PGPR) significantly improves the growth of shoots and roots of grape seedlings, content of photosynthetic pigments in leaves, intensity of photosynthesis. This is a consequence of improving the conditions of plant nutrition. The application of PGPR makes possible to improve the quantity and quality of planting material and to reduce the amount of fertilizers (half of recommended dose of trace elements complex) and chemical pressure on the environment.*
Key words: plant growth promoting rhizobacteria, grape seedlings, pigments, intensity of photosynthesis, Microcom-VA

Rezumat. *S-a stabilit posibilitatea aplicării în comun a unei suspensii sau metaboliți ai rizobacteriilor ce promovează creşterea plantelor (PGPR) şi a unui complex de microelemente Microcom-VA pentru îmbunătățirea creşterii şi dezvoltării butaşilor viței-de-vie. Analiza datelor experimentale obținute în condiții controlate şi de câmp a arătat că fertilizarea foliară a plantelor cu o jumătate de doză de Microcom-VA împreună cu produse bacteriene (suspensii de două sau trei tulpini de PGPR) îmbunătăţeşte în mod semnificativ creşterea lăstarilor şi rădăcinilor butaşilor viței-de-vie, conținutul de pigmenți fotosintetici în frunze, intensitatea fotosintezei. Aceasta este o consecință a îmbunătățirii condițiilor de nutriție a plantelor. Aplicarea PGPR contribuie la creşterea cantității şi calității materialului săditor, reducerea cantității de îngrăşăminte (jumătate din doza recomandată de complex de microelemente) şi scăderea presingului chimic asupra mediului ambiant.*
Cuvinte cheie: rizobacterii ce promovează creşterea plantelor, butaşi de viță-de-vie, pigmenți, intensitatea fotosintezei, Microcom-VA

INTRODUCTION

When cultivating perennial crops (vineyards, orchards), intensive removal of nutritive elements from the soil and contamination of soil with heavy metals due to

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multiple treatments of plants against diseases and pests occurs. In agrocenosis are formed the microorganism complexes, poor in species diversity and less resistant to unfavorable environmental factors (Меренюк, 2009). Modern technologies of planting material production must be supplemented by new links and new processes, which will significantly improve the quality and quantity of seedlings.

Studies conducted in recent years show that new biotechnologies, in particular the use of growth-promoting rizobacteria (PGPR), allow a new look at the possibilities of regulating plant nutrition in ontogenesis and plant resistance to unfavourable growing conditions (Bhardwaj *et al*., 2014; Rojas-Tapias *et al*.; Veliksar S. *et al*., 2014; 2015). This is especially important for growing a healthy planting material for the laying of new perennial vineyard plantations.

It is known that the quality of seedlings is largely dependent on the mineral status of the soil and plants. The use of microorganisms can significantly improve plant nutrition, root formation and, accordingly, the quality of seedlings. The mechanisms of the action of PGPB on plants have not yet been fully explored, but their main effect is related to the production of biologically active substances and effects on plant growth (Ahemad and Kibret, 2014).

This paper presents the results of studying the effect of suspensions and metabolites of three strains of saprophytic bacteria on the content of chlorophyll in the leaves of grapes seedlings, photosynthetic activity, growth of shoots and roots, which ultimately determines the quantity and quality of the planting materials obtained in the nursery for the new vineyards.

MATERIAL AND METHOD

The cuttings of 2 grape cultivars (Codrinskii and Presentable) were rooted in distilled water in darkened vessels. After rooting they were placed in 11 L plastic pots filled with soil and grown on the growing platform (vegetation complex) before the autumn. Soil – chernozem carbonate loamy. Another experiment was carried out in the production conditions - in a vine nursery on the same type of soil.

Two-day suspensions of strains of *Azotobacter chroococcum, Bacillus subtilis* and *Pseudomonas fluorescens*, with a titre of 107 cfu / mL were applied to the soil during the cuttings plantation. For foliar fertilization of plants in the process of vegetation the products of the metabolism of bacteria, obtained by centrifugation of concentrated suspensions, were used. The plants were sprayed three times during vegetation period (interval – 15-17 days) by metabolites of microorganisms separately and together with a half of the recommended dose of specially created for grape complex of trace elements Microcom-VA. Microcom-V contains 6 most needed for grape plants trace elements in optimal ratio, and is recommended for foliar fertilization of grape at the critical phases of plants development. Intensity of photosynthesis was determined using a portable LCI device, the content of photosynthetic pigments - in acetone extract.

RESULTS AND DISCUSSION

The content of photosynthetic pigments in leaves is one of the important indications of the plants status during the growing season. Foliar fertilization of plants by Microcom-V increased the amount of chlorophyll (a + b) in the leaves by 119.6 %

compared to the control (tab. 1). The introduction of a suspension of bacteria *Azotobacter chroococcum* and *Pseudomonas fluorescens* (1:1) into the soil together with 0,5 dose of the trace element complex Microcom-V was more effective, sum of the photosynthetic pigments composed 1.34 mg /g f.w. (120.8% compared to the control). The amount of chlorophyll increased mainly due to chlorophyll b. The ratio of the forms *a* and *b* varied according to the variants of the experiments in the range from 3.5 (control) to 2.64 – 2.88 (variants with foliar treatment). The quantity of carotenoides, as a rule, was at the level of the control variant or below it.

Table. 1

Influence of microelements, suspension and metabolites of rhizobacteria on the content of photosynthetic pigments in leaves of grape seedlings, cv. Codrinschii, growing platform , mg /g f.w.

Foliar fertilization	clor. *a*	clor. *b*	*a+b*	carotinoides
Control (H_2O)	0.86 ± 0.01	0.25 ± 0.01	1.11 ± 0.02	0.36± 0.01
Foliar fertilization by Microcom-V 0.5	0.98 ±0.01	0.34 ± 0.02	1.32 ± 0.05	0.35± 0.02
Suspension of *Ag. chroococcum*+ *Ps. fluorescens* (into the soil)	0.92 ±0.03	0.34 ± 0.03	1.27 ± 0.01	0.34± 0.01
Suspension of *Ag. chroococcum*+ *Ps. fluorescens* (into the soil) + Microcom-V 0.5 (foliar)	0.97 ±0.01	0.37 ± 0.01	1.34 ± 0.02	0.35± 0.02
Metabolites of *Ag. chroococcum*+ *Ps. fluorescens* (foliar) + Microcom-V 0.5 (foliar)	0.94 ±0.02	0.37 ± 0.01	1.31 ± 0.05	0.34± 0.04

An increase in the intensity of plants transpiration on the growing platform was noted, especially when the suspension of PGPR was introduced into the soil, followed by foliar fertilization of plants by Microcom-V 0.5. In the same variants a higher stomatal conductance was noted, the lowest - in the control variant.

The intensity of transpiration and the stomatal conductance of plants are closely related to the intensity of photosynthesis and the productivity of plants. The intensity of the photosynthesis of leaves of seedlings in control variant was 6.89 ± 2.31. It was evident higher after the fertilization and varied within 7.71 ± 0.52 and 9.29 ± 2.57 Mmol / M^2 / sec in dependence of applied substances.

In the table 2 the results of the determination of photosynthetic pigments content in leaves of seedlings in a grape nursery (experience in field conditions) are presented, where a consortium of three strains of rizobacteria and a half dose of trace elements complex was applied foliar three times during vegetation. The metabolites of microorganisms *Azotobacter chroococcum* + *Pseudomonas fluorescens* + *Bacillus subtilis* (1: 1: 1) together with Microcom-V in a half dose contributed to an increase in the total amount of chlorophyll (a + b) in leaves by 118.6% compared to control. The ratio of the forms *a* and *b* was in control plants 2.68, and 2.11- in treated plants. The sum of carotenoides was lower than in the control variant.

Table 2

Influence of metabolites of rhizobacteria and trace elements on the content of photosynthetic pigments in the leaves of grape seedlings in the nursery, cv. Codrinskii, mg / g fresh weight

Variants	**clor. *a***	**clor. *b***	***a+b***	**carotinoi-des**
Control	1.02±0.01	0.38±0.01	1.40±0.02	0.20±0.001
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + *B.subtilis* (1:1:1) + Microcom-V, 0.5 (foliar)	1.12±0.002	0.53±0.002	1.66±0.01	0.15±0.002

Improvement of photosynthetic activity of plants is closely connected with the best growth and maturation of shoots of seedlings. Analysis of the main indicators of seedlings quality during their digging (September-October) shows that in both our experiments in all variants with application of bacteria and trace elements the weight and total length of the roots were higher in comparison with control plants. As a rule in the greatest extent the growth and development of the root system was stimulated after fertilization. In the experiment on the growing platform reduced dose of the complex Microcom-V and metabolites of bacteria increased the average length of roots by 186.3 % compared to control (tab. 3). It is also important that these seedlings develop more intensively small roots (of 3 and 4 order), contributing to better nutrition of plants. More evident increase of shoots length was noted after the foliar fertilization of plants by suspension and metabolites of microorganisms together with trace elements (respectively 154,5 and 131.7% to the control). This effect is due to the fact that the main mechanism of action of PGPR on plants is the production of phytohormones, which play the role of chemical messengers and act as regulators of plant growth and development (Martínez-Viveros *et al*, 2010; Rojas-Tapias *et al*, 2012).

Table 3

Influence of trace elements, suspension and metabolites of rhizobacteria on growth and development of grape seedlings, variety Presentable, vegetative complex (average of 20 plants)

Variants	Average length of roots/1 plant, cm	Average length of shoots/1 plant, cm	% to control	
			roots	shoots
Control	246.8±53.87	26.6±2.98	100	100
Foliar fertilization by Microcom-V 0,5	448.5±59.43	33.0±4.23	181.9	123.9
Suspension of *Ag. chroococcum*+ *Ps. fluorescens-(into the soi)l*	358.1±30.26	34.1±2.67	145.1	128.2
Suspension of *Ag. chroococcum*+ *Ps. fluorescens (into the soli)* + Microcom-V 0.5 (foliar)	387.5±47.88	41.1±7.39	157.1	154.5
Metabolites of *Ag. chroococcum*+ *Ps. fluorescens* (foliar) + Microcom-V, 0.5 (foliar)	459.6±24.22	35.1±4.17	186.3	131.7

In the experiment conducted in the grape nursery, the difference in the development of the aboveground part of the seedlings and the root system between the variants (control and three-time fertilization with a complex of trace elements and metabolites of three strains of bacteria) was much more pronounced (tab. 4).

Table 4

Effect of trace elements and PGPR on plant growth in field conditions, cv. Codrinschii, vine nursery

Variants	shoots		roots	
	M±m, cm	% to control	M±m, cm	% to control
Control	26.3	100	232.7	100
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + *B.subtilis* (1:1:1) + Microcom-V, 0,5 (foliar)	54.7	207.9	517.3	222.4

Thus, fertilization of grape seedlings with a small amount of trace elements together with the suspension or metabolites of growth-stimulating bacteria improves their photosynthetic activity and plant growth, which contributes to the improvement of the quality of planting material of grapes. In many cases, application of a suspension of bacteria is more effective and a more technological way to improve the quality of the planting material.

CONCLUSIONS

1. The possibility of joint application of a suspension or metabolites of plant growth promoting bacteria and a complex of trace elements Microcom-V for improving the growth and development of roots and shoots of grape seedlings was established, which is very important for increasing the quantity and quality of planting material.

2. The application of metabolites of rizobacteria makes possible to decrease the total amount of trace elements in the Microcom-V complex (halve of the recommended dose) when growing seedlings, which reduces the possible pollution of the environment by reducing the amount of chemical fertilizers used for plant nutrition.

REFERENCES

1. **Ahemad Munees, Mulugeta Kibret, 2014** - *Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective*. Journal of King Saud University – Science, 26, 1–20
2. **Bhardwaj Deepak, Mohammad Wahid Ansari, Ranjan Kumar Sahoo, Narendra Tuteja, 2014** - *Biofertilizers function as key player in sustainable agriculture by*

improving soil fertility, plant tolerance and crop productivity. Microbial Cell Factories, 13:66.

3. **Fuentes-Ramires L.E., Caballero-Mellado J., 2006** - *Bacterial biofertilizers.* In: Z.A. Siddiqui (ed). PGPR: Biocontrol and Biofertilization. Springer, Netherlands, pp:143-172.
4. **Martínez-Viveros O., Jorquera M.A., Crowley D.E., Gajardo G., Mora M.L., 2010** - *Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria*. J. Soil Sci. Plant Nutr. V.10, n.3.
5. **Rojas-Tapias D., Andrés Moreno-Galván, Sergio Pardo-Díaz, Melissa Obando, Diego Rivera, Ruth Bonilla, 2012** - *Effect of Inoculation with Plant Growth-Promoting Bacteria (PGPB) on Amelioration of Saline Stress in Maize (Zea Mays)*. Applied Soil Ecology 6, 264–272.
6. **Veliksar Sofia, Lemanova N., Toma S., David T., Gladei M., 2014** - *Influence of micronutrients and metabolites of microorganisms on nutritive status of grape. 2nd International Conference on Microbial Biotechnology.* Chisinau, p.93-98.
7. **Veliksar Sofia, Toma S., Lemanova Natalia, Bratco D., Gladei M. 2015** - *Improving vine performance under unfavorable conditions of growth by use of trace elements and microorganisms*. Lucrări ştiințifice, seria Horticultură.
8. **Меренюк Г.В**., **2009 -** *Деградация почв, вовлеченных в сельскохозяйственный оборот, решение проблемы с микробиологических позиций //* Probleme actuale de microbiologie si biotehnologie. Chisinau, p. 25-78.

THE INFLUENCE OF ORGANIC FERTILISER NOVA® ON THE OENOLOGICAL POTENTIAL OF FETESCĂ REGALĂ AND ALIGOTÉ IN IASI VINEYARD

INFLUENȚA FERTILIZANTULUI ECOLOGIC NOVA® ASUPRA POTENȚIALULUI OENOLOGIC AL SOIURILOR FETESCĂ REGALĂ ŞI ALIGOTÉ, CULTIVATE ÎN PODGORIA IAŞI

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Abstract. *The necessity of vineyard fertilization is imposed by the fact that the vine is a perennial plant and it extracts for a long time large amounts of nutrients. In this context, this paper also includes a study on the use of a NOVA® organic fertilizer in establishing the oenological potential of vine varieties that are highly cultivated in Moldova, which allow the consumer to appreciate a wide range of wines. The technological grape indices of the studied varieties have demonstrated the superiority of applying biofertilisers, making them larger, less dense in berries that have a higher mass and must capacity. The grape yield is slightly larger, namely: 73.7% for the Aligoté variety, compared to 72.5% untreated variety, while for Fetească regală variety the increase is up to 65.9%, compared to 64.9%. The quality of the grapes has also been improved by application of the NOVA® foliar biofertiliser, the accumulation in sugar being higher while the acidity is more balanced. Accumulation in sugars allows the production of white table wines with geographical indication (Aligoté) or quality wines (Fetească regală).*
Key words: Fetească regală, Aligoté, grapevine variety, growth biostimulator, production

Rezumat. *Necesitatea administrării îngrăşămintelor în cultura viței de vie este impusă de faptul că fiind plantă perenă, extrage timp îndelungat cantități mari de substanțe nutritive. În acest context se include şi lucrarea de față, care constituie un studiu privind folosirea unui fertilizant de proveniență ecologică NOVA®, în stabilirea potențialului oenologic al unor soiuri de viță de vie, cu areal mare de cultură în Moldova şi care permit obținerea unei game largi de vinuri apreciate de consumatori. Indicii tehnologici ai strugurilor la soiurile luate în studiu au demonstrat superioritatea aplicării tratamentului cu biofertilizator, aceştia devenind mai mari, mai lacşi în boabe, cu boabele mai grele şi capacitate mai mare de formare a mustului, randamentele la prelucrarea strugurilor fiind uşor mai mari, şi anume: la soiul Aligoté 73,7%, față de 72,5%, iar la soiul Fetească regală de 65,9%, față de 64,9%. Calitatea strugurilor a cunoscut şi ea o îmbunătățire prin aplicarea tratamentului cu biofertilizantul foliar NOVA® , acumulările în zaharuri fiind mai mari, iar aciditatea mai echilibrată.. Acumulările în zaharuri din must permit obținerea vinurilor de masă albe cu indicație geografică (Aligoté), sau a celor de calitate (Fetească regală).*
Cuvinte cheie: Fetească regală, Aligoté, soi de viță de vie, biostimlator de creştere, producție

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INTRODUCTION

Obtaining quantitative and qualitative agricultural and horticultural productions, in the context of soil fertility growth or maintenance while taking in consideration a real protection of agroecosystems, is one of the major objectives of modern agriculture (Rotaru and Stoleru, 2011). Each farmer must understand the need for accurate assessment and regular monitoring of plant nutrient requirements based on realistic forecasts, depending on local technological conditions, soil, climate and expected yield (Burzo, 2014). In this way, excesses can be avoided and nutrient deficiencies can be corrected (Davies, 2004).

Rational fertilization with products admitted in organic viticulture, which are administered directly by spraying on the plant, facilitates their direct introduction into its metabolism and is a prerequisite for obtaining superior crops in terms of quantity and quality (Amiri and Fallahi, 2007).

MATERIAL AND METHOD

The determinations were made on two varieties for white wines characteristic to Iasi vineyard, namely Aligotė and Fetească regală. NOVA® is a complete organic fertilizer that has been made from compounds free of heavy metals and toxic substances, harmful to humans. This is a fertilizer with amino acids and organic enzymes intended to be applied by foliar and dripping fertilization. NOVA® product contains 14% organic nitrogen (N), 9% phosphorus (P), 8% potassium (K) and trace elements (iron 0.04%, manganese 0.04%, magnesium 0.32% 02% zinc 0.03%, copper 0.01% and 0.02% molybdenum). Three treatments with this product were carried out, the first before flowering (June 6th), the second after flowering (June 25th), and the last during intensive berry growth (July 19th) at 15 liters / ha.

The application of the biostimulator was carried out in 2016 on a group of vines located in the Ampelographic Collection of the Faculty of Horticulture, on a land with a slope of about 5%, on a chernozem cambic soil. The graft used is Berlandieri x Riparia Kober 5 BB with planting distances of 2.2 / 1.2 m, with a vertical monoplane support system and an average of 3700 vines / ha. The trellising system in the plantation is semi-high, trunck of 0.75 m, bilateral cord with cutting into medium lenghth elements (spur + 4-7 buds cane) and 40-42 buds / vine, about 14-16 buds / m^2. Soil and vine maintenance works are those specific to the industrial viticultural ecosystem.

RESULTS AND DISCUSSIONS

Regarding the average number of grapes grown per vine (tab. 1), it was found that for both varieties, there were more grapes in the untreated variant, namely 53.52 for the Aligoté variety and 50.78 for the Fetească regală variety, while at the variants that were treated with the NOVA® biofertilizer, the numbers were slightly lower, ie 50.78 for the Aligoté variety and 37.03 for the Fetească regală.

Instead, the average mass of a grape had higher values for the grapes treated with the NOVA® biofertiliser: 115 grams for the Aligoté variety and 135 grams for the Fetească regală variety. Consequently, the production levels achieved were

different, being influenced by both the genetic nature of the variety and the application of the NOVA® biofertiliser treatment.

Table 1

Productive potential of studied varieties

Variant	Average no. of grapes per vine	Average mass of grape (g)	Average no. of berries per grape	Average production per vine (kg/vine)	Production per ha (calculated) (t/ha)
Aligoté-control.	53.52	105	132	5.62	20.79
Aligoté-treated	50.78	115	114	5.84	21.61
Fetească regală-control	39.42	120	88	4.73	17.50
Fetească regală-treated	37.03	135	75	5.00	18.50

Regarding the average number of berries formed on clusters, they are higher in the case of the untreated variant, while the version treated with the NOVA® biofertiliser shows lower values. In the Aligoté variety, 132 berries were formed on average on bunches of untreated varieties, while in the treated variant 114 berries were formed. At Fetească regală control, on average, 88 berries were formed, compared to 75 berries formed on average at the variant with NOVA® treatment.

Average production was slightly superior in the case of the NOVA® biofertiliser treatment so that, the production per hectare was superior with 820 kg/ha for Aligoté and 1000 kg/ha for Fetească regală.

The production per hectare was for the control variant was 20.79 t/ha for the Aligoté variety and 17.5 t/ha for Fetească regală. Following the application of the NOVA® Biostimulator treatments, the achieved yields were 21.61 t/ha for the Aligoté variety and 18.50 t/ha for Fetească regală.

Table 2

Technological indices for studied grape varieties

Variant	Structural index of the grapes	Compositional index of the berry	Berry index	Yield index	Must yield (%)
Aligoté-control	31.1	7.4	64.0	4.4	72.5
Aligoté-treated	33.6	8.6	61.9	4.8	73.7
Fetească regală-control	24.6	6.2	53.6	4.2	64.9
Fetească regală-treated	26.3	6.8	50.6	4.6	65.9

In the case of the studied varieties it is found that for the structural index of the grapes the higher values were for the treated variants (tab. 2). Therefore, following the application of the NOVA® biofertiliser, the weight gain of the berries, the values of this index were higher, being 33.6 for the Aligoté variety and 26.3 for the Fetească regală variety.

Also, in the case of the berry composition, the values were higher for the variants where NOVA® biofertiliser was applied, so the mass of the pulp was higher than that of the skins and seeds. Grape variety wise, the highest values were for Aligoté, 8.6, compared to those registered for Fetească regală, with 6.8.

The berry index was higher for the Aligoté variety, where the grains are smaller than those for the Fetească regală variety, which has medium berries. It is found that in the case of the variant where the NOVA® biofertiliser was applied due to the increase in grain sizes, the highest values are recorded for fertilized variants, namely 61,9 for Aligoté and 50,6 for Fetească variety royal.

The yield index had values specific to wine varieties being higher for variants where the NOVA® biofertiliser was applied, due to their larger berry size and more succulent berries. The values obtained were 4.8 for the Aligoté variety and 4.6 for the Fetească regală variety.

The must yield was higher for the Aligoté variety, 73.7%, at the limit of quality wines and table wines and for Fetească regală variety it was specific for quality wines, namely 65.9%.

Table 3

Results regarding the attack of main diseases for vines in the case of Aligoté

Studied diseases	Treated			Control sample		
	I %	F %	G.A. %	I %	F %	G.A. %
Downy mildew -leaves	4.42	16.87	0.75	8.78	14.86	1.30
Downy mildew -grapes	5.06	16.66	0.84	6.79	13.65	0.92
Powdery mildew -leaves	18.23	33.98	6.19	20.41	41.10	8.39
Powdery mildew - grapes	6.69	41.12	2.75	8.52	52.23	4.45
Grey rot on grapes	3.98	10.12	0.41	4.55	16.6	0.75

Table 4

Results regarding the attack of main diseases for vines in the case of Fetească regală

Studied diseases	Treated			Control sample		
	I %	F %	G.A. %	I %	F %	G.A. %
Downy mildew -leaves	5.08	17.32	0.88	9.95	15.32	1.52
Downy mildew -grapes	5.69	18.21	1.04	7.14	17.14	1.22
Powdery mildew -leaves	21.73	46.55	10.11	35.65	74.14	26.36
Powdery mildew -grapes	7.89	52.42	4.13	16.02	68.08	11.04
Grey rot - grapes	5.27	11.49	0.61	7.88	28.49	2.32

Following application of the NOVA® biofertiliser, there is an improvement in the resistance to the main diseases of the vine. Thus, in the case of the Aligoté variety, the degree of downy mildew attack is less than one and a half times at the treated variant, and relatively similar in the case of attack on grapes (tab. 3). In Fetească regală variety, the highest values are recorded at leaf attack 1.04%, double compared to the values present in the fertilised variant 1.52% (tab. 4). In powdery mildew, the degree of attack was stronger in both varieties, the highest

values being recorded for leaf attack, Fetească regală variety, untreated variant, of 26.36%. The lowest values were for Aligoté grape variety, only 2.75%. A very good resistance is also recorded in the case of grey rot, so one of the major gains in using NOVA® biofertilizers is to increase disease resistance.

Table 5

Mass of 100 berries (g) in grapes during maturation

Grape variety	Grape véraison		After 15 days		After 25 days		Grape maturation	
	T	N	T	N		T	N	T
Aligoté	125	114	132	129	141	136	152	144
Fetească regală	163	152	169	159	174	166	181	174

T-tratat cu biofertilizator N- netratat cu biofertilizator

The mass of 100 berries has increasing values since veraison, but was superior to the treated variant (tab. 5). Thus, in the Aligoté variety, it ranged from 125 g during veraison to 152 g at full maturation, in the treated variant, while in the control variant the limits were from 114 g during veraison to 144 g at full maturation. In the case of Fetească regală variety, the treated variant, the limits ranged from 163 g during veraison to 181 g at full maturation. For the control samples, the mass of 100 berries ranged from 152 grams to 174 grams, at full maturation.

At the time of veraison, the accumulation of sugars in grapes was small in both varieties (tab. 6). In the control variant Aligoté variety, sugars were registered at 99 g/L, reaching full maturity at 187 g/L, which allows the obtaining of wines with geographical indication, whereas in the treated variant, the accumulations in sugars allowed the production of quality wines, at 195 g/L.

In the case of the Fetească regală variety, the accumulations in sugars were superior in both variants and even higher after application of fertilization. Thus, the limits for the treated variant ranged from 116 g/L at veraison and reached 226 g/L at full maturation. At the same vegetation phenophases, the untreated lot values ranged from 115 g/L to 220 g/L, in both cases, quality white wines being able to be obtained.

Table 6

Sugars evolution (g/L) in grapes during maturation

Grape variety	Grape véraison		After 15 days		After 25 days		Grape maturation	
	T	N	T	N		T	N	T
Aligoté	102	99	128	123	159	150	195	187
Fetească regală	116	115	154	149	199	192	226	220

T-treated with biofertiliser N- control

The titratable acidity of the must was lower in the variant treated with the biostimulator, but not significantly, so that balanced wines can be obtained by using the NOVA® fertilizer (tab. 7). The variation limits for the Aligoté variety were, in the fertilized variant, ranging from 18.3 g/L to 5.2 g/L tartaric acid at full

maturation. For Fetească regală, at the same phenological data, these values were between 13.7 g/L tartaric acid to 4.9 g/L tartaric acid at full maturation.

Table 7

Acidity evolution (g/L tartaric acid) in grapes during maturation

Grape variety	Grape véraison		After 15 days		After 25 days		Grape maturation	
	T	N	T	N	T	N	T	N
Aligoté	18.3	19.7	9.9	10.7	7.8	8.1	5.2	5.6
Fetească regală	13.7	14.2	9.1	10.4	6.4	6.6	4.9	5.1

T-treated with biofertiliser N- control

CONCLUSIONS

1. By applying the NOVA® foliar biofertiliser, the Aligoté and Fetească regală varieties have increased their productive potential, by increasing the average weight of a grape and the mass of 100 berries. The studied Technological Grape Indices have demonstrated the necessity of applying biofertilizers, making them larger, denser, with heavier grains, and higher must production capacity, the yields for grape processing being slightly higher.

2. Resistance to disease has been greatly improved by the use of NOVA® foliar biofertiliser, the degree of attack being almost halved compared to untreated variants.

3. The quality of grapes has also been improved by applying the NOVA® foliar biofertilizer, the accumulations in sugars is higher and with a more balanced acidity, so that the gluco-acidimetric index has values close to those specific to wine grapes. Accumulation of sugars in musts allows the production of white table wines with geographical indication (Aligoté) or quality wines (Fetească regală).

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REFERENCES

1. Amiri M. E., Fallahi E., 2007 - *Influence of mineral nutrients on growth, yield, berry quality, and petiole mineral nutrient concentrations of table grape*. J. of Plant Nutrition, 30 (3): 463-470.

2. Burzo I., 2014 - *Modificări climatice şi efectele asupra plantelor horticole*. Ed. Sitech, Craiova.

3. Davies P. J., 2004 -.*Plant hormones: Biosynthesis, signal transduction, action!*, Ed. Kluwer Academic Publishers, Netherlands

4. Rotaru Liliana, Stoleru V., 2011 - *Bazele producției viticole în sistem ecologic*. Ed. Performatica, Iaşi

EVALUATION OF THE CONSERVATION OF BIODIVERSITY OF ECOSYSTEMS IN THE WINE CENTER OF COPOU IAŞI

EVALUAREA STĂRII DE CONSERVARE A BIODIVERSITĂȚII ECOSISTEMELOR DIN CENTRUL VITICOL COPOU IAŞI

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Abstract. *In order to assess the positive impact of the implementation of bio-resources, the greening system and multifunctional protection areas, on the functional biodiversity in the vineyard ecosystems of the vine plantations under the administration of the Research Station for Viticulture and Enology Iasi, six experimental plots were selected, which have native varieties, older and newer, recent creations, varieties of table grapes and wine grapes. The conservation status of biodiversity was assessed by means of two indicators, namely the quantity of semi-natural elements in the landscape of the vineyard holding and their quality. Following the assessment of the conservation status of the agroecological infrastructures (IAE) within the studied wine perimeter, it was found that these have on average a medium to good status, being able to apply corrective measures.*
Key words: biodiversity, agroecological infrastructures, vineyard

Rezumat. *Pentru evaluarea impactului pozitiv al implementării bio-resurselor, a sistemului de înverzire şi a zonelor multifuncţionale de protecţie, asupra biodiversităţii funcţionale în ecosistemele viticole din plantaţiile de viţă de vie aflate în administraţia Staţiunii de Cercetare Dezvoltare pentru Viticultură şi Vinificaţie Iaşi, au fost selectate şase loturi experimentale, cu soiuri autohtone, mai vechi şi mai noi, creaţii recente, soiuri de struguri pentru masă şi vin. Starea de conservare a biodiversităţii a fost apreciată cu ajutorul a doi indicatori, respectiv cantitatea elementelor seminaturale din peisajul exploataţiei viticole şi calitatea acestora. În urma evaluării stării de conservare a infrastructurilor agroecologice (IAE) din cadrul perimetrului viticol studiat, s-a constat că acestea prezintă o stare generală medie spre bună, existând posiblilitatea aplicării unor măsuri corective.*
Cuvinte cheie: biodiversitate, infrastructură agroecologică, plantaţii viticole

INTRODUCTION

Conservation of biodiversity as a scientific area has emerged as a necessity to reduce the dangers that threaten living organisms and their living environments (Billeter *et al.,* 2008). Protection of nature in general and of biodiversity in particular has as its main objective the unhindered preservation of the natural ecosystems (ecofond) and the genetic fund at global and regional level in order to

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ensure the balance between the natural components of the environment, on the one hand and between them and human society, on the other (Le Roux, 2008).

Conservation of biodiversity in wine ecosystems is an objective national strategy (Tomoiaga *et al.,* 2016) and involves the following actions: biodiversity assessment based on crop technologies used, especially for disease and pest control; controlling diseases and pests by less polluting methods by using low-toxicity substances; increasing the ratio of useful organisms / pathogens to meet the requirements of modern farming practices regarding the health status of human populations, soil and biodiversity conservation; creating a quick diagnosis on how, the time and the products needed to perform sanitary phytosanitary treatments, as well as the monitoring of the effects after treatment; increasing the level and quality of agricultural produce by improving plant protection systems in line with the concept of sustainable development; making agriculture sustainable and competitive in the context of preventing environmental damage through anthropogenic activities.

MATERIAL AND METHOD

In order to evaluate the positive impact of the implementation of the bio-resources, the greening system and the multifunctional protection zones on the functional biodiversity in the vineyard ecosystems of the vine plantations under the administration of the Viticulture and Oenology Research and Development Station in Iasi, six experimental lots with areas ranging from 1.60 to 1.74 ha and a number of 8 varieties representative of the Copou wine center (tab. 1).

Table 1

Identification data of experimental lots - VORDS Iaşi

Culture	Plot	Latitude N	Longitude E	Altitude, m	Variety	Locality
Vine	Plot 1	47°12'59.12"	27°32'05.29"	119	Fetească albă	Iaşi
	Plot 2	47°12'45.10"	27°32'05.29"	153	Fetească regală	Iaşi
	Plot 3	47°12'27.44"	27°32'04.57"	195	Aromat de Iaşi	Iaşi
	Plot 4	47°12'22.28"	27°32'04.92"	192	Aligote	Iaşi
	Plot 5	47°13'05.86"	27°32'08.35"	118	Fetească regală	Iaşi
	Plot 6	47°12'12.35"	27°31'41.22"	172	Golia, Gelu, Paula	Iaşi

The structure and morphology of microhabitats (vineyards, terraces, hedges, trees, wooded areas, etc.) and their conservation status were established in each batch.

REZULTATS AND DISCUSSIONS

In order to assess the conservation status of biodiversity in wine ecosystems, two indicators were taken into account, namely the quantity of semi-natural elements in the landscape of the vineyard holding and their quality.

The *quantitative indicator* represents the share of the total surface area of the component elements (artificial landscape and infrastructure) in relation to the surface of the vineyard.

In the case of the six experimental lots, the area actually occupied by vines (UAE) is 9.94 ha and the agroecological infrastructure (AEI) represented by terraces, hedges, tree trunks, isolated trees, wooded areas and flower strips, occupies 0.67 ha (tab. 2).

Table 2

Structure and morphology of micro habitats vineyard ecosystem Copou Iaşi

Nr. crt	Specification	Surface of experimental lots, ha						Total vineyards, ha
		Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	
1	Vineyards	1.62	1.63	1.73	1.74	1.60	1.62	9.94
2	Terraces	0.048	0.13	-	-	0.16	0.08	0.418
3	Hedges	-	-	-	0.05	-	-	0.05
4	Tree trunks	-	-	-	0.005	0.01	-	0.015
5	Isolated trees	-	-	0.02	-	-	-	0.02
6	Wooded areas	0.05	0.03	-	-	-	0.06	0.14
7	Flower strips	-	-	0.01	0.015	-	-	0.025
	Total, ha	**1.72**	**1.79**	**1.76**	**1.81**	**1.77**	**1.76**	**10.61**

Under these circumstances, the ratio between AEI and UAE is 7% and artificiality rate are 93% (tab. 3).

Table 3

Participation of the seminatural elements in the experimental lots

Nr. crt	Experiment lots	UAE (area actually occupied by the vine, ha)	AEI (agro-ecological infrastructure, ha)	AEI/ UAE, %	Artificiality rates, %
1	**Lot 1**	1.62	0.10	6	94
2	**Lot 2**	1.63	0.16	10	90
3	**Lot 3**	1.73	0.03	2	98
4	**Lot 4**	1.74	0.07	4	96
5	**Lot 5**	1.60	0.17	11	89
6	**Lot 6**	1.62	0.14	9	91
	Total, ha	**9.94**	**0.67**	**7**	**93**

The qualitative indicator reflects the conservation status of the landscape elements. Quality is evaluated based on several criteria defined for each type of AEI: structure, composition and assimilated functions, respectively degradations. This allows to obtain a radial structure diagram showing the IAE in good conservation status, medium and unfavorable (tab. 4 to tab. 9).

At farm level, quality is assessed by aggregating all conservation status obtained for all agroecological infrastructures on the vineyards.

The assessment of the conservation status of agroecological infrastructures (AEI) within the studied viticultural area shows that 61% of them have a good overall status, 36% average and 3% unfavorable, with the possibility of applying corrective measures.

Table 4

The conservation status of the terraces within the experimental lots

Criteria	Indicators	Conservation status			Observ.
		Good	Medium	Unfavorable	
Structure	width, m	<5	5 - 2	>2	
	the presence of uncultivated soil, %	<10	10 - 20	>20	
	the recovery of trees or bushes (<30 cm), %	<25	25 - 50	>50	
Compozition	exotic species, %	<1	1 - 10	>10	not evaluated
	ruderal species, %	<1	1 - 10	>10	
	number of species of plants with visible flower, no	<10	5 - 10	>5	
	recovery of perennial species	<80	50 - 80	>50	
Degradation	surface degradation, %	<1	1 - 10	>10	burning plant remains

Table 5

The state of conservation of live hedges within experimental lots

Criteria	Indicators	Conservation status			Observ.
		Good	Medium	Unfavorable	
Structure	the width of the fence, m	>2	1 - 2	<1	
	the fence distance to the treated surface	>1	0,5 - 1	<0,5	
	flooring at the edge of the fence	current shifting	without overlapping		
	types of associated structures: bunch of branches, stones, walls, fallen trees	<3	1 - 2	0	
Compozition	number of species of shrubs with thorns	<1	1 - 10	>10	
	recovery of exotic species	<1	1 - 10	>10	
Degradation	surface degradation, %	<1	1 - 10	>10	

Table 6

The state of conservation of tree strata in experimental lots

Criteria	Indicators	Conservation status			Observ.
		Good	Medium	Unfavorable	
Structure	height, m	>4	2 - 4	<2	
	the distance from the trunk to the edge of the treated / cultivated area, m	>1	0,5 - 1,0	<0,5	
	small associated structures: a bunch of branches, stones, walls, fallen trees	<3	1-2	absence	
Compozition	exotic species except platanus and trees, %	absence	<5	>5	
Degradation	surface degradation, %	<1	1 - 10	>10	

Table 7

The state of conservation of wooded areas within the experimental lots

Criteria	Indicators	Conservation status			Observ.
		Good	Medium	Unfavorable	
Structure	the width of the fence, m	>2	1 - 2	<1	
	the fence distance to the treated surface	>1	0,5 - 1	<0,5	
	number of wood layers (arboricola:> 3m, bushy high from 1.5 to 1.5 m, bushy down <1.5 m)	3	2	2	
	flooring at the edge of the fence	current shifting	without overlapping		
	trees with trunks or trees> 30 cm in diameter	1	absence		
	types of associated structures: bunch of branches, stones, walls, fallen trees	<3	1 - 2	0	
Compozition	number of species of shrubs with thorns	<1	1 - 10	>10	
	recovery of exotic species	<1	1 - 10	>10	not evaluated
Degradation	surface degradation, %	<1	1 - 10	>10	

Table 8

The state of conservation of isolated trees within the experimental lots

Criteria	Indicators	Conservation status			Observ.
		Good	Medium	Unfavorable	
Structure	height, m	>4	2 - 4	<2	
	distance from the trunk to the edge of the treated / cultivated area, m	>1	0,5 - 1,0	<0,5	
	small associated structures: a bunch of branches, stones, walls, fallen trees	<3	1-2	absence	
Compozition	exotic species except platanus and trees, %	absence	<5	>5	
Degradation	surface degradation, %	<1	1 - 10	>10	

Table 9

The state of conservation of the flower bands in the experimental lots

Criteria	Indicators	Conservation status			Observ.
		Good	Medium	Unfavorable	
Structure	width, m	<5	5 - 2	>2	
	the presence of uncultivated soil, %	<10	10 - 20	>20	
	number of layers	<3	2	1	
Compozition	exotic species, %	<1	1 - 10	>10	not evaluated
	ruderal species, %	<1	1 - 10	>10	
	number of species of plants with visible flower	<10	5 - 10	>5	
	recovery of perennial species	<80	50 - 80	>50	
Degradation	surface degradation, %	<1	1 - 10	>10	burning plant remains

Among the measures for improving the conservation status we mention: creating green corridors connecting green areas inside and on the periphery of the farm; expanding flower bands with melliferous plants as pollen and nectar source for pollinating insects or seeds (*Vicia sp., Lotus corniculatus*); the extension of *Prunus spinosa* and *Rosa canina* fruit bands for the useful entomofauna shelter; protection of meadows and natural meadows, which are only mown if necessary and in any case will not till; protection of large solitary trees and existing shrubs because they can provide food and shelter to wildlife; providing structural elements such as stones or woods that offer a good habitat for insects; rebuilding soil retention structures such as terraces on sloping land.

CONCLUSIONS

1. Following the assessment of the conservation status of agroecological infrastructures (AEI) within the studied wine-growing perimeter, it was found that they have a medium to good overall state, with the possibility of applying corrective measures: creation of green corridors, extension of honey and bushes, restoration of terraces on slope lands.

2. The researches revealed a significant positive correlation between the state of biodiversity of the viticultural ecosystem and the semi-natural elements of the agroecological infrastructure.

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REFERENCES

1. Billeter R., Liira J., Bailey D., Bugter R., Arens P., Augenstein I., Aviron S., Baudry J., Bukacek R., Burel F., Cerny M., De Blust G., De Cock R., Diekötter T., Dietz H., Dirksen J., Dormann C., Durka W., Frenzel M., Hamersky R., Hendrickx, F., Herzog F., Klotz S., Koolstra B., Lausch A., Le Coeur D., Maelfait J. P., Opdam P., Roubalova M., Schermann A., Schermann N., Schmidt T., Schweiger O., Smulders M.J.M., Speelmans M., Simova P., Verboom J., Zobel M., Edwards P.J., 2008 - *Indicators for biodiversity in agricultural landscapes : a pan-European study*. Journal of Applied Ecology, no 45, pp: 141-150.

2. Le Roux X., Barbault, R. Baudry J., Burel F., Doussan I., Garnier E., Herzog F., Lavorel S., Lifran R., Roger-Estrade J., Sarthou J.P., Trommetter M. (éditeurs), 2008 - *Agriculture et biodiversité. Valoriser les synergies.* Expertise scientifique collective, rapport, INRA (France).

3. Tomoioagă Liliana, Ficiu Lidia, Tăbăranu G., Argatu Cosmina, Zaldea Gabi, Vizitiu Diana, 2016 - Studies on Biodiversity Diagnosis of Vineyard Farms at the Agro - ecological Infrastructure Level. Bulletin UASVM Horticulture 73 (2), pp: 205 -209.

NEGATIVE INFLUENCE OF TEMPERATURE OVER FLAVOR COMPOUNDS FROM WINE

INFLUENŢA NEGATIVĂ A TEMPERATURII ASUPRA COMPUŞILOR DE AROMĂ DIN VIN

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Abstract. *The aromatic profile of wine is offered by the variety of volatile chemical compounds, especially esters, alcohols, carboxylic acids and nitrogen compounds. These substances can determine different aromas depending on concentration. One of the very important factors in forming aromas is the fermentation temperature whose which has an optimal value between 15-17 °C. This study evaluated wines samples which were fermented without temperature control, reaching an average temperature in between 21-24 °C. Following this experiment it was observed how the compounds that give floral and fruity aromas can give unwanted sensations in high concentrations. At the same time, several esters of the fatty acids were observe which give a heavy smell and cause the sensation of fats. The aromatic profile of the sample which was fermented at higher temperature is more affected, even becoming repulsive once the temperature is rising.*
Key words: wine, faults, GC-MS

Rezumat. *Profilul aromatic al unui vin este oferit de varietate de compuşii chimici volatili, în special esteri, alcooli, acizi carboxilici şi compuşi ce conţin azot, care pot imprima diferite arome în funcţie de concentraţia în compuşi. Un factor foarte important în formarea percepţiei aromatice îl reprezintă temperatura de fermentare a cărei valoare optimă trebuie să fie cuprinsă între 15-17 °C. În acest studiu s-au evaluat o serie de probe de vin care au fost fermentate spontan fără control de temperatură, ajungându-se la o medie a fermentaţiei de 21-24 °C. În urma acestui experiment s-a putut observa cum compuşii care dau arome florale, fructate, la concentraţii ridicate conduc la apariţia unor senzaţii generale nedorite. Au fost identificaţi mai mulţi esteri ai unor acizi graşi care imprimă un miros greu, dând astfel o senzaţie de gras. În plus, s-a observat că profilul aromatic al probelor fermentate la temperatură mai ridicată este mai afectat, devenind chiar repulsiv odată cu creşterea temperaturii.*
Cuvinte cheie: vin, defecte, GC-MS

INTRODUCTION

The fact that at the base of the transformation from must to wine it is the alcoholic fermentation is the core of one of the most famous beverage in the wold. The specialists in this area have established certain temperature intervals in which

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the fermentation can bring advantages or disadvantages for the wine quality. A wine's aroma consists of several classes of chemical compounds, each with its own sensation or particularities. For the wine to get as rich as possible in aromas, the ideal fermentation temperature has to be between 15 and 18 °C. A temperature above 20 °C certainly will affect the wine's quality and aromatic profile (Țârdea *et al.*, 2000; Cotea *et al.,* 2009).

A sensorial analyse of wine has a varied vocabulary of descriptors classified into categories and sub-categories which indicate the primary attributes or aromas (which are given by the raw material – generally grapes), middle aromas (or secondary that are given by compounds obtained during alcoholic fermentation) and tertiary dominant aromas (which are given by the wine maturation processes). Among these we note in the primaries: flower, fruit, vegetal, spice, caramel, wood, earth, oxidized or even chemical product, fermentative aromas where the latter are in the category of unwanted aromas that can be flaws. A more clear and detailed classification was offered by Ann Noble in 1984 through what is called the "aromas' wheel" (www.winearomawheel.com). Helping ourselves with the "wine's nose" (www.lenez.com) and with the aromas' wheel still does not make it easy to completely and correctly identify the aromas found in a wine sample. For this, a chromatographic gas analysis is usually needed. This gives us a more complex view due to the chemical compounds that it can identify and whose aroma is approximately known.

A problem in characterizing these volatile chemical compounds is their perception threshold and the fact that most of them can have different sensory perceptions based on the concentration in which they are found in an environment (Yusen *et al*., 2016). The threshold at which a chemical substance is perceived as pleasant or unpleasant is based on each person's olfactory sensitivity and the level of awareness of the smell (Monique *et al.,* 2008). Therefore, an evaluation of the aromatic profile using chromatography was tried taking into account the possibility of the presence of different attributes even for the same compound.

MATERIAL AND METHOD

The samples used in the experiment were wines obtained spontaneously in fermentation, with no temperature control, in which an average of 21-24 °C was reached. The first spontaneously fermented samples were at a maximum of 28 °C and the second one is a maximum of 32 °C.

The analysis method for determining the volatile compounds is gas chromatography coupled to mass spectrometry detection which was performed in this study using a Shimadzu HS 20trap-GC 2010plus-MS040TQ. Seven millilitres of wine where taken from which sample and analysed. The analysis method used as well as the details about the device's components and its parameters are described in literature (Tarțian *et al.,* 2017).

The results were qualitatively processed by comparing them with tree databases for mass spectrums. In order to obtain quantitative results the samples were processed by benchmark against an internal standard of 4-metil-2-pentanol. The area of the chromatographic peak corresponding to the internal standard was used to

compute the concentrations of the other identified compounds, which were expressed in µval eq. SI (microval equivalent of internal standard).

RESULTS AND DISCUSSIONS

The volatile chemical compounds obtained are in variety of categories: alcohols, esters, carboxylic acids, aldehydes and ketones. Among these, normally found also in wine in different quantities comparative to samples which were fermented at normal temperatures. The fermenting temperature of the wines was different and as expected the concentration of the compounds is different as well or even new compounds to appear. The following table shows the chemical compounds that were identified in the samples analysed using gas chromatography (tab. 1).

Table 1

The concentrations of chemical compounds identified using gas chromatography

Class	Chemical compounds	Concentration (µval eq. SI)		Particularities of flavour
		ferm. 32 °C	ferm. 28 °C	
Alcohols	2-methyl-1-butanol	186.33	20.46	green, malt, onion, fish oil
	3-methyl-1-butanol	1072.6	762.97	floral, cocoa, malt, burnt
	2-nonanol	46.03	ND	cucumber
Alcohols	3-methyl-1-pentanol	66.33	83.00	floral
	1-hexanol	2653.3	ND	characteristic, sweet, pleasant
	2-heptanol	ND	80.45	citrus, earth, fried, mushroom
	3,3,5-trimethylcyclohexanol	81.61	83.87	mint, cool
	2,6-dimethyl-7-octen-2-ol	12.24	10.56	fresh, citrus, lime, floral, clean, weed, woody
	1-octanol	ND	95.39	bitter almonds, floral fat
	2,3-butanediol	203.89	333.01	buttery, rancid butter, plastic, rubber
	1-nonanol	11.08	ND	floral, green. fat, oil
	1-decanol	32.09	16.07	fatty
	benzyl alcohol	9.03	4.76	boiled cherries, musk, rose
	phenethyl alcohol	1503.2	800.80	fruit, honey, lilac, rose
	phenol	6.44	4.44	rubber, plastic, heavy
	1-nonadecanol	87.50	ND	combustible
	phenethyl acetate	42.18	28.65	floral, rose, honey
	isoamyl acetate	10.48	99.02	banana and pear
	ethyl lactate	999.52	404.43	butter, in large quantities: lactic souring
	hexyl formate	ND	98.30	fruity
	amyl formate	ND	14.52	fruity
	ethyl pelargonate (wine ether)	ND	27.12	fruits, banana, apple, rose, tropical, cognac, wax
	ethyl 3-hydroxybutanoate	ND	62.99	red fruit, fresh fruit (marshmallow, roasted nuts)

	ethyl benzoate	53.47	43.96	flower, fruit, chamomile, celery, fat
	diethyl succinate	359.01	ND	floral, fruity, cotton, fabric, wine
	ethyl dodecanoate	ND	27.32	floral, fruit, leaves
	ethyl octanoate	340.78	4.85	apricot, pineapple, floral, brandy, fat
	ethyl decanoate	607.30	755.90	sweet, fruit, pear, apple, cognac, wax
	isopropyl myristate	54.83	23.68	dairy
	ethyl pentadecanoate	14.30	23.33	wax
	methyl isopalmitate	7.76	5.81	fruity
	ethyl palmitate	142.82	134.40	wax
	ethyl stearate	5.60	8.89	wax
Carboxylic acids	benzoic acid	40.77	49.73	fat, convulsive
	octanoic acid	286.56	777.13	cheese, fat, grass, oil
	acid acetic	2438.3	2521.6	irritating, pungent
	isobutyric acid	ND	128.24	burned, butter, cheese, sweat
	butanoic acid	347.38	246.88	butter, cheese, sour
	hexanoic acid	138.26	581.69	cheese, oil, viscous, sour
Aldehydes and ketones	acetophenone	ND	46.86	almonds, flower, meat, must
	benzophenone	6.17	68.47	geranium, sweet, rose
	benzaldehyde	62.88	16.72	bitter almond, burnt sugar, cherry, malt, burnt pepper

ND-not detected

As the table shows, a large part of the chemical compounds that are part of a wine's aroma give it floral and fruity notes. These are specific to wines, although it depends on the concentration in which they are present. An example is ethyl octanoate which in low concentration can give a fruity aroma (pineapple, apricot), floral, but it can also give a fatty note if it is present in larger concentrations. This difference was also observed in the analysed samples. Another fruity ester which in large concentrations gives unwanted waxes notes is ethyl decanoate. Diethyl succinate has an aroma specific to a floral wine, fruity but there can also be notes of cotton, fabric. Among these floral-fruity compounds that can be repelling at the same time there are other compounds which keep their pleasant aromas. Some examples of these are the esters hexyl format, amyl format, ethyl pelargonat, ethyl 3-hydroxybutanoate and isoamyl acetate. Sweet, fruity and pleasant aromas are also given by the 3-methyl-1-pentanol and 1-hexanol, these being the middle alcohols (with the number of carbon atoms between 4 and 6) known in literature for having a pleasant smell. An important alcohol is the phenethyl alcohol – a compound that is found in different quantities in the two samples. Its concentration in the sample fermented at 32 °C is 0.183 g/L which is above the 0.1 g/L threshold that signals a stingy, herby aroma, while the concentration in the sample that was fermented at 28 °C is 0.097 g/L and is within the threshold that gives it a pleasant aroma such as honey, fruity, floral like lilac or rose (Cotea *et al.,* 2009).

Others prevailing aromas that were observed in both samples and in rather high concentrations are the fatty aromas of oil, or wax, buttery and cheesy. A clear example is the ethyl lactate which determined the lactic souring that was present in high concentrations. Other compounds are butanoic, hexanoic and octanoic acids whose smell is cheesy, sour or even oily. The wax smell can be sense because of the ethylic esters of the hexadecanoic and pentadecanoic acids respectively. The fatty sensation is also given by 1-decanol which is found in both samples, although in a higher concentration in the sample where the fermentation reached 32 °C.

A clear observation is the presence in high concentrations of the acetic acid which indicates that the wine vinegary process. It is known the fact that this happens during the alcoholic fermentation of the must when the temperature reaches 28-30 °C and instead of the continuation of the alcoholic fermentation, an acetic fermentation starts. Due to the fact that in our experiment the fermentation reached the temperatures of 28-30 °C it was expected that the prevailing smell of the wine to have such a sensorial shift, being more unpleasant in the sample fermented until 32°C. Aside from acetic acid which is prevailing in the wine vinegary process there is also hexanoic acid which is found in the analysed wines. We observed that in the case of the sample fermented at 28 °C the quantity of hexanoic acid is greater than in the case of the sample fermented at 32 °C. Ethyl lactate is a compounds that in large quantities determines lactic souring, behaviour observed especially at the sample fermented at 32 °C (Cotea *et al.,* 2009).

From the category of the compounds that give strange, unpleasant aromas, are the 2,3-butanediol which has a smell of plastic, rubber and was found in relatively large quantities in both samples. (www.odour.org.uk). Hydroxybenzene gives the same heavy rubber smell and 1-nonadecanol which can give a sensation of fuel. In the same category is the 3-methyl-1-butanol alcohols which give a cocoa (which in wine is not a sensorial advantage), malt or burnt aromas and its isomer, the 2-methyl-1-butanol whose common aroma descriptor is the malt to which onion or fish oil are added (www.thegoodscentscompany.com). Aside from these, another compound which attracts attention through its various aromas is the 2-heptanol. This is usually found in wine and it gives citric aroma when it is found in trace quantities (www.pubchem.ncbi.nlm.nih.gov). If the concentration is, however, greater than usual for a wine sample, then the 2-heptanol gives earthy, mushroom or fried notes which can be sometimes disturbing (www.phenol-explorer.eu; www.leffingwell.com).

Analysing each compound class we can see that the alcohols are largely responsible for the unpleasant and repelling aromas in the samples, especially the methylic isomers of butanol. Aside from these, but also giving unwanted aromas, are the carboxylic acids which significantly contribute to the sensations of sour, cheese, butter or oil. Their proportions are relatively close to each other with the exception of the octanoic acid which was found in higher quantities in the sample fermented near 28 °C. Generally known for their wide palette of pleasant aromas, the esters give in our case fruity-floral notes, especially through the isoamyl acetate and ethyl octanoate. Contributing in a positive manner is the class of

aldehydes and ketones through the sweet, pleasant aromas of the benzophenone and benzaldehyde. Aside from the volatile compounds presented in the table there were also others identified that belonged to these classed, but for which the literature does not give information regarding their aroma or smell.

CONCLUSIONS

By carefully consideration the concentrations of the compounds that give fruity-floral pleasant aromas, but also at the same time give unwanted aromas, we see that the sample fermented at a maximum of 28 °C shows stronger pleasant aromas in contrast to the one fermented at a maximum of 32 °C which has more repelling sensations. In this study, the pleasant and positive notes are generally given by esters, aldehydes and ketones and the repelling notes which prevail are contributing to the unpleasant aroma by the carboxylic alcohols and acids that are resulted mostly from the fermentation process. Furthermore, in this study the fact that a fermentation at high temperatures, that are above 20 °C causes the appearance of unwanted aromas in the wine which greatly decrease its sensorial quality by processes of acetic fermentations, lactic and tartaring souring, and also cheesy, fatty, waxy sensations.

REFERENCES

1. **Cotea D.V., Zănoagă C., Cotea V.V., 2009 -** *Tratat de oenochimie* Vol. I and Vol. II, Editura Academiei Române.
2. **Monique A.M. Smeets Hendrik N.J. Schifferstein S. R. Boelema G. L.-M., 2008 -** *The Odor Awareness Scale: A New Scale for Measuring Positive and Negative Odor Awareness*, Chemical Senses, vol. 33, 8, p. 725–734.
3. **Tarțian A.C., Cotea V. V., Niculaua M., Zamfir C.I., Colibaba C. L., Moroşanu A. M., 2017** - *The influence of the different techniques of maceration on the aromatic and phenolic profile of the Busuioacă de Bohotin wine*, BIO Web of Conferences, 9, 02032.
4. **Țârdea C., Sârbu Gh., Țârdea Angela, 2000** - *Tratat de vinificație*, Editura "Ion Ionescu de la Brad", Iaşi, p 329-337.
5. **Yusen W., Shuyan D., Liping Z., Zhen G., Meng L., Shiren S., Wenping X., Caixi Z., Chao M., Shiping W., 2016** - *Aroma characterization based on aromatic series analysis in table grapes*, Scientific RepoRts, 6, 31116.
6. ******* http://www.winearomawheel.com
7. ******* https://www.lenez.com/en/kits/wine
8. ******* https://pubchem.ncbi.nlm.nih.gov
9. ******* http://www.thegoodscentscompany.com
10. ******* http://phenol-explorer.eu
11. ******* http://www.leffingwell.com/odorthre.htm

INFLUENCE OF PHASAL FERTILIZATION ON THE SOIL FERTILITY, NUTRITIVE BALANCE AND PRODUCTION, AT A CROP OF CUCUMBERS PRODUCED IN VEGETATION POTS

INFLUENŢA FERTILIZĂRILOR FAZIALE ASUPRA STĂRII DE FERTILITATE A SOLURILOR, BALANŢEI NUTRITIVE ŞI PRODUCŢIILOR, LA O CULTURĂ DE CASTRAVEŢI, PRODUSĂ ÎN VASE DE VEGETAŢIE

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Abstract. *A primary factor in achieving higher production to crops, along with other vegetation factors, is the fertilization. For the vegetable crops where production system is intensive, it is frequently used the additional fertilization, using solid or liquid fertilizers as radicular or foliar application. The supplementary fertilizations made in the period of intensive growth and on the background of a basic fertilization judiciously set, create in soil the stabilization of the nutritive balance, resulting in increased production. In this context, at the culture of cucumbers under study, on an plot of 250 Kg / ha active substance NPK, the use of F_{221} foliar and of the urea administered to the soil, in 2 successive pheno-phases led to a content of the accessible forms of nutrients of 47 ppm nitrate ammonia nitrogen and, 45 ppm accessible phosphorus and 204 ppm accessible potassium in the soil. These insurance levels in the soil correlate with content of total forms of nutrients from the plant in the bloom phenol-phase, 5.1, 0.45 and 5.1% nitrogen, total phosphorus and potassium. The productions obtained with the same formula fertilization is situated at 51000 kg / ha, statistically assured. The comparative results are obtained by using F_{221} and ammonium nitrate in the same pheno-phases, respectively crops of 49800 kg / ha, but with slightly lower production increases.*
Cuvinte cheie: foliar fertilizer, plot, nutritive elements

Rezumat. *Un factor primordial în obținerea unor producții superioare la culturile agricole, alături de ceilalți factori de vegetație, îl reprezintă fertilizarea. Pentru culturile legumicole, unde sistemul de producție este unul intensiv, se recurge în mod deosebit la fertilizări suplimentare, utilizând îngrăşăminte solide sau lichide, administrate radicular sau foliar. Fertilizările suplimentare, practicate în perioadele de creştere intensă şi pe fondul unei fertilizări de bază judicios stabilite cantitativ, crează în sol stabilizarea elementelor de nutriție în jurul valorilor optime iar în plantă, conduc la echilibrarea balanței nutritive, soldate cu creşterea producțiilor. În acest context, la cultura de castraveți luată în studiu, pe un agrofond de 250 Kg/ha subtanță activă NPK, utilizarea foliarului F_{221} şi a ureei administrate la sol, în 2 fenofaze succesive, a condus în sol la un conținut al*

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formelor accesibile de elemente nutritive de 47 ppm azot nitric şi amoniacal, 45 ppm fosfor accesibil şi 204 ppm potasiu accesibil. Aceste nivele de asigurare în sol se corelează cu conţinutul de forme totale de elemente nutritive din plantă în fenofaza de inflorire, de. 5,1; 0,45 şi 5,1 % azot, fosfor şi potasiu total. Producţiile obţinute cu aceeaşi formulă de fertilizare se situează la 51000 kg/ha, asigurate statistic. Rezultate comparative se obţin şi prin utilizarea F_{221} şi a azotatului de amoniu, în aceleaşi fenofaze, respectiv recolte de 49800 kg/ha, însă cu sporuri de producţie uşor mai scăzute.

Cuvinte cheie: îngrăşământ foliar, agrofond, elemente nutritive

INTRODUCTION

The varying requirements of plant crops for nutrients during the growing season requires the application of fertilizers in several phases, taking into account the rhythm of assimilation and the amount of nutrients required by some phenol-phases, the root system and not least the increase of the degree of fertilizers used. The mineral nutrition during the growing season can become, in some cases, one of the main ways of meeting the requirements in nutrients (Davidescu, 1992). In particular at the cucurbit crops, the vegetation can be administered 4-5 additional fertilization in different phenological phases such as early flowering, early binding of fruits, early ripening fruit first, etc. In these phenological phases and at these species, the nitrogen and potassium requirements are quite high (Budoi, 2001). Correcting the nutrition deficiencies through foliar diagnosis but also by analyzing the status of soil fertility in conjunction with the specific consumption of plants but also with a series of agri-eco-pedological factors, constitutes systemic approaches of establishing the dosage, reports and varieties of fertilizers, within a culture technology (Volf, 2008).

MATERIAL AND METHOD

Experience was conducted in the green house of the Department of Agricultural Chemistry, UASVM Iasi, along three years, 2013-2015, using cucumbers, Korinda F_1 hybrid. The experimental scheme was designed in blocks with three repetitions on variations and had two impact factors into study:

- Factor A – plot of fertilization, with graduations:
  a_0 – Mt, unfertilized, a_1 – plot 150 kg/ha active substance, a_2 – plot 200 kg/ha active substance, a_3 – plot 250 kg/ha active substance.
- Factor B – additional fertilization with graduations:
  b_0 – Mt, nefertilizat, b_1 – F_{221}, b_2 - F_{221}+NH_4NO_3, b_3 - F_{221} + urea, b_4 - NH_4NO_3, b_5 – urea, b_6 – Fertcomplex, b_7 – Kristalon

For basic fertilization was used ammonium nitrate (34% a.s.), superphosphate concentrate (50% etc.) and calcium bicarbonate (40%), administered in a complexing report of 1: 0.4: 1.8 NPK, similar to that required balance ratio of NPK in the soil, needed for this species (Davidescu, 1992).

For the additional soil and foliar fertilizations there were used NH_4NO_3 and urea (100 kg / ha) and F_{221}, Fertcomplex and Kristalon (3-7 L / ha a. s.) with a varied chemical composition, administered in two divided phenol-phases of vegetation, at 5-7 leaves and before blooming (tab. 1).

Table 1

Chemical composition of foliar fertilizers

Foliar fertilizer	CSA %	pH	N g/L	P g/L	K g/L	B g/L	S g/L	Mn g/L	Mg g/L	Zn g/L	Cu g/L	Fe g/L	Mo g/L
F_{221}	16	6.5-7	70	70	48	0.2	9	0.2	0.5	0.1	0.05	0.2	-
Fertcomplex	16	6.5	80	80	75	0.3	0.4	0.4	0.04	0.04	0.1	0.3	0.03
Kristalon	16	6.6	180	180	180	0.025	-	0.04	-	0.025	0.01	0.07	0.004

The soil used in the vegetative pots was a cambic chernozem with a light texture, a slightly acid pH (6.7) and a state of medium fertility.

Prior to setting up the cultures and at the end of vegetation, soil samples were taken and subjected to laboratory analyses. For the foliar diagnosis, before flowering there were taken samples of limb that were prepared and analyzed in the laboratory. At harvest there were performed various biometric measurements, namely the average weight of fruits / plant (g), average plant height (cm), the average diameter of plant (cm).

For the statistic calculation of yields, it was established a density of 24,000 plants / ha.

The working methods were in accordance with the ones elaborated by I.C.P.A. Romania for agrochemical analyses. There were made the following determinations:

For soil: content of assimilable nitrogen (ammoniacal) in KCl 0.1 N extract, Colourimetric method, with Nessler reactive; content of nitric nitrogen (NO_3^-), in KCl 0.1 N extract, Colourimetric method with fenoldisulfonic acid; content of potentially assimilable phosphor, Egner - Riehm - Domingo (P-AL) Method; content of assimilable potassium, in plugged solutions extract, Egner - Riehm - Domingo (K - AL) Method.

For plants: dosage of total nitrogen, variant mineralized with sulfuric acid, distillation and titration with H_2SO_4; dosage of total phosphorous – mineralization on wet way, with ammonium molibdate and reduction with stanium colure, colorimetric dosage (after Nicolov, 1976); dosage of total potassium, by mineralization with a mixture of sulfuric and perchloric acid.

RESULTS AND DISCUSSIONS

The dosage of nutritive elements in accessible forms revealed that their quantitative level in the soil is in close accordance with the plot used but also with the chemical composition of fertilizers used at the additional fertilization (tab. 2).

Due to the increase of doses at the basic fertilization and by administering phasal fertilizations in the formula F_{221} + NH_4NO_3 and F_{221} + urea, it was noticed an intake of mobile forms in soil, reaching for the plot of 250 kg / ha a.s. at 44 and 47 ppm nitric nitrogen + ammonium nitrogen, 46 and 45 ppm P-AL and respectively 201 and 202 ppm K-AL. The values are considered optimal for nitrogen, phosphorous and normal moderate to high for potassium.

Table 2

Influence of fertilization on the accessible forms of nitritive elements

Variant/plot	150 kg/ha active substance			200 kg/ha active substance			250 kg/ha active substance		
	NH_4+NO_3 ppm	P_2O_5 ppm	K_2O ppm	NH_4+NO_3 ppm	P_2O_5 ppm	K_2O ppm	NH_4+NO_3 ppm	P_2O_5 ppm	K_2O ppm
Control	8	11	102	8	11	102	8	11	102
F_{221}	18	23	165	28	33	175	32	40	195
$F_{221}+NH_4NO_3$	22	25	172	35	35	183	44	46	201
F_{221} + urea	23	24	169	36	34	182	47	45	204
NH_4NO_3	20	24	163	32	36	179	43	44	202
urea	21	23	165	33	32	184	45	45	200
Fertcomplex	20	20	174	31	34	180	36	43	198
Kristalon	18	22	173	29	32	181	34	45	199

The trend of evolution of nutritive elements form the plant under the influence of fertilizations somehow follows the balance of determined accessible elements in the soil. It was noticed a considerable increase in the content of macronutrients in the plants from the plot of 150 kg/ha a.s/. and to the plot of 250 kg/ha a.s., in all variants of additional fertilization. In the same trend there are registered the increases compared to the unfertilized control variant, thus finding a progression of these values in comparison to the doses of fertilizers used and their chemical composition (tab. 3). The formulas of additional fertilization F_{221} and F_{221} + NH_4NO_3 + urea attract a content of 4.8, 0.40 and 4.9% nitrogen, total phosphorus and potassium, and respectively 5.1, 0.45 and 5.1% in plant material for the plot of 250 kg/ha a.s. but also the administration of singular Kristalon, on the same plot reaches values of 5.2, 0.45 and 4.9% nitrogen, phosphorus and potassium, total forms, values that, in all cases, falls in the optimum – slightly elevated category.

Table 3

Influence of fertilization on the evolution of total forms of nutritive elements from the vegetal material

Variant/ plot	150 kg/ha active substance			200 kg/ha active substance			250 kg/ha active substance		
	N_t %	P_t %	K_t %	N_t %	P_t %	K_t %	N_t %	P_t %	K_t %
Control	2.9	0.35	3.7	2.9	0.35	3.7	2.9	0.35	3.7
F_{221}	3,9	0.35	3.9	4.1	0.41	4.1	4.5	0.40	4.1
$F_{221}+NH_4NO_3$	4.4	0.40	4,2	4.5	0.45	4.5	4.8	0.40	4.9
F_{221} + urea	5.2	0.47	4.7	5.0	0.45	4.9	5.1	0.45	5.1
NH_4NO_3	4.7	0.42	4.1	4.9	0.40	4.6	5.0	0.40	4.5
urea	4.6	0.42	4.0	4.6	0.46	4.1	4.9	0.39	4.1
Fertcomplex	4.8	0.44	3.9	4.9	0.43	4.2	5.0	0.42	4.3
Kristalon	5.0	0.49	4.2	5.0	0.44	4.5	5.2	0.45	4.9

The biometric characteristics determined point out notable increases in comparison to the plot used and type of fertilizer used at the additional

fertilizations (tab. 4). It stood out the F_{221} + urea variant, on the plot of 250 kg/ha a.s. NPK, at all biometric indicators analyzed, respectively 4125 gr fruit/pl, a average length of the plant of 110 cm and a average fruit diameter of 5.5 cm.

Table 4

Influence of fertilizations on the biometric characteristics

Variant/plot	150 kg/ha active substance			200 kg/ha active substance			250 kg/ha active substance		
	Av. weight fruit/pl (g)	Av. length. pl (cm)	Ø fruit (cm)	Av. weight fruit/pl (g)	Av. length. pl (cm)	Ø fruit (cm)	Av. weight fruit/pl (g)	Av. length. pl (cm)	Ø fruit (cm)
Control	1125	22	3.5	1125	22	3.5	1125	22	3.5
F_{221}	1255	26	3.9	1315	59	4.5	1390	90	4.8
F_{221}+NH_4NO_3	1530	32	4.8	1860	55	5.2	2075	98	5.4
F_{221} + urea	1600	33	5.0	1925	56	5.3	4125	110	5.5
NH_4NO_3	1410	31	4.4	1675	54	5.1	1890	77	5.3
urea	1450	32	4.7	1780	55	5.1	1925	78	5.4
Fertcomplex	1320	28	4.3	1405	49	4.7	1540	61	5.0
Kristalon	1290	27	4.5	1430	49	4.8	1530	69	5.1

The production obtained in the three years of experimentation are statistically assured, at significant and distinctly significant level, for all three plots, by using additional fertilization, in formulas F_{221} +NH_4NO_3, F_{221} + urea, NH_4NO_3 and urea (tab. 5).

Table 5

Influence of basic and additional fertilization on production

Variant/ plot.	150 kg/ha active substance				200 kg/ha active substance				250 kg/ha active substance			
	Prod. kg/ha	%	Dif. ±kg/ha	Sem.	Prod. kg/ha	%	Diff. ±kg/ha	Sem	Prod. kg/ha	%	Diff. ±kg/ha	Sem
Control	27000	100	-	-	27000	100	-	-	27000	100	-	-
F_{221}	30120	112	+3120	-	31560	117	+4560	-	33360	123	+6360	-
F_{221}+ NH_4NO_3	36720	136	+9720	*	44640	165	+17640	**	49800	184	+22800	**
F_{221} + urea	38400	142	+11400	*	46200	171	+19200	**	51000	189	+24000	**
NH_4NO_3	33840	125	+6840	-	40200	149	+13200	*	45360	168	+18360	**
urea	34800	129	+7800	*	42720	158	+15720	*	46200	171	+19200	**
Fertcomplex	31680	117	+4680	-	33720	125	+6720	-	36960	137	+9960	-
Kristalon	30960	115	+3960	-	34320	127	+7320	-	36750	136	+9750	-

DL 5% = 7435 kg/ha = 9870 kg/ha = 11340 kg/ha
DL 1% = 14320 kg/ha = 16200 kg/ha = 17565 kg/ha
DL 0.1% = 22210 kg/ha = 24535 kg/ha = 26540 kg/ha

In the variants of additional combined fertilization (radicular + foliar) F_{221} + NH_4NO_3 F_{221} + urea there are obtained productions of 49800 respectively 51000 kg/ha cucumber, with an increase of production compared to the unfertilized plot of 22800 kg and 24000 kg, to the variants of additional radicular fertilization only with NH_4NO_3 and urea, in which the productions although statistically insured at a significantly distinct level, it registered lower values of 45 360 and 46200 kg/ha, respectively.

CONCLUSIONS

1. The efficiency of the fertilization system, reflected in the assurance status of the soil with nutrients, in accordance with the balance nutritious plant but especially in production levels is higher when there are taken into consideration the chemical characteristics of fertilizers used and their differentiated application, depending on the condition of soil fertility and biological and physiological peculiarities of the species.

2. In all fertilization variants we could notice an increasing trend of insurance of the soil condition with NPK accessible forms, a condition of the total NPK forms in the plant but also of the yields compared to unfertilized control variant in relation to the dose of basic fertilizer and with the nature of administered fertilizer.

3. At the culture of cucumbers, on a ground of nutritive elements judiciously set, the fractional administration of doses of supplementary fertilizers, for the critical moment of nutrition, leads at getting impressive increases of production, regardless of how it is applied alone or combined.

4. The F_{221} foliar, in combination with nitrogen fertilizers radicularly administered, determines the largest productions in the conditions of a basic fertilization of 250 kg/ha NPK a.s, respectively 49800 and 51000 kg/ha cucumber.

REFERENCES

1. Budoi Gh., 2001 – *Agrochimie,* Editura Didactică şi Pedagogică, Bucureşti.
2. Davidescu D., Davidescu Velicica, 1992 – *Agrochime horticolă***,** Editura Academiei Romane, Bucureşti.
3. Volf Mariana, 2008 – *Agrochimie,* Editura Renaissance, Bucureşti.-

VARIATION OF ELEMENTARY CHEMICAL COMPOSITION AT A CULTURE OF CHEMICHALLY FERTILIZED POTATO IN AN AREA BELONGING TO TCE 3 BRAZI, GIROV, NEAMT COUNTY

VARIAȚIA COMPOZIȚIEI CHIMICE ELEMENTARE LA O CULTURĂ DE CARTOF FERTILIZATĂ CHIMIC, ÎNTR-UN AMPLASAMENT APARȚINÂND TCE 3 BRAZI, GIROV, JUDEȚUL NEAMȚ

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Abstact : *The elementary composition of plants varies in relation to several factors, but fertilization is a restrictive and limiting factor. The study has in view this aspect and highlights the impact of fertilizer doses, but also their chemical composition, on elementary chemical composition, on a potato culture produced in climatic and soil conditions in Neamt county. A fond fertilization with 375 kg / ha with NPK and an additional fertilization in vegetation with a formula of 16-20+18-46+Kristalon, achieves an accumulation in haulm of 0.43; 0.56 and 3.76% N, P, K in d. s. (ensuring medium to normal), and in tubers an accumulation of 1.04; 0.59 and 1.80% NPK in d.s. (ensuring normal to very good). Corroborated with soil fertility status as well as with other eco-pedo-climatic factors, these results are recorded in a register of the favorability of culture in the context of this scheme of fertilization.*
Key words : elementary chemical composition, fertilization, fertilizers

Rezumat : *Compoziția elementară a plantelor variază în raport cu o serie de factori, însă fertilizarea reprezintă un factor restrictiv şi limitativ. Studiul intreprins se raportează la acest aspect şi reliefează impactul dozelor de îngrăşăminte dar şi compoziția chimică a acestora, asupra compoziției chimice elementare, la o cultura de cartof, produsă în condiții climatice şi de sol din județul Neamț. O fertilizare de fond cu 375 kg/ha s.a NPK şi o fertilizare suplimentară în vegetație cu o formulă de tip 16-20+18-46+Kristalon, reuşeşte o acumulare în vreji de 0,43; 0,56 şi 3,76 % N,P,K din s.u (asigurare medie spre normală), iar în tuberculi, o acumulare de 1,04; 0,59 şi 1,80 % NPK din s.u.(asigurare normală spre foarte bună). Coroborate şi cu starea de fertilitate a solului dar şi cu alți factori eco-pedo-climatici, aceste rezultate se înscriu într-un registu al favorabilității culturii, în contextul acestei scheme de fertilizare.*
Cuvinte cheie: compoziție chimică elementară, fertilizare, fertilizanți

INTRODUCTION

Creating certain nutritional preferences appropriate to crops, is a prime factor in obtaining large and constant quantitative and qualitative production. The

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use of fertilizers in balanced reports for satisfying the metabolic processes of plants is reflected in the elementary chemical composition of the vegetative parts, but especially of the recoverable parts. In potatoes, nitrogen fertilizers have moderate efficacy, with potassium being the most effective and less influenced by climatic conditions, followed by phosphorus with the least efficiency (Ianosi, 1980). The process of vegetative formation as well as the additional consumption of fertilizing elements can be stimulated by foliar treatments, thus maintaining the plant active over a longer period of time (Loon, 1984). An inadequate supply with only one of the essential elements produces a worsening of the assimilation of other elements but also of the conditions of plant growth and development (Davidescu, 1980).

MATERIAL AND METHOD

The research is a continuation of the studies undertaken within TCE 3 Brazi, Girov, Neamt County, in 2015, on a potato culture. The experience with chemical fertilizers was organized on an area of 0.5 ha, following the subdivision blocks method, in 4 rehearsals. As a precursor plant, the potato had a corn crop, the variety being Castrum.

The climate in which the potato was produced is a pronounced continental temperate climate in which the average temperature is 8.4^0C and the sum of the average annual rainfall is 635.5 mm distributed in a number of 89.5 days, favourable conditions for potato cultivation.

The soil on which the crop is located is a gleic proxicalcarus aluvisol with a pH of 5.54-5.8, a hydrolytic acidity of 3.12-4.32 me/100g of soil, a humus content of 1.86-2 .58% and a saturation degree in bases of 71-76%.

The basic fertilizations were made with urea (46.6%), concentrated superphosphate (50% sa) and potassium salt (40%), administered in progressive doses of 110 kg/ha a.s. to 75 kg/ha a.s of P_2O_5 and 45 Kg/ha of K_2O, respectively 135 kg/ha a.s. of N, 95 kg/ha a.s. of P_2O_5 and 70 kg/ha a.s. of K_2O and the third level of fertilization 160 kg/ha a.s. of N, 120 kg/ha a.s. of P_2O_5 and 95 kg/ha a.s. with K_2O.

Phaseally, two solid complex fertilizers Cx 16-20-0 and Cx 18-46-0 produced by SC Arvi Agro SRL were used at 25 kg/ha a.s. for fertilization I and 50 kg/ha a.s. for fertilization II, and two foliar fertilizers, Fertcomplex and Kristalon in doses of 5 L/ha a.s. fertilization I and 7 L/ha a.s. fertilization II. The moments of phase fertilization were at the beginning of vegetation, respectively 10-15 leaves and before flowering.

The complexes 16-20 and 18-46 have a granulometric structure of 3% (fraction of the total mass of granules of size ≤ 1 mm)

Fertcomplex and Kristalon foliar fertilizers have a pH of 6.6 and 6.5 and a content of 80,80 and 75 g/L N, P, K - Fertcomplex and 180,180 and 180 g/L N, P, K - Kristalon. They also contain a number of other macro and micro elements such as S, Mg, Mn, Mo, Zn, Fe.

Samples of whole plants were taken at technological maturity, according to the standard methodology in force, on analytical units. The laboratory analyzes have been prepared and subjected separately, the recoverable part from the non-worthable part (haulm/tubers) The following were determined:

- total nitrogen dosing, sulfuric acid mineralization, distillation and ammonium titration with H_2SO_4;

- total phosphorus dosing - wet mineralization, ammonium molybdate and chlorine reduction, colorimetric dosing (after Nicolov, 1976);
- total potassium dosing by mineralization with sulfuric acid and perchloric acid mixture and flame photometry dosing.

RESULTS AND DISCUSSIONS

From the researches carried out, which aimed at the effectiveness of the basic and phasic chemical fertilization, on the accumulation of fertilizing elements in the potato plants, it results that, under the conditions studied, the fertilizers have a major impact on the nutrient accumulations in the plant, both in the profitable part and in the unvalued one (tab. 1, tab. 2).

The elementary chemical composition in haulm varied in relation to the fertilizer doses used as agrofonds as well as the chemical composition of fertilizers used additionally during vegetation. The maximum total nitrogen, phosphorus and potassium content was recorded in the case of the three agrofonds, by using during the vegetation the additional fertilizations in the combination 16-20 + 18-46 + Kristalon, with insignificant differences from one agrofond to another.

Table 1

Elementary chemical composition (% N, P and K from d.s.) - haulm

Var/agrofond	110-75-45 kg/ha NPK s.a			135-95-70 kg/ha NPK s.a			160-120-95 kg/ha NPK s.a		
	Nt %	Pt %	Kt %	Nt %	Pt %	Kt %	Nt %	Pt %	Kt %
Control	0.20	0.30	2.20	0.20	0.30	2.20	0.20	0.30	2.20
16-20-0	0.29	0.33	2.29	0.30	0.35	2.34	0.33	0.38	2.56
18-46-0	0.31	0.38	2.28	0.35	0.41	2.36	0.35	0.44	2.60
16-20+18-46	0.33	0.43	2.30	0.38	0.46	2.35	0.39	0.50	2.62
Fertcomplex	0.30	0.35	2.50	0.35	0.37	2.69	0.37	0.40	2.72
Kristalon	0.35	0.40	2.90	0.35	0.44	3.10	0.40	0.49	3.30
16-20+18-46+ Fertcomplex	0.35	0.40	2.55	0.38	0.46	2.71	0.38	0.52	2.70
16-20+18-46+ Kristalon	0.37	0.45	3.10	0.40	0.50	3.40	0.43	0.56	3.67

In the case of elementary chemical composition NPK in tubers, the same tendency is noticed, with the observation that, from one agrofond to another, the increases are significant for the same additional fertilization, ie the use of 16-20 + 18-46 + Kristalon combination.

Table 2

Elementary chemical composition (% N, P and K from d.s.) - tubers

Var. /agrofond	110-75-45 kg/ha NPK s.a			135-95-70 kg/ha NPK s.a			160-120-95 kg/ha NPK s.a		
	Nt %	Pt %	Kt %	Nt %	Pt %	Kt %	Nt %	Pt %	Kt %
Control	0.26	0.15	0.55	0.26	0.15	0.55	0.26	0.26	0.55
16-20-0	0.30	0.25	0.57	0.35	0.29	0.60	0.44	0.30	0.75
18-46-0	0.34	0.30	0.60	0.38	0.40	0.59	0.45	0.45	0.77
16-20+18-46	0.45	0.39	0.61	0.50	0.49	0.63	0.60	0.50	0.77
Fertcomplex	0.37	0.37	0.55	0.45	0.46	0.59	0.59	0.47	0.69
Kristalon	0.44	0.40	0.65	0.49	0.49	0.75	0.60	0.51	0.80
16-20+18-46+ Fertcomplex	0.59	0.45	0.70	0.61	0.50	0.82	0.75	0.55	0.99
16-20+18-46+ Kristalon	0.70	0.46	0.85	0.87	0.55	1.33	1.04	0.59	1.80

CONCLUSIONS

1. The potato produced under intensive conditions within SC TCE 3 Brazi Girov and benefiting from the ecological conditions of the area, is responsible for their fertilization, certified by the results of the analysis of the elementary composition of the plants.

2. By providing a sustained and balanced NPK agrofond and the use of phasial fertilizers, we note the improvement of the elementary chemical composition, both in the unvaluable and the recoverable part, the dosages being within the limits of a medium-to-normal insurance quoted by the literature, for the essential macroelements, nitrogen, phosphorus and potassium.

3. Additional fertilization with Kristalon, for all three agrofonds, brings in nitrogen, phosphorus and potassium content compared to additional fertilization 16-20 + 18-46 + Kristalon.

4. With the variant 16-20 + 18-46 + Kristalon, on the agrifond 160-120-95 kg/ha a.s. with NPK, there are obtained the potato tubers with maximum nitrogen, phosphorus and potassium content, i.e. 1.04; 0.59 and 1.80% of d.s.

REFERENCES

1. Davidescu D., Calancea I, Davidescu Velicica, Lixandru Gh., Ţârdea C., 1981 – *Agrochimie*, Ed. Didactică şi Pedagogică, Bucureşti.

2. Ianoşi Maria, Copony W., Pamfin Gh., 1980 – *Contribuţii la interpretarea şi prognoza eficacităţii fertilizării culturii cartofului prin modelul matematic de optimizare a fertilizării în zona Braşov.* Lucrări ştiinţifice, Analele ICPC Braşov, vol XI., p.165.

3. Loon van C.B., 1983 – *Hollands Kartoffelprofis*- Top Agrar das Magazin fur Moderne Landwirtscaft K 6144 E

INFLUENCE OF SUPPLIMENTARY FERTILIZATION ON STATUS OF SOIL FERTILITY AND PRODUCTION, AT A CROP OF POTATOES IN POSITION BELONGINGTO TCE 3 BRAZI, GIROV, NEAMT COUNTY

INFLUENŢA FERTILIZĂRII SUPLIMENTARE ASUPRA STĂRII DE FERTILITATE A SOLURILOR ŞI ASUPRA PRODUCŢIEI, LA O CULTURĂ DE CARTOF, ÎNTR-UN AMPLASAMENT APARŢINÂND TCE 3 BRAZI, GIROV, JUDEŢUL NEAMŢ

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Abstact: *Fertilization of soils is a very important agrochemical measure, in the chain of the technological links of a culture. The harmonization of the use of fertilizers in the ground, plant and cultivation technology and agronomic efficiency, are steps required in the case of using crops in an intensive system. The potato produced in the appropriate climate zones, is one of the cultures that have a favourable response to fertilization. The paper presents results of a study using conventional fertilizers as well as additional fertilizer on a crop of potato. The results showed that together with basic fertilization, the additional fertilization with a complex of fertilizers administered radicularly improved the fertility of the soil, providing nitrogen-form equivalent to an average towards the normal; the amount of phosphorus digestible and assimilable potassium is normal levels towards higher level. But the production is influenced by the foliar fertilizers, situated in the case of the agro-fond160-120-95 kg / ha a.s. NPK at 34,570 kg for Fertcomplex to 34,400 for Kristalon. The maximum productionis reached on the same agrofond of fertilization, in the version of combined administration of phasial, root and foliar fertilization, i.e. in the variant 16+20 + 18+46 + Kristalon.*
Key words: agronomic efficiency, fertilization, fertility

Rezumat: *Fertilizarea solurilor este o măsură agrochimică deosebit de importantă, în lanţul verigilor tehnologie ale unei culturi. Armonizarea folosirii îngrăşămintelor în raport cu solul, planta şi tehnologia de cultură şi eficientizarea agronomică, reprezintă paşi obligatoriu de parcurs, în cazul practicării unor culture în sistem intensiv. Cartoful, produs în zone cu climat adecvat, este una din culturile care au un răspuns favorabil la fertilizare. Lucrarea prezintă rezultatele unui studiu, utilizând fertilizatori convenţionali la o cultură de cartof, administraţi ca agrofond dar şi ca fertilizatori suplimentari. Rezultatele obţinute au evidenţiat că alături de fertilizarea de bază, fertilizările suplimentare cu îngrăşăminte complexe administrate radicular, îmbunătăţesc starea de fertilitate a solurilor, situând asigurarea cu azot-forme asimilabile, la medie către normală, conţinutul în fosfor asimilabil şi potasiu asimilabil, la nivelul normal către ridicat. Producţiile însă sunt influenţate şi de*

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fertilizările foliare, situându-se în cazul agrofondului 160-120-95 kg/ha s.a NPK la 34400 kg pentru Fertcomplex şi 34570 pentru Kristalon. Producţia maximă se obţine totuşi pe acelaşi agrofond de fertilizare, în varianta administrării combinate a fertilizărilor faziale, radicular şi foliar, respectiv în varianta 16-20+18-46+Kristalon.
Cuvinte cheie: eficentizare agronomică, fertilitate, fertilizare

INTRODUCTION

The fertilizations during the growing season, are designed to complete the nutrients applied before the vegetation startup till the optimal economic dose and taking into consideration the dynamic requirements of plant nutrients (Budoi, 2001).The simultaneous application to the root and vegetative system, two, three, or more essential nutrients during the vegetation period of the plant, proves itself to be an efficient practice to stimulate production, superior to the application of simple fertilizers (Volf, 2008). Amid the fund fertilization, judiciously set, in gradually increasing doses, is distinguished at all species of crops, the production levels being higher than the unfertilized sample (Volf, 2003). Numerous surveys of crops done in the last decade, both nationally and internationally certify the need for this type of use.

MATERIAL AND METHOD

The study undertaken was done within TCE 3 Brazi, Girov, Neamt County, in 2015, on a potato crop. The area taken into study was of 0.5 ha, being organized an experience, according to the method of sub-divided blocks in four repetitions. The potato had as prior plant maize, the previous year. The variety of potato was Castrum, a variety with high production potential, resistance to drought and high temperatures but also with good resistance manna.

The area where the site is located has a pronounced continental climate, where the average temperature is 8.4^0C and annual average precipitation amount is of 635.5 mm distributed in a number of 89.5 days, which gives good condition for the growth and development of potato crop.

The soil on which the culture is located is a aluvisol proxicalcaric gleic, with pH of 5.54 - 5.8, an acid number of 3.12- 4.32 me/100 g soil, a humus content of 1.86 to 2.58% and a degree of base saturation of 71-76%.

For basic fertilization there was used urea (46.6% a.s.), superphosphate concentrated (50% a.s.) and potassium salt (40%) administered in progessive doses of 110 kg/ha N, 75 kg/ha a.s. P_2O_5 and 45 kg/ha K_2O, namely 135 kg/ha a.s., N, 95 kg/ha P_2O_5, 70 kg/ha K_2O and the third level of fertilization 160 kg/ha a.s. N, 120 kg/ha a.s. P_2O_5 and 95 kg/ha K_2O.

For phasal fertilizations there were used two solid complex fertilizers, respectively Cx 16-20-0 and Cx 18-46-0, produced by SC Arvi Agro SRL and administered in dose of 25 kg/ha a.s. fertilization I and 50 kg/ka a.s. fertilization II and two foliar fertilizers Fertcomplex and Kristalon in doses of 5 L / ha a.s. fertilization I and 7 L/ha fertilization II. The chemical composition and physico-chemical characteristics of these fertilizers are varied (tab. 1, tab. 2, tab. 3). The phasal fertilization, with both liquid and solid fertilizers were performed at the beginning of vegetation, at 10-15 leaves and before flowering.

Table 1

Chemical composition of foliar fertilizers

Foliar fertilizer	CSA %	pH	N g/L	P g/L	K g/L	B g/L	S g/L	Mn g/L	Mg g/L	Zn g/L	Cu g/L	Fe g/L	Mo g/L
Fertcomplex	16	6.5	80	80	75	0.3	0.4	0.4	0.04	0.04	0.1	0.3	0.03
Kristalon	16	6.6	180	180	180	0.025	-	0.04	-	0.025	0.01	0.07	0.004

Table 2

Physical-chemical indicators of NPK 16-20-0 ferilizer

(source: S.C. ARVI AGRO S.R.L.)

Name of indicators	Specifications	Values obtained according to the analysis
Total nitrogen (N, %)	16	15.5
Ammonia nitrogen (N-NH_4, %)	16	15.5
Total phosphorus (P_2O_5)	20	19.4
Soluble phosphorus in NACS (P_2O_5)	20	19.2
Soluble phosphorus in water (P_2O_5)	18	18.3
Soluble sulfur in water (S)	12	12.0
Content of water (H_2O) %, max	2	1.3
Granulometric structure %		
< 1%	3	max 3

Table 3

Physical-chemical indicators of NPK 18-46-0 fertilizer

(source: S.C. ARVI AGRO S.R.L.)

Name of indicators	Specification
Total nitrogen (N, %)	18
Content of water (H_2O) %, max	1.8
Fosfor total (P_2O_5)	46
Free flow.	100
Granulometric structure % (fraction of the total mass of granules on dimensions)	
< 1 mm	3

The soil samples were taken at the end of the vegetation in the previous year, for the dosage of initial forms of macro-elements in the soil and at the end of vegetation in the analyzed year, for the same dosage forms and their analysis conducted after fertilization. The harvesting depth was of 0-20 cm.

We have conducted a series of analyzes of soil samples, using the methods of RISSA dosing, namely:

- colorimetric dosage forms of ammoniacal and nitric nitrogen, the method with Nessler's reagent and phenol acid -2,4 disulfonic
- content of phosphorus in the soil, extacted with a solution of ammonium lactate acetate of pH = 3.7 to 3.8 determined by Egner-Riehm-Domingo method or in the case of fertilized soils with non-activated phosphorites by extraction with a solution of 0.3% ammonium molybdate in 0.001 N $CaCl_2$ at pH = 4.4;
- content of mobile potassium in the soil, extracted with phosphorus and the same solutions as determined by flame photometry method.
- degree of saturation in base;
- humus content, Schollenberger version.
- hydrolytic acidity - Kappen method.

RESULTS AND DISCUSSIONS

The fertilizing elements administered in the form of fertilizera influenced the accesible forms of nutrients in the soil, development which had effect, regardless the time of application and the dosage used. Thus, the results reflect a fairly wide range of values, emphasizing the fact that they grow, in relation to nature and chemical composition of the fertilizing material (tab. 4).

The content of nitrogen accessible forms registered progressive growth rates from the control variant towards the variants with additional complex solid fertilizer but also in relation to agro-fund. The 20+18-46 si 16-20+18-46+ Kristalon variants, recorded on a NPK 160-120-95 ha and a content of 25 ppm NO_3 + NH_4, which falls to a ground of state average supply.

The accessible phosphorus registers increases from 28 ppm to 46 ppm at theunfertilized control sample, 48 ppm and 56 ppm P-AL for 16-20 + 18-46 + Kristalon sample, the agrofunds 110-75-45, 135-95-70 and 160-120-95 kg/ha a.s. NPK respectively, values that fall in a state of high assurance of this element for the soil.

The potassium in assimilable forms reaches comparable values, at the variants that were additionally fertilized with complex fertilizers but also at variants of combined suplimetary fertilization, clearly being also influenced by the agrofund. The 160-120-95 kg/ha a.s. NPK agrofund, bringing in the soil a content of 235 and 237 ppm of K-AL, for the variants 16-20 + 18-46 and 16-20 + 18-46 + Kristalon, which means that it brings a state of normal provision of the soil with potassium.

Table 4

Influence of fertilization on accessible forms of nutritive elements

Var./agrofund	110-75-45 kg/ha NPK a.s.			135-95-70 kg/ha NPK a.s.			160-120-95 kg/ha NPK a.s.		
	NH_4+ NO_3 ppm	P-AL ppm	K-AL ppm	NH_4+ NO_3 ppm	P-AL ppm	K-AL ppm	NH_4+ NO_3 ppm	P-AL ppm	K-AL ppm
Mt . unfert	10	28	176	10	28	176	10	28	176
16-20-0	11	34	185	19	37	190	22	41	194
18-46-0	15	44	180	19	47	192	23	49	220
16-20+18-46	23	49	183	23	49	195	25	55	235
Fertcomplex	10	27	176	11	27	178	11	29	175
Kristalon	11	28	175	12	27	177	10	29	174
16-20+18-46+ Fertcomplex	24	46	182	25	48	199	24	56	229
16-20+18-46+ Kristalon	23	47	180	24	47	200	25	55	237

The potato yields obtained after fertilization significantly increase, especially for the supplementary fertilized variants with complexes administered radicularly and foliarly, on all agrofunds (tab. 5)

On the agrofund 110-75-45 kg/ha a.s. NPK, the yields reach 33400 and 34500 kg/ha at the variants 16-20 + 18-46 + Fertcomplex and 16-20 + 18-46+Kristalon, with differences in comparison to the control sample of +11400 and +12500 kg, respectively.

Using the agrofund 135-95-70 kg/ha a.s. NPK, the comparable production variations are obtained in 16-20 + 18-46 of 34450 kg/ha potatoes and Kristalon with 33280 kg/ha potatoes. But the maximum yield is obtained with version 16-20 + 18-46 + Kristalon of 37800 kg/ha potatoes with a difference of +15800 kg/ha from the unfertilized control sample.

On the agrofund of 16 0-120-95 kg/ha a.s. NPK, the maximum production of potatoes are obtained in 16-20 + 18-46 + Kristalon variant of 39850 kg/ha potato, with an increase + 17850 kg compared to the control sample.

Table 5

Influence of basic and supplementary fertilization on production

Var./agrofund	110-75-45 kg/ha NPK a.s			135-95-70 kg/ha NPK a.s.			160-120-95 kg/ha NPK a.s.		
	Yield kg/ha	%	Diff. ±kg/ha	Yield kg/ha	%	Diff. ±kg/ha	Yield kg/ha	%	Diff ±kg/ha
Mt . unfert	22000	100	-	22000	100	-	22000	100	-
16-20-0	25050	114	+3050	29100	132	+7100	33120	151	+11120
18-46-0	28550	130	+6550	32250	146	+10250	33950	154	+11950
16-20+18-46	30150	137	+8150	34450	156	+12450	35450	161	+13450
Fertcomplex	29220	133	+7220	31250	142	+9250	34400	156	+12400
Kristalon	32000	145	+10000	33280	151	+11280	34570	157	+12570
16-20+18-46+ Fertcomplex	33400	152	+11400	34900	159	+12900	36850	168	+14850
16-20+18-46+ Kristalon	34500	157	+12500	37800	172	+15800	39850	181	+17850

CONCLUSIONS

1. The potato is successful in this climatic zone taking also advantage of a soil adequate for its culture, high productions, influenced on the one hand by an agrofund rich in nutrients and on the other hand, by the complex of fertilizers used.

2. The state of providing the fertilizing elements to the soil, using the proposed fertilizers, is situated in the medium towards optimal levels for nitrogen, medium towards optimal levels for phosphorus and potassium and certifies the impact of fertilization, especially the radicular one on the fund of nutritive elements in the soil.

3.The most efficient fertilization variants in terms of preserving the status of the soil, were the variants 16-20 + 18-46, 16-20 +18-46 Fertcomplex and 16-20 + 18- 46 + Kristalon, on the agrofund 160-120-95 kg/ha a.s. NPK.

4. On an agrofund of nutritive elements in increasing doses and usinf combined phasal fertilization, simultaneously applied radicularly and foliarly, there could be obtained remarkable yields.

5. Comparable productions are obtained both in the foliar fertilization on the 160-120-95 kg/ha a.s. NPK agrofund, as well as in the combined fertilizations (radicular + foliar) on the 135-95-70 kg/ha a.s. NPK agrofund, which proves equally important to ensure fertility status of the soil and also thge impact which the phasal fertilizations have.

6. Ensuring an agrofund of 160-120-95 kg/ha a.s. NPK, when administered phasally the16-20-18-46 + Kristalon complexes, leads to maximum increases of yield.

REFERENCES

1. Budoi Gh., 2001 – *Agrochimie,* Editura Didactică şi Pedagogică, Bucureşti.

2. Volf Mariana, **Avarvarei I., Lisnic T., Avarvarei Teona, 2003** - *Influența fertilizării organo-minerale asupra nivelului producțiilor la cultura de varză de Bruxelles (Brassica oleracea var. gemmifera)*, Lucr. st., seria Horticultură, vol.45. USAMV Iaşi.

3. Volf Mariana, 2008 – *Agrochimie,* Editura Renaissance, Bucureşti.

INFLUENCE OF FERTILIZATION WITH ZINC PRODUCTS ON THE PRODUCTIVITY AND QUALITY OF APPLE FRUITS

EFECTUL TRATĂRII CU FERTILIZANȚI FOLIARI PE BAZĂ DE ZINC ASUPRA PRODUCTIVITĂȚII ŞI CALITĂȚII FRUCTELOR DE MĂR

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Abstract. *The study subject of the experience was Idared apple variety grafted on M 9, trees were trained as Slender Spindles for distance 3.5 x 0.8 m. To study the influence of fertilization with zinc products on the fructification of apple plantation was experimented the following variants: 1. Control – no treatment; 2. Basfoliar Flo Zn - 1.0 L/ha; 3. Nertus Zinc - 2.0 L/ha; 4. Nertus Zinc - 3.0 L/ha. The Basfoliar Flo Zn fertilizer was applied once by spraying in the intensive growth phase of the fruit. The first treatment with Nertus Zinc was given in the pink button phase, the second - after flowering, and the third - the intensive growth of the fruits. The research was conducted during the period of 2015 year. In the present research work, we demonstrated that Nertus Zinc may be included in the technology system, applied 3 sprays at 2.0 L/ha.*
Key words: apple, *foliar fertilizer*, production, color.

Rezumat. *Ca obiect de studio a fost luat soiul de măr Idared altoit pe portaltoiul M9, pomii au fost conduşi ca fus zvelt ameliorat, distanța de plantare 3,5 x 0,8 m. Pentru a studia influența fertilizării cu produse pe bază de zinc asupra fructificării plantației de măr au fost montate următoarele variante: 1. Martor - fără tratare; Basfoliar Flo Zn – 1,0 L/ha; 3. Nertus Zinc – 2,0L/ha;4. Nertus Zinc – 3,0 L/ha. Fertilizantul Basfoliar Flo Zn a fost aplicat o singură dată, în faza de creştere intensivă a fructelor. Primul tratament cu Nertus Zinc a fost administrat în faza butonul roz, al II-lea – după înflorire, iar al III-lea – în faza creşterea intensivă a fructelor. Cercetările au fost efectuate pe parcursul anului 2015. Cercetările, au demonstrat, că Nertus Zinc poate fi inclus în sistemul tehnologic aplicat în 3 etape în doza de 2,0 L/ha.*
Cuvinte cheie: măr, fertilizant foliar, producție, culoare.

INTRODUCTION

Foliar fertilization is an agro technical procedure that complements, without replacing the root fertilization. It consists of spraying branches, shoots and leaves with different solutions of liquid fertilizers (Babuc, 2012; Babuc *et al*., 2013; Ghena *et al,* 2004).

Foliar fertilization allows for rapid compensation of deficient items and it is easy to apply and in some cases can be used with some phytosanitary products (Cimpoieş, 2012; Orphanas, 1982).

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Most often, the zinc deficiency occurs in the apple and occurs primarily on soils with high pH and with a large reserve of Ca; with humidity excess and low temperatures (Swietlik, 2002; Toma, 2008).

The absorption of zinc by trees corresponds to the pink button period, the intensive growth of shoots and the formation of fruits, and then decreases towards the end of the summer (Cimpoieş, 2012; Voiculescu *et al*., 2005).

MATERIAL AND METHOD

The research was conducted during 2015 year in apple orchard founded in the autumn of 2012 at the company „Codru-ST" Ltd. The subject of the experience was Idared apple variety grafted on M9 rootstock. The trees were as Slender Spindle. The distance plantation is 3.5 x 0.8 m.

To study the influence of fertilization with zinc products on the fructification of apple plantation was experimented the following variants: 1. Control – no treatment; 2. Basfoliar Flo Zn - 1.0 L/ha; 3. Nertus Zinc - 2.0 L/ha; 4. Nertus Zinc - 3.0 L/ha.

The Basfoliar Flo Zn fertilizer was applied once by spraying in the intensive growth phase of the fruit. The first treatment with Nertus Zinc was given in the pink button phase, the second - after flowering, and the third - in the binding phase - the intensive growth of the fruits.

The treatment was made with portable sprayer in the early morning hours without wind. The quantity of solution per tree was 0.3 liters considering the amount of trees per unit area and the quantity of water needed which is 1000 L/ha.

The average weight of a fruit, production per tree and per unit area and their redistribution in diameter were established during harvest.

The evaluation was performed using apple firmness penetrometer FT 327, which secures ingress resistance of pulp a piston area of 1 cm^2. Were conducted 10 assessments of firmness, being recorded media.

The chemical composition of the fruit was assessed by the content of soluble solids and titratable acidity.

The color intensity of fruit area was determined by the method described by Alina Basak a 5-point scale. First gradation fruits colored from 1 - 25%, second – 26 - 50%, third – 51 - 75%, forth – 76 - 90% and fifth – 90% or more from the fruit surface.

RESULTS AND DISCUSSIONS

The investigations carried out (tab. 1) show that treatment with the Nertus Zinc foliar fertilizer positively influenced the weight of zinc in the leaves of Idared apple trees.

If, in the control variant, as a result of the foliar diagnosis, zinc content in the leaf is 25 ppm, then the variants treated with foliar fertilizers increased and constituted 32-41 ppm. It was basically recorded that zinc in the plant increased by 28.0 - 64.0%, compared to the control variant.

The increase of zinc content in the plant is favorable because for a normal course of physiological processes in the apple trees, it is recommended that the zinc supply level is 25-50 ppm.

The results obtained show that in the case of control variant the zinc content is at the lower limit (25 ppm). Basfoliar Flo Zn fertilizer treatments

increased zinc in the plant to 32 ppm, or 28.0% compared with the control variant. This increase of the element in question is explained by the fact that its share in the composition of the foliar fertilizer is 635 g/l, or 43.0%.

In the case of the Nertus Zinc foliar fertilizer treatment, the weight of the element in the plant was higher than the recommended minimum standard with 36.0-64.0%, representing 34-41 ppm.

Table 1

The influence of foliar fertilizer on zinc content in the leaves of Idared apple trees, ppm

Variants	Zinc content
Control	25
Basfoliar Flo Zn, 1.0 L/ha	32
Nertus Zinc, 2.0 L/ha	34
Nertus Zinc, 3.0 L/ha	41

Studying the influence of the treatment dose on the zinc content in the plant, we note that with its increase the value of the studied index increases. In the case of treatment with Nertus Zinc at the rate of 2.0 L/ha, the amount of zinc in the leaf constituted 32 ppm and with the increase of the dose to 3.0 L/ha, the studied index increased by 20.5% compared with the previous variant. The higher content of zinc in the plant during that period has a significant influence on the growth of shoots, blooming and the degree of fruit binding.

Fruit production is the final index, which demonstrates how all agro-technical measures have been carried out in the Idared apple plantation.

The investigations show that the number of fruits in the crown of the trees included in the research differs according to the variants in the study (tab. 2). If, in the control variant, without treatment, the number of fruit was 51 pcs/tree, then in the variant treated with the Basfoliar Flo Zn fertilizer were 52 pcs/tree. When treating the trees with Nertus Zinc in the dose of 2.0 l/ha the studied index was 57pcs/tree, and in the variant treated with Nertus Zinc foliar fertilizer in dose of 3.0 L/ha - 56 pcs/tree.

This partial increase in the number of fruit in the tree crown was due to a higher degree of fruit binding after treatment with the Nertus Zinc foliar fertilizer until and after the flowering period.

The difference between the control variant and the Basfoliar Flo Zn variant was 1 pcs/tree, between the Nertus Zinc variant at the dose of 2.0 L/ha - 6pcs/tree, and from the Nertus Zinc variant in the dose 3.0 L/ha -5 pcs/tree.

The average weight of a fruit on the variants in the study did not show large changes under the influence of the fertilizers used compared to the number of fruits per tree. The lowest average fruit weight was recorded in the variant treated with Nertus Zinc in dose of 2.0 L/ha - 174.1 g followed in ascended order by the

control variant - 174.2 g, the variant Nertus Zinc in dose of 3.0 L/ha - 174.7 g and the variant treated with the Basfoliar Flo Zn fertilizer, where the average weight of a fruit was 176.4 g, or an increase of 2.2 g compared to the control variant. This slight difference in average weight of fruits in variants 3 and 4 is due to the negligible number of fruits and under the influence of the Nertus Zinc foliar fertilizer. The results outlined above are also confirmed by statistical processing.

Table 2

The influence of the zinc fertilization on production and quality parameters of Idared apple variety

Variants	Number of fruits, pcs/tree	Average weight, g	Production		Comparing with control variant, %
			kg/tree	kg/tree	
Control	51	174.2	8.88	31.71	100.0
Basfoliar Flo Zn, 1.0 L/ha	52	176.4	9.17	32.74	103.2
Nertus Zinc, 2.0 L/ha	57	174.1	9.92	35.42	111.7
Nertus Zinc, 3.0 L/ha	56	174.7	9.78	34.92	110.0
DL 5%	2.4	7.3	0.44	1.46	-

The fruit production on a tree and on a surface unit correlates directly with the number of fruits and their average weight. The smallest fruit production was recorded in the control variant, constituting 8.88 kg / tree or 31.71 t/ha.

In the variant treated with the Basfoliar Flo Zn fertilizer, the fruit production constituted 9.17 kg/tree or 32.74 t/ha, or an increase of 3.2% compared to the control variant, without treatment.

The highest fruit production was recorded in the variant treated with the Nertus Zinc foliar fertilizer in dose of 2.0 L/ha, constituting 9.92 kg/tree or 35.42 t/ha. Slightly lower values were recorded in the variant treated with Nertus Zinc foliar fertilizer in dose of 3.0 L/ha - 9.78 kg / tree or 34.92 t/ha. The insignificant difference between Nertus Zinc variants at 2.0 l/ha and Nertus Zinc at 3.0 L/ha is also demonstrated by statistical data.

Statistical data on fruit production on a tree and on a surface unit shows a statistical difference between the control variant and the Nertus Zinc variants at 2.0 L/ha and Nertus Zinc at a dose of 3.0 L/ha.

The results show that the Nertus Zinc foliar fertilizer in dose of 2.0 l/ha and 3.0 L/ha used to prevent zinc deficiency influenced the amount of fruits and the productivity of the plantation.

It is very important that apples are harvested at the optimum time. The data of the investigations carried out (tab. 3) show that the firmness of the apple pulp on the variants in the study at the time of the harvest constituted 7.2 - 7.5kg/cm^2.

The smallest firmness of the pulp was recorded in the control variant without treatment - 7.2 kg/cm^2.

When treatments with foliar fertilizers were cared out, there is an insignificant increase in pulp firmness. In the standard variant where Basfoliar Flo Zn foliar fertilizer treatment was performed, the firmness of the pulp increased and constituted 7.4 kg/cm^2, or by 0.2 kg/cm^2 higher compared to the control variant.

In variants treated with Nertus Zinc, it was recorded approximately the same values of pulp firmness as in the Basfoliar Flo Zn variant, but larger as in the control variant. In the given case, the studied index constituted 7.3 - 7.5 kg/cm^2, or an increase of 0.1 - 0.3 kg/cm^2 compared to the control variant. The increase in the treatment dose did not significantly affect the firmness of the pulp.

The content of soluble dry substance depends of the variety, after which can be determined the optimum harvesting time. The investigations show that the quantity of soluble dry substances in the Idared variety on the variants in the study constituted 13.6 - 13.9%.

Table 3

The influence of zinc fertilization on biochemical and coloring indexes in apple fruit of Idared variety

Variants	Firmness, kg/cm^2	Soluble dry substance,%	Titratable acidity, %	Coloration index
Control	7.2	13.6	0.51	2.1
Basfoliar Flo Zn, 1.0 L/ha	7.4	13.8	0.48	2.5
Nertus Zinc, 2.0 L/ha	7.3	13.8	0.49	3.7
Nertus Zinc, 3.0 L/ha	7.5	13.9	0.49	3.8

The smallest value of the shear of the soluble dry substances was recorded in the control variant - 13.6%. In the case of foliar fertilizers treatment, we record an increase in the studied index to 13.8 - 13.9%. That is, when treating with the Basfoliar Flo Zn fertilizer, the amount of fruit-soluble dry substance increased by 0.2% compared to the control variant.

Treatments with Nertus Zinc foliar fertilizer on both variants increased by 0.2 - 0.3% compared to the control variant and by 0.1% compared to the standard variant. Therefore, foliar fertilizer treatments have had an insignificant effect on increasing the amount of soluble dry substance.

The share of titratable acids in fruits is in direct dependence on the amount of soluble dry substance. Simultaneously, with decreasing the quantity of dry soluble substance in fruits, the titratable acids weight increased, with the highest value in the control variant - 0.51%.

In the case of the Basfoliar Flo Zn variant, the amount of titratable fruit

acids was 0.48%, or a 0.03% decrease compared to the control variant.

Treatments with the Nertus Zinc foliar fertilizer reduced the titration of the titrated acids to 0.49%, or a 0.03% decrease compared to the control variant.

Color is a major feature for assessing fruit quality, and an indicator for determining maturation for harvesting. Color is a characteristic of the variety and is determined by the presence of pigments in epidermal cells.

Conducted investigations show that the lowest coloration index was recorded in the control variant being 2.1 points.

Basfoliar Flo Zn fertilizer treatments intensified slightly the coloring index, representing 2.5 points, or an increase of 0.4 points compared to the control variant.

In the variants treated with the Nertus Zinc foliar fertilizer the index of coloring improved and constituted 3.7 - 3.8 points, or an increase of 76.2 - 80.9% compared to the control variant and 48.0% compared to Basfoliar Flo Zn.

CONCLUSIONS

1. The Nertus Zinc foliar fertilizer has positively influenced the prevention of zinc deficiency, fruit production compared to the control variant and Basfoliar Flo Zn.

2. Same values of pulp firmness, soluble dry substance, titratable acidity and fruit coloring index are recorded when treatment with the Nertus Zinc foliar fertilizer in dose of 2.0 L/ha and at dose 3, 0 L/ha were made.

3. When foliar fertilizer was used to prevent Zinc deficiency in plants, higher yields and superior quality were recorded in the Nertus Zinc variant in dose of 2.0 L/ha, applied three times by foliar spraying. The first fertilization should be made in the pink button phase, the second after the flowering, and the third in the binding phase - the intensive growth of fruits.

REFERENCES

1. Babuc V., 2012 - *Pomicultura.* Chişinău, p. 348-354.
2. Babuc V., Peşteanu A, Gudumac E., Cumpanici A., 2013 - *Producerea merelor.* Ed. Bonss Office, Chişinău, p.158-169.
3. Cimpoieş Gh., 2012 - *Cultura mărului.* Chişinau, p. 303-337.
4. Ghena N., Branişte N. Stănică Fl., 2004 - *Pomicultura generală.* Ed. MatrixRom, Bucureşti,, p. 391-422.
5. Orphanos P.I., 1982 - *Spray and soil application of zinc to apples.* Hortscience 57(3), p. 259-266.
6. Swietlik D. 2002 - *Zinc Nutrition of Fruit Crops.* HortTehnology, 12(1), p. 56-60.
7. Toma S., 2008 - *Aplicarea îngrăşămintelor în agricultura durabilă.* Chişinău, p 136-153.
8. Voiculescu N., Hoza D., Spiță V., 2006 - *Nutriția cu elemente minerale la pomi şi arbuşti fructiferi.* Partea I, Bucureşti, 263 p.

THE BIODIVERSITY STUDY OF THE ENTOMOFAUNA (superfamily PENTATOMOIDEA - HETEROPTERA) FROM BĂNEASA FOREST, BUCHAREST

STUDIUL BIODIVERSITĂŢII ENTOMOFAUNEI (superfamilia PENTATOMOIDEA - HETEROPTERA) DIN PĂDUREA BĂNEASA, BUCUREŞTI

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Abstract. *Among the factors that cause biodiversity loss, human activity in the sensitive ecosystem of forests can be easily monitored. The research carried out during 2016 focused on the study of Heteroptera,* superfamily *Pentatomoidea fauna in the Baneasa forest, where the natural environment was modified by human intervention through both recreational activity and constructions, insect collection being made by mowing with the entomological net, determining the structure of the systematic groups of the Heteroptera identified in the Baneasa forest, and a characterization of the zoogeographical origin of the species. In the Baneasa forest, the area hardly affected by the human activity, but less researched in terms of Heteroptera fauna, 52 species of Pentatomoidea were found, in our opinion 12 seem to originate from Manchurian refuge Usuric subcenter, 37 of the Mediterranean arboreal refuge, 2 come from the Caucasian arboreal refuge and one species could originate from the eremial Aralo-Caspic refuge (Turanic).*
Key words: Heteroptera-Pentatomoidea, biodiversity, forest Băneasa

Rezumat. *Printre factorii ce determină pierderi în cadrul biodiversitatii, activitatea omului în ecosistemul sensibil al pădurilor poate fi cu uşurinţă monitorizată. Cercetările, efectuate în cursul anului 2016, s-au axat pe studiul faunei de heteroptere din pădurea Băneasa, acolo unde mediul natural a fost modificat de intervenţia omului atât prin activitatea de recreaţie cât şi construcţiile realizate, colectarea insectelor făcându-se prin cosire cu fileul entomologic, determinându-se structura grupurilor sistematice ale heteropterelor identificate în pădurea Băneasa, şi s-a facut o caracterizare a originii zoogeografice a speciilor. În pădurea Băneasa, zonă relativ puternic afectată de activitatea umană, dar mai puţin cercetată din punct de vedere al faunei de heteroptere, au fost identificate 52 de specii ale suprafamiliei Pentatomoidea, după părerea noastră, 10 par să fie originare din refugiul Manciurian subcentrul Usuric, 37 din refugiul arboreal Mediteranean, 2 provin din refugiul arboreal Caucazian şi o specie ar putea fi originară din refugiul eremial Aralo-Caspic (Turanic).*
Cuvinte cheie: Heteroptera-Pentatomoidea, biodiversitate, pădurea Băneasa

INTRODUCTION

Emphasizing the vital importance of ecosystems for the health and well-being of people, the EU is launching a very clear message on the severity of

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biodiversity losses, recalling among the main factors determining the loss of biological diversity and inadequate water management water resources. They have to show the deforestation of the forests, the steppes have exerted a particular influence on the heteroptera, especially by changing the natural conditions of existence, the number of species, individuals, the evolution of these, may include the group as an indicator of biodiversity. Landscape fragmentation is one of the major reasons which lead to the drastic degression or loss of biodiversity over the world. It blocked gene flow among populations, caused inbreeding depression; and altered bio-geographical environments the species depended on, and minished the living space of the species (Liu *et. al*., 2005). Biodiversity measurement is commonly used to evaluate and monitor the health of ecosystems and as a tool for conservation planning (Magurran, 1988; Hoffmann, 2010). Like any other biologically successful group of organisms, the Heteropteran are prolific and diverse and have adapted to a variety of habitats. Heteropteran have complex and important roles in the balance of nature, the majority of them occupy an intermediate position in the ecological food chain; they use food producers (plants) and serve as food sources for parasites and other animals, Heteropteran are essentially nonsocial insects, a few species utilize plant-feeding insects as food (Froeschner, 2017). Morrison *et al.* (2012) identified Hemiptera and Coleoptera as effective indicator taxa of greater invertebrate richness in old field habitats in Michigan and the Great Lakes region. Duelli and Obrist (1998) showed that heteropteran diversity is a strong indicator of arthropod richness in semi natural and cultivated habitats in Switzerland.

MATERIAL AND METHOD

Insect collection was carried out by mowing with the entomological net in some areas of the Băneasa forest, where the natural environment was less or even altered by human intervention. In the entomological mesh, large species were caught by hand, and the small ones with the entomological vacuum cleaner were then placed in chloroform killing containers and then sorted. After sorting, some heteropteran species were stored in 70º alcohol, others were prepared on entomological needles and preserved in insect collection boxes and determined in the laboratory. Considering that not all specimens could be determined at the species level, their classification was gender and family. The determination of the heteropteran species was carried out with the help of determiners books or papers (Kiritshenko, 1951; Wagner, 1967; Benedek, 1969; Puchkov, 1961, 1965, Fuente, 1971, 1972 a, b, 1973 a, b; Kis B., 1984; Stichel, 1955-1962).

Characterization of the zoogeographical origin of the Heteropteran collected species was done taking into consideration papers of different authors (De Lattin, 1967; Hoberlandt, 1955; Banarescu, 1970; Banarescu and Boşcaiu, 1973; Roşca, 1979). The centers for the spreading of the faunistic elements, namely the glacial refuges of the arboreal and eremial fauna in the Holarctic region are presented in figure 1.

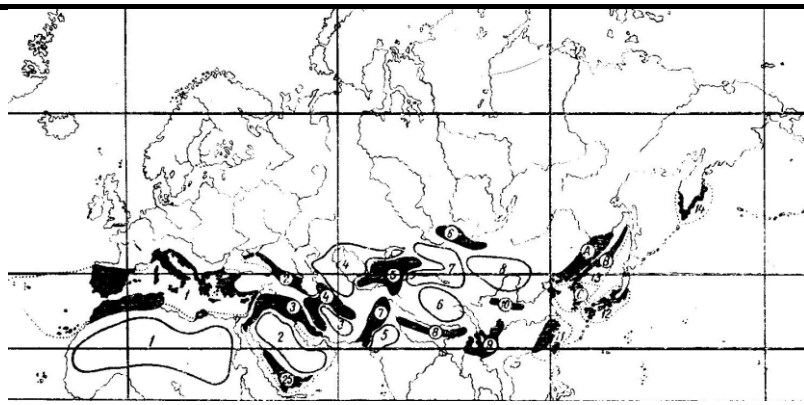

Fig. 1 The centers for the spreading of the faunistic elements, the glacial refuges of the arboreal and eremial fauna in the Holarctic region (Black=arboreal refuges: 1, Mediterranean; 2, Caspian (Caucasian); 3, Syrian; 4, Iranian; 5, Turkestanic; 6, Mongol; 7, Sinic; 8, Nepal; 9, Yunnan; 10, Sino-tibetan; 11, Sino-Pacific; 12, Japanese; 13, Manchurian (with 3 sub-centers, the main one being Usuric); 14 Kamciatkan; 16, 25, Yemenite; White=eremial centers: 1, Afro-eremial; 2, Siro-eremial (Arabic); 3, Irano-eremial; 4, Turano-eremial; 5, Sino-eremial; 6, Tibetan-eremial; 7, Mongolo-eremial; 8, Sino-eremial) (de Lattin, 1967)

RESULTS AND DISCUSSIONS

The superfamily Pentatomoidea, REUTER, 1910, has been and is relatively well studied today, and from a systematic point of view it is well known both in Romania and abroad the five families of the group: Scutelleridae, Pentatomidae, Acanthosomatidae, Cydnidae and Plataspidae which were the subject of special attention from us, in this study. Under the circumstances of our country importance of studying the Heteropteran order, it is particularly important if it is considered that in present there is any specialist deals with the study of Heteropteran species. Heteropteran species of superfamily Pentatomoidea species which were found in the Băneasa forest are presented below.

Superfamily PENTATOMOIDEA, Reuter, 1910

Family SCUTELLERIDAE Leach, 1815

Subfamily Odontoscelinae

1. *Odontoscelis dorsalis* F., 1803, comes from the Mediterranean arboreal refuge.

Subfamily Odontotarsinae

2. *Odontotarsus purpureolineatus* R., 1790, originates from the Mediterranean arboreal refuge.

Subfamily Eurygasterinae

3. *Psacasta exanthematica* Scop., 1763 originates from the Mediterranean arboreal refuge.

4. *Psacasta* (Cryptodonus) *neglected* H-S, 1837, originating from the Mediterranean arboreal refuge, more precisely from the Ponto-Mediterranean sub-center, extending westward along the southern continent of Europe.

5. *Eurygaster integriceps* Pt., 1881, originating from the Caucasian arboreal refuge.
6. *Eurygaster maura* L., 1758, originating from the Mediterranean arboreal refuge.
7. *Eurygaster austriaca* Schr., 1778, originated from the Mediterranean arboreal refuge, more precisely from the Atlantic-Mediterranean sub-center, limited to the original area because it is not extended to the north and east.
8. *Eurygaster testudinaria* G., 1875, originated from the Mediterranean arboreal refuge, but extended to the north and especially to the Far East.

Family PENTATOMIDAE Lech, 1815

Subfamily Podopinae

9. *Vilpianus galli*, W. 1802, a faunistic element originating from the Mediterranean arboreal refuge, withdrawn from the southern part of the initial area and now only spread in the northern Mediterranean.
10. *Ancyrosoma leucogrames* Gml. 1789, an element from the Mediterranean arboreal refuge, extending eastward to the Carpathians Altai, and westward to the Canary Islands.
11. *Graphosoma lineatum* L. 1758. That is why we consider Wagner to be an element from the Mediterranean arboreal refuge, which is very extensive, although Kis considers it Palearctic.
12. *Derula flavoguttata* Ms. et Rey, 1857, species originating from the Mediterranean arboreal refuge.

Subfamily Pentatomidae

Tribe Sciocorini

13. *Sciocoris cursitans* F. 1794, Mediterranean species, withdrawn from North Africa and extended to the north and especially to the east.
14. *Sciocoris helferi* Fb. 1851, Faunistic element from the Mediterranean arboreal refuge extended to the east until Kopet-Dag mountains.
15. *Sciocoris deltocephalus* Fb. 1861, a faunistic element from the Caucasian glacial arboreal refuge, extending north through the Black Sea to the west to Hungary, and east to south of Kazakhstan and the mountainous region of Russia as Hoberlandt shows us, is a species characteristic of steppe formations with rare vegetation.
16. *Sciocoris sulcatus* Fb. 1851, a species from the Mediterranean arboreal refuge, so most authors regard it as a Holomediterranean or Mediterranean species extending eastwards to Turkestan.
17. *Sciocoris* (Aposciocoris) *macrocephalus* Fb. 1851, a species originating from the Mediterranean arboreal refuge, and Hoberlandt describes it as a Holo-mediterranean species.
18. *Sciocoris* (Aposciocoris) *microphthalmus* Fl. 1860, the species originates from the Mediterranean refuge, but being adapted to a cold climate, it is withdrawn from North Africa, Western Europe, Asia Minor and the Near East.

19. *Dryoderes umbraculatus* F. 1775, originates from the Mediterranean arboreal refuge.

Tribe Aelini

20. *Aelia acuminata* L. 1758. Wagner regards it as a Holo-Paleartic, Hoberlandt as Euro-Siberian and Kis as Palearctic species. Species could originate from the eremial Aralo-Caspic (Turanic) refuge.
21. *Aelia rostrata* Bh. 1852, originating from the Mediterranean arboreal refuge, is actually an Aralo-Caspic (Turanic) faunistic element.
22. *Neottiglossa bifida* C. 1847, element derived from the Mediterranean arboreal refuge.
23. *Neottiglossa pusilla* Gml. 1789, element derived from the Mediterranean arboreal refuge.

Tribe Eysarcorini

24. *Stagonomus amoenus*, Br. 1832, element of the Mediterranean arboreal refuge.
25. *Stagonomus pusillus*, H-S. 1830, a member of the Mediterranean arboreal refuge.
26. *Stagonomus bipunctatus*, L. 1758, element derived from the Mediterranean arboreal refuge.
27. *Eysarcoris fabricii*, Kirk. 1904 (E. venustisimus Schrck), originating from the Manchurian arboreal refuge, Usuric subcenter.
28. *Eysarcoris inconspicuus*, H-S. 1844, an element from the Mediterranean arboreal refuge.
29. *Eysarcoris aeneus*, Scop. 1844 entered in our fauna through the extension of the species from Manchurian arboreal refuge, the Usuric subcenter, to the west.

Tribe Cappaeini

30. *Halyomorpha halys* Stål 1855, a species considered to be invasive, first reported in Romania in 2015, originating in the Far East, Manchurian arboreal refuge (Usuric subcenter)

Tribe Carpocorini

31. *Staria lunata* Hahn. 1835, comes from the Mediterranean arboreal refuge.
32. *Rubiconia intermedia* W. 1811, a species derived from the Manchurian arboreal refuge, the Usuric subcenter.
33. *Holcostethus vernalis* W. 1804, originates from the Mediterranean glacial shelter, partially withdrawn from the south of the initial area and extended to the north and especially to the east.
34. *Holcostethus* (Drycoris) *sphacelatus* F. 1794, species originating from the Mediterranean arboreal refuge, with less ecological valences than the previous species, as it is less extended to the north and especially to the east.
35. *Carpocoris purpureipennis* Deg. 1773, the species has expanded from the Manchurian arboreal refuge, the Usuric subcenter, to the west.
36. *Carpocoris fuscispinus* Boh. 1849, a species derived from the Manchurian arboreal refuge, the Usuric subcenter.

37. *Carpocoris pudicus* Pd. 1761, a faunistic element from the Mediterranean arboreal refuge.
38. *Dolycoris baccarum* L. 1758, the species originates from the Mediterranean arboreal refuge.
39. *Palomena viridissima* Pd. 1701, the species originates from the Manchurian arboreal refuge, the Usuric subcenter.
40. *Palomena prasina* L. 1761, species derived from the Manchurian arboreal refuge, Usuric subcenter.

Tribe Eurydemini (Strachini)

41. *Eurydema ornatum* L. 1758, species originating from the Mediterranean arboreal refuge.
42. *Eurydema oleraceaum* L. 1758, species originating from the Mediterranean arboreal refuge.

Tribe Pentatomini

43. *Piezodorus lituratus* F. 1794, the native species was a Manchurian, Usuric element, possibly re-adapted to the Mediterranean arboreal refuge.
44. *Rhaphigaster nebulosa* Pd. 1761, species originating from the Mediterranean arboreal refuge.

Subfamily Asopinae

45. Arma custos F. 1794, faunistic element derived from Manchurian arboreal refuge Usuric subcenter.
46. *Rhacognatus punctatus* L. 1758, faunistic element derived from Manchurian arboreal refuge Usuric subcenter.
47. *Zicrona coerulea* L. 1758, the species originated from the Mediterranean arboreal refuge, the refuge from which a large part of the Heteropteran species existed in Romania are coming.

Family ACANTHOSOMATIDAE Stäl, 1865

48. *Acanthosoma haemorrhoidale* L. 1758, originates from the eastern region where it has expanded its area northwards into the Manchurian arboreal refuge, the Usuric subcenter,

Family PLATASPIDAE Dallas, 1851

49. *Coptosoma scutelleatum* Geoffr. 1785, species originating from the Mediterranean arboreal refuge.

Family CYDNIDAE Billiberg, 1820

50. *Aethus nigritus* F. 1794, species originated from the Mediterranean arboreal refuge.
51. *Cydnus aterrimus* Forst. 1771, a faunistic element originally from the eastern region of the Mediterranean arboreal refuge.
52. *Tritomegas bicolor* L. 1758, an element originating from the Mediterranean arboreal refuge with the Euro-Siberian spreading, withdrew from the southern areas of the area and extended along the mountain ranges to the east.

In the Băneasa forest, the relatively hardly affected area of the human activity, but less researched from the point of view of the Heteropteran fauna, 52

species of the superfamily Pentatomoidea family were identified, in our opinion 12 seem to originate from Manchurian refuge Usuric subcenter, 37 of the Mediterranean refuge, 2 come from the Caucasian arboreal refuge and one species could originate from the Aralo-Caspic (Turanic) refuge.

Bugs (Heteropteran species), like any other species, responding to the action of all environmental factors, can avoid by adapting their unfavorable influence. This adaptation is made by behavioral features, widening ecological amplitude and enhancing ecological valency, migrations, restructuring of the development cycle, to some species of different hemocromy, mimetism, even mymecophilia and arachnofilia. We must show that the deforestation of the forests, the desertification of the steppes exerted a particular influence on the heteroptera, especially by changing the natural conditions of existence. It can be said that with all the activity of studying the Heteroptera in Romania, there is at present a large field of activity, both in terms of the systematic of this order and in terms of their ecology and biology.

CONCLUSIONS

1. Characterization of the zoogeographical origin of the Heteropteran collected species from forest Băneasa was done.

2. 52 species of the superfamily Pentatomoidea family were identified in Băneasa forest, in our opinion 12 seem to originate from Manchurian refuge Usuric subcenter, 37 of the Mediterranean refuge, 2 come from the Caucasian arboreal refuge and one species could originate from the Aralo-Caspic (Turanic) refuge.

REFERENCES

1. Banarescu P., 1970 - *Principii si Probleme de Zoogeografie*. Editura Academiei R.S.R, 260 pp.
2. Banarescu P., Boscaiu N., 1973 – *Biogeografie*. Editura Ştiintifică, Bucuresti, 302 pp.
3. Benedek P., 1969 - *Heteroptera VII*. In: Magyarország állatvilága (Fauna Hungariae) XVII/7. Akadémiai Kiadó, Budapest, 86 pp.
4. De Lattin R.F., 1967 – *Grundriss der Zoogeographie*. Gustave Fischer, Verlag, Stuttgart, Jena, 602 pp.
5. Duelli P., Obrist M. K., 1998 - *In search of the best correlates for local organismal biodiversity in cultivated areas*. Biodiversity and Conservation, 7, p. 297-309.
6. **Froeschner Richard Charles, 2017** – *Heteropteran - Insect order*. Encyclopædia Britannica, inc., https://www.britannica.com/animal/heteropteran
7. Fuente de la J.A., 1971 - *Revisión de los pentatómidos ibéricos (Hemiptera). Parte I. Tribus Sciocorini Stål, 1872, y Strachiini Stål, 1872*. Eos, Revista española de entomología, XLVII, p. 71–135.
8. Fuente de la J.M., 1972 a - *Revisión de los Pentatárnidos ibéricos. Familia Cydnidae Billberg, 1820*. Boletín de la Real Sociedad Española de Historia Natural, sección Biológica, 70, p. 33-78.
9. Fuente de la J.A., 1972 b - *Revisión de los Pentatómidos ibéricos (Hemiptera). Parte II. Tribus Aeliini Stål, 1872, Stagonomini nov. nom. (=Eysarcorini Auct.) y Carpocorini Distant, 1902.* Eos, Revista española de entomología, XLVIII, 1-4, p. 115-201.

10. Fuente de la J.A., 1973 a - Revisión de los pentatómidos ibéricos (Hemiptera). Parte III. Tribu Pentatomini s.str. Eos, Revista española de entomología, Eos, Revista española de entomología, XLIX, 1-4, p. 113-129.

11. Fuente de la J.A., 1973 b - *Revisión de los Pentatómidos ibéricos. Familia Scutelleridae Leach, 1815, con adiciones y correcciones a un trabajo anterior*. Boletín de la Real Sociedad Española de Historia Natural, sección Biológica, 71, p. 235-270.

12. Hoberlandt L., 1955 - *Results of the Zoological Scientific Expedition of the National Museum in Praha to Turkey-18: Hemiptera IV: Terrestriale Hemiptera-Heteroptera of Turkey*. Acta ent. Mus. Nat. Pragae, Suppl. 3, 274 pp.

13. Hoffmann B.D., 2010 - *Using ants for rangeland monitoring: global patterns in the responses of ant communities to grazing*. Ecological Indicators, 10, p.105-111.

14. Kiritshenko A.N., 1951 – True bugs of the European part of the USSR (Hemiptera); Key and bibliography. Opredeliteli po Faune SSSR, 42, 423 pp.

15. Kis B., 1984 - *Heteroptera. Partea generală şi suprafamilia Pentatomoidea*. Fauna Republicii Socialiste România. Insecta, 8, 216 pp.

16. Liu Jian-feng, XIAO Wen-fa, JIANG Ze-ping, FENG Xia, LI Xiu-ying, 2005 - *A Study on the Influence of Landscape Fragmentation on Biodiversity.* Forest Research, http://en.cnki.com.cn/Journal_en/D-D049-LYKX-2005-02.htm

17. Magurran A.E., 1988 - *Ecological Diversity and Its Measurement*. Princeton University Press, Princeton, NJ., 179 pp.

18. Morrison III William R., Waller Joseph T., Brayshaw Alyssa C., Hyman David A., Johnson Michael R., Fraser Ann M., 2012 - *Evaluating Multiple Arthropod Taxa as Indicators of Invertebrate Diversity in Old Fields.* The Great Lakes Entomologist, 45, 1-2, p. 56-68.

19. Puchkov V.G., 1961 - *True Bugs*. Fauna of the Ukraine, 21, 1, Academy of Sciences of the Ukrainian SSR. Kiev. 338 pp.

20. Puchkov V.G., 1965 - *Pentatomoidea of the Central Asia*. Ilim, Frunze. 329 pp.

21. Roşca I., 1979 - *Contribuții la studiul sistematic şi zoogeografic al heteropterelor terestre din R.S.Romania*. Teza de doctorat, Institutul Central de Biologie-Bucuresti, 205 pp.

22. Stichel W., 1955-1962 - *Illustrierte Bestimmungstabellen der Wanzen. II. Europa (Hemiptera-Heteroptera Europae) Pentatomorpha*. Berlin-Hermsdorf, 4, 1-27, 838 pp.

BIOCHEMICAL AND MICROBIOLOGICAL STUDY CONCERNING IDENTIFICATION ROLE OF HYDROLASE ACTIVITIES FROM *TRICHODERMA HARZIANUM* AND *TRICHODERMA KONINGII* IN PATHOGENIC FUNGUS *F. OXYSPORUM* INHIBITION

STUDIU BIOCHIMIC ŞI MICROBIOLOGIC PRIVIND IDENTIFICAREA ROLULUI ACTIVITĂŢILOR DE HIDROLAZĂ DIN *TRICHODERMA HARZIANUM* ŞI *TRICHODERMA KONINGII* ÎN INHIBAREA CIUPERCII PATOGENE *F. OXYSPORUM*

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Abstract. *The genus Trichoderma are a very large group of microorganisms that play a significant role in plant protection. Several Trichoderma spp. like Trichoderma harzianum and Trichoderma koningii strongly affected plants by stimulating plant growth, and protecting plants from fungal and bacterial pathogens such as Fusarium oxysporum. They are used as a biological plant protection as bio fungicides. Members of the Trichoderma spp. are also utilized in different branches of industry - principally in the areas of enzymes, antibiotics, and other metabolites. In this study we focus in the effect of T. harzianum strain ICCF 417 and T. koningii strain ICCF 418 on F. oxysporum (ZUM 2407) by microbiologic and enzymatic tests. Where the results of fungal growth speed of the malt medium showed that the fungus T. koningii was the fastest in growing, followed by T. harzianum and F. oxysporum after 72 hours of culture. While the degree of antagonism was 1 according to Bell scale in petri dish on the PDA medium the ability of fungi T. harzianum and T. koningii to overcome on fungus F. oxysporum. The results of the study showed the susceptibility of bio-fungi on production of an enzyme FPase was 32.3 % in T. harzianum comparative to T. koningii after 14 days of fermentation, the amylase was 84.5% in T. harzianum comparative to T. koningii while the CMCase was 36.6 % in T. harzianum comparative to T. koningii. Our results showed that hydrolase activities studied in this experiment play an important role in pathogenic fungus F. oxysporum inhibition and the degree of effect is different.*

Key words: *Trichoderma harzianum, Trichoderma koningii, F. oxysporum* hydrolases

Rezumat. *Genul Trichoderma este un grup foarte mare de microorganisme care joacă un rol semnificativ în protecţia plantelor. Mai multe specii ale genului Trichoderma cum ar fi Trichoderma harzianum şi Trichoderma koningii, afectează puternic plantele prin stimularea creşterii plantelor şi protejarea acestora de agenţii*

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patogeni fungici şi bacterieni cum ar fi Fusarium oxysporum. Ele sunt utilizate ca protecție biologică a plantelor, ca fungicide bio. Specii ale genului Trichoderma sunt, de asemenea, utilizate în diferite ramuri ale industriei, în principal domeniul enzimelor, antibioticelor şi altor metaboliți. În acest studiu ne concentrăm asupra efectului tulpinii T. harzianum ICCF 417 şi tulpinii T. koningii ICCF 418 asupra F. oxysporum (ZUM 2407) prin teste microbiologice şi enzimatice. În cazul în care rezultatele vitezei de creştere a fungilor din mediul de malț au arătat că fungul T. koningii a fost cel mai rapid în creştere, urmat de T. harzianum şi F. oxysporum după 72 de ore de cultură. În timp ce gradul de antagonism a fost 1 în funcție de scala Bell în vasul Petri pe mediul PDA capacitatea fungiilor T. harzianum şi T. koningii de a depăşi pe ciuperca F. oxysporum. Rezultatele studiului au arătat că susceptibilitatea bio-fungilor la producerea unei enzime FP-a fost de 32,3% în T. harzianum comparativ cu T. koningii după 14 zile de fermentație, amilaza a fost de 84,5% în T. harzianum comparativ cu T. koningii în timp ce CMCaza a fost de 36,6% în T. harzianum comparativ cu T. koningii. Rezultatele noastre au arătat că activitățile de hidrolază studiate în acest experiment joacă un rol important în inhibarea fungicidelor patogene F. oxysporum, iar gradul de efect este diferit.

Cuvinte cheie: *Trichoderma harzianum, Trichoderma koningii, F. oxysporum,* enzime hidrolitice

INTRODUCTION

The genus *Fusarium* spp. is known for a long time as important plant pathogens, *Fusarium* spp. is a one fungal species belonging to class Deuteromycota as it includes *Fusarium* spp more than 90 species, and these species have adapted to live within range of environmental and broad stretches across the world, in addition the types that include in it described as the important causes of pathogen of the plant (Booth, 1971). The wilt disease caused by *F. oxysporum* is an important tomato crop diseases and the disease was knew for the first time in the world in 1895 in the English Channel Islands (Walker, 1971). Recently scientists interested in biological control, including the fungus *Trichoderma*, this genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. A lot of methods of action have been proposed to clarify the biocontrol of plant pathogens by *Trichoderma*; these methods consist of the production of antibiotics and cell wall de grading enzymes, competition for key nutrients, parasitism, stimulation of plant defense mechanisms and combination of these possibilities. The double role of antagonistic action against plant pathogens and plant growth promoter make *Trichoderma* strains attractive alternatives to severe fumigants and fungicides. The aim of the study inhibition factors from in live and dead cells of *T. harzianum*, strain ICCF 417 and *T. koningii* strain ICCF 418 against pathogenic fungus *F. oxysporum* strain ZUM 2407.

MATERIAL AND METHOD

Liquid medium of malt extract was prepared by adding 20 grams of ready medium for a liter of distilled water, then it was sterilized and left to cool down, after

that antibiotic chloramphenicol was added to it (1 mg/100 mL). The density of 0.05 for 120 mL was prepared for each fungi using dilution law and mixing well and distributed by 10 ml of 12 tube and then was measured by the change in density within 12 hours. Total FPase activity in the culture filtrate was determined according to the standard method of Hankin and Anagnostakis (1975). CMCase activity was measured using a reaction mixture containing 1 ml of 1% carboxymethyl cellulase (CMC) in 0.5 M citrate acetate buffer (pH 5.0) and aliquots of suitably diluted filtrate. Cellobiose activity was measured using a reaction mixture containing 1 mL of 0.02 cellobiose in 0.5 M citrate acetate buffer (pH 5.0) and aliquots of suitably diluted filtrate. Reducing sugar produced was determined by DNS method (Murao *et. al.*, 1988). Amylase activity was determined by measurement of maltose released from starch according to the method of Miller (1959). The enzymatically liberated reducing sugar was calculated from a standard curve using maltose. The protein concentration (mg/mL) was determined by the method of Lowery method (Lowery *et. al.*, 1951), using bovine serum albumin (BSA) as a standard. The enzymes activity and protein were measured after 1, 2, 3 and 4 weeks of culture. In order to determine the optimum pH value for the enzyme obtained after fermentation, the activity of the enzyme was assayed between the pH values of 3.0-9.0. The ability of fungi *T. koningii* and *T. harzianum* to produce enzymes in synthetic medium, fungi's were grown in 250 mL Erlenmeyer flask that contained 100 ml of synthetic medium SM then we add the enzymes inducer for each enzyme, the cultures were incubated and the ability of production was determined after 1, 2, 3 and 4 weeks, also enzymes were used diluted to 100%, 50% and 10%. The fungi weight was determined by weighting an empty tube, then put (1 mL) of the fungi from each period of culture into the weighted tube after that drying and centrifuging and taking the precipitate which dried at 50^0 C for 3 days the weighted again. Preparation of cell lysate was done by taking (10 mL) of each culture and centrifuged, the precipitate re-suspended in 5 mL of phosphate buffer (pH 7.4) with vortex, then adding some of sand to the tube, put it in IKA® ULTRA TURRAX device at 6000 rpm for 30 s then put it at ice for 1 min, replay for three times and centrifuged at 10000 rpm for 10 min at 4^0 C. Filtered by Millipore 0.22µm, aliquots of the supernatant were used for next assays. All cultures were put at 100^0 C for 15 min, filtered by micro filter, aliquots of the supernatant were used for next assays. Characterization of enzymes that produced from *T. koningii* and *T. harzianum* that effect on *F. oxysporum* was done in PDA medium using a petri dish divided into 6 sections, at the first one we put 10 µL synthetic medium SM, the second 10 µL of dead cell of enzymes culture, the third one 10 µL of living cell of enzymes culture, the forth section 10 µL of dead cell from fungi culture, the fifth with 10 µL of dead lysate, the last one with 10 µL living lysate. And for control we put a petri dish of *T. koningii*, *T. harzianum* and *F. oxysporum* alone and a petri dishes divided into two part one with *Trichoderma* and the other with *F. oxysporum.* All experiments were in triplicate.

RESULTS AND DISCUSSIONS

The results regarding fungi growth in liquid malt medium extract after 12 hours from culture, showed that *F. oxysporum* reached the highest growth (0.42) of the fungus followed by fungus us *T. harzianum* and *T. koningii* (0.33) and (0.31) respectively, after 72 hours, *F. oxysporum* reached the highest growth (1.3) of the fungus followed by fungus us *T. harzianum* and *T. koningii* (0.95) and (0.87) respectively. The fungi weight/1 mL was 0.0097 for *T. harzianum* 0.009

for *F. oxysporum* and 0.0016 for *T. koningii*. Our results of the optimum pH for the enzymes showed that the optimum pH for all enzyme, for all fungi, was at pH=6 (fig. 1), Lee *et al.*, (2002) found that the best pH for FPase and CMCase was at the range of 4-7, also Refaz *et al.*, (2013) mention that the best production of FPase and CMCase enzymes was at pH= 5.6, Yassien *et al.*, (2014) proved that the best productivity of FPase enzyme was at pH= 5.5-7.5.

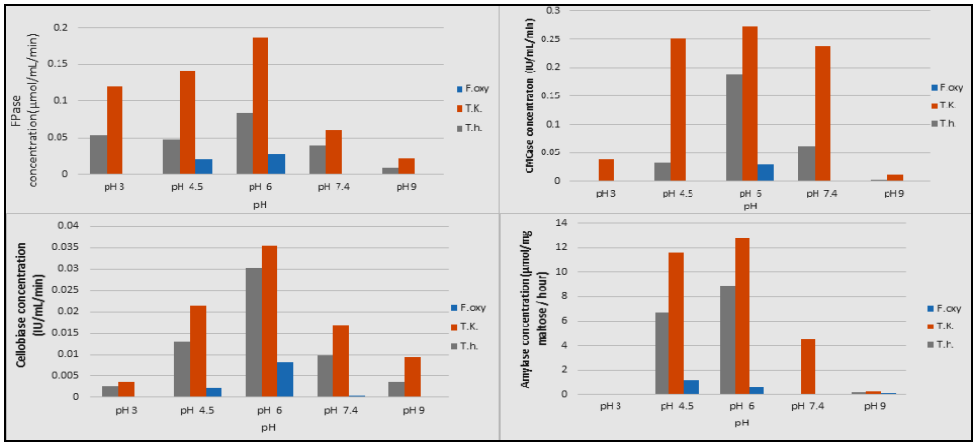

Fig. 1 The optimum pH for FPase, CMCase, Cellobiose and amylase enzymes in *T. harzianum*, *T. koningii* and *F. oxysporum*

The results of FPase enzyme showed that the two fungi can produce the FPase but its level on *T. koningii* was higher than *T. harzianum*, and for the two fungi the period of 14 days was the highest production, also the dilution ratio 100% was the highest, as shown in figure 2. The levels of FPase enzyme was 0.24 and 0.07 µmol/mL in *T. koningii* and *T. harzianum* respectively, several studies have relied on the values of this enzyme in production, (Ojumu *et al.*, 2003).

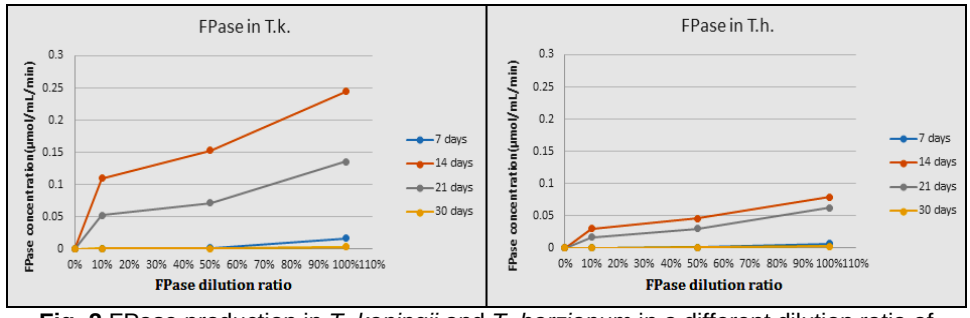

Fig. 2 FPase production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The production of CMCase enzyme in synthetic medium in *T. koningii* was higher than *T. harzianum* and the second week was the highest period of production, as shown in figure 3.

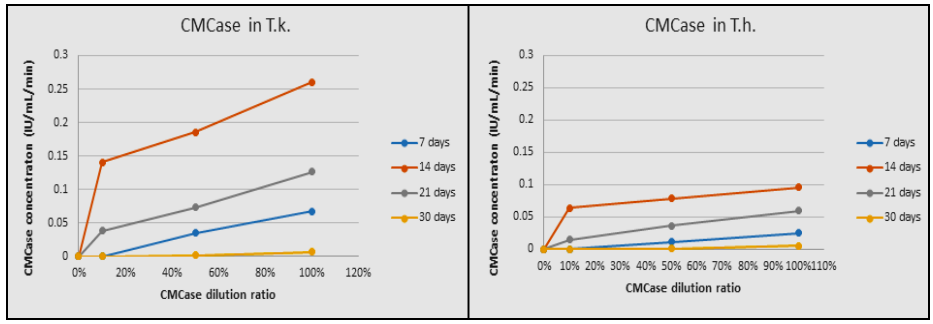

Fig. 3 CMCase production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

In figure 4 are presented the results of cellobiose production in *T. koningii* and *T. harzianum* respectively, cellobiose levels on *T. koningii* was higher than *T. harzianum*, and for the two fungi the period of 14 days was with the highest production, also the dilution ratio 100% was the highest increasing.

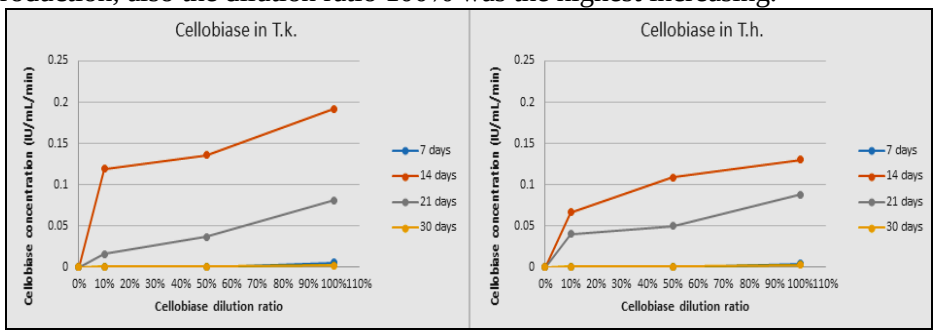

Fig. 4 Cellobiose production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The production of amylase enzyme in synthetic medium in *T. koningii* was higher than *T. harzianum* and the second week was the highest period of production, as shown in figure 5. Generally, the protein levels were in out cell higher than the lysate for all enzymes.

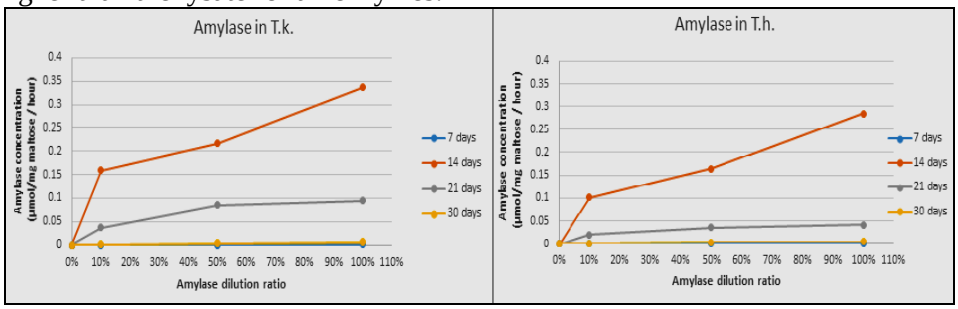

Fig. 5 Amylase production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The protein levels in FPase enzyme were in *T. koningii* higher than the *T. harzianum*, and the second week had the highest levels of protein in the two fungi, as shown in figure 6.

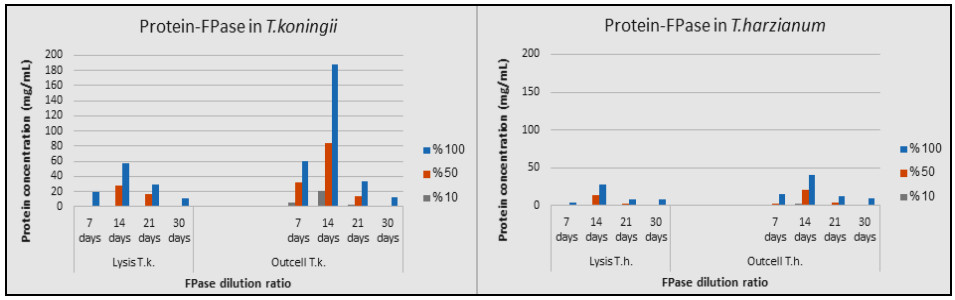

Fig. 6 Protein levels in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The highest protein level in the CMCase enzyme medium was *T. koningii* in comparison with its level in *T. harzianum*, but also the period of 14 days was the highest level of protein production in comparison with the rest periods, as shown in figure 7.

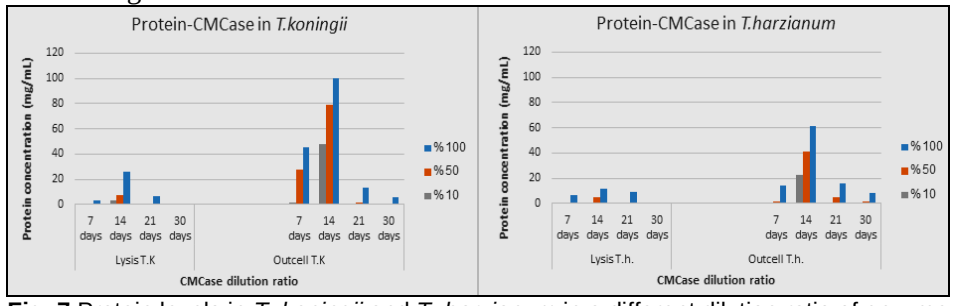

Fig. 7 Protein levels in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The result of the level of protein from the cellobiose enzyme are the same as the results of protein levels in FPase enzyme which was the highest level of protein in the second week and in the fungus *T. koningii*. The cellobiose activity was 0.19 IU/mL in *T. koningii* and 0.13 IU/mL in *T. harzianum*, *Trichoderma* spp. are known to produce FPase, the cellobiose in one of the FPase enzymes complex, this enzyme works on the hydrolysis of cellobiose to glucose (De Marco *et. al.*, 2003). The amylase enzyme production was 0.33 IU/mg in *T. koningii* and 0.28 IU/mg in *T. harzianum*, *T. harzianum* produces substantial amounts of lytic enzymes, including amylases, the protein levels in enzymes' medium were proportional proportionality with the levels of enzymes, also the levels in out cell were higher than the cell lysate and the second week had the highest level of protein for all out cell and the lysate.

In figure 8 are presented the levels of protein in amylase enzyme synthetic medium in *T. koningii* and *T. harzianum* respectively, protein levels on *T. koningii* was higher than *T. harzianum*, and for the two fungi the period of 14 days was the highest production. The levels of protein in *T. koningii* were 187.54, 100.24, 28.59 and 86.95 mg/mL for FPase, CMCase, cellobiose and amylase respectively in out of cell, while in cell lysate were in FPase 57.40 mg/mL, CMCase 25.88 mg/mL, cellobiose 16.24 and amylase 34.82 mg/mL. In *T. harzianum* the protein levels were 39.98, 61.30, 21.98 and 72.96 mg/mL for FPase, CMCase m cellobiose and amylase respectively in out of cell, while in cell lysate were in FPase 28.53 mg/mL, CMCase 11.75 mg/mL, cellobiose 10.33 and amylase 25 mg/mL.

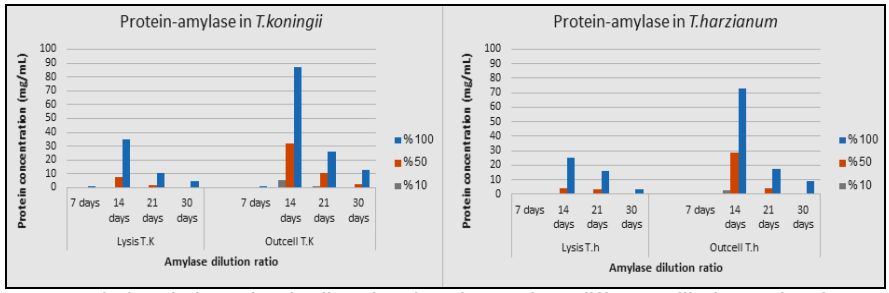

Fig. 8 Protein levels in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The results of the effect of *T. harzianum* and *T. koningii* on *F. oxysporum* showed that these fungi have a high ability against the pathogenic fungi *F. oxysporum,* the ratio reached to 1, according to the scale of (Bell *et al.*, 1982). While the cellobiose and the amylase enzyme were not effective on *F. oxysporum* for all period of incubation, also the first, the third and the forth weeks of FPase and CMCase enzymes were not effective. Also table 1 shows the weight results of fungi in the synthetic medium of the enzymes.

Table 1

The weight of fungi in the synthetic medium of the enzymes

	T. koningii			
	Week 1	Week 2	Week 3	Week 4
FPase	0.0032	0.0151	0.01185	0.00135
CMCase	0.0048	0.1138	0.10865	0.00394
Cellobiose	0.00125	0.00355	0.00255	0.000992
Amylase	0.00115	0.01055	0.00755	0.001058
	T. harzianum			
	Week 1	Week 2	Week 3	Week 4
FPase	0.001775	0.00925	0.06245	0.001276
CMCase	0.0026	0.0221	0.012	0.0015
Cellobiose	0.001	0.00315	0.00105	0.000875
Amylase	0.0021	0.00535	0.0039	0.001008

CONCLUSIONS

1. There is an inhibitory effect of *T. harzianum*, strain ICCF 417 and *T. koningii* strain ICCF 418 against pathogen *F. oxysporum* strain ZUM 2407 by production of protein and extracellular enzymes which may effect on the action *F. oxysporum*.

2. The CMCase has the highest weight in comparison with the others enzymes, while the cellobiose and the amylase enzyme were not effective on *F. oxysporum* for all period of incubation.

REFERENCES

1. **Bell D.K. Wells C.D., Mirkham C.R., 1982** - *In vitro antagonism of Trichoderma species against six fungal plant pathogens*. Phytopathology. 72, 379-382.
2. **Booth C., 1971** - *The Genus Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, United Kingdom. 237 pp..
3. **De Marco J.L., Valadares-Inglis M.C., Felix C.R., 2003** - Production of hydrolytic enzymes by Trichoderma isolates with antagonistic activity against Crinipellis perniciosa, the causal agent of witches' broom of cocoa. Brazilian Journal of Microbiology, 34, p. 33-38.
4. **Hankin L., Anagnostakis S.L., 1975** - The use of solid media for detection of enzyme production by fungi. Mycologia, 67, p 597-607.
5. **Lee R.L., Paul J.W., Van Zyl W.H., Pretorius I.S., 2002** - *Microbial cellulase utilization: Fundamentals and biotechnology*. Microb. Mol. Biol. Review 66 (3), p 506-577.
6. **Lisboa De Marco Janice, Valadares-Inglis Maria Cleria, Roberto F.C.,** 2003 - *Production of hydrolytic enzymes by Trichoderma isolates with antagonistic activity against Crinipellis perniciosa the causal agent of witches' broom of cocoa*. Brazilian journal of microbiology. 34, p 33-38.
7. **Lowery O.H., Roserbrough N.J., Fair A.L., Randall R.J. 1951** - *Protein measurement with the Folin phenol reagent*. J. Biol. Chem. 193 (1), p 255-257.
8. **Miller G.L., 1959** - Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. chem. 31 (3), p 426-428.
9. **Murao S., Sakamoto R., Arai M., 1988** - *FPase of Aspergillus aculeatus*. In: Methods in Enzymology, Wood, WA and Kellog, ST (Eds) Academic Press Inc, London, 160, p 275-284.
10. **Ojumu T.V., Solomon B., Betiku E., Layokun S.K., Amigun B., 2003** - *Cellulase Production by Aspergillus flavus Linn Isolate NSPR 101 fermented in sawdust, bagasse and corncob*. African Journal of Biotechnology 2 (6), p 150-152.
11. **Refaz A.D., Saba Iram, Shahnawaz M., Sangale M.K., Avinash B.A., Rather S.A., Qazi P.H., 2013** - *Isolation, purification and characterization of carboxymethyl FPase (CMCase) from endophytic Fusarium oxysporum producing podophyllotoxin*. Advances in Enzyme Research 1 (4), p 91-96.
12. **Walker J.C., 1971** - *Fusarium wilt of tomato*. Published by the American Phytopathological Society. St. Paul, Minnesota. Monograph No 6, 56 pp..
13. **Yassien M.A.M., Jiman-Fatani A.A.M., Asfour H.Z., 2014** - *Production, purification and characterization of cellulase from Streptomyces sp..* African Journal of Microbiology Research. 8 (4), p 348-354.

RESEARCH ON BIODIVERSITY CONSERVATION AND MANAGEMENT IN THE VITICULTURAL AGROECOSYSTEM IN THE DEALUL BUJORULUI VINEYARD

CERCETĂRI PRIVIND CONSERVAREA ŞI GESTIONAREA BIODIVERSITĂŢII ÎN AGROECOSISTEMUL VITICOL DIN PODGORIA DEALUL BUJORULUI

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Abstract. *The paper presents the research carried out at the Bujoru Viticulture and Wine Research and Development Station between 2015 and 2016. Research has focused on conservation and enhancement of functional and planned biodiversity through the implementation of all bio-resources of the greenhouse system and multifunctional protection areas, which are conducive to reducing the pathological risks and reducing external inputs (diesel, pesticides). Assessment of the state of conservation of biodiversity in the viticultural ecosystem of pogoria Dealul Bujorului. Biodiversity is a specific feature of our planet that ensures the optimal functioning of ecosystems, the existence and development of the biosphere in general. Lately, the issue of protecting biodiversity at ecosystems, species and populations has become increasingly vital to reducing the human impact on the biosphere. The viticultural ecosystem is defined as the functional unit of biosphere created and controlled by man in order to obtain high yields of grapes, of high quality and in more economical and socially advantageous conditions.*
Key words: grapevine, biodiversity, ecosystems

Rezumat. *Lucrarea prezintă cercetările efectuate la Staţiunea de Cercetare şi Dezvoltare pentru Viticultură şi Vinificaţie Bujoru în perioada 2015-2016. Cercetările au vizat studii privind conservarea şi consolidarea biodiversităţii funcţionale şi planificate, prin implementarea tuturor bio-resurselor a sistemului de înverzire şi a zonelor multifuncţionale de protecţie, favorabile reducerii riscurilor patologice şi diminuării inputurilor externe (motorină, pesticide). Evaluarea stării de conservare a biodiversităţii în ecosistemul viticol din podgoria Dealul Bujorului. Biodiversitatea reprezintă o particularitate specifică a planetei noastre, care asigură funcţionarea optimă a ecosistemelor, existenţa şi dezvoltarea biosferei în general. În ultima perioadă, problema protejării biodiversităţii la nivel de ecosisteme, specii şi populaţii a devenit tot mai vitală pentru reducerea impactului uman asupra biosferei. Ecosistemul viticol este definit ca fiind acea unitate funcţională a biosferei creată şi controlată de către om, în vederea obţinerii unor producţii ridicate de struguri, de calitate superioară şi în condiţii economice şi sociale tot mai avantajoase.*
Cuvinte cheie: viţă de vie, biodiversitate, ecosistem

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INTRODUCTION

Sustainable growth of production and income in vineyard culture requires extensive measures to combat pathogens and pests. Beside the beneficial effect of the phytosanitary measures on the vine, these may have negative effects on the preservation of biodiversity in wine ecosystems.

At the same time, reducing damages caused by diseases, pests and herbage, must be achieved by reducing the dependence of culture on conventional resources (Ball *et al.,* 1986; Tălmaciu *et al.*, 1996).

MATERIAL AND METHOD

Research was conducted between 2015 and 2016 in the vineyard plantations of the Bujoru Viticulture and Wine-growing Research and Development Station.

In order to evaluate the positive impact of implementation of bio-resources, of greening systems and of multifunctional protection areas on functional biodiversity in vineyard ecosystems in vineyards were identified and installed six experimental variants located on terraces located on the level cubes with a width of about 20 m and a length of 600 m (tab. 1).

Table 1

Identification data of experimental lots - SCDVV Bujoru

Culture	Parcel	The variety	Soil maintenance system	Lat. N	Long. E	Altitude (m)
Vine	Variant 1	Rkatiteli	black field	45.50.01.59	27.55.25.06	40
	Variant 2	Rkatiteli	string mulch chopped	45.50.01.20	27.55.24.22	45
	Variant 3	Fetească albă	black field	45.50.00.46	27.55.22.22	49
	Variant 4	Fetească albă	string mulch chopped	45.50.00.29	27.55.21.18	53
	Variant 5	Babească gri	black field	45.49.58.94	27.55.15.60	66
	Variant 6	Babească gri	string mulch chopped	45.49.58.60	27.55.14.97	71

RESULTS AND DISCUSSIONS

In order to assess the conservation status of biodiversity in wine ecosystems two indicators were taken into account, namely the amount of semi-natural elements in the landscape of the vineyard holding and their quality.

The quantitative indicator represents the share of the total surface area of the component elements (artificial landscape and infrastructure in relation to the surface of the vineyard).

The case of the six experimental lots, the surface actually occupied by vine is 17.11 ha, and the agro-ecological infrastructure represented by grasshoppers, isolated trees and other crops occupy 9.01 ha. Under these circumstances the ratio between IAE and UAE is 53% and the artificialism rate is 47%.

The structure and morphology of the viticultural habitat in the Bujoru ecosystem: Total wine-growing = 26.12 ha of which:
Surface occupied by multifunctional protection areas = 9.01 ha:

- grasshoppers: 13500 m x 5 m = 6.75 ha;
- isolated trees: 10 m x 10 m = 0.01 ha;
- trenches: 2m x 1250m = 0.25ha;
- wetland specific vegetation = 0.50 ha;
- squid = 1,00 ha and alfalfa = 0.50 ha.

Total surface actually occupied by vineyard culture: 17.11 ha
17.11 ha => IAE/UAE= 53% Rate of artificialization= 100-53= 47%

The qualitative indicator reflects the conservation status of the landscape elements. Quality is evaluated based on several indicators defined for each tipe of IAE. Indicators are divided into three categories: structure, composition and functions assimilated here to degradations. These indicators are being deducted for each type of IAE in rating grids and are then classified into three categories: good, medium, unfavorable, depending on their condition. Farm level, quality is assessed by aggregating all the conservation status obtained for all IAEs on the holding. This allows to obtain a radial pattern diagram showing the IAE share of good conservation, medium and unfavorable.

Barber soil traps, filled 2/3 with formalin solution (formaldehyde) 4%, 3/ each variant, have been installed on the vineyard rows in order to establish the quantitative and qualitative structure of entomofauna from the soil surface between May-August.

With Barber soil traps, entomological material (specimens of insects - juveniles, adults) was collected. Traps were disposed randomized in the experimental lot trying to cover as many ecological niches as possible (habitats). The entomological material collected and labeled was transported to the laboratory washed under water jet and then passed into a solution of ethyl alcohol 7%. The identification and counting of the entomofauna was done with the trinocular magnifier (KRÜSS) with two WF 10x20 magnifiers.

The relative numerical abundance (A.r.%) of a population is defined as the proportion represented by the number of individuals of a species or group compared to the total number of individuals belonging to all species in the sample.

In the observations on entomological material collected at ground level using Barber soil traps (May - August) we found the following (tab. 2):

Table 2

Structure of the species caught in the Bujoru agroecosystem

Systematic classification (species, family, order)	The percentage (%) main culture												Total catches
	Vine												
	V1		V2		V3		V4		V5		V6		
	Number of catches	A %	Number of catches	A %	Number of catches	A %	Number of catches	A %	Number of catches	A %	Number of catches	A %	
Alopecosa pulverulental fam. Lycosidae/	133	*24.44*	120	*21.98*	79	*17.67*	135	*19.07*	139	*22.10*	118	*18.97*	**724**
Formica rufa / fam. Formicidae	139	*25.55*	120	*21.98*	158	*35.35*	255	*36.01*	158	*25.12*	177	*28.46*	**1007**
Epicometis hirta Poda./fam. Scarabeidae	2	*0.38*	0	*0.00*	0	*0.00*	0	*0.00*	0	*0.00*	0	*0.00*	**2**
Apis sp./fam. Apidae	18	*3.31*	38	*6.96*	20	*4.74*	24	*3.39*	10	*1.59*	16	*2.57*	**126**
Carabus sp./fam. Carabidae	32	*5.88*	64	*11.72*	44	*9.84*	53	*7.49*	79	*12.56*	97	*15.60*	**369**
Eurigaster Maura, Subordinul Geocoridae	2	*0.37*	2	*0.37*	0	*0.00*	0	*0.00*	1	*0.16*	0	*0.00*	**5**
Gryllus campestris/fam. *Gryllidae*	1	*0,18*	0	*0.00*	0	*0.00*	6	*0.85*	3	*0.48*	0	*0.00*	**10**
Fam. *Vespidae*	20	*3.68*	31	*5.68*	16	*3.58*	17	*2.40*	16	*2.54*	12	*1.93*	**112**
C. septempunctata/fam. Coccinellidae	6	*1.10*	8	*1.46*	16	*3.58*	14	*1.98*	16	*2.54*	2	*0.32*	**62**
Cicada viridis/fam. Cicadidae/	32	*5.88*	28	*5.13*	15	*3.36*	26	*3.67*	31	*4.93*	22	*3.54*	**154**
Ord. Diptera	33	*6.07*	46	*8.42*	27	*6.04*	52	*7.34*	61	*9.70*	53	*8.52*	**272**
Myriapoda	3	*0.55*	0	*0.00*	0	*0.00*	0	*0.00*	3	*0.48*	0	*0.00*	**6**
Tettigonia viridissima/ fam.Tettigonidae	6	*1.10*	10	*1.83*	4	*0.90*	21	*2.97*	7	*1.11*	5	*0.80*	**53**
Lepidoptere	1	*0.18*	0	*0.00*	0	*0.00*	0	*0.00*	0	*0.00*	0	*0.00*	**1**
Forficula auricularia	0	*0.00*	0	*0.00*	0	*0.00*	0	*0.00*	2	*0.32*	0	*0.00*	**2**
Other species	116	*21.33*	79	*14.47*	68	*15.21*	105	*14.83*	103	*16.37*	120	*19.29*	**591**
TOTAL	**544**	***100***	**546**	***100***	**447**	***100***	**708**	**100**	**629**	***100***	**622**	***100***	3496

Variant 1: 14 arthropod species or groups belonging to the *Myriapoda*, *Insecta* and *Arachnida* classes were identified, but most belong to the *Insectae* class. The highest relative abundance was recorded by the species of the *Formicidae* family (25.55%), followed by the *Lycosidae* (24.44%), *Carabidae* (5.88%), *Vespidae* (3.68%), *Diptera* 6.07%) and other species (21.33%). The *Coccinellidae* family recorded (1.10%).

Variant 2: Ground fauna consisted of 11 species or groups of arthropods belonging to the classes: *Insecta* and *Arachnida*. The largest population was represented by the *Formicidae* family and the *Lycosidae* family with (21.98%), *Carabidae* (11.77%), *Vespidae* (5.68%) and other species (14.47%). The *Coccinellidae* family recorded (1.47%).

Variant 3: Ground fauna consisted in 9 species or groups of arthropods belonging to the classes: *Insecta* and *Arachnida*. The most numerous population was the *Formicidae* family (35.35%), *Lycosidae* family (17.67%) and *Carabidae* family (9.84%). The *Coccinellidae* family recorded (3.58%).

Variant 4: The fauna at the ground level consisted in 10 species or arthropod groups belonging to the classes: *Insecta* and *Arachnida*. The largest population was represented by the *Formicide* family (36.01%), the *Lycosidae* family (19.07%), the *Carabidae* family (7.49%) and other species (14.83). The *Coccinellidae* family recorded (1.98%).

Variant 5: 13 arthropod species or groups belonging to the *Insecta* and *Arachnida* classes were identified, but most belong to the *Insecta* class. The highest relative abundance was recorded by the species of the *Formicidae* family (25.12%), followed by the *Lycosidae* family (22.10%), the *Carabidae* (12.56%), the *Diptera* Order (9,70%) and other species (16.37%). The *Coccinellidae* family recorded (2.58%).

Variant 6: 9 arthropod species or groups belonging to the classes of *Insecta* and *Arachnida* have been identified, but most of them belong to the *Insecta* class. The highest relative abundance was recorded by the species of the *Formicide* family (28.46%), followed by the *Lycosidae* (18.97%), the *Carabidae* (15.60%), the *Diptera* Order (8.53%) and other species (19.29%). The *Coccinellidae* family recorded (0.32%).

To manage biodiversity in order to protect and preserve it, it is necessary to measure it. In this respect, the following biodiversity characterization indices were used to quantify the biodiversity of the studied agroecosystem: the number of species (species richness), the Simpson index (D), the Shannon (H) diversity index, the Shannon-Weaver index H) and equity (E) (tab. 3).

Table 3

Simpson Diversity Index (D); Shannon-Weaver (H) and Equality (E) diversity index for the Bujoru viticultural agroecosystem

Agroecosystem	**Number individuals of a species**	**Number total catches in the perimeter analyzed**	**Proportion of representation**	**Simpson index (D)**		**Diversity Index (H)**	**Equity (E)**
the species	ni	N	pi=ni/N	(pi)2	lnpi	-pi*lnpi	E=H/ln (S)
1	724	3496	0.2071	0.0429	-1.5746	0.3261	0.1176
2	1007	3496	0.2880	0.0830	-1.2446	0.3585	0.1293
3	2	3496	0.0006	0.0000	0.0000	0.0000	0.0000
4	126	3496	0.0360	0.0013	-3.3231	0.1198	0.0432
5	369	3496	0.1055	0.0111	-2.2486	0.2373	0.0856
6	5	3496	0.0014	0.0000	0.0000	0.0000	0.0000
7	10	3496	0.0029	0.0000	0.0000	0.0000	0.0000
8	112	3496	0.0320	0.0010	-3.4409	0.1102	0.0398
9	62	3496	0.0177	0.0003	-4.0322	0.0715	0.0258
10	154	3496	0.0441	0.0019	-3.1224	0.1375	0.0496
11	272	3496	0.0778	0.0061	-2.5536	0.1987	0.0717
12	6	3496	0.0017	0.0000	0.0000	0.0000	0.0000
13	53	3496	0.0152	0.0002	-4.1891	0.0635	0.0229
14	1	3496	0.0003	0.0000	0.0000	0.0000	0.0000
15	2	3496	0.0006	0.0000	0.0000	0.0000	0.0000
16	591	3496	0.1691	0.0286	-1.7776	0.3005	0.1084
Sum = 16	***0.1765***		***1.9237***	***0.6938***	***0.1765***		***1.9237***

CONCLUSIONS

The area actually occupied by vineyards is 17.11 hectares, and the agroecological infrastructure represented by grassy slopes, isolated trees and other crops, occupies 9.01 hectares. Under these circumstances, the ratio between the IAE and the UAE is 53% and the artificialism rate is 47%.

The most useful insect species were from *Formicidae* (28.80%) and *Lycosidae* (20.71%) family. The results show that in the vineyards there is a rich fauna, both as a number of species and as a number of individuals.

REFERENCES

1. Baal D., Tapp L., Gordon F., Potter D.A., 1986 - *Effects of pesticides on earhworm populations"* in Kentucky bluegrass Insescticide and Acaricide Tests, p. 395.

2. **Tălmaciu M., Georgescu T., Mitrea I., Filipescu C., Bădeanu Marilena, Radu C., 1996** - *Contributions to the knowing of the carabid fauna of the vine plantation in Husi vineyard, Vaslui District,* Lucrări stiintifice, vol. 39, Seria Horticultură, USAMV Iasi, p. 267 – 271.

EFFICACY EVALUATION OF CERTAIN "BIO" PRODUCTS ON POWDERY MILDEW PATHOGENS ON TOMATOES AND MELONS IN LABORATORY CONDITIONS

EVALUAREA EFICACITĂŢII UNOR PRODUSE „BIO" FAŢĂ DE AGENŢII PATOGENI CARE PRODUC FĂINAREA LA TOMATE ŞI PEPENI GALBENI ÎN CONDIŢII DE LABORATOR

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Abstract. *The purpose of this experiment was to study the biological efficacy of "bio"products Mimoten 0.3% (80% Mimosa tenuifolia extract) and Zytron 0.15% (20% citric seeds extract), on tomatoes and melons against Erysiphe* sp. *and Sphaerotheca fuliginea, respectively. The experiments were carried out in "wet chambers" (in thermostat at 26± 2^{0}C and 60 -70% RH) on detached leaves with similar levels of the attack degree from tomato and melons plants attacked by Erysiphe* sp. *on tomatoes and Sphaerotheca fuliginea on melons. The combination of Mimoten 0.3% + Zytron 0.15% products had the best efficacy in controlling of both pathogens Erysiphe* sp. *on tomatoes (76.3%) and Sphaerotheca fuliginea on melons (65.4%).*
Key words: tomatoes, melons, *Erysiphe* sp., *Sphaerotheca fuliginea*

Rezumat. *Scopul acestei experienţe a fost evaluarea eficacităţii biologice a produselor „bio" Mimoten 0,3% şi Zytron 0,15%, la tomate şi pepeni galbeni faţă de agenţii patogeni Erysiphe* sp. *şi respectiv Sphaerotheca fuliginea. Experienţele s-au efectuat în „camere umede" (la termostat 26± 2^{0}C şi 60 -70% umiditate atmosferică), pe frunze detaşate, care prezentau atac de Erysiphe* sp. *la tomate şi Sphaerotheca fuliginea la pepeni galbeni, cu niveluri asemănătoare ale gradului de atac. Combinaţia de produse Mimoten 0,3% + Zytron 0,15% a avut eficacitatea cea mai bună atât în combaterea agentului patogen Erysiphe* sp. *la tomate (76,3%) cât şi Sphaerotheca fuliginea la pepenii galbeni (65,4%).*
Cuvinte cheie: tomate, pepeni galbeni, *Erysiphe* sp., *Sphaerotheca fuliginea*

INTRODUCTION

Powdery mildew diseases are one of the most important plant pathological worldwide problems. Important crops, including cereals, grapevine, a number of vegetables species and ornamentals are among their major targets (Agrios, 2005). The need for new control strategies for the management of powdery mildews has led researchers and growers to explore suitable environmentally friendly alternatives or complementary to chemicals, biological control being the most

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investigated of these approaches (Bélanger and Labbé, 2002). The strains of *Bacillus subtilis* provided disease control for powdery mildew disease, caused by *Podosphaera fusca* on melon seedlings similar to that achieved with the mycoparasite-based products AQ10® (*Ampelomyces quisqualis*) and Mycotal® (*Lecanicillium lecanii*), or the fungicide azoxystrobin (Romero *et al*., 2007).

MATERIAL AND METHOD

There was studied the biological efficacy of "bio" products Mimoten 0.3% (80% *Mimosa tenuifolia* extract) and Zytron 0.15% (20% citric seeds extract), on tomatoes and melons against *Erysiphe* sp. and *Sphaerotheca fuliginea*, respectively.

Mimoten is a natural extract of Mimosa tenuifolia for controlling pathogens such as *Sphaerotheca fuliginea, Botrytis cinerea* and *Alternaria* spp. It is applied as foliar during the vegetation period. It does not require a pause between application and harvest.

Zytron is a natural extract of citrus seeds with preventive and curative action in the control of pathogens *Sphaerotheca fuliginea, Erwinia* sp. and *Sclerotinia* sp. The ionic activity of the extract allows penetration of the cell walls of the microorganisms, destroying them without affecting the plants and fruits. In the post-harvest period it increases the shelf life of fruits and vegetables.

The experiments were carried out in "wet rooms"(in thermostat at 26± 2^0C and 60 -70% RH) on detached leaves with similar degrees of attack from tomato and melons plants attacked by *Erysiphe* sp. and *Sphaerotheca fuliginea*.

The experimental variants, both for tomatoes and melons, were:

1. Mimoten 0,3%
2. Zytron 0,15%
3. Mimoten 0,3% + Zytron 0,15%
4. Untreated check

Treatments were applied as foliar spraying on 10.09.2016 on melons and 20.09.2016 on tomatoes. Observations on the frequency and severity of the attack, on the basis of which the degree of attack was calculated, were made before and 7 days after treatments. The efficacy of the products was calculated using the Abbot formula.

RESULTS AND DISCUSSIONS

The best results were obtained with the combination of Mimoten 0.3% + Zytron 0.15% for both tomatoes and melons (tab. 1). In tomato the degree of attack of pathogen *Erysiphe* sp. decreased from 49.4% to 12.2%, and in melons the attack of the pathogen *Sphaerotheca fuliginea* decreased from 67.5% to 33.7%, 7 days after application of the treatments with this combination of products.

In tomatoes, after 7 days of treatment, the degree of attack decreased from 54.3% to 21.4% for the variant with Mimoten 0.3% and from 52.6% to 19.5% for Zytron 0.15% (fig. 1 and fig. 2).

Table 1

Efficacy of some biological products for controlling *Erysiphe* sp. on tomatoes and *Sphaerotheca fuliginea* on melons

Variant	Tomatoes - *Erysiphe* sp.			Melons - *Sphaerotheca fuliginea*		
	Degree of attack before treatments (%)	Degree of attack, 7 days after treatments (%)	Efficacy (%)	Degree of attack before treatments (%)	Degree of attack, 7 days after treatments (%)	Efficacy (%)
Mimoten 0,3%	54,3	21,4	58,4	61,3	40,4	58,6
Zytron 0,15%	52,6	19,5	62,1	62,5	43,7	55,2
Mimoten 0,3% + Zytron 0,15%	49,4	12,2	76,3	67,5	33,7	65,4
Untreated check	48,5	51,5	-	63,4	97,5	-

Fig. 1 Degree of attack of pathogen *Erysiphe* sp. on tomatoes leaves, before appling the bioproducts

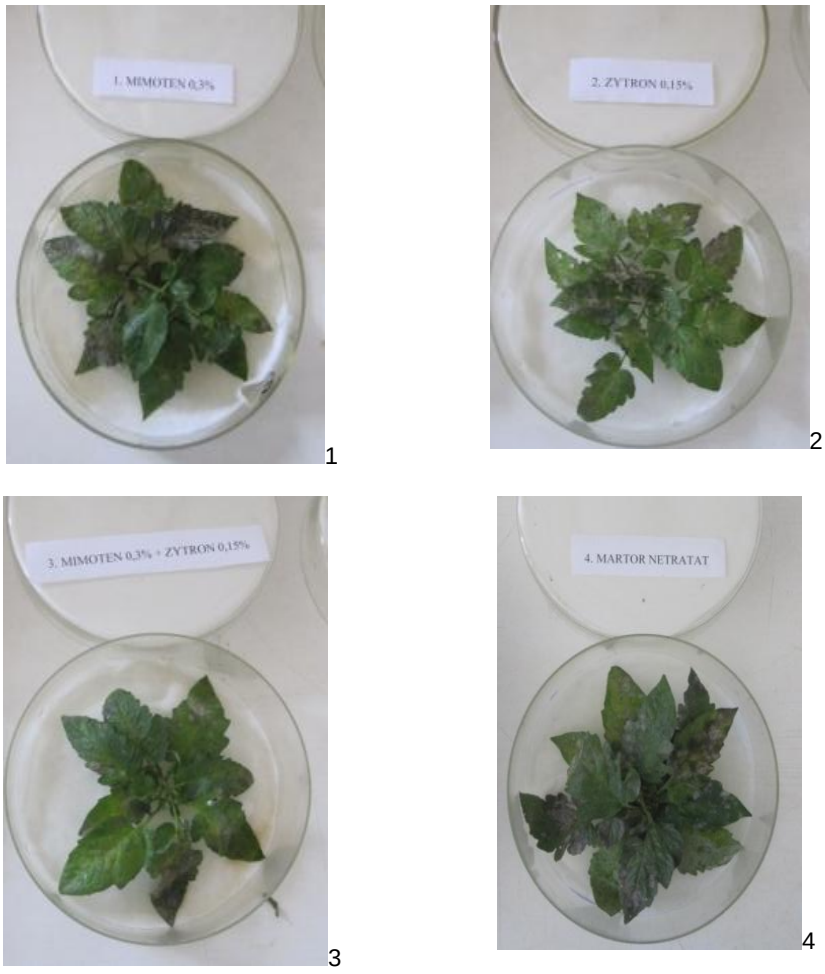
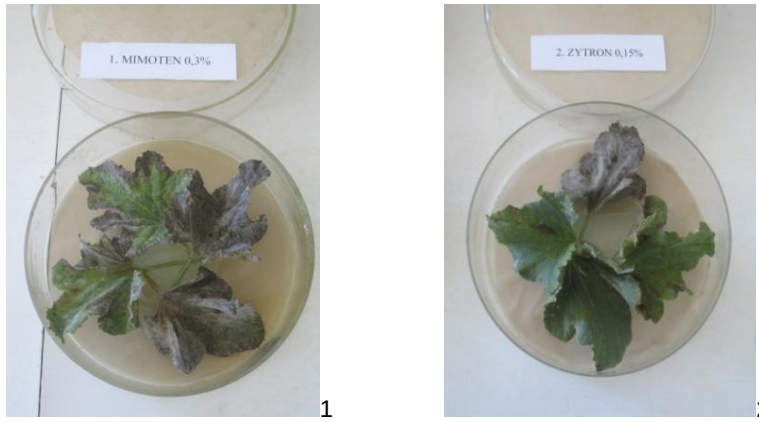


Fig. 2 Degree of attack of pathogen *Erysiphe* sp. on tomatoes leaves, 7 days after appling the bioproducts (1. Mimoten 0,3%; 2. Zytron 0,15%; 3. Mimoten 0,3% + Zytron 0.15%; 4. Untreated check)

In melons, after 7 days of treatment, the attack decrease from 61.5% to 40.4% at Mimoten 0.3% and from 62.5% to 43.7% at Zytron 0.15% (fig. 3 and fig. 4).

At the some time degree of attack on untreated check increase from 48.5% to 51.5% on *Erysiphe* sp. and from 63.4% to 97.5% on *Sphaerotheca fuliginea*.

Fig. 3 Degree of attack of pathogen *Sphaerotheca fuliginea* on melon leaves, before appling the bioproducts



1 2

3

4

Fig. 4 Degree of attack of pathogen *Sphaerotheca fuliginea* on melon leaves, 7 days after appling the bioproducts (1. Mimoten 0,3%; 2. Zytron 0,15%; 3. Mimoten 0,3% + Zytron 0.15%; 4. Untreated check)

CONCLUSIONS

The combination of Mimoten 0.3% + Zytron 0.15% products had the best efficacy in both controlling *Erysiphe* sp. in tomatoes (76.3%) and *Sphaerotheca fuliginea* in melons (65.4%).

The combination Mimoten 0.3% + Zytron 0.15% products can be an alternative solution for the using of chemical products for control of powdery mildew diseases.

REFERENCES

1. Agrios, G.N., 2005 - *Plant Pathology.* 5th edn. Amsterdam, the Netherlands*: Elsevier.*

2. Bélanger, R.R., and Labbé, C., 2002 - *Control of powdery mildews without chemicals: prophylactic and biological alternatives for horticultural crops in the powdery mildews. A Comprehensive Treatise.* Minnesota, USA: APS Press, pp. 256–267.

3. Romero D. , Vicente A., Zeriouh H., Cazorla F. M., Fernández-Ortuño D., J. A. Torés J. A. Pérez-García A., 2007 - *Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon.* Plant Pathology 56, 976–986

RESEARCH CONCERNING THE PATHOGENS CONTROL ON MELONS CROPS IN THE FIELD

ASPECTE PRIVIND CONTROLUL AGENŢILOR PATOGENI LA CULTURILE DE PEPENI GALBENI DIN CÂMP

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Abstract *The melons crops in the field crops are frequently attacked by Pseudoperonospora cubensis (downy mildew) Sphaerotheca fuliginea (powdery mildew) and Alternaria cucumerina (alternaria leaf spot). For controlling of these pathogens there were experimented different mixture between fungicides: Melody Compact 49 WG, Ortiva 250 SC, Bravo 500 SC and Dithane M 45 WP with Score 250 SC or Orius 25 EW. For controlling of pathogen Pseudoperonospora cubensis the best results were obtained with following mixtures: Melody Compact 49 WG 0.2 % + Score 250 SC 0,05 % and Melody Compact 40 WG 0,2 % + Orius 25 EW 0.05 %, with 97.7 % efficacy and respectively 95.2 %. For controlling of pathogen Sphaerotheca fuliginea the best results were obtained with following mixtures: Ortiva 250 SC 0,075 % + Score 250 SC 0,05 % and Ortiva 250 SC 0,075 % + Orius 25 EW 0.05 % with 100 % efficacy. The same variants gave good results in controlling of pathogen Alternaria cucumerina, with 91.8 % efficacy and respectively 89.8 %.*
Key words: melons, *Pseudoperonospora cubensis, Sphaerotheca fuliginea, Alternaria cucumerina*

Rezumat *Culturile de pepeni din câmp sunt frecvent atacate de agenţii patogeni Pseudoperonospora cubensis (mana) Sphaerotheca fuliginea (fainare) şi Alternaria cucumerina (alternarioza). Pentru controlul acestor agenţi patogeni au fost experimentate diferite amestecuri de fungicide: Melody Compact 49 WG, Ortiva 250 SC, Bravo 500 SC şi Dithane M 45 WP cu Score 250 SC sau Orius 25 EW. Pentru controlul agentului patogen Pseudoperonospora cubensis, cele mai bune rezultate au fost obţinute folosind următoarele amestecuri: Melody Compact 49 WG 0,2% + Score 250 SC 0,05% şi Melody Compact 40 WG 0,2% + Orius 25 EW 0,05%, cu o eficacitate 97,7% şi, respectiv, 95,2%. Pentru controlul patogenului Sphaerotheca fuliginea, cele mai bune rezultate au fost obţinute folosind următoarele amestecuri: Ortiva 250 SC 0,075% + Score 250 SC 0,05% şi Ortiva 250 SC 0,075% + Orius 25 EW 0,05% cu eficacitate 100%. Aceleaşi variante au dat rezultate bune în controlul agentului patogen Alternaria cucumerina, cu o eficacitate de 91,8%.*
Cuvinte cheie: pepeni galbeni, *Pseudoperonospora cubensis, Sphaerotheca fuliginea, Alternaria cucumerina*

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INTRODUCTION

Melon crops occupied an important surface worldwide, the most cultivated area with melons are on China, with over 400 thousand ha followed by Turkey, Iran, India and Egypt. High productions are obtained in warm climates such as Puerto Rico, Cyprus and Bahrain. In Europe, Romania ranks 4th as an area (4288 ha) after Spain, Italy and France (FAO, 2013).

Yellow melons crops on field are frequently attacked by the pathogens *Pseudoperonospora cubensis* (downy mildew) *Sphaerotheca fuliginea* (powdery mildew) şi *Alternaria cucumerina* (alternaria leaf blight).

Foliar diseases on melons have a negative impact on fruit yield and quality (Egel, 2016). In the USA, in the main areas where yellow melons are grown, it is recommended to apply fungicides for the control of pathogens *Pseudoperonospora cubensis* (Bravo, Echo, Aprovia Top, Cataraman, Mancozeb, Previcur Flex, Ranman), *Sphaerotheca fuliginea* (Aprovia Top, Fontelis, Luna Sensation, Inspire Super, Pristine) and *Alternaria cucumerina* (Aprovia Top, Convertible EG, Fontelis, Gavel, Inspire Super, Satori, 2017).

The objectives of the experience were to experiment some combinations of products and to establish the most effective control of the main pathogens that cause major damage to the yellow melon crop in the field.

MATERIAL AND METHOD

At RDIVFG Vidra was organized a monofactorial experience, with 10 variants, in 4 repetitions, placed in randomized complete blocks. The plot area was 10m2. The biological material was represented by the melon variety Festiv.

Planting date was on June 2, 2016, with seedlings produced in protected crops, on soil mulched with black polyethylene foil, one row per furrow at a distance of 1.5m between rows. The distance between plants per row was 50 cm.

For the simultaneous control of the three pathogens (*Pseudoperonospora cubensis, Sphaerotheca fuliginea and Alternaria cucumerina*) were experimented several combinations of fungicides Melody Compact (8.4% iprovalicarb + Cu 40.6% copper oxychloride) Ortiva 250 SC (azoxystrobin 250 g/L) 0.075%, Bravo 500 SC (chlorothalonil 500 g/L) 0.2%, Dithane M 45 WP (mancozeb 80% 250 g/L) 0.05% or Orius 25 EW (tebuconazole 250 g/L) 0.05%.

During the vegetation period, were applied 5 treatments (July 21, July 29, August 10, August 22, and August 30).

There were made observations regarding the occurrence and evolution of pathogen attack in correlation with climatic factors, the frequency and severity of the attack, and finally was calculated the degree of attack. The yield data was processed by variance analysis. The effectiveness of the treatment variants has been assessed according to the value of the degree of attack and the production obtained.

RESULTS AND DISCUTIONS

The attack of the three pathogens (*Pseudoperonospora cubensis, Sphaerotheca fuliginea* and *Alternaria cucumerina*) started relatively late (in the

third decade of July) because of unfavorable climatic factors (especially the maximum high temperatures of 31.2 – 32.6 ºC, relatively low humilities 76.3 - 77.5% and lack of rainfall). Due to the heavy rainfall from the second decade of August (34.5 mm) and the third decade of the same month (72.5 mm), the downy mildew attack produced by *Pseudoperonospora cubensis* had a rapid evolution: from a value of 0.9% in the third decade of July reached 68.7 in the first decade of September (tab. 1).

Table 1

Influence of climatic factors on the occurrence and evolution of the pathogens attack to yellow melon culture in the field (Vidra, 2016)

Pathogens and climatic factors	month / decade									
	June			July			August			September
	I	II	III	I	II	III	I	II	III	I
Pseudoperonospora cubensis	0	0	0	0	0	0.9	1.3	15.6	45.7	68.7
Sphaerotheca fuliginea	0	0	0	0	0	0.3	1.0	5.4	9.7	13.8
Alternaria cucumerina	0	0	0	0	0	0.2	2.9	8.9	11.7	14.7
Minimum temparature (ºC)	13.7	15.5	18.7	16.6	16.4	18.0	18.8	16.0	17.8	15.7
Medium temperature (ºC)	18.5	22.0	25.1	22.4	23.4	24.9	26.2	22.6	23.2	21.9
Maximum temperature (ºC)	23.7	29.3	31.5	28.8	31.2	32.6	32.3	29.8	30.0	29.7
Minimum relative humidity (%)	57.0	60.3	51.2	56.0	46.8	43.1	44.4	58.0	52.3	45.3
Medium relative humidity (%)	72.8	72.4	68.5	66.9	59.1	54.4	56.1	62.6	62.1	56.5
Maximum relative humidity (%)	90.0	90.2	88.4	85.1	77.5	76.3	73.6	79.8	77.5	75.5
Rains (mm)	17.0	6.5	7.0	0	0	2.0	0	34.5	72.5	0

For the control of *Pseudoperonospora cubensis* pathogen, the best results gave Melody Compact 49 WG 0.2% + Score 250 EC 0.05% (variant 1, fig. 1) and Melody Compact 49 WG 0.2% + Orius 25 EW 0, 05% (variant 2, fig. 2) with an efficacy of 97.2% and 95.2%, respectively (tab. 2).

In the case of the *Sphaerotheca fuliginea* fungus, the best results were obtained with Ortiva 250 SC 0.075% + Score 250 EC 0.05% (variant 3) or Ortiva 250 SC 0.075% + Orius 25 EW 0.05% (variant 4) with an efficacy of 100.0%.

Also, variants 3, 4 and 6 gave good results in controlling the *Alternaria cucumerina* pathogen with an efficacy of 91.8% and respectively 89.8%.

In untreated check the degree of attack on *Pseudoperonospora cubensis* was 68.7%, *Sphaerotheca fulginea* 13.8% and *Alternaria cucumerina* 14.7%.The frequency of the attacked fruits was 0 (variants 1, 2 and 9: Melody Compact 49 WG 0.2% + Score 250 SC 0.05%, Melody Compact 49 WG 0.2% + Orius 25 EW 0.05% and Dithane M 45 WP 0.2% + Score 250 EC 0.05% alternative to Orius 25 EW 0.05%) and 3.6% (Variant 6: Bravo 500 SC 0.2% + Orius 25 EW 0.05%, tab.

3) compared to 10.9% in the untreated check variant. Damaged fruits have rotted due to the attack of *Phytophthora* sp. and *Fusarium* sp. Regarding the yield / mp (tab. 3) the best results were obtained to the variant 1 (Melody Compact 49 WG 0.2% + Score 250 SC 0.05% with 2.582 kg / m^2.

Table 2

Effectiveness of some combinations of fungicides in the control of pathogens *Pseudoperonospora cubensis, Sphaerotheca fuliginea* and *Alternaria cucumerina* in yellow melon culture in the field (Vidra, 2016)

Nr. crt.	Variant	Degree of attack (%)					
		P. cubensis	Efficacy (%)	*S. fuliginea*	Efficacy (%)	*A. cucumerina*	Efficacy (%)
1	Melody Compact 49 WG 0.2 % + Score 250 SC 0.05 %	1.9	97.2	2.3	83.3	2.4	83.7
2	Melody Compact 49 WG 0.2 % + Orius 25 EW 0.05 %	3.3	95.2	2.0	85.5	2.0	86.4
3	Ortiva 250 SC 0.075 % +Score 250 SC 0.05 %	33.0	52.0	0	100.0	1.5	89.8
4	Ortiva 250 SC 0.075 % + Orius 25 EW 0.05 %	40.1	41.6	0	100.0	1.2	91.8
5	Bravo 500 SC 0.2 % + Score 250 SC 0.05 %	4.9	92.9	0.3	97.8	1.7	88.4
6	Bravo 500 SC 0.2 % + Orius 25 EW 0.05 %	7.9	88.5	0.1	99.3	1.5	89.8
7	Dithane M 45 WP 0.2 % + Score 250 SC 0.05 %	4.8	93.0	1.7	87.7	2.5	83.0
8	Dithane M 45 WP 0.2 % + Orius 25 EW 0.05 %	8.0	88.3	1.2	91.3	2.2	85.0
9	Dithane M 45 WP 0.2 % + Score 250 SC 0.05 % alternativ cu Orius 25 EW 0.05 %	7.0	89.8	0	100.0	1.9	87.1
10	Check untreated	68.7	-	13.8	-	14.7	-

Fig. 1 Variant 1 treatment with Melody Compact 49 WG 0.2 % + Score 250 SC 0.05 %

Fig. 2 Variant 2 treatment with Melody Compact 49 WG 0.2 % + Orius 25 EW 0.05 %

Fig. 3 Variant 10 - Check untreated

Table 3

Frequency of diseased fruit and yield at yellow melon crop in the field (Vidra. 2016)

Variant	Frequency of diseased fruit	Efficacy (%)	yield		
			kg/m^2	t/ha	%
1.	0	100.0	2.582***	25.820	134.0
2.	0	100.0	2.532***	25.320	131.4
3.	2.4	78.0	2.190***	21.900	113.6
4.	2.4	78.0	2.205***	22.050	114.4
5.	2.5	77.1	2.235***	22.350	116.0
6.	3.6	67.0	2.180***	21.800	113.1
7.	1.2	89.0	2.045***	20.450	106.1
8.	1.1	89.9	2.055***	20.550	106.6
9.	0	100.0	2.070***	20.700	107.4
10.	10.9	-	1.927	19.270	100.0

DL5% = 0.041 kg/mp; DL 1% = 0.055 kg/mp; DL 0.1% = 0.073kg/mp

CONCLUSIONS

For the control of the pathogen *Pseudoperonospora cubensis* (downy mildew). the product combinations Melody Compact 49 WG 0.2% + Score 250 SC 0.05% or Orius 25 EW 0.05% gave the best results with very good efficacy of 97.2% and respectively 95.2%.

For the control of the pathogen *Sphaerotheca fuliginea* (powdery mildew). the best results were obtained by using Ortiva 250 SC 0.075% + Score 250 SC 0.05% or Orius 25 EW 0.05% product combinations with 100.0% efficacy. Also. the same combinations realized good results in controlling the *Alternaria cucumerina* pathogen with an efficacy of 91.8% and respectively 89.8%.

REFERENCES

1. **Egel D., 2016 -** *Fungicide Spray Schedule for Cantaloupe and Watermelon - Issue: 612 Vegetable crop hotline* - A newsletter for commercial vegetable growers prepared by the Purdue University Cooperative Extension Service
2.*,** http://www.fao.org/statistics/en
3.***, https://ag.purdue.edu/btny/midwest-vegetable guide/ Documents/ 2017/01 *_MWVegGuide_2017*.pdf

STUDIES ABOUT THE FERTILIZATION REGIME ON SOME ORNAMENTAL SPECIES CULTIVATED IN INDOOR GREEN WALLS SYSTEMS

STUDII ASUPRA REGIMULUI DE FERTILIZARE AL UNOR SPECII ORNAMENTALE CULTIVATE ÎN SISTEM DE PERETE VERDE

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Abstract. *Green walls have been promoted in particular for the benefits they bring in the climate parameters, such as heat and sound insulating effects or pollutants and particulate filter (European Commision, 2012, Iliescu 2003, Nita, 2016). As the green walls are still relatively uncommon and used in Romania, the main objective of this research was to study the behavior of several ornamental indoor, decorative habitus and leaf species, on a fertilization regime with different fertilizers, reflected in the evolution of the main characteristics defining their decorative potential in the specific limiting conditions of a plant wall culture. The range of ornamental indoor decorative habitus and foliage species was as follows: Hedera helix, Ophyopogon jaburan, Tillandsia cyanea, Nephrolepis cordifolia, Tradescantia zebrina, Clorophytum comosum, Spatiphylium walisii.*
Key words: fertilization regime, green walls, indoor ornamental plants

Rezumat. *Pereţii verzi au fost promova şi în special pentru beneficiile pe care le aduc la nivelul parametrilor climatici, precum efectele de izolator termic şi fonic sau de filtrare a poluanţilor şi pulberilor (European Commision, 2012, Iliescu, 2003, Niţă, 2016). Deoarece pereţii vegetali sunt destul de puţin cunoscuţi şi utilizaţi în România, obiectivul principal al acestor cercetări a fost de a studia comportamentul mai multor specii ornamentale de interior, decorative prin habitus şi frunze, supuse unui regim de fertilizare cu diferite îngrăşăminte, reflectat în evoluţia principalelor caracteristici ce le definesc potenţialul decorativ, în condiţiile spcific-limitative ale sistemului de culturi în perete verde. Sortimentul de specii ornamentale de interior, decorative prin habitus şi frunze ales a fost următorul: Hedera helix, Ophyopogon jaburan, Tillandsia cyanea, Nephrolepis cordifolia, Tradescantia zebrina, Clorophytum comosum, Spatiphylium walisii.*
Cuvinte cheie: regim de fertilizare, plante ornamentale de interior, pereţi verzi.

INTRODUCTION

The vertical gardens known as ***green walls*** are vegetable compositions with vertical arrangement. These innovations regarding the arrangement of

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gardens occurred both because of limited space and the need for near nature and fresh air.

In advanced countries in the European Union, such as France, Italy, Spain, England, the various corporations have set up indoor wall systems or building façades, starting from two important aspects: their appearance and contribution to improving the environment and microclimate.

Their arrangement within companies is also seen as a way to improve the image of the company and brand by promoting openness to European ecological strategies by using systems that consume as little energy as possible.

Vegetable walls fit perfectly into both private and public spaces (the headquarters of a company, banks, hotels, restaurants, cafes, beauty parlors, shops, cinemas, hospitals, medical practices), with the choice of environmentally friendly plants respectively.

If previously the cultivation of species brought from distant areas was the exclusive benefit of the very rich, it has now become accessible to the less well-off. With the availability of a wide range of plants and the knowledge of successful cultivation possibilities, indoor plants have become one of the most widespread passions. Their expansion was made possible by enriching knowledge on plant claims to environmental factors and crop routing by specific technologies.

Do plants really need soil? No, the soil is simply a mechanical support. Only the water and the minerals contained in it are essential to the plants, together with the light and the carbon dioxide needed for photosynthesis.

Throughout the year it is necessary to analyze the aesthetic evolution of the plants in order to preserve their decorative qualities. In order to obtain optimal results in this respect, it is a good practice to select plant plants in the composition of the vegetation walls by a person skilled in the art.

Another important aspect of plants is their maintenance.

Due to the fact that these plants are perennial and their culture in this system implies a low volume of substrate, it is necessary to apply a sustained fertilization regime. The recipe for fertilization differs by the proportions of the constituents and the requirements of the plants.

In order to achieve green wall on interior spaces there exist systems of structures with self-support or simply attached to the wall. Facilities are equipped with recirculation water and nutrient management.

Since 1982 Patrik Blanc continuously developed the concept of vertical vegetable gardens and in 1996 and it has patented.

Considering the above, we considered it equally important to identify an assortment of ornamental species, adapted to the vertically system and responding to such a system, in a many seasonal projection in the same time with identifying an available fertilization regime to ensure the healthy life for the plants.

MATERIAL AND METHOD

The experience was carried out in a greenhouse compartiment of the experimental floral field, within USAMV Bucharest. This 1000 m² floral greenhouse is the teaching base for university students for floral plant research.

The vertical walls were made in two versions, on the same panel placed "back to back", using the followed ornamental species for interior decoration (habitus and leaves): *Hedera helix, Ophyopogon jaburan, Tillandsia cyanea Nephrolepis cordifolia, Tradescantia zebrina, Clorophytum comosum, Spatiphylium walisii.*

Nephrolepis cordifolia Presl. The leaf bush can reach 150 cm in diameter, with large leaves, sect, arched, colored in intense green (Amăriuței and Vâşcă-Zamfir, 2015).

Tradescantia zebrina is a plant with spiked shoots, which can reach a length of 30-60 cm. The foliage is spectacular due to the striped, green, silver and violet on the top, with the purple reverse (Şelaru, 2006).

Chlorophytum comosum Bak. forms a bush that can reach 40-50 cm in diameter. The leaves are beautifully arched, green in different shades or cloaks with white or cream. From the middle of the bush grow thin flexible stems, beautiful arched pendent, up to 1 m long, bearing here and there small plants (rosette leaves), very decorative (waterfall effect) (Şelaru, 2006).

Spathiphyllum walisii grows like a rich bush of brightly colored, bright green leaves (rarely more than 30 cm). Inflorescence is a typical spade, with creamy white creams, slightly scented and white, oval-elliptical. (Şelaru, 2006).

Hedera helix L. as an indoor plant does not exceed 1.5 m long. Flexible stems have adventive root knots that favors clamping. The leaves are palmate-lobed, deep green or green colored board / stained with various shades of yellow, beige, white, depending on the variety (Amariutei sticky-Zamfir, 2015).

Tillandsia cyanea hort. The species belongs to the large group of Bromelles. The leaves, numerous (60), long (max. 35 cm) and narrow are arranged in the form of a rosette. The solitary inflorescences with blue flowers appear among the pink bracts at the tip of a slender peduncle (Preda, 1989)

Ophyopogon jaburan Lodd. It is a small species (30-40 cm). The persistent, linear-lanceolate, green leaves form a dense bush (Amăriuței, Vâşcă-Zamfir, 2015).

It has been ensured drip watering system, separately for each panel (different wetting times).

The culture substrate was made up of **garden soil + peat + perlite** (1: 1: 0.5) placed in plastic wall pots with a volume of 1.5 liters (fig.1).

The green wall (panel), after populated with plants is presented in the figure 1(b). Also in the figure 3 (c) there can be seen the aspect at the end of the experimental period with plant at the maximum of their decorative potential.

It were applied the specific maintenance work spaces (conducting the microclimate factors).

It has pursued the development of decorative potential in dynamic compared to the same range of species grown in conventional systems.

Plants were fertilized weekly, using 1 fertilizer cap (10 mL) per 1 liter of water, resulting a 0.1% concentration solutions (usual for ornamental indoor plants).

The experimental variants were:

V_m Unfertilized plants on greenhouse clasic system
V_1 Unfertilized plants on green wall system
V_2 Fertilization with Substral 0,1% solution
V_3 Fertilization with Biopon 0,1% solution

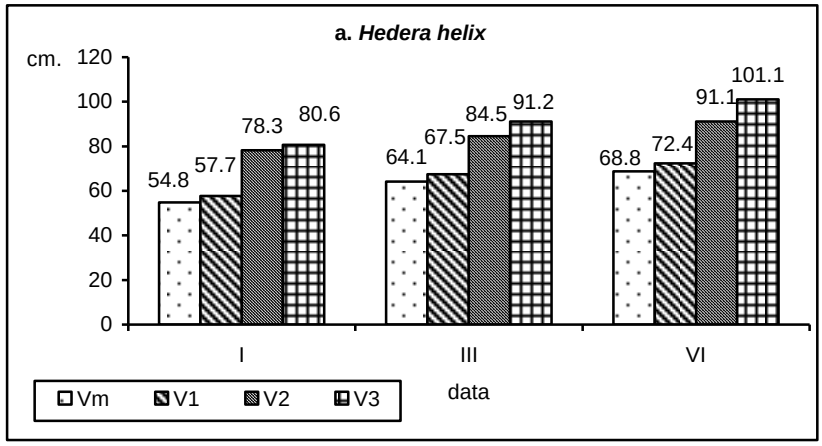
a) b) c)

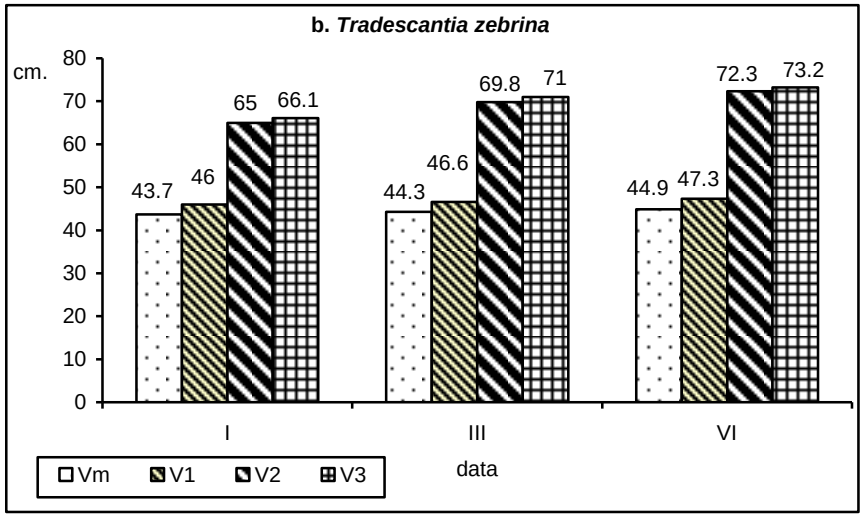
Fig. 1 The panell before (a) and after (b) planting and at the final stage (c)

RESULTS AND DISCUSSIONS

In order to study the effect of the nutrition regime on the growth of shoots and the diameter of the plant, we used one-factor ANOVA for each month and for different species (Steel *et al.,* 1997; Wiliam *et al.,* 2006).

For the study of the growth of the shoots, there were made measurements on *Hedera helix*, *Chlorophytum comosum*, *Tradescantia zebrina*, at three stages in the experimental perioad, in January (I), March (III) and June (VI) (fig. 2 a,b,c).





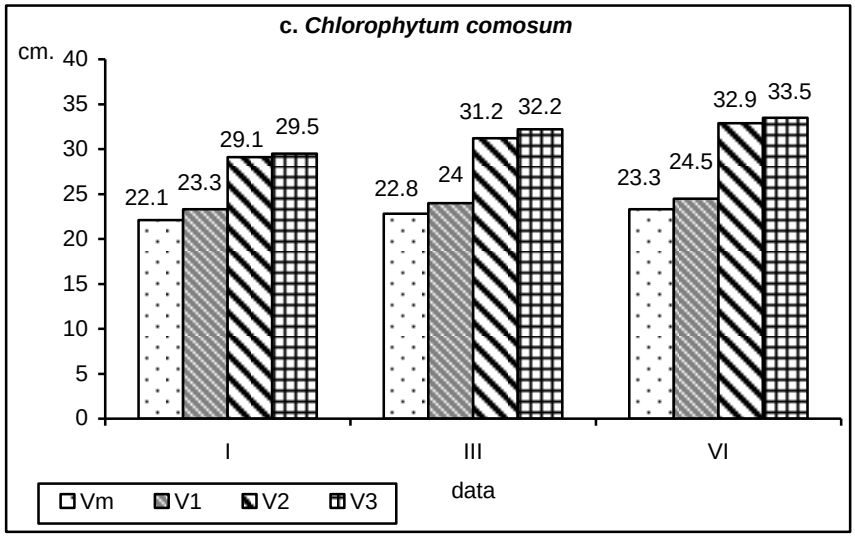


Fig. 2 Shoots lenght(*cm*) on different experimental variants

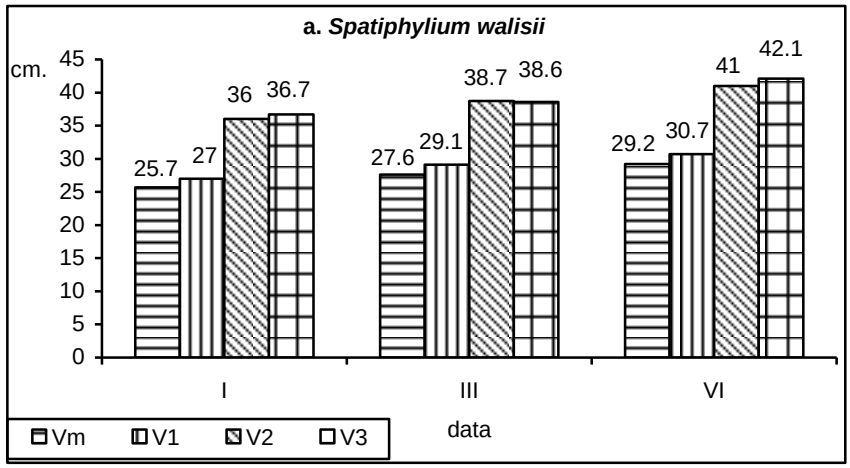
For the study of the diameter of the plant, there were made measurements on *Spatiphylium walisii, Nephrolepis cordifolia, Tillandsia cyanea, Ophiopogon jaburan*(fig. 3 a,b,c,d), on the same data as above.

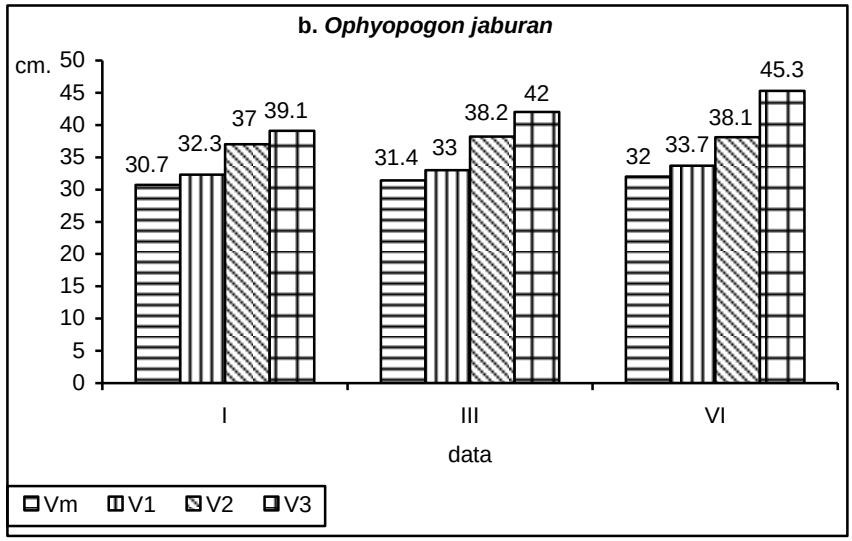
For each month, for each species, significant differences were obtained between the plants grown in the different media [both the F (3,12) values calculated were higher than the tabular value scorres ponding to $\alpha = 0.05$ and the p values were less than 0.05)].

As shown in the Figures 2 and 3, for all species the best values were obtained for V_3 plants. It is generally observed that regardless of the measurement

month, irrespective of the measured characteristic (growth of the shoots or the diameter of the plant) and regardless of the species, the decreasing order of the averaging variants remained the same V_3, V_2, V_1, V_m.

Experimental data showed a normal development during the active vegetation season (spring-summer), with a visible differentiation between plants whose growth has benefited from the intake of fertilizers and whose growth was based only on the nutrient intake in the culture substrate.





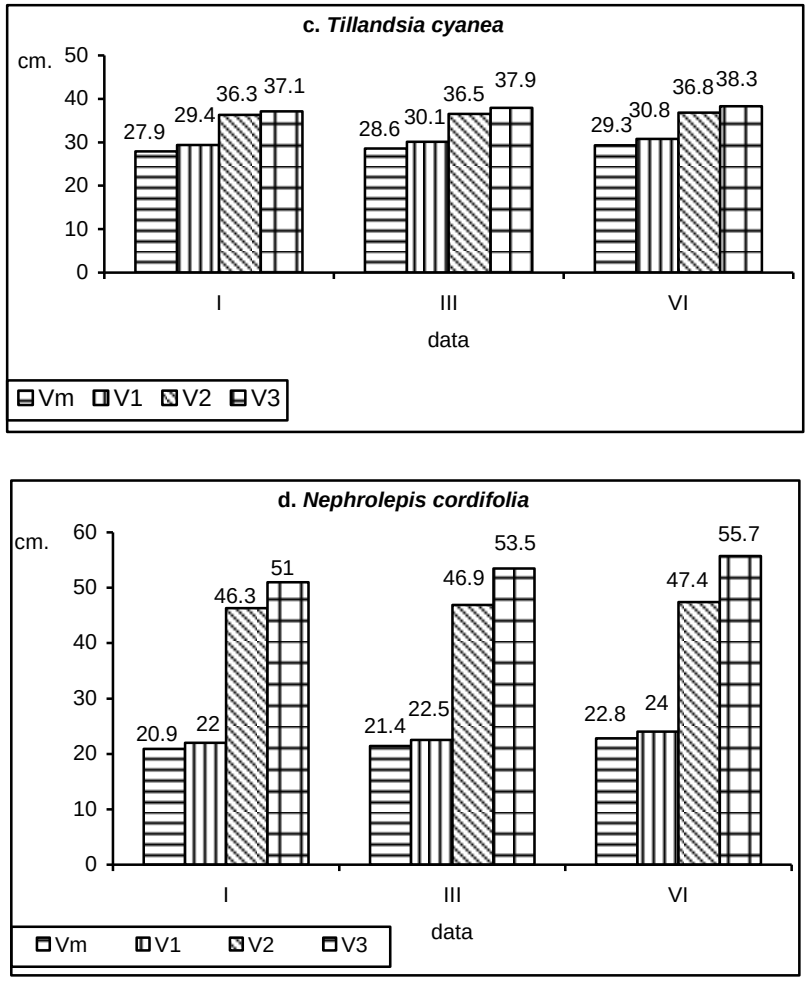


Fig.3 Plant diameter (cm) on different experimental variants

The differences between the two experimental variants applied to fertilizers (V_2 and V_3) in favor of V_3 can be attributed to the different nitrogen content of the two products used for fertilization (8% versus 6%).

CONCLUSIONS

The ornamental studied species have confirmed that are suitable for vertical cultivation systems.

The results, taking into account the restrictions (limited space development/nutrition, exhibition, watering regime etc.) and the intake of fertilizers, are comparable to those obtained in cultures in the classical system.

We consider necessary to continue research in order to extend the assortment, given the development prospects of the green walls system.

REFERENCES

1. **Amăriuței A., Vâşcă-Zamfir Diana, 2015** - *Plante de interior decorative prin frunze*, Ed. ELISAVAROS, Bucuresti
2. **Iliescu Ana Felicia, 2003** - *Arhitectură peisageră*. Editura Ceres, Bucureşti.
3. **Niță M. R., 2016** - *Infrastructuri verzi – o abordare geografică*. Editura Etnologică, Bucureşti.
4. **Preda M., 1989 -** *Dicționar dendroflori*col. Ed Ştiințifică şi Enciclopedică, Bucureşti.
5. **Steel R. G. D., Torrie J. H., Dickey D. A., 1997 -** *Principles and procedures of statistics: a biometrical approach* 1997 3rd ed New York, NY McGraw-Hill
6. **Şelaru E., 2006 -** *Plante de apartament*, (Ediția a II- a), Editura Ceres, Bucureşti
7. **Williams E. R., John J. A., Whitaker D., 2006** - Construction of resolvable spatial row-column designs. Biometrics 62: 103-108
8. ***media.murvegetalpatrickblanc.com/dl/9614 pt. southeast-asia-building-mars-2016
9. ***http://www.verticalgardenpatrickblanc.com/node/1386
10. ***European Commision, 2012. The Multifunctionality of Green Infrastructure. Bruxelles.

LITHUANIAN PROTECTED LANDSCAPE

PEISAJUL LITUANIAN PROTEJAT

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Abstract. *Lithuania has 1020162 hectares of protected areas, which account for 15.64% of total surface of the country. The Lithuanian natural heritage comprise the countryside and natural environment, including flora and fauna (scientifically known as biodiversity), as well as geological elements (scientifically known as geodiversity). These kind of heritage sites often serve as an important component in a country's tourist industry, attracting many visitors from abroad as well as locally. Heritage can also include cultural landscapes, meaning natural features that may have cultural attributes. Some of the cultural features of these protected areas are inherited from past generations, maintained in the present and bestowed for the benefit of future generations.*
Key words: Lithuania, protection, landscape, future

Rezumat. *Lituania are 1020162 hectare de arii protejate care însumează 15.64% din suprafaţa totală a ţării. Patrimoniul natural lituanian cuprinde zone din mediul rural şi natural, din care fac parte flora şi fauna ce constituie biodiversitatea, precum şi elementele geologice ce constituie geodiversitatea. Acest tip de zone de patrimoniu servesc adesea ca importante componente ale industriei turismului lituanian, atrăgând mulţi vizitatori din alte ţări, dar şi localnici. Patrimoniul acesta poate include de asemenea şi peisaje culturale, adică peisaje cu trăsături naturale având atribute culturale. Câteva din trăsăturile culturale ale acestor arii protejate sunt moştenite de la generaţiile trecutului, fiind prezervate în prezent pentru a fi transmise spre beneficiul generaţiilor viitoare.*
Cuvinte cheie: Lituania, protecţie, peisaj, viitor

INTRODUCTION

The protected areas of Lithuania are: national parks, state parks, national reserves, reserve area, biosphere reserves, biosphere polygons, state and local nature reserves, etc. State Office of Protected Areas in Lithuania survey 1020162 hectares of protected areas, which account for 15.62% of Lithuania surface (European Landscape Convention and Explanatory Report, 2001). For applying the state protection of all these areas, the main Legal Acts are the following:

-Republic of Lithuania law on environmental protection (1992, 2003)

-Republic of Lithuania law on protected areas (1993, 2001)

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-Republic of Lithuania law on territorial planning (1994, 2003)
-Republic of Lithuania law on construction (1995, 2003)

MATERIAL AND METHOD

The paper focus on the analysis of the all protected areas of Lithuania and on creating a synthesis of the situation of the natural landscape heritage - comprising the countryside and natural environment, including the biodiversity as well as geodiversity, but also of the cultural landscape heritage - meaning natural features that may have cultural attributes (Deveikis, 2003).

RESULTS AND DISCUSSIONS

The statistic of the Lithuania protected areas categories (tab. 1) show that there are five main categories of protected areas each one having some specific areas, as following:

1. STATE STRICT RESERVATION
- Natural
- Cultural
- Small Strict Reservation
2. RESERVATION
- State Reservation
- Municipal Reservation
3. STATE PARKS
- National parks
- Regional parks
4. BIOSPHERE RESERVATION
5. BIOSPHERE POLYGONS

Table 1

STATISTIC OF THE PROTECTED AREAS CATEGORIES

Category	Amount	Area (ha)	Part of country are(%)
STATE STRICT RESERVATION	**6**	**18772.09**	**0.29**
Natural	3	18406.72	0.28
Cultural	2	245.85	0.004
Small Strict Reservation	1	119.52	0.002
RESERVATION	**390**	**156114.88**	**2.39**
State Reservation	279	143229.46	2.19
Municipal Reservation	111	12885.42	0.20
STATE PARKS	**35**	**593700.35**	**9.09**
National parks	5	144338.50	2.21
Regional parks	30	449361.85	6.88
BIOSPHERE RESERVATION	**1**	**18489.69**	**0.28**
BIOSPHERE POLYGONS	**28**	**233518.73**	**3.58**
		1021440.20	**15.64**

The *State Strict Reserves* are the most strictly controlled protected areas that are established to facilitate the protection and research of Lithuania's landscapes with scientific environmental or cultural value. All economic activity in strict reserves is forbidden.

Objectives of establishment of strict reserves:

1. to ensure the unaffected course of natural processes or maintenance of authenticity of territorial complexes and objects (properties) of cultural heritage;
2. to preserve a typical or unique natural or cultural landscape and the objects of heritage located therein;
3. to preserve valuable natural ecosystems, habitats, gene pool of wild flora, fungi and fauna species;
4. to organise continuous scientific research and monitoring as well as museum work;
5. to promote territorial complexes and objects (properties) of natural or cultural heritage.

As seen in table 1, according to the type of protected properties, strict reserves shall be classified as follows:

1. Natural - for the preservation of particularly valuable complexes of natural landscape.
2. Cultural (museums-reserves) - for the preservation of particularly valuable complexes of cultural landscape (Archives of the Land Survey Institute, 1987 – 1993).

Within Lithuania there are three state strict nature important reserves:

- Čepkeliai
- Kamanos
- Viešvilė

The state strict cultural reserves are two:

- Kernavė
- Vilnius Castles

But also there are 36 nature and three cultural strict reserves within complex protected areas and one small strict reserve-Dubrava.

Regarding the *Protected Reservation* there are 390 protected areas: 279 state reservation and 111 municipal reservation.

Other very important protected areas are the Lithuania *State Parks* in total 35, of which there are 5 national parks and 30 regional parks.

There are some important objectives of establishment of state parks:

- to preserve a naturally and culturally valuable landscape;
- to preserve typical or unique ecosystems;
- to restore destroyed and damaged natural and cultural complexes and objects (properties);

- to provide conditions for scientific research in the field of protection of natural and cultural heritage;
- to promote and support the ethno-cultural traditions of Lithuanian regions;
- to provide conditions for recreation, primarily cognitive tourism;
- to develop environmental education, promote ecological farming;
- to implement other objectives of their establishment provided for in statutes of the state parks.

The fifth category of Lithuania protected areas are the *Biosphere Reservations* (1) and *Biosphere Polygons* (28). Biosphere grounds shall be established with a view to conducting national and regional environment monitoring in the areas of particular geoecological importance.

Objectives of establishment of areas of biosphere monitoring:

- to create a representative system of complex ecological monitoring – to monitor, control and forecast changes in natural systems;
- to conduct experiments and research of the use of biosphere;
- to develop environmental education and propaganda;
- to ensure protection of natural complexes.

CONCLUSIONS

All these Lithuanian protected heritage sites often serve as an important component in a country's tourist industry, attracting many visitors from abroad as well as locally. Some of the cultural features of these protected areas are inherited from past generations, maintained in the present and bestowed for the benefit of future generations.

Related to the actual situation of these protected areas, a stringent need has been highlighted: the importance of finding new ways to increase the search for curative and preventive solutions for the preservation, restoration and regeneration.

REFERENCES

1. Deveikis S., 2003 - *Le patrimoine dans les anciens domaines /manoirs dans un processus de la planification territoriale en Lituanie*. Proceedings of 33 International Symposium of the European Faculty of Land Use and Development, Gediminas Technical University, Vilnius, p. 62 – 70.

2. *, 1987 – 1993** – *Archives of the Land Survey Institute. Archives of the Inventory of manors.* Land Survey Institute, Vilnius.

3. *, 2001** - *European Landscape Convention and Explanatory Report*, Ministry of Environment, Vilnius.

KINETICS STUDY OF Pb(II) AND Hg(II) REMOVAL FROM AQUEOUS MEDIA ON RAPESEEDS BIOMASS

STUDIUL CINETIC AL ÎNDEPĂRTĂRII IONILOR DE Pb(II) ŞI Hg(II) DIN MEDII APOASE PE BIOMASĂ DE RAPIȚĂ

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Abstract. *In this study, the removal of Pb(II) and Hg(II) ions from aqueous solution using rapeseeds biomass was examined in batch systems as a funtion of contact time. The results obtained for the removal of each studied metal ion were analyzed using three kinetics models: prseudo-first order, pseudo-second order and intra-particle diffusion, in order to elucidate the mechanism of the removal process. For both studied metal ions, the experimental data are well described by the pseudo-second order kinetics model. These results can be used for to highlight the potential applicability of rapeseeds biomass as low-cost biosorbent in the clean-up of aqueous effleunts, containing toxic heavy metals.*
Key words: metal ions, rapeseed biomass, biosorption, kinetics.

Rezumat. *În acest studiu a fost examinată îndepărtarea ionilor de Pb(II) şi Hg(II) din soluții apoase utilizând biomasă de rapiță, în sisteme discontinui în funcție de timpul de contact. Rezultatele obținute pentru îndepărtarea fiecărui ion metalic în parte au fost analizate utilizând trei modele cinetice: modelul cinetic de ordin pseudo-unu, modelul cinetic de ordin pseudo-doi şi modelul de difuzie intra-particulă, în scopul de a elucida mecanismul procesului de îndepărtare a ionilor metalici. Pentru ambii ioni metalici studiați, datele experimentale sunt cel mai bine descrise de modelul cinetic de ordin pseudo-doi. Aceste rezultate pot fi utilizate pentru a evidenția aplicabilitatea biomasei de rapiță in procesele de îndepărtare a ionilor metalici toxici din efluenți industriali.*
Cuvinte cheie: ioni metalici, biomasă de rapiță, biosorbție, cinetică.

INTRODUCTION

Nowadays anywhere in the world, an important issue is the contamination of environment with heavy metals. This because the heavy metals are not biodegradable and due to their toxic effect and bioaccumulation tendency through food chain, can seriously affect many life forms (Fu and Wang, 2011). The main source of environmental pollution with heavy metals is industrial activities. The wastewaters resulted from various technological processes often contains significant concentrations of heavy metals, and from this reason their discharge into environmental must be done only after a rigorous treatment. Romanian

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legislation impose that the concentration of Pb(II) and Hg(II) ions in industrial effluents which are discharged into natural water sources to not exceeds 0.2 mg/L and 0.05 mg/L, respectively (NTPA 001/2005).

The biosorption of metal ions from aqueous solution has receive considerable attention in the recent years as potential treatment method of industrial effluents containing heavy metals, mainly because it is cost-effective, easy of operate (even at industrial scale) and can use as biosorbents various materials which are considered as waste in other agricultural or industrial activities (Gogate and Pandit, 2004; Barakat, 2011).

The use of various agricultural wastes as biosorbents for the removal of heavy metals from aqueous solution has been extensively studied in literature (Demirbas, 2008; Krika et al., 2016), because these materials are cheap and available in large quantities in many regions. In addition, due to their chemical composition (cellulose, lignin, polysaccharides, etc.), most of agricultural wastes have rather large cation exchange capacities, what makes these materials to be effective biosorbents in the heavy metals removal processes (Demirbas, 2008). The rapeseeds can be such example of biosorbent for heavy metal ions. Cultivated on increasingly large surfaces in our country due to his industrial utilizations, the rapeseeds biomass can be valorized and as biosorbent in the treatment of industrial effluents containing heavy metals.

In this study, the kinetics of Pb(II) and Hg(II) ions biosorption from aqueous solution on rapeseeds biomass was examined. The influence of contact time on the biosorption efficiency was studied in batch systems, under optimal experimental conditions (pH 5.5, 8.0 g biosorbent/L) established previously (Arsenie *et al.,* 2017; Arsenie and Bulgariu, 2017). The experimental results were analyzed using three kinetic models: pseudo-first order, pseudo-second order and intra-particle diffusion model. On the basis of these models, the kinetic parameters were calculated for each case.

MATERIAL AND METHOD

The chemical reagent used for experiments were of analytical grade and were used without further purifications. The stock solutions of Pb(II) and Hg(II) ions (10^{-2} mol/L) were obtained by metal nitrate dissolving in distilled water. All working solutions were then obtained from the stock solutions by dilution. The initial solution pH was adjusted at required value with 0.1 N HNO_3 solution.

The rapeseeds used as biosorbent in this study were purchased form a local farm (Iaşi, Romania). The rapeseeds were first, washed several times with distilled water and dried in air (4-5 days, room temperature), and then crushed and sieved until the particles grain-size was less than 1.0 mm.

The kinetics experiments were performed by batch technique, at room temperature (22 ± 2 °C), mixing biosorbent quantities of 0.2 g with 25 mL of Pb(II) and Hg(II) ions solution, with initial concentration of 0.2 mmol M(II)/L, in 100 mL conical flasks. Each sample was intermittent stirred for various time intervals (from 5 to 180 min), and then filtered on quantitative filter paper. The Pb(II) and Hg(II) ions concentration in filtrate was analyzed spectrophotometric (Digital Spectrophotometers

S104 D, 1 cm glass cell), using an adequate method (Dean, 1995) and a prepared calibration graph.

The biosorption capacity (q, mg/g) of rapeseeds biomass for Pb(II) and Hg(II) ions was calculated from experimental results using the equation:

$$q = \frac{(c_0 - c) \cdot V}{m} \quad (1)$$

where: c_0, c – initial and equilibrium concentration of metal ions in aqueous solution (mg/L), V – volume of aqueous solution (L), m – the mass of biosorbent (g).

RESULTS AND DISCUSSIONS

The kinetics studies are important in the adaptation of a given biosorption process at industrial scale. This because the parameters, calculated from kinetics modeling, provide useful information about how take place the interactions between metal ions from aqueous solution and functional groups from biosorbent surface and the efficiency of biosorption process under well defined experimental conditions (Rao and Khan, 2009). For the kinetics modeling of a biosorption process, the pseudo-first order, the pseudo-second order and intra-particle diffusion models are most widely used to describe the experimental data (Gerente *et al*., 2007), and the mathematical equation of these three kinetics models are summarized in table 1.

Table 1

Mathematical equations of kinetics models used in this study (Ho and McKay, 1999; Gerente et al., 2007)

Kinetics model	Equation	Notations
Pseudo-first order model	$\log(q_e - q_t) = \log q_t - \frac{k_1}{2.303} \cdot t$	q_e, q_t - the biosorption capacity at equilibrium and at time t, (mmol/g); k_1 - pseudo-first order rate constant (1/min)
Pseudo-second order model	$\frac{t}{q_t} = \frac{1}{k_2 \cdot q_e^2} + \frac{t}{q_e}$	k_2 - rate constant of pseudo-second order kinetic model (g/mmol min)
Intra-particle diffusion model	$q_t = k_{dif} \cdot t^{1/2} + c$	k_{diff} - intra-particle diffusion rate constant (mmol/g $min^{1/2}$) ; c - concentration of metal ions from solution at equilibrium (mmol/L)

The selection of these three kinetics models for the mathematical analysis of the experimental results is justified by the nature of the information provided by each model. Thus, the pseudo-first order and pseudo-second order kinetics models are based on the assumption the rate limiting step in the biosorption process is the chemical interaction between metal ions from aqueous solution and superficial functional groups of biosorbent, and that the retention of the metal ions on biosorbent surface requires one or respectively two binding sites (Ho and McKay, 1999). Unlike these, the intra-particle diffusion model is generally used to highlights the importance of the elementary diffusion processes in the metal ions uptake onto a considered biosorbent (Gerente *et al.,* 2007). Therefore, the finding of

the most adequate kinetics model for to describe the experimental results will also provide an overview on the studied biosorption process.

The experimental results obtained at the study of the effect of contact time on the biosorption efficiency of Pb(II) and Hg(II) ions from aqueous solution on rapeseeds biomass are illustrated in Fig. 1. It can be observed that in considered experimental conditions, the removal efficiency of Pb(II) and Hg(II) ions on rapeseeds biomass increases with increase of contact time.

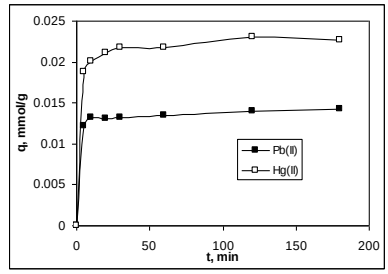
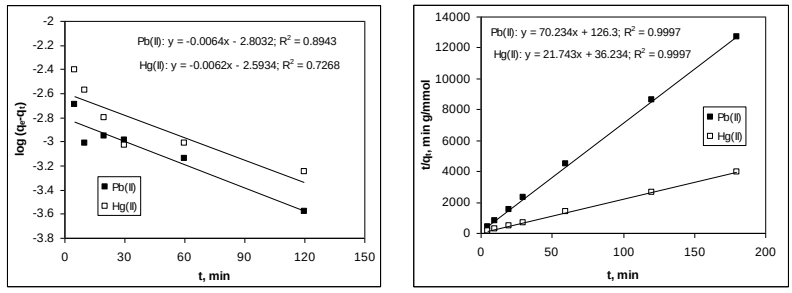


Fig. 1 Effect of contact time on the biosorption efficiency of Pb(II) and Hg(II) ions on rapeseeds biomass

The biosorption process is very fast in the initial step, when in the first 20 min are retained almost 65 % from initial Pb(II) ions and over 89 % from initial Hg(II) ions, respectively. In the second step, the biosorption process becomes slower, and the equilibrium is attained after 30 min, for both metal ions.

The kinetics parameters calculated from the linear representations of the pseudo-first order (Fig. 2a) and the pseudo-second order (Fig. 2b) are summarized in Table 2.



(a) (b)

Fig. 2 Linear representations of the pseudo-first order (a) and pseudo-second order (b) kinetics models.

The compatibility between experimental data and the pseudo-second order kinetics model indicate that the biosorption process is limited by the chemical interactions between metal ions and functional groups of biosorbent, and these interactions involve two binding sites. In addition, the comparison of the kinetics

parameters obtained for Pb(II) and Hg(II) ions shows that the Pb(II) ions are more easy removed, while the Hg(II) ions are more efficient retained.

Table 2

Kinetics parameters for the biosorption of Pb(II) and Hg(II) ions on rapeseeds biomass

Metal ion		Pb(II)	Hg(II)
	q_e^{exp}, mmol/g	**0.0141**	**0.0227**
Pseudo-first order kinetic model	R^2	0.8943	0.7368
	q_e, mmol/g	0.0016	0.0025
	k_1, 1/min	0.0028	0.0027
Pseudo-second order kinetic model	R^2	0.9997	0.9997
	q_e, mmol/g	0.0142	0.0249
	k_2, g/mmol min	39.0563	13.0473

The linear representation of the intra-particle diffusion model for Pb(II) and Hg(II) ions biosorption on rapeseeds biomass is presented in fig. 3, and the kinetics parameters calculated in this case are summarized in table 3.

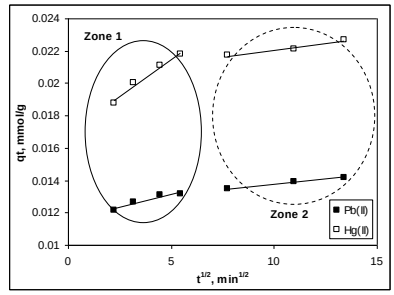


Fig. 3 Linear representation of the intra-particle diffusion kinetic model.

Table 3

Kinetics parameters of the intra-particle diffusion model for the biosorption of Pb(II) and Hg(II) ions on rapeseeds biomass

Metal ion	Pb(II)		Hg(II)	
	Zone 1	Zone 2	Zone 1	Zone 2
R^2	0.9351	0.9933	0.9759	0.9719
k_{diff}, mmol/g $min^{1/2}$	0.0003	0.0001	0.0009	0.0002
c, mmol/L	0.0116	0.0125	0.0169	0.0204

It can be observed from figure 3 that for none of studied metal ions, the linear dependence does not go through the origin, which means that the intra-diffusion particle process is not the rate limiting step, but that the diffusion controls the metal ions biosorption up to a certain degree. In addition, the higher slope of the first zone in comparison with second zone obtained in case of both metal ions (tab. 3), suggests that the active sites are located on the biosorbent surface and are readily accessible for the chemical interactions. The analysis of

the results obtained from kinetics modelling shows that the chemical interactions play an important role in the first stage of Pb(II) and Hg(II) ions biosorption on rapeseeds biomass, while the diffusion of metal ions to the pores of biosorbent becomes important in the second stage of biosorption process.

CONCLUSIONS

1. The removal of Pb(II) and Hg(II) ions from aqueous solution using rapeseeds biomass was studied in batch systems as a funtion of contact time.

2. The experimental results were analyzed using three kinetics models: pseudo-first order, pseudo-second order and intra-particle diffusion.

3. For both studied metal ions, the experimental data are well described by the pseudo-second order kinetics model.

4. These results can be used for to highlight the potential applicability of rapeseeds biomass as low-cost biosorbent in the heavy metals removal processes.

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REFERENCES

1. Arsenie T.D., Bulgariu L., 2017- *Rapid and efficient removal of Pb(II) ions from aqueous media using low quality rapeseeds biomass as biosorbent*. Process Engineering Journal, 1, 34-40.

2. Arsenie T.D., Nemeş L., Nacu G., Bulgariu L., **2017-** *Quantitative removal of Hg(II) ions from aqueous media by biosorption on rape waste biomass*. Proceeding of the *6th IEEE International Conference on E-Health and Bioengineering.*

3. Barakat M.A., 2011- *New trends in removing heavy metals from industrial wastewater*. Arabian Journal of Chemistry, 4, 361-377.

4. Dean A.J., 1995 - *Hadbook of Analytical Chemistry*. Mc-Grow Hill Inc., New York.

5. Demirbas A., 2008 - *Heavy metal adsorption onto agrobased waste materials: A review*. Journal of Hazardous Materials, 157, 220-229.

6. Fu F., Wang Q., 2011 - *Removal of heavy metal ions from wastewaters: A review*. Journal of Environmental Management, 92, 407-418.

7. Gerente C., Lee V.K.C., Lee P., McKay G., 2007 - *Application of chitosan for the removal of metals from wastewaters by adsorption - mechanisms and models review*. Critical Reviews in Environmental Science and Technology, 37(1), 41–127.

8. **Gogate P.R., Pandit A.B., 2004 -** *A review of imperative technologies for wastewater treatment II: hybrid methods*. Advanced Environmental Research, 8, 553-597.

9. **Ho Y.S., McKay G., 1999 -** *Pseudo-second-order model for sorption processes*. Process Biochemistry, 34, 451-465.

10. **Krika F., Azzouz N., Ncibi M.C., 2016 -** *Adsorptive removal of cadmium from aqueous solution by cork biomass: Equilibrium, dynamic and thermodynamic studies*. Arabian Journal of Chemistry, 9, S1077-S1083.

11. **Rao R.A.K., Khan M.A., 2009 -** *Biosorption of bivalent metal ions from aqueous solution by an agricultural waste: kinetics, thermodynamics and environmental effects*. Colloids Surfaces A, 332, 121-128.

12. *, NTPA 001/2005**: https://www.scribd.com/document/35652703/NTPA-011-28-02

DEGREE OF THE RIPARIAN ENVIRONMENT IN THE TROTUS HYDROGRAPHIC BASIN AFTER THE FLODS IN THE YEARS 2016

DEGRADAREA MEDIULUI RIVERAN ÎN BAZINUL HIDROGRAFIC AL RÂULUI TROTUŞ ÎN URMA VIITURILOR DIN ANUL 2016

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Abstract. *The paper presents an analysis of the degradation phenomena of the riparian environment in the Trotuş river basin following the floods produced in the summer of 2016. The Trotuş River has been affected by many floods over the past 28 years, 2004, 2005, 2011 and 2016. The riverine environment was affected by the degradation of shore defence works, the morphological modification of the riverbed, the destruction of the roads, the erosion of the agricultural land, the deposition of pollutants on the land adjacent to the river, etc. The June 2016 flood in the Trotuş River basin resulted in the degradation to the total destruction of over 318 km of bank defence hydrotechnical works. The floods caused the degradation of 1337 ha of agricultural land, the degradation of 355 km of road and 180 bridges. The floods affected over 376 homes in the studied area, from which it destroyed 76.*
Key words: discharges, floods, defence banks works, roads, bridge

Rezumat. *Lucrarea prezintă o analiză a fenomenelor de degradare a mediului riveran în bazinul hidrografic al râului Trotuş în urma viiturilor produse în vara anului 2016. Bazinul hidrografic al râului Trotuş a fost afectat de multiple viituri în ultimii 28 ani, dintre care se remarcă cele din 1991, 2004, 2005, 2011 şi 2016. Mediul riveran a fost afectat prin degradarea lucrărilor de apărare de mal, modificarea morfologică a albiei râului, distrugerea drumurilor, eroziunea terenului agricol, depunerea de poluanţi pe terenul adiacent râului etc. Viitura din iunie 2016 în bazinul hidrografic al râului Trotuş a produs degradarea până la distrugerea totală a peste 318 km de lucrări hidrotehnice de apărare a malurilor. Viitura a produs degradarea a 1337 ha de teren agricol, degradarea a 355 km de drum şi 180 de poduri. Viitura a afectat peste 376 de locuinţe în zona studiată, din care a distrus 76.*
Cuvinte cheie: debite, inundaţie, apărare de mal, drum, pod

INTRODUCTION

Globally, a series of climatic changes have occurred over the past 30 years and have significantly influenced the environment. Climate change has affected Romania's territory by influencing the monthly distribution of temperatures and precipitation. The high value of changing meteorological parameters creates a hydroclimatic risk in the evolution of flows and river levels. Hydrological

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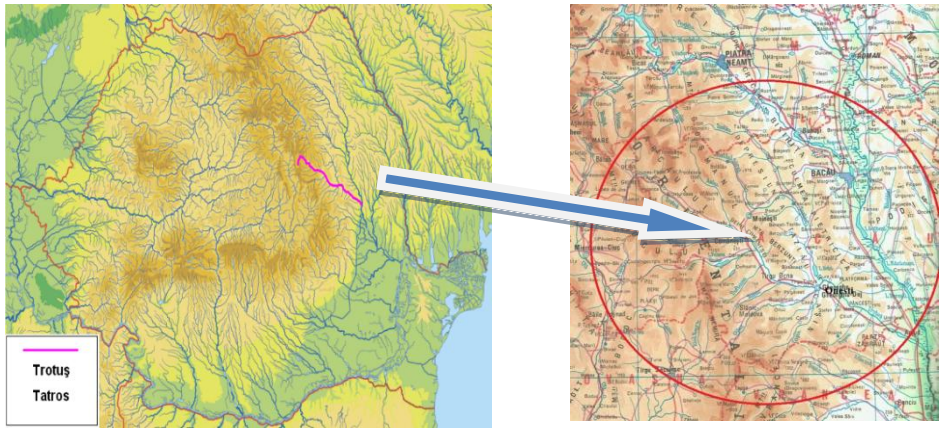
parameters directly influence the morphological evolution of the river and, implicitly, the stability of existing buildings in the riverbed and the riparian. The hydrological regime of the rivers in the Trotuş River Basin is characterized in the last period of time by the high frequency of the floods. Floods have caused major economic damage and human losses in recent years.

Elements of hydrological risk modify intensively the morphology of riverbed in cross-section and longitudinal section. The stability of bed constructions (bridges, adjustments) and shore (shore defence works, dikes) is influenced by the high frequency of floods. Hydrological risk elements affect the existing habitat in the minor and major river bed. The amount of flood damage has become very high, which has forced the allocation of significant investments to restore destroyed targets. Effects of changes in the riverbed are immediately detected, or may occur after a longer period of time.

STUDY AREA AND RESEARCH METHOD

The research was conducted in the Trotuş River basin. The hydrographic basin of the Trotuş River is located in the relief area of the Oriental Carpathians. The river passes through the geomorphological units of the Ciucului, Tarcăului, Comăneştii and Tazlău - Caşin depressions. The Trotuş River flows into the Siret River downstream of Adjud. The Trotuş River has the XII-1-69 cadastral code. The Trotuş River has its springs in the Ciuc Mountains at an altitude of 1360 m. The course of the river has a main north-south direction.



a b

Fig. 1 Framing of the study area in the Siret catchment area: a - location of the area on the territory of Romania; b - the location of the area in the East Carpathians.

The inhabitants of the Trotuş River on the sector considered are (Ujvari I., 1972):
- on the right: Ciugheş, Sulita, Ciobănuş, Uz, Dofteana, Slănic, Oituz, Caşin;
- on the left: Brusturoasa, Agăş, Ciungi, Asău, Urmeniş, Vâlcele, Tazlău.

The hydrographic basin of the Trotuş stream has an area of 4.440 km^2. The length of the river is 158 km. The average altitude of the river basin varies from springs to springs in the range 1140 - 734 m. The Trotuş River and the tributaries are

monitored by 21 hydrometric stations. Monitoring parameters have been determined flow parameters (liquid and solid flows, levels, ice, etc.) for a period of 40-60 years (table 1, ABA Bacau 2016).

Climatic, hydrological, hydraulic, topographic, geotechnical studies were collected and elaborated in the area of analysis. The state of exploitation of shore regulation and defence buildings was analyzed by technical expertise and field inspections. Research has included meteorological and hydrological data over a period of about 50 years. Several aspects have taken place over 10 to 25 years. The theoretical and experimental research was also based on a series of technical expertise produced after the floods.

Primary data was processed using the statistical calculation programs and the hydrological and hydraulic calculation programs applicable to this study.

RESULTS AND DISCUSSIONS

The study of the degradation of the buildings located in the riverbed and the riparian area was carried out on the watercourses in the Trotuş river basin.

Hydrological parameters were taken from hydrometric stations located in characteristic sections in the Trotuş River basin and its tributaries (tab. 1). The data from the hydroclimatic risk periods were analyzed by considering variable time periods (10-65 years). The analysis considered the average, maximum and minimum multiannual flows between 1950 and 2016 (Avram, 2016). The research analyzed the variation of the average and maximum annual flows from the multiannual average. The frequency of overflowing flows with the probability of calculation on the studied rivers was also analyzed.

Table 1

The hydrometric monitoring network of the Trotuş catchment area (A.B.A. Siret, 2016)

Nr. crt.	Hidrometric station	Area HB (km^2)	H_m (m)	Q_{med} (m^3/s)	Q_{max} (m^3/s)	$Q_{max, p}$ (m^3/s)			
						1%	2%	5%	10
1	Lunca de Sus	88	1140	0.805	23.2	210	165	113	77,5
2	Ghimeț-Făget	381	1116	3.65	127	500	410	300	220
3	Goioasa	781	1052	6.59	353[1]	750	625	460	340
4	Tg. Ocna	2091	924	17.5	1490[2]	1200	1025	795	625
5	Oneşti	2836	830	25.1	2294[3]	1620	1390	1075	840
6	Vrânceni	4092	734	35.0	2845[4]	2345	2095	1580	1255

H_m - Average altitude

From the analysis of the multi-annual average flow values, with a seasons distribution, the following aspects can be mentioned (Avram, 2016):

- the minimum flow takes place in winter and autumn, where the value of the winter flow is 8.7 - 11.6% of the annual volume and the autumn 12 - 15.5%; the two periods accumulate over 23% of the annual flow;

- the maximum flow occurs in spring and summer, where the value of the spring flow represents 39.9 - 49.3% of the annual volume and the summer season 27.1 - 34.7%; the two periods accumulate over 60% of the annual volume.

Flood from 02.06-05.06.2016 in B.H. Trotus was formed following the precipitations that fell on 02 and 03 06.2016. On 02.06.2016 there were precipitations with values above 80 L/m^2. Precipitation continued on 03.06.2016 and on the night of 3-4.06.2016, where the peak reached 40 L/m^2. The analysis on the studied area shows that large quantities of precipitation accumulated throughout the period 02.06.-03.06-04.06.2016. The measured values at the meteorological stations were 108 L/m^2 at Goioasa, 82.9 l/m^2 at Onesti, 68.1 L/m^2 in Sulta, 87.5 L/m^2 in Cremenea, 61.4 L/m^2 at Ciresoaia , 93.6 L/m^2 in Tazlau, 85.9 L/m^2 in Lucăceşti on the Tazlăul Sărat River, etc. (ABA Bacau, 2016).

Table 2

Parameters of floods formed in BT Trotuş in the period 02.06-04.06.2016

Nr. crt.	River	Hidrometric station	H_{max} (cm)	*p* (%)	*Pp.tot* l/m^2	*Q* (m^3/s)
1	Trotuş	Tg. Ocna	382	5-10	48,5	686
2		Oneşti	455	10	82,9	846
3		**Vrânceni**	525	**1**	58,3	**2525**
4	Asău	Asău	220	20	71,1	89,0
5	Slănic	Cireşoaia	260	5	61,4	118
6	Dofteana	Dofteana	285	5	64,6	134
7	Tazlău	**Helegiu**	410	**2**	69,6	**1280**
8	Tazlăul Sărat	**Lucăceşti**	360	**3**	85,9	**342**

H_{max}- Water share; p - probability; Pp.tot - Total rainfall

a b

Fig. 2 Degradation on the Asau River in June 2016: a - shore erosion; B- defense of damaged riverbank in the locality of the locality (A.B.A. Siret, 2005).

The analysis of the data results in the delimitation of an area with exceptional precipitation and flows located in the Tazlău Depression and on the Trotuş corridor downstream of the Tg Ocna section. The flood flows in this area have shown probabilities of exceeding the calculation values for defence constructions (tab. 3).

a b

Fig. 3 The evolution of the flood in June 2016 on the river Caşin in the commune of the Monastery of Caşin: a-shore erosions; b-degradation of roads and dwellings (Avram M., 2016)

Table 3

Shore defense works works degraded by floods in June 2016 in the Basin Hydrographic River Trotuş, Bacau county (selection Synthesis report - I.S.U Bacau, 2016)

Nr. crt.	Name of the work/county	**Location**	**Date[1]**	**EVD** (Tho. lei)	**Impaired capacities**
1	Regularization of Tazlău Sărat R. Zemes county	Zemes	02-04 .06.2016	27,00	Concrete support wall L=40m destroyed concrete pore L=60 m
2	Arrangement of the Slănic creek, Slănic Moldova and Tg. Ocna (PIF 2009)	Slanic Moldova	02-04. 06.2016	920,00	Wall damaged damage over a length of approx. 220 m
3	River Regularization Trotuş Palanca commune	Palanca	02-04. 06.2016	728,0	Defense of the left bank of gabons on L = 260 m
4	Landscaping of Trotuş and tributaries Object Brusturoasa, Camenca	Brusturosa	02-04. 06.2016	315,0	Defense of gabion shore on L = 80 m; 2 bottom thresholds
5	Arrangement of the Trotus river River and tributaries, Object Brusturoasa – Camenca river (PIF 2015)	Brusturosa	02-04 .06.2016	916,0	Gabion mattresses - 194 pcs. Gabions - 5 pcs. Reproduction of the whale-L = 1850m
6	Regularization river Helegiu in Helegiu commune (PIF 2005)	Helegiu	02-04 .06.2016	1069,0	Reprofilation of the bed l = 475 m, consolidation on L = 650 m; Shore consolidation L = 150 m

Date1 - the date the calamity occurred. EVD - Estimate value damage (impaired capacity)

The inventory of the July flood damages on the Trotuş River highlighted the large number of destroyed social and economic objectives. The works of regulating the Trotus river bed and the defences of the shore were partially and totally degraded on a large scale (synthesis, tab. 4) (Avram, 2016; ABA Siret, 2016).

Table 4

The main damages caused by floods in Trotuş Hydrographic Basin,in the years 1991-2016 (A.B.A. SIRET, 1991-2016, Avram M., 2017)

Nr. crt.	Object name	1991	2004	2005	2016
1	Impaired or destroyed hydrotechnical works (km)	5,64	13,4	35.46 out of which 28.61 in the area considered	318.14 in Bacău County
2	Dwellings and households affected / destroyed by floods (no)	2602	25/ 762	3149/922	376/64
3	Agricultural land flooded (ha)	11.460	280	5453,5	1337
4	Flooded / destroyed roads (km)	15	26	83,75	355
5	Flooded/destroyed bridges (pieces)	32	309	117	180
7	Flooded economic targets (no.)	11	9	51	4
8	Dead people (no.)	76	3	5	1

The hydrotechnical works were strongly affected by the 2016 floods in the Trotuş catchment area, where 318.14 km of partially and totally degraded works in Bacău County (ABA Siret, 2016) centralized.

CONCLUSIONS

1. The territory of the hydrographic basin Trotuş has been affected in the last 25 years by disastrous hydrological phenomena, which have greatly influenced the hydrotechnical regulation and shore defence.
2. The floods produced in June 2016 on the Trotuş River and its tributaries recorded flows with probabilities of 5 ... 1%, where the effects were extremely destructive on the riverbed and the riparian.
3. The degradation of shore regulation and defence works registered the highest value in the calendar period 1991-2016 (about 318 km in Bacău County).

REFERENCES

1. **Avram M., 2016** - *Cercetări privind impactul factorilor de risc hidroclimatic asupra proiectării, tehnologiilor de realizare şi exploatării lucrărilor de regularizare a râurilor - Studiu de caz. Raport 2 de cercetare.* Universitatea Tehnică „Gheorghe Asachi" din Iaşi.
2. **Luca M., Stoenescu, I.**, **2007** - *Current Issues Concerning Regularization Works Behavior under Disaster Conditions,* International Conference „Disaster and Pollution Monitoring – IC.DPM. 3". 1-2 nov. Iaşi, pp. 93-98.
3. **Luca M., Avram M., Lateş I., 2016** - *The influence of hidroclimatic risk factors on the evolution of natural sites*. Lucrări Ştiinţifice Seria Horticultură, An LIX, 59 (1)/2016, Universitatea de Ştiinţe Agricole şi Medicină Veterinară Iaşi, p. 207-212.
4. **Ujvari I.**, **1972** - *Geografia apelor României.* Edit. Ştiinţifică, Bucureşti.
5. **Vamanu E., Olariu P., 2002** - *Riscuri hidroclimatice în Spaţiul hidrografic Siret în contextul modificărilor geografice. Culegere de lucrări,* Ses. Ştiinţ. INMH Bucureşti.
6. ******* A.B.A. SIRET-Bacău - *Rapoartele de sinteză privind apărarea împotriva inundaţiilor, fenomenelor hidrometeorologice periculoase, accidentelor la construcţii hidrotehnice şi poluărilor accidentale din judeţul Bacău*, pentru anii 1991, 2004, 2005, 2010, 2012, 2016.

BIOSORPTION POTENTIAL OF VARIOUS WASTE BIOMASSES FOR Cu(II) IONS REMOVAL FROM AQUEOUS SOLUTION

POTENȚIALUL BIOSORPTIV AL UNOR DEŞEURI DE BIOMASĂ PENTRU ÎNDEPĂRTAREA IONILOR DE Cu(II) DIN SOLUȚII APOASE

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Abstract. *In this study, was analyzed the biosorption potential of some waste biomasses in the removal process of Cu(II) ions from aqueous solution. Three types of biosorbents have been used in experiments, namely: marine algae waste, mustard waste and lignin. All these materials are resulted from different industrial activities, and their use for the metal ions removal from aqueous solution is in agreement with the principles of circular economy. The experimental results obtained for the influence of initial Cu(II) ions concentration and contact time on the removal efficiency from aqueous media were modelled using various isotherm and kinetics models. The parameters obtained from modelling have permitted the evaluation of biosorptive potential of these three types of waste biomasses in the removal processes of Cu(II) ions from aqueous solution.*

Key words: metal ions, waste biomass, biosorption, aqueous solution.

Rezumat. *In acest studiu a fost analizat potențialul biosorptiv al unor deşeuri de biomasă, pentru îndepărtarea ionilor de Cu(II) din soluții apoase. Trei tipuri de biosorbenți au fost utilizați pentru realizarea experimentelor, şi anume: deşeuri de mustar, deşeuri de alge marine şi lignină. Toate aceste materiale rezultă din diferite activități industriale, iar utilizarea lor pentru îndepărtarea ionilor metalici din soluții apoase este în concordanță cu principiile economiei circulare. Datele experimentale obținute în urma studiului influenței concentrației inițiale a ionilor de Cu(II) şi a timpului de contact asupra eficienței îndepărtării acestuia din medii apoase au fost modelate utilizând diferite modele cinetice şi ale izotermelor de biosorpție. Parametrii obținuți în urma modelării au permis evaluarea potențialului biosorptiv ale celor trei tipuri de deşeuri de biomase în procesele de îndepărtare a ionilor de Cu(II) din soluții apoase.*

Cuvinte cheie: ioni metalici, deşeuri de biomasă, biosorbție, soluții apoase.

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INTRODUCTION

Copper is a heavy metal with large industrial applications, such as electroplating, mining, alloys and conductors manufacturing, automobiles industry, pigments and painting, etc. But regardless of the industrial activity in which it originates, the directly discharge of the industrial effluents in the environment is prohibited by current legislation. This is because the copper is non-biodegradable and at high concentration is harmful for humans and other life forms (Barakat, 2011; Fu and Wang, 2011).

The retention of metal ions from aqueous solution on solid materials has begun to play an important role in the pollution control of industrial effluents, in special when these materials are inexpensive (Demirbas, 2008; Barakat, 2011). Thus, numerous studies from literature describe the utilization of various low-cost materials (such as peat, algae biomass, various agricultural residues, industrial by-products, etc.) (Gogate and Pandit, 2004; Demirbas, 2008) for the Cu(II) ions removal from aqueous media. The main advantages for the utilization of such materials in the metal ions biosorption are: (i) their very low cost and large availability, (ii) requires only few steps for the preparation and (iii) do not require complicated regeneration procedures (Demirbas, 2008). But, most of these low-cost materials have already other consecrated economic uses, which make that their eventual utilization as biosorbents to required rigorous economic and technological arguments. From this perspective, more appropriate could be the use of waste biomasses for the removal of metal ions from aqueous effluents. In this case beside that such utilization is in agreement with the principles of circular economy, can also be an ecological alternative for the environment protection.

In this study, the biosorption potential of some waste biomasses for Cu(II) ions removal from aqueous solution was examined in batch systems. The waste biomasses used for the experiments have been: marine algae biomass, mustard waste biomass and lignin, and these are derived from biofuels production, or pulp and paper manufacturing industry. The experimental data obtained from the influence of initial Cu(II) ions concentration and contact time on the biosorption efficiency on each type of biomass were modelled using two isotherm models (Langmuir and Freundlich) and two kinetics models (pseudo-first order and pseudo-second order). The parameters calculated for each model have allowed the evaluation of biosorption potential of these three types of waste biomasses in the removal processes of Cu(II) ions from aqueous solution.

MATERIAL AND METHOD

The chemical reagent used for experiments were of analytical grade and were used without further purifications. The stock solution of Cu(II) ions (10^{-2} mol Cu(II)/L) was prepared from copper sulfate dissolving in distilled water. The working solutions were freshly prepared by dilution from the stock solution. 0.1 N HNO_3 solution was used for the adjusting of initial solution pH at desired value (6.0). The waste biomasses used as biosorbents in this study were obtained as follows: marine algae biomass and mustard

waste biomass were prepared from marine algae biomass and mustard biomass after oil extraction in a Soxhlet extractor with n-hexane for 24 hour, while the lignin was obtained by precipitation, in acid media, from black liquor resulted from woodworking. All the biosorbents were dried in air at 50-55 °C, mortared and stored in desiccators for further use. The biosorption experiments were performed in batch systems, at room temperature (22 ± 2 °C), by mixing biosorbent samples (0.125 g) with 25 mL of Cu(II) ions solution of known concentration, in 100 mL conical flasks. The influence of initial Cu(II) ions concentration was studied in the concentration range between 12.41 and 257.54 mg/L and a contact time of 24 hours. In case of contact time, the same amount of biosorbent (0.125 g) was mixed with 25 mL of 51.51 mg Cu(II)/L solution for different times intervals (between 5 and 180 min). At the end of biosorption experiments, the phases were separated by filtration, and the Cu(II) concentration in solution was analyzed spectrophotometrically with rubeanic acid (Digital Spectrophotometer S104 D, 1 cm glass cell, λ = 390 nm, against blank solution). The biosorption capacity (q, mmol/g) and percents of removal (R,%) of each waste biomass for Cu(II) ions were calculated according with their definition.

The mathematical equation of the isotherm models (Langmuir and Freundlich) and kinetics models (pseudo-first order and pseudo-second order) used for the modeling of the experimental data were taken from the literature (Gerente *et al.*, 2007).

RESULTS AND DISCUSSIONS

Our previous studies (Bădescu *et al.*, 2015; Todorciuc *et al.*, 2015) have shown that the optimum initial solution pH for the Cu(II) biosorption on these waste materials was around 6.0. Even if in case of marine algae waste biomass and mustard waste biomass the initial pH established as optimum was 5.5, the final pH measured after biosorption was around 6.0. In order to evaluate the biosorption potential of these three waste biomass for Cu(II) ions, this value of pH was maintained, in all the experiments.

The effect of initial Cu(II) ions concentration on the biosorption performances of considered waste biomasses is illustrated in figure 1. As can be observed, the increase of Cu(II) ions concentration determined the increase of the biosorption capacities of each biosorbent, and follows the order: lignin > mustard waste > marine algae waste.

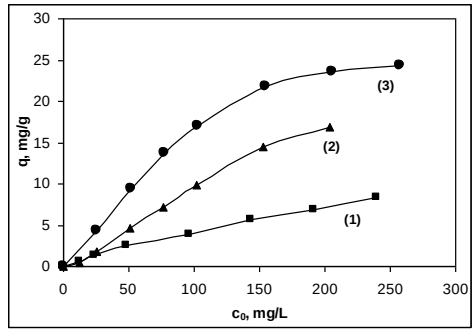
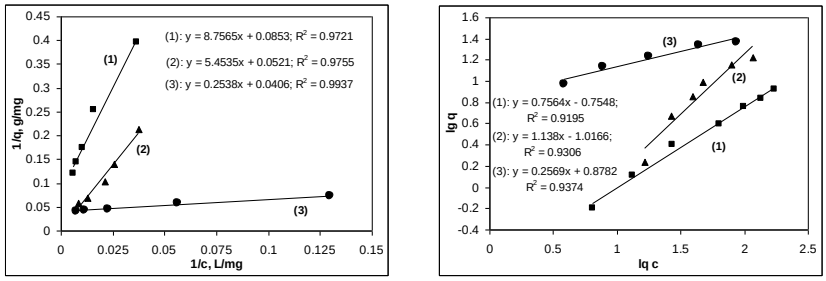


Fig. 1 Influence of initial Cu(II) ions concentration on the biosorption efficiency on (1) marine algae waste, (2) mustard waste and (3) lignin.

Such behavior is a consequence of the fact that the increase of initial Cu(II) ions concentration determine the increase of collision probability between metal ions and functional groups of biosorbents (Cruz *et al.*, 2004). On the other hand, the higher biosorption capacity of lignin for Cu(II) ions in comparison with marine algae waste and mustard waste, indicates that this biosorbent has a high number of functional groups, which are available for interactions with metal ions from aqueous solution.

In order to obtain a quantitative measure of the biosorption potential of these three biosorbents for Cu(II) ions, the experimental data were analyzed using Langmuir and Freundlich isotherm models. The linear representations of each isotherm model are presented in figure 2, and the calculated isotherm parameters are summarized in table 1.



(a) (b)

Fig. 2 Linear representations of Langmuir (a) and Freundlich (b) isotherm models for Cu(II) biosorption on (1) marine algae waste, (2) mustard waste and (3) lignin.

Table 1

Isotherm parameters for Cu(II) ions biosorption on the studied biosorbents

Isotherm model(*)		Marine algae waste	Mustard waste	Lignin
Langmuir	R^2	0.9721	0.9755	0.9937
	q_{max}, mg/g	11.7233	19.1938	24.6305
	K_L, g/L	0.0097	0.0095	0.1599
Freundlich	R^2	0.9195	0.9306	0.9374
	1/n	0.7564	1.1380	0.2569
	K_F, g/L	5.6859	0.0962	7.5544

On the basis of correlation coefficients (R^2) it can be noted that the Langmuir model has the higher applicability in the description of Cu(II) ions biosorption on the considered biosorbents. Therefore, the biosorption process takes place until to the formation of monolayer coverage on the biosorbent surface (Gerente *et al.*, 2007). Also, the maximum biosorption capacity (q_{max}, mg/g) increase in the order: marine algae waste < mustard waste < lignin, in agreement with the previous observations, which have suggest the higher biosorption potential of lignin in comparison with the other two biosorbents.

The second parameter, important in the evaluation of biosorption potential is the contact time. The experimental results obtained at the study of contact time effect on the biosorption efficiency of Cu(II) ions on considered biosorbents is presented in figure 3.

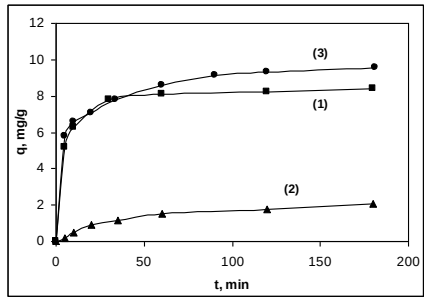
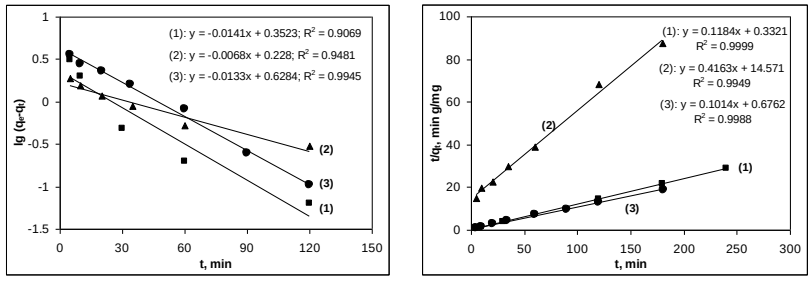


Fig. 3 Influence of contact time on the Cu(II) biosorption efficiency on (1) marine algae waste, (2) mustard waste and (3) lignin.

The experimental results from figure 3 indicates that the biosorption efficiency of Cu(II) ions on considered biosorbents increase with the contact time, and attains the maximum after 30 min in all cases. In this time interval the most efficient biosorbent is lignin (R > 83 %), followed by marine algae waste (R > 79 %) and mustard waste (R > 11 %).

The kinetics modelling of the experimental data illustrated in figure 3 was done using the pseudo-first order and the pseudo-second order kinetics models. The linear representations of these two models are illustrated in Fig. 4, and the calculated kinetics parameters are summarized in table 2.



(a) (b)

Fig. 4 Linear plots of pseudo-first order (a) and pseudo-second order (b) kinetics models, for Cu(II) biosorption on (1) marine algae waste, (2) mustard waste and (3) lignin.

Table 2

Kinetics parameters for Cu(II) ions biosorption on the studied biosorbents

Kinetics model		Marine algae waste	Mustard waste	Lignin
Pseudo-first order kinetics model	R^2	0.9069	0.9481	0.9945
	q_e, mg/g	2.2506	1.6904	4.2501
	k_1, 1/min	0.0061	0.0029	0.0057
Pseudo-second order kinetics model	R^2	0.9999	0.9949	0.9988
	q_e, mg/g	8.9459	2.4021	9.8621
	k_2, g/mg min	0.0163	0.0191	0.0152

It can be observed from figure 4 that the Cu(II) ions biosorption on all considered biosorbents is best described by the pseudo-second order kinetics model, and in consequence it can say that the biosorption process is limited by the chemical interactions between Cu(II) ions and functional groups of biosorbents, and these interactions involve two binding sites. On the other hand, comparing the values of the rate constants (Table 2) it can be noted that the Cu(II) ions are easier retained onto lignin, even if the difference between the rate constants calculated for this biosorbent and for the other two, is not significant.

CONCLUSIONS

1. The biosorption potential of some waste biomasses for Cu(II) ions removal from aqueous solution was examined in batch systems as a funtion of initial concentration and contact time.

2. The experimental results were modeled using two isotherm models (Langmuir and Freundlich) and two kinetics models (pseudo-first order and pseudo-second order)

3. The obtained results have indicate that lignin has the higest biosorption potential for Cu(II) ions from aqueous solution, in comparison with marine algae waste and mustard waste.

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REFERENCES

1. Barakat M.A., 2011- *New trends in removing heavy metals from industrial wastewater*. Arabian Journal of Chemistry, 4, 361-377.

2. Bădescu I.S., Negrilă L., Nacu G., Bulgariu L., 2015 - *Removal of Cu(II) ions from aqueous solution by biosorption on marine algae biomass*. Bulletin of the Polytechnic Institute of Iasi, LXI, 57-66.

3. Cruz C.C.V., da Costa A.C., Henriques C.A.O., Luna A.S., 2004 - *Kinetic modeling and equilibrium studies during cadmium biosorption by dead Sargassum sp. biomass*. Bioresource Technology, 91, 249-257.

4. Demirbas A., 2008 - *Heavy metal adsorption onto agro-based waste materials: A review*. Journal of Hazardous Materials, 157, 220-229.

5. Fu F., Wang Q., 2011 - *Removal of heavy metal ions from wastewaters: A review*. Journal of Environmental Management, 92, 407-418.

6. Gerente C., Lee V.K.C., Lee P., McKay G., 2007 - *Application of chitosan for the removal of metals from wastewaters by adsorption - mechanisms and models review*. Critical Reviews in Environmental Science and Technology, 37(1), 41–127.

7. Gogate P.R., Pandit A.B., 2004 - *A review of imperative technologies for wastewater treatment II: hybrid methods*. Advanced Environmental Research, 8, 553-597.

8. Todorciuc T., Bulgariu L., Popa V.I., 2015 - *Adsorption of Cu(II) from aqueous solution on wheat straw lignin: Equilibrium and kinetic studies*. Cellulose Chemistry and Technology, 49(5-6), 439-447.

ANALYSIS OF THE MOLDAVIAN PLATEAU POTABLE WATER SOURCES QUALITY PARAMETERS

ANALIZA PARAMETRILOR DE CALITATE LA SURSELE DE APĂ POTABILĂ DIN PODIŞUL MOLDOVENESC

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Abstract. *Potable water sources worldwide have started to decline in volume and deteriorate in quality. This situation is present in Romania, in the plain and plateau area of Moldova. This area has a small number of viable drinking water sources from groundwater, rivers and lakes. Rural settlements in this area are the most affected by the lack of unpolluted water sources. The natural pollution occurs due to the nature of the rocks from the water sources' site, where the presence of sulphates and chlorides is predominant. The researches show exceedings to almost the entire nutrient group. The highest concentrations of nitrates are recorded in the Jijia and Bahlui hydrographic basin. The analyses carried out in Iaşi area regarding the groundwater quality parameters have highlighted high concentration of sulphates. This exceeds the standardised value so that the groundwater source is improper for catchment and usage as drinking water for the population.*
Key words: water scarcity, pollution, hydrological parameters

Rezumat. *Sursele de apă potabilă la nivel mondial au început să se diminueze ca volum, iar calitatea să se degradeze. Această situaţie este prezentă în România, în zona de câmpie şi podiş a Moldovei. Acest areal dispune de un număr redus de surse de apă potabilă viabile provenite din subteran, râuri şi lacuri. Localităţile rurale din acest areal sunt cele mai afectate de absenţa surselor de apă nepoluate. Poluarea naturală este dată de natura rocilor din amplasamentul surselor de apă, unde prezenţa sulfaţilor şi clorurilor este preponderentă. Cercetările efectuate arată depăşiri la aproape toată grupa de nutrienţi. În bazinele hidrografice ale râurilor Jijia şi Bahlui se înregistrează concentraţiile cele mai mari de azotaţi. Analizele efectuate în zona municipiului Iaşi privind parametri de calitate ai apei subterane au evidenţiat concentraţia ridicată în sulfaţi. Aceasta depăşeşte valoarea standardizată, astfel că sursa subterană este improprie captării şi folosirii ca apă potabilă pentru populaţie.*
Cuvinte cheie: reducerea surselor, poluare, parametri hidrologici

INTRODUCTION

The quality parameters of water sources from the Moldavian Plateau are a constant concern in the water supply systems management. The way in which available water resources are being used, the human activity carried out and the natural factors present in the catchment area directly affect the quality of water

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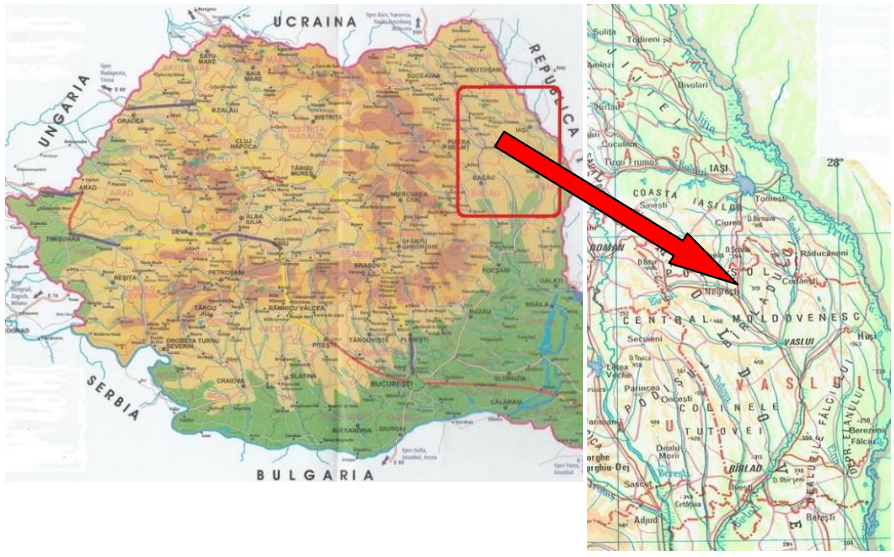
from aquifers and rivers downstream sections.

Among the elements with degradation potential for water quality parameters it can be mentioned: mining activities, action of hydrotechnical works (river regulation works, dams, dikes, micro hydro power plants, intakes), wastewater discharge from localities and industry, road structure works, the animal husbandry farms density, the use of pesticides and fertilisers in agriculture, the lack of rural sewerage systems, inadequate waste management etc.

MATERIAL AND METHOD

The research material consists of the characteristics which define the Moldavian Plateau (fig.1) and how they affect the water sources confined in this geographic space.



a b

Fig. 1 Framing of study areas in the Moldavian Plateau and Plain:
a - location of the study area; b - physical map of the study area

In the analysed area relief forms resulted from erosive slope processes (landslides, layers caving-in, drainage or torrential bodies formation in the shape of ravines) are found, which chemically influence the quality of confined underground and surface water.

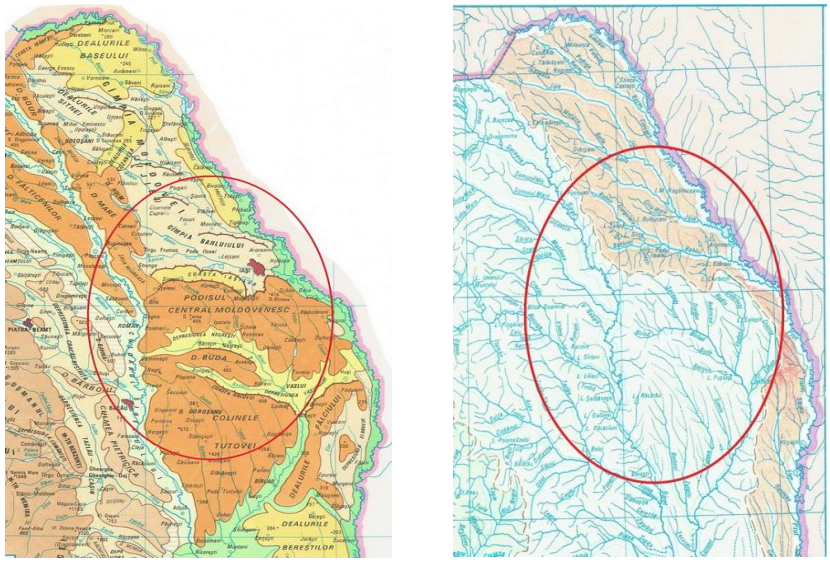
The Moldavian Plateau geomorphology determines the collection and chemical quality of water from groundwater and surface sources. In the analysed area, the relief consists of "low hills", which are hilly surfaces characterised by altitudes less than 200 m and "medium hills", for which the altitude is between 350 and 200 m (fig. 2). The hills are bordered by numerous versants with ridge aspect which allow errosion formation and the transport of alluvial material (Encicl. Geogr., 1982).

The research method aims to highlight and analyse the parameters which influence the water sources quality in the studied area. The geotechnical and

hydrogeological characteristics of the Moldavian Plateau influence the water resources from their way of formation, through usable volumes, physico-chemical and bacteriological parameters. Geotechnical characteristics also influence the depth of catchment facilities, the complexity of treatment plants, the investment costs and the final price of water distributed to consumers.

RESULTS AND DISCUSSIONS

The Moldavian Plateau area extends over two large hydrographic basins: Prut and Siret (fig. 2). The only surface water sources with acceptable quality and quantity parameters in this area are Prut, Moldova and Siret rivers. However, these rivers do not meet quality requirements imposed by the standards in force in all the catchment sections or during a whole year. Groundwater and surface water resources in the Prut River basin are characterised by a significant and variable content of chemicals.



a b

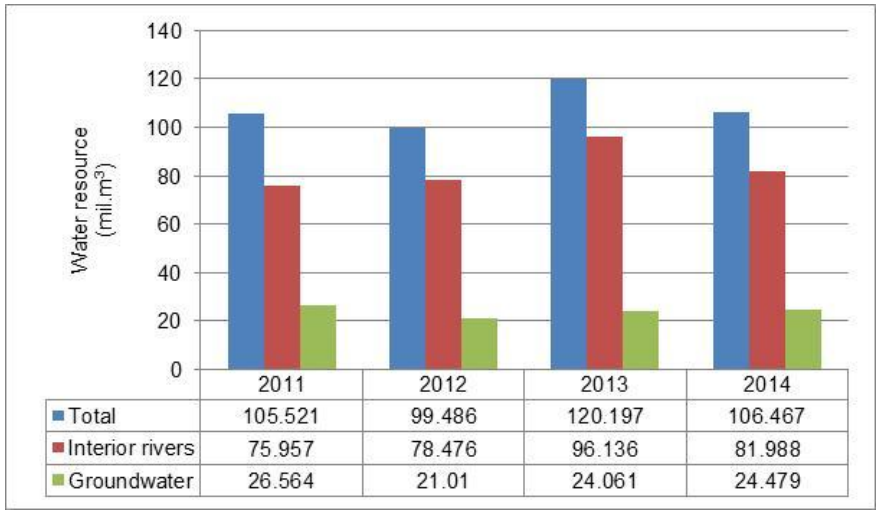
Fig. 2 Study area framing in the Moldavian Plateau and Plain relief and hydrography: a – relief features in the study area; b – study area hydrography.

The groundwater and surface water quality parameters result from the monitoring activity. The data from continuous monitoring of water sources indicates the state, consequences and possible directions of action which can be taken for the qualitative remediation of water bodies. Monitoring results often reveal the risk situation in which water sources fall, largely due to the degradation of quality parameters under natural and anthropic actions. The lack of continuous surveillance of groundwater and surface water leads to the amplification of this phenomenon.

Prut - Bârlad hydrographic area is characterised by surface water sources amounting to 3661 mil. m^3/year, of which approximately 960 mil. m^3/year can be used. It includes 72 storage lakes with a total usable volume of 614.85 mil. m^3. The estimated groundwater resources are 251.4 mil.m^3, of which phreatic ones total 35.7 mil. m^3 and the depth ones 216.7 mil. m^3. The water volumes confined in this hydrographic space are low and have an uneven temporal and spatial distribution, which is below the national average (ABA Prut-Bârlad, 2016).

Prut River basin groundwater is found in sandy deposits with clayey intelayers and gypsic horizons. The lithological conditions, correlated with the presence of salts from soils washed by precipitation and infiltrated in the groundwater, determine the groundwater's sulphated character, with high mineralization and hardness degree. Some water resources are found in river beds but have a low flow rate.

Approximately 75% of the available water resources in Iaşi County come from surface sources and the remaining 25% from groundwater sources. The total potential and technical resources usable from 2011 to 2014 varied annually with values between 6 and 20 million m^3 (fig. 3).



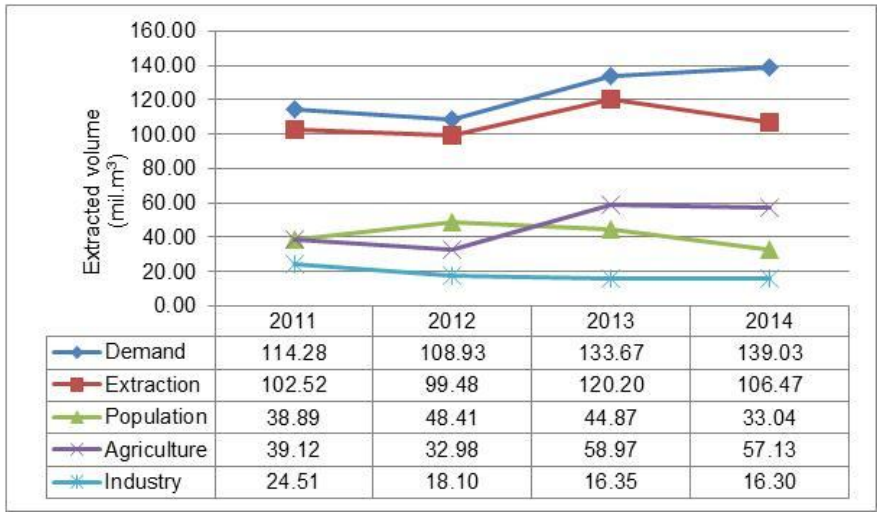
	2011	2012	2013	2014
Total	105.521	99.486	120.197	106.467
Interior rivers	75.957	78.476	96.136	81.988
Groundwater	26.564	21.01	24.061	24.479

Fig. 3 Iaşi County - available water resources (ABA Prut-Bârlad, 2016)

The year 2013 is marked by the largest recorded value of the total water resource used in the urban and rural water supply systems. However, out of 120.197 million m^3, only 24.061 million m^3 are groundwater resources, the remaining 96.136 million m^3 coming from interior rivers. In all the years studied it is noticed that the volume of groundwater varies in a tight range compared to the evolution of volumes from interior rivers. This phenomenon occurs irrespective of the total water resource's ascending or descending evolution.

The water demand evolution during 2011-2014 shows a steady increase, with

a slight decrease in 2012. However, the extraction fails to cover the water requirement of all consumption categories. The water volumes extracted for population and industry are decreasing. At the same time, those for agriculture have increased by about 45% in 2013 and have remained constant around 57-58 million m^3. Figure 4 shows the evolution of water volumes extracted for population, agriculture and industry. The values evolution is justified on the one hand by investments in water infrastructure, which have significantly reduced the losses (reflected in the decrease in consumption for the population and industry) and on the other hand by the irrigation systems' expansion which have led to increased agricultural water consumption.



	2011	2012	2013	2014
Demand	114.28	108.93	133.67	139.03
Extraction	102.52	99.48	120.20	106.47
Population	38.89	48.41	44.87	33.04
Agriculture	39.12	32.98	58.97	57.13
Industry	24.51	18.10	16.35	16.30

Fig. 4 Volumes extracted by activity type (ABA Prut-Bârlad, 2016)

From the total of seven groundwater bodies monitored, only four are characterised by good chemical status, the other three having a poor chemical status. In terms of surface water, the quality of lakes' water is classified as "not meeting the quality objective" in a moderate degree, and in terms of ecological potential, only two water bodies out of six "meet the quality objective". The ecological status of Prut basin rivers, assessed in relation to the total length, shows that 48.85% are in good ecological status, 36.12% in moderate status and 20.03% in poor status (Administrația Națională "Apele Române", 2016).

The lack of investments in the extension of sewerage systems and in the upgrading and rehabilitation works for the waste water treatment plants is felt significantly in the Moldavian Plateau space. These elements are risk sources which affect the quality parameters of water confined in the study area. Prut - Bârlad hydrographic basin is characterised by a high risk potential caused by the human settlements not connected to sewerage systems (77.03% of the 1341 identified negative impact factors), followed by the risk of waste water evacuation

from treatment plants (6.64%) (ABA Prut-Bârlad, 2016). In addition to these hazards, geomorphological pressure factors (regulation works, dykes, dams construction) sum up to 14.62% of the 1341 identified factors.

CONCLUSIONS

1. Worldwide drinking water sources have begun to decrease in volume and their quality to deteriorate, situation also present in Romania, especially in the Moldavian Plateau.

2. Human factors, such as the lack of sewerage systems, the animal husbandry farms development, and natural ones, such as the characteristics of the rocks found in the establishment areas, have led to significant changes in the quality of water bodies with potential for human consumption.

3. The situation of drinking water sources in the Moldavian Plateau is a continuous challenge in the water supply systems management, in terms of optimal use of good quality available volumes.

4. In the context of the continuous development of the water supply systems in the rural areas of the Moldavian Plateau, it is necessary to make complementary investments in centralised sewage systems and waste water treatment plants in order to protect the quality parameters of water sources.

REFERENCES

1. **Alexandrescu Simona Adina, 2013** - *Contribuții la optimizarea hidraulică şi funcțională a sistemelor regionale de alimentare cu apă*. Teză de doctorat. Universitatea Tehnică „Gheorghe Asachi" din Iaşi.
2. **Alexandrescu Simona Adina, Luca M., Mănescu Andreea, 2012** - *The influence of drinking water quality on population health in the Moldavian area*. Conf. Ştiinț. „Present Environment and Sustainable Development", Vol. 6 nr. 2, pp. 159-166, ISSN (electronic) 2284 – 7820, ISSN (print) 1843-5971.
3. **Luca M., Tămăşanu F., Luca Al. L., 2010** - *Proiectarea sistemelor de alimentare cu apă în condițiile Podişului Moldovenesc*. Conferința Tehnico-Ştiințifică ARA „Dezvoltarea sistemelor de alimentare cu apă şi canalizare în comunități rurale", Bucureşti, 15-16 iunie 2010, pp. 15 - 20.
4. *****, 1982** - *Enciclopedia geografică a României*. Edit. Ştiinț. şi Encicl. Bucureşti.
5. ***** Agenția de Mediu Iaşi, 2015** - *Raport privind starea mediului în județul Iaşi*
6. ***** Administrația Bazinală de Apă Prut-Bârlad, 2016** - *Planul de management al spațiului hidrografic Prut-Bârlad-ciclul al II-lea 2016-2021*.
7. ***** Administrația Națională „Apele Române", 2016** - *Sinteza calității apelor din România în anul 2015 (extras)*, Bucureşti.

PROTECTION OF DISTRIBUTION NETWORKS WATER QUALITY

PROTECȚIA CALITĂȚII APEI POTABILE DIN REȚELELE DE DISTRIBUȚIE

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Abstract. *This paper presents a number of issues regarding the protection of drinking water quality in conveyance and distribution networks. Studies and research have shown that the physical and hydraulic integrity of the conveyance and distribution system influences the drinking water quality parameters. The state of pipelines, tanks, and hydraulic installations' physical integrity may influence the influx of contaminants into drinking water. The physical integrity factors considered in the research are: pipe material, geometric parameters, interior and exterior protection quality, corrosion, structural failure, degradation. The hydraulic integrity factors considered in the research are: variation of flow and pressure, velocity, sedimentation phenomenon, cavitation. Water integrity factors are: disinfectant dose, storage and movement time, physical and chemical reactions with the pipe, contaminant infiltration, age, biological stability. The case study for Iaşi city confirms the evolution of water quality parameters along the pipe network.*
Key words: physical integrity, hydraulic parameters, pollutants

Rezumat. *Lucrarea prezintă o serie de aspecte privind protecția calității apei potabile din rețelele de transport şi distribuție. Studiile şi cercetările efectuate au arătat că integritatea fizică şi hidraulică a sistemului de transport şi distribuție influențează parametrii de calitate ai apei potabile. Starea integrității fizice a conductelor, a rezervoarelor, a instalațiilor hidraulice pot influența afluxul de contaminanți în apa potabilă. Factorii de integritate fizică considerați în cercetare sunt: materialul pentru conductă, parametri geometrici, calitatea protecției interioare şi exterioare, coroziunea, cedări structurale, degradări. Factorii de integritate hidraulică considerați în cercetare sunt: variația debitului şi presiunii, viteza, fenomenul de sedimentare, cavitația. Factorii de integritate ai apei sunt: doza de dezinfectant, timpul de stocare şi mişcare, reacțiile fizico-chimice cu conducta, infiltrația contaminanților, vârsta, stabilitatea biologică. Studiul de caz întocmit pentru oraşul Iaşi confirmă evoluția parametrilor de calitate ai apei în lungul rețelei de conducte.*
Cuvinte cheie: integritate fizică, parametri hidraulici, poluanți

INTRODUCTION

Water supply systems provide the consumers' water demand, in accordance

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with the quality and quantity parameters imposed by the standards in force. Drinking water quality parameters are corrected when entering the system through treatment plants. Throughout their layout, in accordance with the networks' size and the recorded consumption, there can be found intermediate chlorination points within the storage tanks or directly into the pipeline distribution network.

Water supply distribution networks can have layouts covering thousands of kilometers, located both in urban and rural areas. Due to the extensive spatial distribution, the situations which can occur and influence the quality parameters of the water conveyed are extremely varied. Factors interfering with distribution networks can substantially change the characteristics of supplied water. An important requirement in the drinking water distribution management is the compliance with the quality parameters values required by the standards in force.

MATERIAL AND METHOD

The material used for research consists of the distribution network components with impact on the quality of drinking water conveyed to consumers. The pipeline distribution network is influenced externally and internally by a series of factors which can change water quality parameters locally and along the pipeline.

External factors which have an impact on water quality depend on the pipeline network's embedding surroundings through the rock's structure and physico - chemical parameters. Physical parameters consist of soil electric conductivity, cohesion and hydraulic conductivity. Chemical parameters include chemical conductivity and dissolved chemical content. Biological parameters interfere through areas with intermittent or continuous bacteriological potential. Other external factors include sewerage networks position in relation to water pipes, land use in the area, density of fittings, joints or branches and their degree of pipe sealing etc.

Internal factors which have an impact on water quality depend on the nature of the pipe material, the lack of disinfectants, the network running time and the consumption degree. These are elements which dictate the water age inside the pipes, the evolution of pathogens through virus and bacterial pollution, biofilm layer formation on pipe walls, the presence of organic or inorganic compounds which can react with the disinfectant used etc. Water quality must be constantly monitored. Delivering water which does not comply with the required standards is a major risk to the health of the population and can have serious consequences.

The research method analyses and evaluates the factors which affect drinking water quality parameters in distribution networks by using statistical calculation and graphic processing programs. The evolution of drinking water quality parameters transported through pipelines is analysed with appropriate computing software (eg EPANET), or developed in MATLAB computer programming environment.

RESULTS AND DISCUSSION

Water from the exterior can enter the pipe distribution network via various paths. The most common entering areas are pores, micro-cracks, cracks, fissures in pipe walls and related installations. Infiltrations from the embedding environment cause changes in the quality parameters of the water transported by pipeline to consumers. Contaminated water may come from damaged sewer networks,

uncontrolled discharges from animal husbandry farms, agricultural land chemical stress, seepage from domestic and industrial waste dumps, accidental pollution etc.

The main water quality parameters are established by standards and norms, in conjunction with those from the European Community. Permissible concentrations are regulated in Romania by Law 458/2002 (tab. 1) and other standards.

Table 1

Potable water quality parameters

Parameters	Unit	Permissible concentration
1. Organoleptic parameters		
Colour	-	Acceptable
Taste	-	Acceptable
Smell	-	Acceptable
2. Disinfection parameters		
Free residual chlorine	mg/L	0.1 – 0.5
3. Physico – chemical parameters		
Ammonium	mg/L	max. 0.5
Nitrite	mg/L	max. 0.5
Nitrate	mg/L	max. 50
Hardness	^{o} Ge	min. 5
Turbidity	UNT	max. 5
Sulphates	mg/L	max. 250
pH	pH units	6.5 – 9.5
Conductivity	µS/cm	max. 2500
Free cyanide	mg/L	max. 0.01
Sodium	mg/L	max. 200
Lead	mg/L	max. 0.01
4. Radioactivity parameters		
α global activity	Bq/L	max. 0.1
β global activity	Bq/L	max. 1
5. Microbiological parameters		
Escherichia coli	no./100 mL	0
Coliform bacteria	no./100 mL	0
Enterococci	no./100 mL	0
Clostridium perfringens	no./100 mL	0

S.C. APAVITAL S.A. Iaşi regional operator constantly monitors the quality of drinking water distributed, from source to consumer. Analyses are carried out at the water's entry point into the system, the treatment plant, the storage tanks, as well as at characteristic points on the distribution network. In addition to the water company's monitoring activity, Public Health Department supervises in parallel the water quality parameters provided to consumers.

The data gathered by DSP Iaşi from the analyses carried out to evaluate the quality parameters of the drinking water distributed through the regional water supply system can be found in figure 1. Parameters which showed non-compliant values were chemical and bacteriological ones. The analysis of radioactivity parameters did not show any values exceeding the permitted limits.

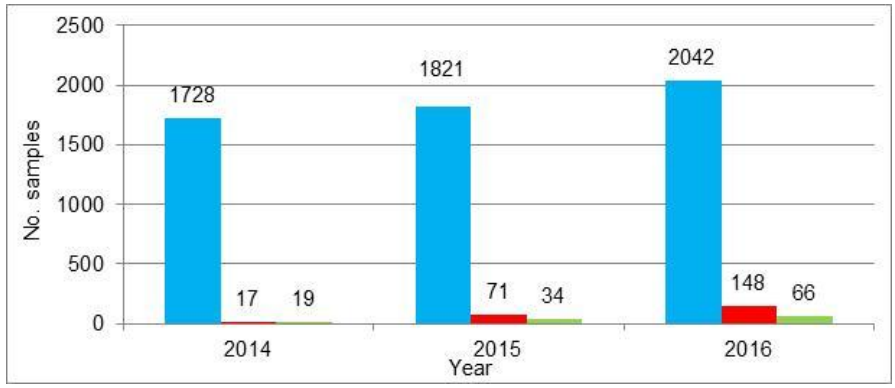


Fig. 1 Quality of drinking water in Iaşi County distribution networks (the color code represents: blue - the number of water samples, red - unsatisfactory chemical samples, green - unsatisfactory bacteriological samples (DSP Iaşi, 2017).

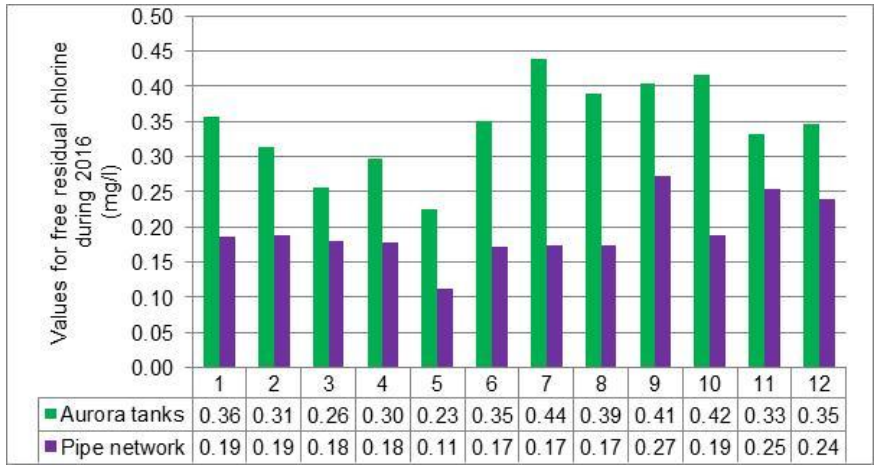
The number of samples taken during 2014 - 2016 has steadily increased. This is related to the expansion and establishment rate of water supply systems, especially in rural areas. The analyses revealed many unsatisfactory chemical and bacteriological samples. From 2042 samples taken in 2016, 7.25% did not meet the chemical requirements and 3.23 the bacteriological ones.

According to the Drinking Water Quality Law 458/2002, the permitted free residual chlorine value at the system's entry point is 0.5 mg/l and at the end of the distribution network is 0.1 mg/L. The studies show that some average monthly values obtained by the regional operator in Iaşi City in 2016 for the free residual chlorine parameter are below the permitted limit (fig. 2). Samples taken from Aurora tanks and distribution network are shown in parallel. The difference between the values obtained at the water departure point and those from the distribution network shows that there are reactions and processes inside the pipes which cause the disinfectant concentration to decrease. External contamination, disinfectant reactions with pipe material, accidental contamination, or water loss along the pipe lay-out are the most common factors affecting water quality.

Aurora tanks store the water that reaches Iaşi City from Timişeşti catchment fronts. The free residual chlorine values recorded during 2016 show compliance with the limits imposed by the Drinking Water Quality Law. The lowest value recorded is 0.23 mg/L, and the highest one is 0.44 mg/L. The water volumes distributed from Aurora tanks are chlorinated again, in order to bring the free residual chlorine value to 0.5 mg/L, according to the law.

The average values of free residual chlorine in the distribution network are very close to the minimum allowed by law. Towards the end of the distribution network there is an increased risk that the supplied water does not meet the required quality parameters. Figure 2 shows the value registered in May of 0.11 mg/L exceeds only by 0.01 mg/L the minimum allowed by Law 458/2002. The

highest registered value was 0.27 mg/L in September.



	1	2	3	4	5	6	7	8	9	10	11	12
Aurora tanks	0.36	0.31	0.26	0.30	0.23	0.35	0.44	0.39	0.41	0.42	0.33	0.35
Pipe network	0.19	0.19	0.18	0.18	0.11	0.17	0.17	0.17	0.27	0.19	0.25	0.24

Fig. 2 The evolution of free residual chlorine in the Aurora tanks and the distribution network of Iaşi City in 2016

Measurements conducted in one of the worst case operating points on the distribution network (fig. 3) show the values for the free residual chlorine parameter reach the lowest limit permitted by law for about half of the analysed samples. This requires the development of a research program to highlight the factors which influence the reduction of residual chlorine.

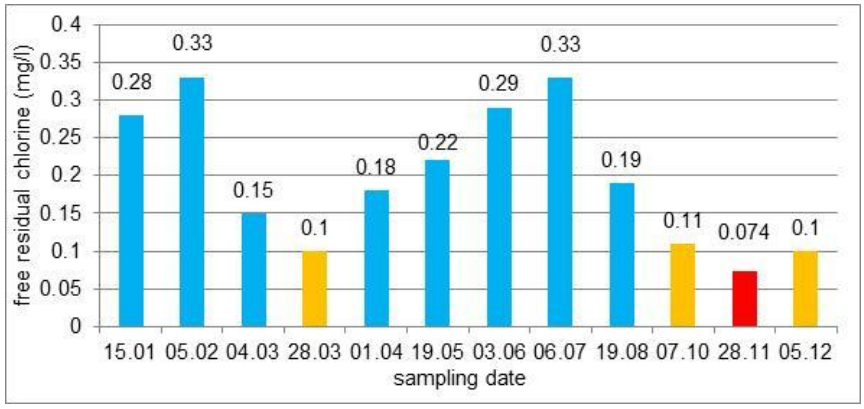


Fig. 3 Values for free residual chlorine in Piața Halei measuring point, year 2016 (the color code represents: blue – values permitted by law, orange – values close to the lowest permitted limit, red – values bellow the permitted limit)

Piața Halei monitoring point analysis show the variation in free residual chlorine concentrations ranges from 0.33 mg/L to 0.074 mg/L. In March, October

and December, values of 0.1 mg/L are recorded, and the November results indicate a value below the permitted potable limit.

Fluctuations of the free residual chlorine value show the action of some temporary factors which do not permanently affect the water quality. Since February, recorded values have declined from 0.33 mg/L to 0.1 mg/L in March, after which the chlorine concentration increases to a maximum of 0.33 mg/l in July. The same phenomenon also occurred in July, when values began to drop steadily to 0.074 mg/L in November, after which the free residual chlorine concentration began to rise again. This pattern suggests the presence of external contaminants, which penetrated the distribution network through the pipes' cracks and pores. Variations can also be caused by water losses along the pipe network up to the measuring point. These elements may cause a decrease in the disinfectant concentration which does not return to normal values until the disturbing factors are removed or remedied. All this confirms the necessity of field studies and research to highlight the factors influencing the reduction of residual chlorine on the transport and distribution network.

CONCLUSIONS

1. Studies and research have shown that the physical and hydraulic integrity of the transport and distribution pipe network significantly influences drinking water quality parameters.

2. The study shows an external and internal type of water contamination, where the external contamination is mainly caused by the infiltration of pollutants and the internal one is represented by the physical and chemical transformations occurring inside the pipes, tanks and hydraulic installations.

3. In-situ analyses have shown that disinfectant concentration decreasing may be the result of external contamination, but this may also be the result of disinfectant reactions with pipe walls and organic matter left in the water.

4. The absence of a residual disinfectant shows that the system's integrity has been compromised and the drinking water contamination rapidly occurs downstream to the consumer, without measures to restore the quality parameter.

REFERENCES

1. **Alexandrescu Simona Adina, Luca M., Mănescu Andreea, 2012** - *The influence of drinking water quality on population health in the Moldavian area*. Conf. Ştiinț. „Present Environment and Sustainable Development", Vol. 6 nr. 2, pag. 159-166.
2. **Mănescu Brînduşa, 2008** – *Managementul calității apei în rețelele de distribuție*. Teză de doctorat, Universitatea Tehnică de Construcții Bucureşti
3. **Țuțuianu Alina Elisabeta, 2013** – *Program de calcul pentru monitorizarea calității apei în rețelele de distribuție a apei*. Teză de doctorat, Universitatea Tehnică de Construcții Bucureşti.
4. **Varduca A., 1999** - *Monitoringul integrat al calității apelor*. Editura H.G.A., Bucureşti.
5. ***** Legea 458/2002** privind calitatea apei potabile.
6. ***** Ministerul Sănătății. Direcția de Sănătate Publică a Județului Iaşi, 2017** – *Raportul de activitate pentru anul 2016*.
7. ***** S.C. APAVITAL S.A., 2017** - *Raport de performanță 2016*.

LIFE CYCLE ASSESSMENT OF CORRUGATED BOARD PACKAGING

EVALUAREA CICLULUI DE VIAŢĂ A AMBALAJELOR DE CARTON ONDULAT

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Abstract. *In this paper corrugated board packaging life cycle was evaluated with life cycle assessment (LCA) methodology. Two LCA methods were considered for the evaluation: CML 2001-Jan.2016 and ReCiPe 1.08, both included in GaBi software. Results showed that corrugated board packaging has negative impacts on the environment even if there were registered low values. Elimination methods like landfilling and incineration of corrugated board packaging waste are increasing the negative impacts on the environment, while recovery of packaging waste and their use in the corrugated board production leads to the reduction of these impacts and natural resources conservation.*
Key words: corrugated board, environmental impacts, life cycle, packaging

Rezumat. *În această lucrare a fost evaluat ciclul de viaţă al ambalajelor din carton ondulat cu ajutorul metodologiei de evaluare a ciclului de viaţă (ECV). Pentru evaluare au fost luate în considerare două metode ECV: CML 2001-Ian.2016 şi ReCiPe 1.08, ambele incluse în instrumentul software GaBi. Rezultatele au arătat că ambalajele din carton ondulat au un impact negativ asupra mediului, chiar dacă s-au înregistrat valori scăzute. Metodele de eliminare, precum depozitarea şi incinerarea deşeurilor de ambalaje din carton ondulat, sporesc impactul negativ asupra mediului, în timp ce recuperarea deşeurilor de ambalaje şi utilizarea acestora în producţia de carton ondulat conduce la reducerea acestor impacturi şi conservarea resurselor naturale.*
Cuvinte cheie: carton ondulat, impacturi de mediu, ciclul de viaţă, ambalaje

INTRODUCTION

The pulp, paper and cardboard industry has gone through the past 200 years through periods of growth and decline in its various segments. At the beginning of mechanized production, the paper industry was dominated by countries such as France, Great Britain and Germany. Then in the 19th century the dominance was taken over by Northern American and Northern European countries and later by countries from the Southern hemisphere and the Far East (Ojala *et. al.*, 2013). The main production process of paper is unchanged for nearly 2000 years. The fibers are mainly obtained from wood and it is considered that twentieth full-grown trees

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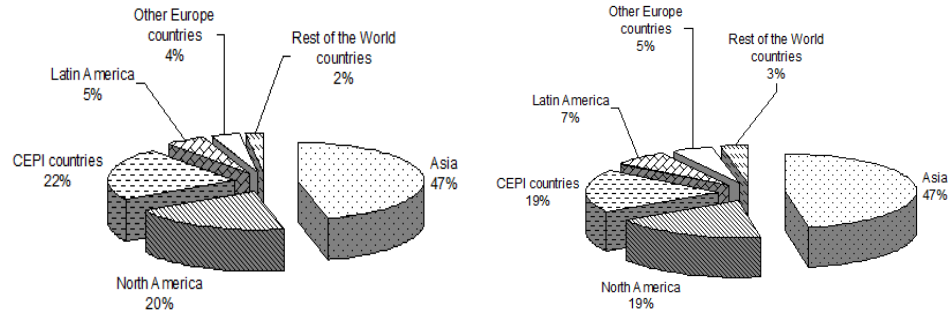
are necessary for the production of one tonne of paper (Clean Up Australia, 2009). According to Statista (2017) the quantities of paper and board produced and consumed in the world has increased from 392.7 million metric tons (mmt) in 2008 to over 410 mmt in 2015. China is the largest producer of paper and cardboard (over 107 mmt in 2015) followed by US (approximate 73 mmt), Japan (with 26 mmt) and Germany (22 mmt) (FAO, 2016). In Europe, the Confederation of European Paper Industries (CEPI) which was founded in 1992 includes 18 countries (Austria, Belgium, Czech Republic, Finland, France, Germany, Hungary, Italy, The Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and United Kingdom) with 623 number of companies in 2016 (153 pulp mills and 750 paper and board mills) having a production capacity for paper and board of 100,374,000 tonnes (CEPI, 2017). The paper and cardboard consumption according to CEPI (2017) in the member countries of this organization was of 77.4 million tonnes (newsprint, other graphic papers, case materials, other packaging (including board), sanitary and household, other paper and board). Production and consumption of paper and board in different regions in 2015 are illustrated in figure 1.



a) b)

Fig. 1 Paper and cardboard a) production, b) consumption in different regions of the world according to CEPI (2017)

CEPI (2017) declared that the raw materials consumed in paper and board production are: woodpulp (39.4%), pulp other than wood (0.4%), paper for recycling (46.2%) and non-fibrous materials (13.7%). A wood volume of 147.3 million m^3 was consumed by CEPI countries in 2016: hardwood (27.6%) such as birch, eucalyptus, beech, aspen, others, and softwood (72.4%) like pine and spruce. It is important to emphasize that almost 50% of raw materials comes from recovered paper and cardboard (EPRS, 2015). According to ERPC (2016) the paper fibre is reused 3.5 times on average in Europe. The recovered paper is used manly to produce newspapers and packaging and is collected from industry, households and offices. EPRS (2015) affirms that one tonne of paper and board can substitute three tonnes of wood. The amount of packaging waste generated in 2014 in the EU countries was 162.9 kg per capita and includes paper and cardboard with the highest percentage, followed by plastic and others (fig. 2).

According to Eurostat (2017) the recycling rate for total packaging waste increased from 54.6 % in 2005 to 65.5 % in 2014. The recycling rates for paper and cardboard packaging in EU27 and Romania are illustrated in figure 3.

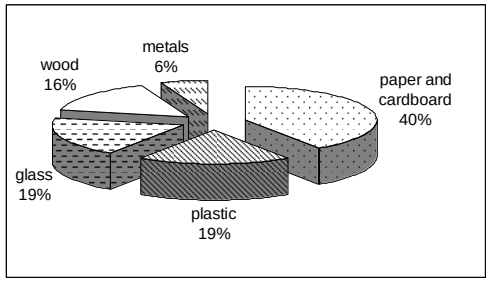


Fig. 2 Packaging waste generated by weight in EU countries in 2014 (adapted upon Eurostat, 2017)

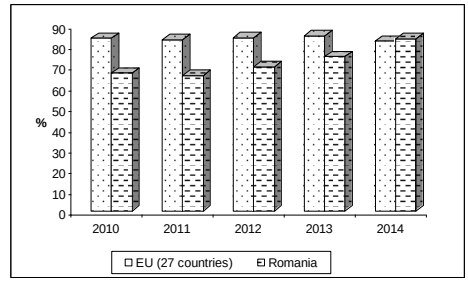


Fig. 3 Recycling rates for paper and cardboard packaging (adapted upon Eurostat, 2017)

The aim of this paper was to investigate the environmental impacts of corrugated board packaging boxes using life cycle assessment methodology.

MATERIAL AND METHOD

1. Corrugated board production using primary and recovered fibre

Cardboard is an agglomeration of cellulosic fibers resulting from the processing of vegetable raw materials such as grain straw, corn kernels, reed, fir, pine, beech, poplar etc. This is a compact paper with very little flexibility and it is mainly used for packaging. There are five types of cardboards used for packaging:

- *Flat carton* - is different from paper in that it is thicker and stiffer,
- *Duplex carton* - consists of two different layers of wet bonded fiber material by pressing,
- *Triplex carton* - is made up of three different layers of fibrous material bonded wet,
- *Corrugated cardboard* - consists of four smooth stucco and three wavy layers, joined together by an adhesive,
- *Micro-cardboard* is lightweight, has a good resistance to cracking and is a good duplex and triplex cardboard replacement.

For corrugated cardboard production different systems as Kraftliner, Testliner, Smeichemical Fluting and Wellenstoff are used (FEFCO, 2015).

In this study it was considered 1.05 t of recovered paper and 0.21 t of wood for production of 1 t of corrugated board, which means that were used 83% recycled fibre and 17% virgin fibre. According to FEFCO (2015), it can be obtained 1 t of corrugated board from 1.08 t of recovered paper. CEPI statistics mentioned that 87% of corrugated board used in Europe was collected and recycled (CEPI, 2017). Based on this fact we can assume that 0.87 t of corrugated board from 1 t of cardboard waste produced and used can be recovered and recycled in order to obtain new corrugated products. The remaining 0.13 t of corrugated board can be incinerated or landfilled. Considering that, for boxes with 650 g weight and dimensions 575 x 385 x 225 mm (L x B x H) there are necessary Kraftliner 233 g/box, Wellenstoff 267g/box, Testliner 233 g/box and Glue 13g/box, it results total losses (shavings) of 96 g/box. This means that

at 1 t of corrugated cardboard we will have 129 kg of shavings/tonne and it will be obtained approximately 1340 corrugated cardboard boxes. Figure 4 illustrates the life cycle of corrugated cardboard boxes.

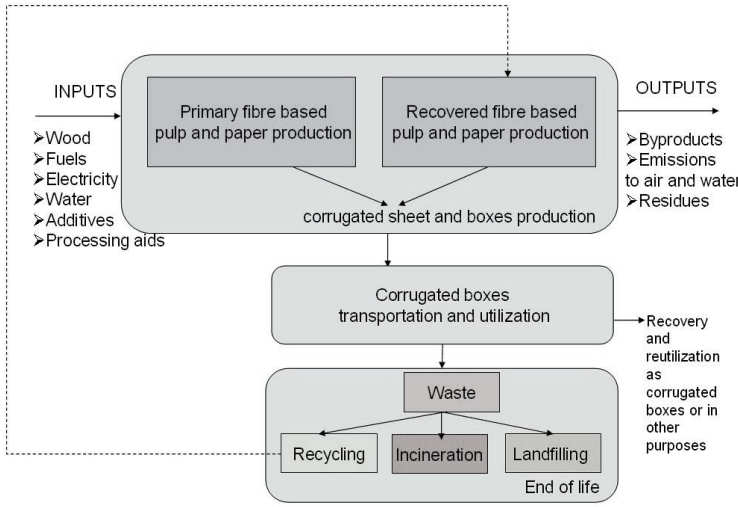


Fig. 4 System boundaries of corrugated boxes

2. Life Cycle Assessment (LCA)

LCA is a standardised tool with four main stages which can be successfully applied for environmental assessment of different product, process, systems, services etc. All LCA studies must include: goal definition and scoping, inventory analysis, impact assessment and interpretation (Ghinea *et. al.*, 2014; Petraru *et. al*., 2011). In this study we used GaBI software as an instrument which includes different LCA methods (CML, Eco-Indicator 95, Eco-Indicator 99, EDIP 1997, 2003, ReCiPe, UBP, TRACI etc.) and supports every stage of the LCA. In our case only CML 2001 and ReCiPe 1.08 LCA methods were selected for the evaluation.

Goal and scope of the study, system boundaries, functional unit:

- the scope of this study was to evaluate the environmental performance of corrugated boxes production, transport, use, landfilling and incineration;
- the system boundaries are illustrated in figure 4;
- 1 t of corrugated board production was considered as the functional unit.

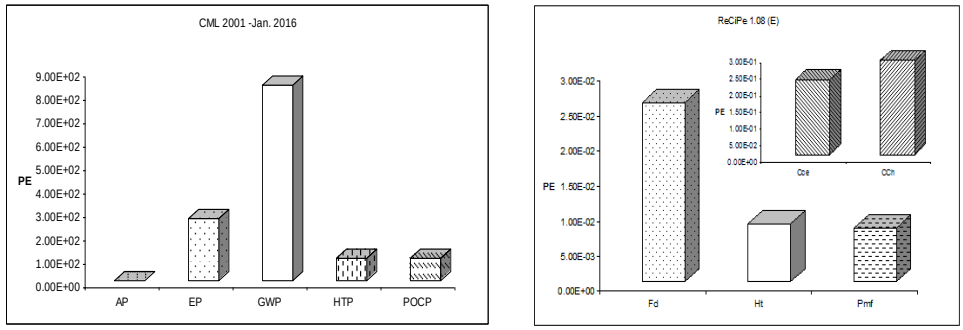
Inventory analysis: in this stage all inputs and outputs data were collected for each phase included in the analysed system. Some of the data were obtained from FEFCO, (2015) CEPI and GaBi database and others were calculated.

Impact assessment: all the data obtained in the previous stage were introduced in GaBi software in order to quantify the impacts categories associated to corrugated cardboard system considered for the analysis. The impact categories considered in this case were as follows: *Acidification Potential* (AP), *Eutrophication Potential* (EP), *Global Warming Potential* (GWP), *Human Toxicity Potential* (HTP), *Photochemical Ozone Creation Potential* (POCP) included in CML method and *Climate change Ecosystems* (CCe), *Climate change Human Health* (CCh), *Fossil depletion* (Fd), *Human toxicity* (Ht) and - *Particulate matter formation* (Pmf) which can found in ReCiPe 1.08 method.

Interpretation stage is presented in the results and discussions section.

RESULTS AND DISCUSSIONS

The results shows that the stages (production, transport, use, landfilling, incineration) included in life cycle of corrugated board generate negative impacts on the environment (the positive values mean negative impacts -fig. 5). All results are expressed in person equivalents (PE). From figure 5a it can be observed that the corrugated board (CB) production influences the *Global Warming Potential* (GWP), followed by EP, POCP and HTP. The highest contribution to the GWP is due to CB production followed by CB landfilling and CB incineration. A hierarchy of the contribution to impact categories of the evaluated processes can be made in order of decreasing contribution: for EP - CB landfilling>CB incineration>CB production; for HTP - CB landfilling>CB production>CB incineration and for POCP - CB production>CB incineration> CB landfilling. Considering the results presented in figure 5b, a hierarchy of categories of impact in order of decreasing environmental impact was performed: CCh>Cce>Fd>Ht>Pmf. It can be observed that the CCh and Cce impact categories included in ReCiPe method are the most affected by the corrugated board production and elimination steps.



a) b)

Fig. 5 Environmental impacts of corrugated board system using a) CML 2001- Jan. 2016 and b) ReCiPe 1.08 methods

CONCLUSIONS

1. The environmental impacts associated with corrugated board life cycle were determined by applying LCA methodology.

2. GaBi software was used for the evaluation and the results obtained by considering LCA methods such as CML 2001 and ReCiPe 1.08 were presented.

3. It can be concluded that corrugated board transportation, landfilling and incineration have significant negative impacts on the environment, while the impacts associated with the production stage can be reduced by using waste paper and cardboard at this stage.

***Acknowledgments**: This work was elaborated with the support of one Grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI: project number PN-III-P2-2.1-BG-2016-0130, contract 64BG/30.09.2016 and GaBi Education: Software and database for Life Cycle Engineering.*

REFERENCES

1. Clean Up Australia, 2009 - *Paper and Cardboard Fact Sheet*, Online at: http://www.cleanup.org.au.

2. Ghinea Cristina, Petraru M., Simion I., Sobariu D., Bressers H.Th. A., Gavrilescu Maria, 2014 - *Life Cycle Assessment of waste management and recycled paper systems*. Environmental Engineering and Management Journal,13, p. 2073-2085.

3. Ojala J., Lamberg J.-A., Peltoniemi M., Särkkä T., Voutilainen M., 2013 – *The Evolution of Global Paper Industry*. O Papel, 74, p. 51-54.

4. Petraru M., Ghinea Cristina, Bressers H.Th.A., Gavrilescu Maria, 2011 - *Analysis of Environmental Impact for Industry Products. Case Study: Paper Manufacturing*. Bulletin of the Polytechnic Institute of Iasi, 57, p. 63-74.

5. *, CEPI, 2017** – *Key statistics 2016 European pulp & paper industry*. Confederation of European Paper Industries (CEPI), Online at: http://www.cepi.org/system/files/public/documents/publications/statistics/2017/KeyStatistics2016_Final.pdf.

6. *, EPRS, 2015** - *Understanding waste streams Treatment of specific waste*. European Parliamentary Research Service (EPRS), Online at: http://www.europarl.europa.eu/EPRS/EPRS-Briefing-564398-Understanding-waste-streams-FINAL.pdf.

7. *, ERPC, 2016** - *Paper recycling, Monitoring Report 2015 Final Report of the 2011-2015 period.* European Recovered Paper Council (ERPC), Online at: http://www.cepi.org/system/files/public/documents/publications/recycling/2016/FinalMonitoringReport2015.pdf.

8. *, Eurostat, 2017** - *Packaging waste statistics*, Online at: http://ec.europa.eu/eurostat.

9. *, FAO, 2016** - *Pulp and paper capacities*. Food and Agriculture Organization of the United Nations (FAO), Online at: http://www.fao.org/3/a-i5946t.pdf.

10. *, FEFCO, 2015** - *European Database for Corrugated Board Life Cycle Studies*. European Federation of Corrugated Board Manufacturers (FEFCO), Online at: http://www.fefco.org/sites/default/files/lca-report-2015.pdf.

11. ***, **STATISTA, 2017 –** *Consumption of paper and cardboard since 2006*. Online at: https://www.statista.com/statistics/270319/consumption-of-paper-and-cardboard-since-2006/.

INFLUENCE OF HYDROCLIMATIC RISK FACTORS ON THE RIVER POLLUTION PHENOMENON

INFLUENŢA FACTORILOR DE RISC HIDROCLIMATIC ASUPRA FENOMENULUI DE POLUARE AL RÂURILOR

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Abstract. *The paper presents an analysis of the river pollution phenomena in the Trotuş River Basin. The Trotuş River area has been affected by multiple floods over the past 28 years (significant in 2004, 2005, 2011 and 2016). The most important categories of pollutant pressures in the Trotuş Hydrographic Basin are punctual, diffuse and hydromorphological. The most significant point sources of pollution in the river basin are of domestic, industrial and agricultural type. The pollutants that affected the watercourses in the Trotuş catchment area during the study period were petroleum products (51.2%) and organic substances (21.4%). The remaining 27.4% of pollutants are ammonium, ammonia, hydrogen sulphide, heavy metals, cyanide, urea, detergents, ash, phosphorus, etc*.
Key words: pollution, riverine environment, floods, degradation

Rezumat. *Lucrarea prezintă o analiză a fenomenelor de poluare a râurilor din bazinul hidrografic al râului Trotuş. Arealul râului Trotuş a fost afectat de multiple viituri în ultimii 28 ani (semnificative cele din 2004, 2005, 2011 şi 2016). Cele mai importante categorii de presiuni poluante din Bazinul Hidrografic Trotuş sunt de tip punctiform, difuze şi hidromorfologice. Sursele punctiforme de poluare cele mai semnificative în bazinul hidrografic sunt de tip menajere, industriale şi agricole. Substanţele poluante care au afectat major cursurile de apă din bazinul hidrografic Trotuş pe perioada de studiu au fost produsele petroliere (51,2%) şi substanţele organice (21,4%). Restul de 27,4% dintre poluanţi sunt reprezentaţi de amoniu, amoniac, hidrogen sulfurat, metale grele, cianuri, uree, detergenţi, cenuşă, fosfor etc.*
Cuvinte cheie: poluare, mediu riveran, inundaţie, degradare

INTRODUCTION

The hydrological regime of rivers in the Siret River basin is characterized in the last period of time by the high frequency of floods. River quality parameters are influenced by a complex of pollutants. For the purpose of establishing appropriate measures for the protection and warning of surface water pollution, the individual impacts of each source of pollution on hydrographic basin, river stretches, or geographic areas shall be taken into account. The

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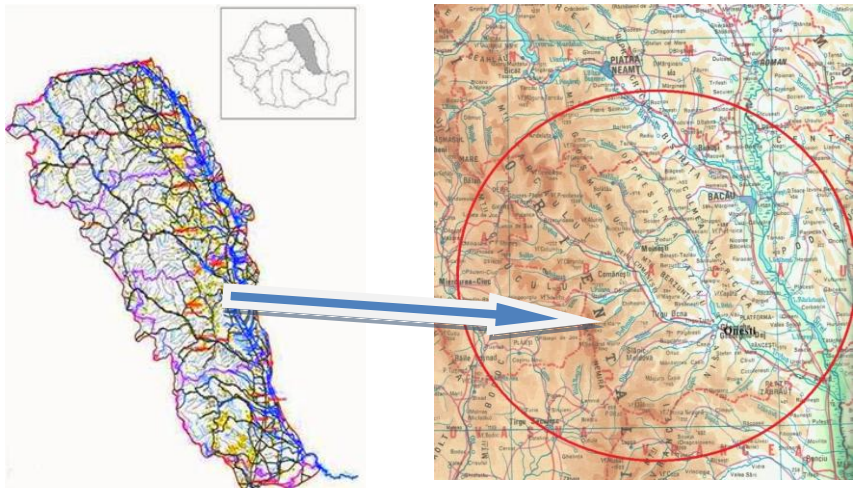
pollutant transport vector in a river, the water velocity in this case, can cause local, regional and even transfrontier pollution (Avram, 2016).

Water quality from surface sources is adversely affected by pollution induced especially by human activity. As a result of the increase in the degree of pollution, the ability to use surface water, which could be used especially for drinking purposes, is restricted. Human health and the equilibrium of aquatic ecosystems are heavily affected by the high water pollution from rivers and lakes. For these reasons, maintaining water quality in surface courses in line with environmental protection requirements is mandatory, through sustainable water management policies and programs (Avram, 2016).

MATERIAL AND METHOD

The research was conducted in the Trotuş River basin. The research material was collected from the Siret basin and customized on the Trotuş River basin. The hydrographic basin of the Trotuş River is located in the relief area of the Oriental Carpathians. It goes through the geomorphological units of the Ciucului, Tarcăului, Comăneştii and Tazlău - Caşin depressions (fig. 1). The Trotuş River flows into the Siret River downstream of Adjud.



a b

Fig. 1 Framing of the study area (BH Trotuş) in the Siret catchment area:
a - the Siret catchment area; b - location of the area in the East Carpathians
(http://www.rowater.ro/dasiret/Sinteza%calitate.20raurilor/Forms/AllItems.aspx.jpg)

The Trotuş River has its springs in the Ciuc Mountains at an altitude of 1360 m and has a main north-south direction. The tributaries of the Trotuş River in the analyzed sector are (Ujvari 1972): on the right: Ciugheş, Cotumba, Grohotiş, Sulita, Ciobănuş, Supan, Uz, Dofteana, Slănic, Nicoreşti, Oituz, Caşin etc.; on the left: Brusturoasa, Camenca, Agăş, Seaca, Asău, Urmeniş, Plopul, Vâlcele, Tazlău.

The hydrographic basin of the Trotuş stream has an area of 4.440 km^2. The length of the river is 158 km. The average altitude of the river basin varies from springs to spills up to 1140 - 734 m. The Trotuş River and tributaries are monitored by 21 hydrometric stations. Monitoring parameters have been determined flow parameters (liquid and solid flows, levels, ice, etc.) for a period of 40-60 years.

The data collected consists of hydrological, hydrological, topographic, geotechnical studies, state of exploitation of the regulation and shore defence structures. Studies and research are conducted over a period of about 20 years. The theoretical and experimental research was carried out in the following areas.

Primary data was processed using the statistical calculation programs and hydrological, hydraulic and pollutant computing programs applicable to the case study.

RESULTS AND DISCUSSIONS

The riparian of river area is vulnerable to pollution from water courses. Floods amplify the intensity of the pollution phenomenon through the transport vector, the water velocity, and the increased capacity of incorporating pollutants from different sources. Pollution sources are classified according to their spatial distribution characteristics as point sources, non-point sources, and internal sources discharged into rivers. Point sources of pollution include mainly industrial and domestic wastewater from urban areas. Non-urban sources include pollutants from surface leakage on farmland, forests, meadows, riparian lands, etc.

For the analysis of water quality data control sections were monitored on the Trotuş River and the Tazlău River for a period of 10 years 2006-2016. These data were taken from the Siret Regional Water Monitoring Program.

The Trotuş River and its tributaries have a major role to play in the pollution, collecting domestic and industrial waste water and leakage from farmland. Pollution sources that influence the quality of the Trotuş River water and the tributaries come from natural and human pollution. A predominant role is played by accidental pollution caused by flood plains. Floods are a transport vector of pollutants, which transforms local pollution into regional pollution. Inappropriate and excessive use of fertilizers and pesticides on agricultural land leads to a phenomenon of aggressive pollution on surface and underground water. The main tributaries of the Trotuş River are urban waste water collectors, which have a negative influence on the quality of the water.

The most important pressure categories in the Trotuş Hydrographic Basin are punctual, diffuse and hydromorphological. The pollution sources of the Trotuş Hydrographic Basin can be classified as follows:

• point sources of significant pollution, represented by the sources: domestic, industrial and agricultural,

• diffuse pollution sources, mainly represented by chemical fertilizers used in agriculture, rural agglomerations and the urban environment with a low percentage of population connection to the sewerage network.

The pollutants that affected the watercourses in the Trotuş catchment area in most cases were petroleum products 51.2% and organic substances 21.4%, the

remaining 27.4% being ammonium, ammonia, hydrogen sulphide, heavy metals, cyanides, urea, detergents, ash, phosphorus, suspensions (ABA Bacau, 2016).

The oil exploitation of the Tazlăul Sărat river basin influences the balance of the pollutants on the Trotuş River (fig. 2). The oil activity in the Moineşti area affects the Tazlăul Sărat, Tazlău and Trotuş watercourses, as well as the groundwaters in the area, through the accidental spills of oil products and salty waters. The Tazlăul Sărat River has frequent accidental pollution with petroleum products, in most cases due to the rupture of transport pipelines. Some of the oil pipelines have an out-of-service life.

Studies and research over the last few years have shown the continued change in water quality parameters in the Trotuş River and its tributaries. In the monitoring sections of the Trotuş River, the value of the main pollutants was analyzed over time intervals (http://apesiret.uvp.ro). The obtained values were compared with the maximum admitted by the national and European standards. Tables 1 and 2, show the values of the concentrations of the water quality chemical indicators determined in the measurement sections Tg. Ocna and Vrânceni on the Trotuş River. The data were compared with the maximum admissible concentrations established by Order no. 161/2006 and the Water Framework Directive 2000/60 / EC (Mănescu, 2013).

Analysis of the measured and processed values in the monitoring section Tg. Ocna indicates annual variation in pollutant concentrations and exceeded the maximum admissible values. The most serious situation is represented by ammonium, nitrates and phosphates, which indicate a domestic and agricultural pollution in the Trotuş catchment area.

a b

Fig. 2 Tazlăul Salat river pollution risk zones: a - oil pipelines exposed by floods in June 2016; b - oil pipelines uncovered by the river infiltration stream (Avram, 2016).

The N-NH4, N-NO2, P-PO4 quality indicators indicate the quality of the Trotus River water quality in the monitoring section Tg. Ocna in grade 4 and 5 during the study period, depending on the hydrological regime of the watercourse. The CBO5 indicator fits the quality class III quality monitoring section within the limit imposed by Order no. 161/2006.

The analysis of the measured and processed values in the monitoring section Vrânceni indicates the annual variation of the concentrations of the pollutant, the exceeding of the maximum admissible values by the norms, but also the influence of the diffusion process on the transport length.

Table 1

Analysis of chemical indicators determined in control section Tg. Ocna, Trotuş River (Mănescu, 2013, ABA Siret Bacău 2010)

Year	OD (mg/L)	CBO_5 (mg/L)	CCO-Mn (mg/L)	CCO-Cr (mg/L)	$N\text{-}NH_4$ (mg/L)	$N\text{-}NO_2$ (mg/L)	N-NO3 (mg/L)	$P\text{-}PO_4$ (mg/L)
2007	14.52	12.41	5.45	13.65	1. 58	0.3	4.64	0.26
2008	13.25	13.61	3.31	11.24	1.76	0.13	5.93	0.40
2009	15.89	13.17	5.12	17.05	1.67	0.52	5.7	0.55
2010	16.17	14.56	6.65	23.3	2.61	0.611	5.9	0.75
2011	16.63	12.58	4.78	14.55	1.65	0.615	5.76	0. 68
Color code for the water quality class: Blue - Class I; Green - Class II; Rose - Class III; Orange - Class IV; Red - Class V								

Table 2

Analysis of chemical indicators determined in control section Vranceni, Trotuş River (Mănescu, 2013, ABA Siret Bacău 2010)

Year	OD (mg/L)	CBO_5 (mg/L)	CCO-Mn (mg/L)	CCO-Cr (mg/L)	$N\text{-}NH_4$ (mg/L)	$N\text{-}NO_2$ (mg/L)	N-NO3 (mg/L)	$P\text{-}PO_4$ (mg/L)
2007	13.75	12.21	5.33	11.67	1. 45	0.26	4.02	0.21
2008	13.46	13.91	3.45	13.44	1.12	0.19	5.36	0.43
2009	15.70	12.37	5.02	16.04	1.49	0.61	5.72	0.31
2010	16.54	13.54	6.81	23.31	1.2	0.59	5.5	0.28
2011	16.69	11.53	4.78	14.59	1.47	0.57	5.34	0.3
Color code for the water quality class: Blue - Class I; Green - Class II; Rose - Class III; Orange - Class IV; Red - Class V.								

The most serious situation is represented by ammonia, nitrates and phosphates, which is the fourth and fifth grade monitoring section during the study period. This shows domestic and agricultural pollution in the Trotuş catchment area. The determined values are: at N-NH4 of 1.12-1.49 mg/L, at N-NO2 0.57-0.61 mg/L, at P-PO4 0.21-0.3 mg/L, etc. Indicates a classification of the water quality of the Trotuş River in the monitoring section Tg. Ocna in grade 4 and 5 during the study period, depending on the hydrological regime of the watercourse.

The presence of human-type pollution is particularly evident in the Trout River basin over the last 25 years. The most obvious pollution processes in the riparian area are represented by (Avram, 2016, Mănescu, 2013):

- pollution with biological material resulting from uncontrolled forest felling;
- pollutant pollution from eroded soil transport from deforested slopes and agricultural degrading soil; oil pollution from oil operations;
- household waste pollution from localities without sewerage systems and treatment plants; pollution with household waste from localities without landfills.

Hydroclimatic risk factors significantly influence the transport of pollutants on rivers and contribute to the decrease or increase of their concentration on the length of the river. Significant hydroclimatic risk factors for the transport of pollutants are:

- reduced capacity of major river transit in case of high-floods; the flood trains waste in the major bed and integrates them into the waterway with their long-distance transport;

- the morphological modification of the river bed, formation of sedimentation zones, erosion of the shore, blocking of the bed, etc.; erosion zones adversely affect the stability of the constructions and installations in the riverbed, with the occurrence of pollution phenomena (the oil pipeline on the Tazlăul Sărat river);

- increasing the transport of biological material in the bed (branches, wood waste, trees, etc.), which causes the flow of the rivers and bridges to be blocked; pollution caused by wood is characteristic of the Trotuş River Basin;

- uncontrolled deforestation that has intensified rainfall-drainage and river water concentration; This situation contributes to a rapid movement of pollutants on the soil surface and their concentration.

CONCLUSIONS

1. The territory of the Trotuş catchment area has been affected in the last 25 years by disastrous hydrological phenomena, which have greatly influenced the morphology of the rivers, with important influences on the riparian environment and, in particular, the transport of pollutants.

2. The sources of pollution in the Trotuş River basin are natural and human, where both types have developed over the last 25 years with a negative influence on water quality parameters in the rivers and on the riparian area.

3. The floods produced over the past 25 years on the Trotuş River and its tributaries have influenced and intensified the transport of pollutants, in which the riparian habitat suffered extremely destructive effects.

REFERENCES

1. **Avram Mihaela, 2016** - *Cercetări privind impactul factorilor de risc hidroclimatic asupra proiectării, tehnologiilor de realizare şi exploatării lucrărilor de regularizare a râurilor - Studiu de caz. Raport 2 de cercetare.* Universitatea Tehnică „Gheorghe Asachi" din Iaşi.
2. **Mănescu Andreea, 2013 -** *Contribuții la monitorizarea şi modelarea fenomenului de poluare a cursurilor de apă*. Teză de doctorat, Universitatea Tehnică „Gheorghe Asachi" din Iaşi.
3. **Ujvari I**., **1972** - *Geografia apelor României.* Edit. Ştiințifică, Bucureşti.
4. *** **A.B.A. SIRET-Bacău** *Rapoarte de sinteză privind apărarea împotriva inundațiilor, fenomenelor hidrometeorologice periculoase, accidentelor la construcții hidrotehnice şi poluărilor accidentale din județul Bacău*", anii 1991, 2004, 2005, 2010, 2012, 2016.
6. ***, *Directiva 2000/60 pentru stabilirea unui cadru al politicii comunitare în domeniul apelor*.
7. *** http://apesiret.uvp.ro
8. ***http://www.rowater.ro/dasiret/Sinteza%calitate.20raurilor/Forms/AllItems.aspx.jpg

RESEARCHES ON GROUNDWATER POLLUTION IN THE AREA OF INDUSTRIAL WASTE DUMPS

CERCETĂRI PRIVIND POLUAREA APELOR SUBTERANE ÎN ZONA DEPOZITELOR DE DEŞEURI INDUSTRIALE

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Abstract. *Industrial waste dumps in exploitation or conservation represent a high risk of groundwater pollution. The Moldovan area has a large number of industrial waste dumps for mine waste, slag and ash from thermal power stations and steel plants, technological waste, etc. The degradation of the constructive structure of the deposits allows the underground infiltration of pollutants and contamination of water sources. The case study highlights the movement of a complex of pollutants from the landfill of a metalworking plant into the underground water. During the research measurements were made in observation drills and laboratory determinations were performed. The processing of the experimental data showed substantial overshoots in the following physical and chemical indicators: total iron (Fe +), ammonium (NH4), hardness, potassium permanganate (KMnO4), sulphates (SO4-) and chlorides (C1-). Contaminants from the landfill polluted groundwater and surface water sources for several localities.*
Key words: industrial waste, pollution, diffusion, groundwater, ground, air

Rezumat. *Haldele de deşeuri industriale aflate în exploatare sau în conservare prezintă un risc ridicat de poluare a apelor subterane. Zona Moldovei prezintă un număr mare de halde deşeuri industriale pentru steril de mină, zgură şi cenuşă de la termocentrale şi combinate siderurgice, reziduuri tehnologice etc. Degradarea structurii constructive a depozitelor permite infiltrarea în subteran a poluanţilor şi contaminarea surselor de apă. Studiul de caz întocmit evidenţiază deplasarea în apa subterană a unei complex de poluanţi proveniţi de la depozitul de deşeuri a unei fabrici de produse metalice. Pe perioada cercetărilor s-au efectuat măsurători în foraje de observaţie şi s-au realizat determinări de laborator. Prelucrarea datelor experimentale au arătat depăşiri substanţiale la următorii indicatori fizico-chimici: fier total (Fe^{+}), amoniu (NH_4), duritate, permanganat de potasiu ($KMnO_4$), sulfaţi (SO_4^{-}), cloruri (Cl^{-}) şi azotiţi (NO_2). Contaminaţii proveniţi din depozitul de deşeuri au poluat sursele de apă subterană şi de suprafaţă pentru câteva localităţi.*
Cuvinte cheie: deşeu industrial, poluare, difuzie, apa subterana, sol, aer

INTRODUCTION

Industrial waste is stored in premises located inside or outside economic objectives. Some deposits were made without considering the risk of environmental pollution. The design did not analyze in detail the nature of the

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waste to be stored and its evolution over time, as well as its influence on the construction structure of the deposit. At present, landfills in exploitation or conservation represent a high risk of pollution of groundwater, soil, air and human health. In this case one can enumerate (Bălan, 2010).

- ash dumps from thermal power plants for the production of electricity and heat;
- slag slags in the steel industry;
- landfills in the chemical and building materials industry,
- landfills in the hydrocarbon industry, etc.

All landfills of this type produce groundwater contamination for small but also long periods. The effect of pollution causes the removal of groundwater sources from the water supply circuit.

The amount of hazardous industrial waste generated in Romania has declined steadily in recent years due to the reduction of the activity of large economic units. In this context, landfills in operation or in the conservation area will have to be systematized and exploited safely. In the polluted areas ecological reconstruction works must be carried out to reduce and prevent the risk of accidental pollution of the environment (Luca, 2004).

A number of industrial deposits must be closed or rehabilitated to meet the new environmental protection standards and standards in the area of waste disposal sites.

MATERIAL AND METHOD

The research material consists of the first industrial waste deposit at S.C. Bearings S.A. Barlad. The Industrial Waste Depot is located in the Barlad River meadow in the north of Barlad. Industrial waste disposal was done outside the factory on the slag dump located to the east and at a distance of 1000 m from the Barlad River and about 600 m from the Simila River. This deposit is a model of environmental pollution in the absence of reconstruction measures for the protection of the underground environment. The landfill has changed over time in shape, waste treatment, environmental protection, etc. Waste storage was done by stacking, on unpolluted and permeable land for liquid and leachate (Bălan, 2010).

The research methods included field studies to take ground and groundwater samples, dusts from the air. The samples taken from the site and the area adjacent to the deposit were analyzed in the laboratory. Experimental results were processed using a set of specialized computing programs.

The forecasting of the pollution phenomenon of the groundwater pollutant concentrations and the depollution phenomenon has been achieved by using a special pollutant transport study program in the underground environment (the FEFLOW program) (Charbeneau, 2000; Bălan, 2010; de Marsily, 1994).

RESULTS AND DISCUSSIONS

In the landfill there are various wastes such as nature and environmental hazards: inert laminae from emulsion treatment, carbide, ion-exchange filters, petroleum products, dirt, ash, refractory brick, etc. The land area occupied by the

deposited material is about 1.6 ha, of which the area adjacent to the heterogeneous landfill of the land is about 0.5 ha.

The landfill of industrial waste with its diverse composition also has a significant pollutant over time (during operation and post-conserving) on the soil. Soil pollutants are trained in the soil, which, under the effect of rainfall, are transported vertically and horizontally at various distances.

Table 1

Indicators used for soil pollution in the landfill area (Balan, 2010)

No. crt.	Point of harvest	H_{rec} (m)	Indicators analyzed (Concentrations, ppm/dry soil)					
			Pb	Fe	Zn	Cu	Mn	Cd
1	Waste storage	5	93	**1684**	86	17	184	1.79
		30	88	**1328**	72	8.3	156	1.24
2	Between the industrial waste landfill and the petroleum product depot	5	84	**1740**	297	85	145	1.86
		15	71	**1417**	235	61	108	1.52
C_{ad}			250/ 1000	250/ 1000	700/ 1500	250/ 500	2000/ 4000	5/10

H_{rec} - harvest depth; C_{ad}- Maximum permissible concentration according to O.M. no. 756/1997 - alert threshold / intervention threshold

During the monitoring process periodic measurements were carried out at different collection points in the area adjacent to the deposit. Field and processed data are presented in table 1 and table 2.

Table 2

Indicators analyzed for soil pollution in the landfill area (Balan, 2010)

No. crt.	Point of harvest	H_{rec} (m)	Indicators analyzed (Concentrations, ppm/dry soil)					
			Cr^{6+}	Cr tot	Ni	Mg	pH	S_{ex}
1	Waste storage	5	0.11	15.8	8.4	111.4	7.28	44.70
		30	0.08	12.5	5.5	120.7	7.52	44.45
2	Between the industrial waste landfill and the petroleum product depot	5	0.26	20.4	11.2	172.0	7.91	**286**
		15	0.22	17.1	9.6	125.0	7.89	**316**
C_{ad}			10/ 20	300/ 600				200/ 500

H_{rec} - harvest depth; C_{ad}- Maximum permissible concentration according to O.M. no. 756/1997 - alert threshold / intervention threshold ; S_{ex}- extractable substances

From the analysis of the processed data (tab. 1, tab. 2) that comprise the measurements performed on the soil at different depths (5 m, 15 m and 30 m) it is observed that for most of the indicators studied the ppm/dry soil concentrations

established by O.M. no. 756/1997, do not exceed the alert and intervention thresholds except the chemical indicator Fe. The research highlighted the following (Bălan 2010):

- the Fe concentration in dry soil at a depth of 5 m, both in and around the warehouse area, exceeds the 6.96 times the alert threshold and 1.74 times the intervention threshold;

- the Fe concentration in dry soil at 15 m depth in the area adjacent to the landfill, respectively between industrial waste landfill and oil product depot exceeds the 5.6 times the alert threshold and 1.41 times the intervention threshold;

- the Fe concentration in dry soil at 30 m depth in the storage area exceeds 5.31 times the alert threshold and 1.32 times the intervention threshold.

At the "extractable substances" indicator, exceedances were recorded at ppm / dry soil concentrations, compared to the alert threshold established by O.M. no. 756/1997, between the industrial waste deposit and the oil products warehouse. The following can be mentioned (Bălan, 2010):

- at a depth of 5 m, the concentration of extractable substances in dry soil exceeds 1.43 times the alert threshold;

- at the depth of 15 m, the concentration of extractable substances in dry soil exceeds the 1.58 times the alert threshold.

The circulation of groundwater contaminated with pollutants from the deposit mass is tributary to the fluctuation of the water level on the Simila and Barlad rivers and the rainfall regime. The analysis shows the following:

- during periods of low water on the two rivers or during abundant rainfall on the landfill, the underground water can move from the deposit to the Simila and Bârlad water courses; the pollution of the groundwater and the water from the two rivers is caused by this pollution;

- during periods of water rise in the two rivers and without abundant precipitation on the landfill, the groundwater movement is made in the opposite direction; thus transporting pollutants from the rivers to the underground of the deposit.

Since 2000, a series of thorough analyzes have been initiated on the influence of the closed industrial waste deposit on environmental factors and especially on groundwater. The number of groundwater quality monitoring drillings has been increased to 14. Drillings have been located at characteristic points of influence of the industrial platform. The study period was 2004-2010.

The processing of water samples taken from drillings highlighted groundwater quality parameters and their variation over time. F7 drilling presented significant parameters on groundwater quality and negative impact of industrial waste landfill. The F7 drilling is located in the northern part of the old industrial waste dumps and was made of PVC pipe with Dn 200 mm at a depth of 4 m. The phreatic layer was intercepted at a depth of 2 m from the natural ground quota. The results of the research are presented in table 3 for data taken from the F7 drilling.

Table 3

Analysis of physic-chemical indicators determined for F7 water (year 2006)

Quarter	pH	Chloride (mg/L)	NH_4 (mg/L)	$KMnO_4$ (mg/L)	NO_2 (mg/L)
I	7.5	**652.2**	**7.84**	*	**7.84**
II	8	90.75	**1.64**	**23.98**	0.05
III	8	207	**1.61**	**26.38**	0.05
IV	8	124.7	**6.75**	**23.10**	**1.73**
$C_{m,an}$	7.87	**268.66**	**4.46**	**24.48**	**2.41**
C_{ad}	**6.5 ÷ 9.5**	**250**	**0.5**	**5**	**0.5**

$C_{m\,in}$ average annual concentrations; C_{ad}- maximum admissible concentrations according to the Drinking Law no. 311/2004

In figure 1 is the graphical variation of the quarterly variation in concentrations of physic-chemical indicators in drilling F7 (chlorides, suspended matter, potassium permanganate and nitrate) compared to the maximum admissible concentrations (Drinking Law No. 311 of 2004; Bălan, 2010).

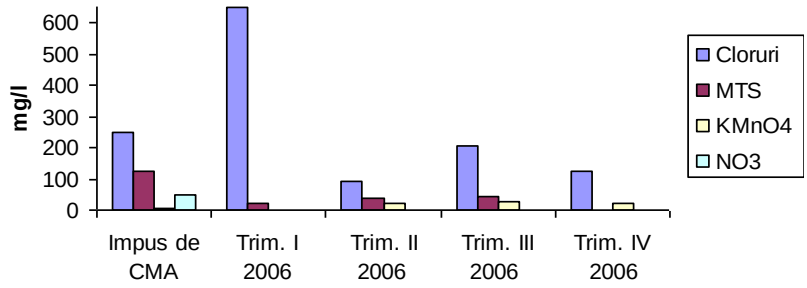

Fig. 1 Quarterly variation of concentrations (mg/L) in monitoring period in drilling F7 for year 2006 (Bălan, 2010).

The analysis and data processing results in the following (Luca *et.al*, 2012):

- in the chlorine indicator, quarterly values were recorded between 90.75 mg/L and 652.2 mg/L; the analysis of the processed data shows that the determined value of 652.2 mg/L, monitored in the first quarter of 2006, exceeds by 250 mg/L and 2.6 times the limit imposed by law;

- the quarterly values between 23.10 mg/L and 26.38 mg/ L were recorded at the potassium permanganate indicator; the analysis of the processed data shows that the values determined over the whole monitoring period (II, III and IV quarters of 2006) do not fall within the limit imposed by the law; the recorded values exceed the required limit (5 mg/ L) about 5 times; the same situation occurs also at the maximum permissible annual concentration (exceeding about 5 times the admissible limit);

- for the indicators of suspended matter and nitrates, the average quarterly values were determined which fall within the normal limits imposed by the Drinking Law no. 311/2004;

- quarterly values were recorded in the ammonium indicator in the range of 1.61mg/L and 7.84 mg/L; from analysis of the results it is observed that the monitored values, in I, II, III and IV quarter 2006, do not fall within the required limit (0.5 mg/L).

From the physic-chemical parameters processing of the samples taken from the drilling, the value of some indicators exceeds the limits imposed by the law. Thus, at F_2 - Albița Simila, F_4 - Stana and F_5 - East waste dumps, there are exceedances of nitrates (NO_2) and ammonium (NH_4) (tab. 4) (Bălan, 2010).

Table 4

Values of the water quality parameters taken from the F2 drilling – Riverbed Simila

CMA cf. Env.Aut.	Cl	NH_4	CCOMn	Extract.	$Cr6^+$	NO_2	pH	MTS
Trim. I	*	0,50	*	*	0,05	0,30	8,5	*
Trim. II	60	0.43	21.67	0	0	**0.53**	6.8	56
Trim. III	48	**0.75**	20.16	0	0	**0.61**	7,0	40
Trim.IV	55	0.21	22	0	0	**0.58**	7,0	50
Av. 2004	54.33	0.463	21.276	0	0	**0.573**	6.93	48.7

CONCLUSIONS

1. Industrial or conservation landfills are a permanent source of air, soil and subsoil pollution in the location area.

2. Research has shown that the landfill industry has negatively influenced the groundwater from the Bârlad River and the Simila River meadow.

3. The soil in the adjacent industrial waste site is polluted mainly by Fe in dry soil at a depth of 15 m where the concentration exceeds the 5.6 times the alert threshold and the 1.41 times the intervention threshold.

4. The underground water from the analyzed industrial waste site is polluted in special mode of the nitrates and ammonium.

REFERENCES

1. Bălan P. R., 2010 - *Contribuții la creşterea siguranței în exploatare a haldelor de steril în privința reducerii potențialului de poluare.* Teză de doctorat, Universitatea Tehnică „Gheorghe Asachi" din Iaşi, p. 267.

2. Charbeneau R., J. 2000 - *Groundwater Hydraulics and Pollution Transport*. Prentice Hall, New Jersey, SUA.

3. Luca M., 2004 - *Considerații privind fenomenul de poluare indus de haldele de steril minier.* Internațional Conference „Disaster and Pollution Monitoring" Universitatea Tehnica „Gh. Asachi" Iaşi.

4. **Luca M., Balan R.P., Mănescu A., Luca Al.L., 2012** - *Modelling pollution transport of the industrial waste underground Conference on Modelling Fluid Flow (CMFF'12).* The 15th Event of International Conference Series on Fluid Flow Technologies, Budapest, Hungary, p. 523-530.

5. **de Marsily G**., **1994** - *Hydrogéologie, comprendre et estimer les écoulements souterrains et le transport des polluants.* Écoles des Mines de Paris (Paris VI).

6. *** O.M. no. 756/1997

7. ***, Drinking Law No. 311/2004

THE CHARACTERISTICS OF FOREST SOILS FROM IAŞI COUNTY

CARACTERISTICILE SOLURILOR FORESTIERE DIN JUDEŢUL IAŞI

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Abstract. *The purpose of this article is to present a description of forest soils from Iasi County, based on the soil analysis realized in the period 1989-2015. As a total, 201 soil profiles and 620 pedo-genetical horizons were analyzed. Amongst the 15 soil types identified in the county, the most widespread are: preluvisol (weakly acid soils, eubasic, with a large total cationic exchange capacity, well supplied with nitrogen and moderately humiferous), eutric cambisol (weakly acid soils, eubasic, with a large total cationic exchange capacity, well supplied with nitrogen and moderately humiferous), luvisol (moderately acid soils, eubasic, with a large total cationic exchange capacity, well supplied with nitrogen and intensely humiferous), phaeozem (weakly alkaline soils, eubasic, with a very large total cationic exchange capacity, normally supplied with nitrogen and intensely humiferous), chernozem (weakly alkaline soils, eubasic, with a very large total cationic exchange capacity, normally supplied with nitrogen and moderately humiferous) and fluvisol.*
Key words: soils, pH, humus, preluvisol, eutricambisol

Rezumat. *Scopul acestui articol este de a prezenta o descriere a solurilor forestiere din judeţul Iaşi, pe baza analizei solului realizată în perioada 1989-2015. În total au fost analizate 201 de profile de sol şi 620 de orizonturi pedogenetice. Dintre cele 15 tipuri de sol identificate în judeţ, cele mai răspândite sunt: preluvisol (soluri slab acide, eubazice, cu o capacitate mare de schimb cationic total, bine aprovizionat cu azot şi humifer moderat), cambisol eutric (soluri slab acid, eubazice, cu o capacitate mare de schimb cationic total, bine aprovizionat cu azot şi humifer moderat), luvisol (soluri moderat acid, eubazice, cu o capacitate mare de schimb cationic total, bine alimentat cu azot şi intens humifer, phaeozem (soluri slab alcaline, eubazice, cu o capacitate foarte mare de schimb cationic, alimentată în mod normal cu azot şi cu umiditate intensă), cernoziomuri (soluri slab alcaline, eubazice, cu o capacitate de schimb cationic totală foarte mare, aprovizionate normal cu azot şi moderat humifere) şi fluvisol.*
Cuvinte cheie: soluri, pH, humus, preluvosol, eutricambosol

INTRODUCTION

The forests from Iasi County occupy a total area of 97.773 ha (www.insse.ro). Romsilva, the National Forest Management Administration, manages through its 8 forest districts a forest area of 66.846 ha (www.rosilva.ro).

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Forest soils are an essential part of silvicultural ecosystems (Târziu *et al.,* 2004, Spârchez *et al.,* 2011). The purpose of this article is to present a description of soils from this county's forest fund.

MATERIAL AND METHOD

At "Marin Dracea" National Institute for Research and Development in forestry from Braşov exists a consistent data base regarding soil analysis realized over time during silvicultural management activities (Crişan *et al*,. 2016). The analyses realized on the soil samples (by using national and international methods - Dincă *et al.,* 2012) were the following: pH, humus content, carbonates content, capacity for basis exchange (Sb), exchange capacity for hydrogen (Sh), total cationic exchange capacity (T), base saturation degree (V), texture, total nitrogen.

The present paper investigates the analysis realizes on soil samples gathered from this County in the period 1989-2015, reaching a total number of 201 soil profiles and 620 pedo-genetical horizons.

RESULTS AND DISCUSSIONS

Types of soils from Iasi Forest District

The most widespread types of soils are the ones from Cernisoil and Luvisoil class, which reach together 71% from all the county's soils. As soil type, the most widespread is preluvisol (22%), followed by eutricambisol (20%), luvisol (18%), phaeozem (17%), chernozem (14%) and fluvisol (8%) (fig. 1).

At the country's level, luvisol occupies the 2nd place (with a total area of 1.440.052 ha, meaning 22%), eutricambisol occupies the 3d place (869.909 ha, meaning 13%), preluvisol the 5th place (335.050 ha, meaning 5%), while phaeozem has 235.282 ha and chernozem 46.026 ha (Dincă *et al*., 2014).

On a surface of 27.470 ha situated on the right side of Prutul Mijlociu basin from Iaşi County, the inventoried soils were the following: Cernisols 53%, Antrisols 28%, luvisols 14%, protisols 5%, hidrisols 0.13%, salsodisols 0.03% (Curea, 2016).

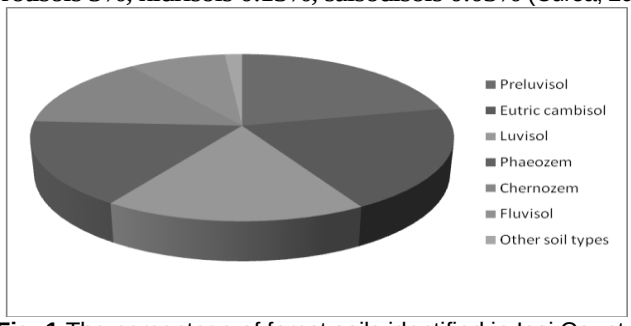

Fig. 1 The percentage of forest soils identified in Iasi County

In Bahluieţului basin, the most widespread soils are chernozems, luvisols, fluvisols and phaeozems (Todosi and Niculiță, 2015).

In the limits area between Copou hillslope and Cacaina floodplain, the typical soil-landscape system is formed by chernozems and regosols affected by

landslides on hillslope and two-generations of soils, phaeozems and fluvisols on the floodplain (Niculiță and Rusu, 2010).

Soil solution reaction

The soils pH was analyzed differentially for the most widespread types of soils (preluvisol, eutricambisol, luvisol, phaeozem and chernozem) (fig. 2).

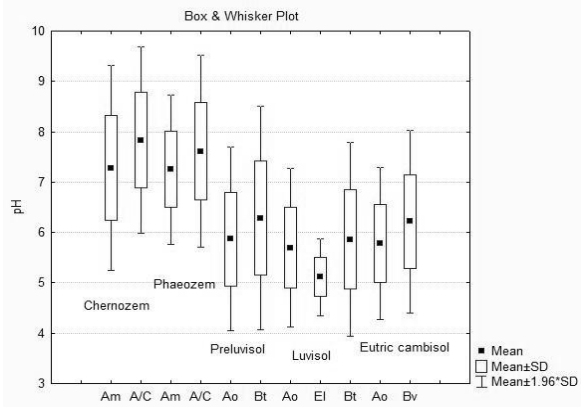

Fig. 2 pH variation of genetic horizons for the most widespread forest soils from Iaşi county

Chernozem and phaeozem, soils characterized by significant quantities of $CaCO_3$, have a very high pH, being weakly alkaline soils. As such, the average pH for chernozem is of 7.37 in the Am horizon, 7.84 in the A/C horizon and 8.35 in Cca, while for phaeozem is of 7.25 in Am, 7.62 in A/C and 8.16 in C.

Luvisol has an average pH of 5.7 in the Ao horizon, 5.11 in El and 5.86 in Bt, being moderately acid in the first two horizons and weakly acid in the third one. Preluvisol has an average pH of 5.87 in the Ao horizon and of 6.29 in Bt, being a weakly acid soil.

Eutricambisol, a type of soil specific to beech stands from the hill areas, has an average pH of 5.78 in the Ao horizon and 6.22 in the Bv horizon, being a weakly acid soil.

Also in Iaşi county, but in Dobrovăț basin, which occupies an area of 196 km^2, the soil acidity increases from south towards norths, which means that soils from areas with higher altitude are more acid, while soils from areas with higher humidity and steep slopes are more alkaline (Patriche *et al*., 2011).

Base saturation degree

As in the case of pH, the average values for each horizon of the most representative soils from the County were calculated for the base saturation degree (fig. 3).

It can be observed that the variation amplitude of this parameter is high for all soils.

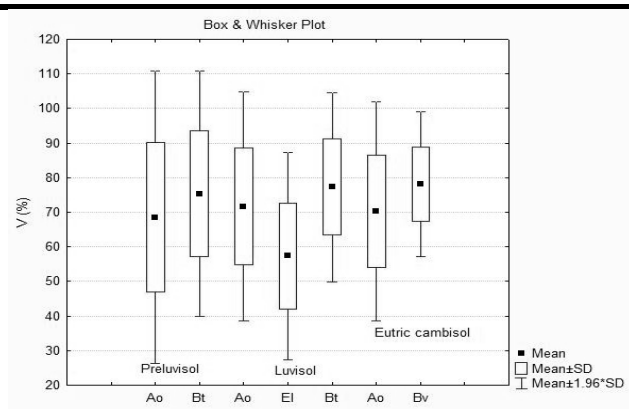

Fig.3 Base saturation degree variation for the most widespread soils from Iaşi county

Chernozem has an average V of 92% in Am and 99% in A/C, phaeozem has a value of 91% in Am and 93% in A/C, luvisol 72% in Ao, 57% in El and 77% in Bt, preluvisol 69% in Ao and 75% in Bt, while eutricambisol has 70% in Ao and 78% in Bv. All soils are eubasic.

Total cationic exchange capacity

An average value per profile was calculated for the total cationic exchange capacity and was rendered as table for each type of soil (tab. 1).

Table 1

Total cationic exchange capacity, average nitrogen content and humus for forest soils from Iaşi County

Chernozem	Phaeozem	Preluvisol	Luvisol	Eutric cambisol
Average total cationic exchange capacity per type of soil (T-me 100 g^{-1} sol)				
28.32	26.39	21.30	21.07	20.72
Average nitrogen content in the A horizon per soil type (%)				
0.17	0.25	0.22	0.27	0.21
Average humus content in A horizon per soil type (H-%)				
3.56	5.04	4.39	5.44	4.15

Charnozem and phaeozem have a very high cationic exchange capacity, while preluvisol, luvisol and eutricambisol are registering high capacities of cationic exchange (fig. 4).

Similar values for the total cationic exchange capacity for forest chernozem in Prisecani (Iaşi) area (24-29 me 100 g^{-1} sol) were also obtained by Bireescu *et al*., 2006.

Nitrogen

Due to the fact that the nitrogen quantity is decreasing on the soil's profile, only the afferent values of this parameter were analyzed for the first horizon of the studied soils (tab. 1).

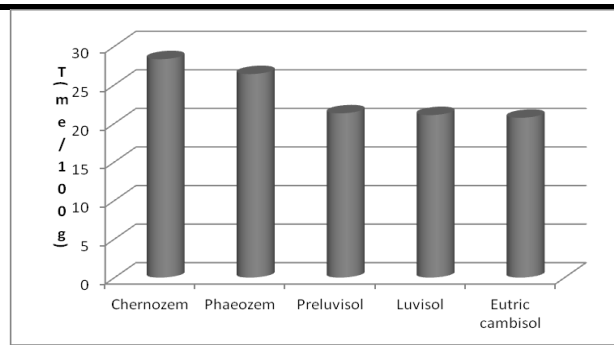

Fig. 4 The variation of total cationic exchange capacity for the most widespread types of forest soils from Iaşi County

Chernozem is normally supplied with nitrogen, while the other soils are well supplied with it.

In Prisecani area from Iaşi County, the realized soil analysis have revealed similar values for the nitrogen quantity in the first horizon of cambic chernozem and vertic ones from the forest area (Bireescu *et al*., 2006).

Humus

As in the case of nitrogen, the values corresponding to the A horizon were analyzed for humus (tab. 1).

The largest quantity of humus is found in the case of luvisoil (5.44%), while the smallest one is found for chernozem (3.56%) (fig. 5).

Phaeozem and luvisol are intensely humiferous soils, while chernozem, preluvisol and eutric cambisol are moderately humiferous soils.

The humus quantities from this county are similar with the average values calculated for the entire country for forest soils (Dincă *et al.,* 2012).

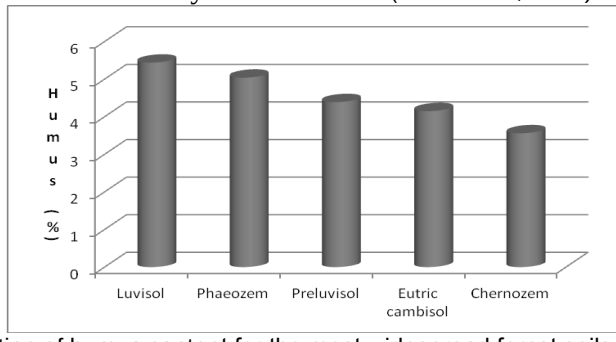

Fig.5 The variation of humus content for the most widespread forest soils from Iaşi County

CONCLUSIONS

1. In Iaşi County, the most widespread soils are the one specific to the field area (chernozem, phaeozem) and low hill one (preluvisol, luvisol, eutric cambisol). The above mentioned 5 types of soils have approximately an equal spread on the county's surface (between 22% and 14%).

2. Chernozem and phaeozem are weakly alkaline soils, while eutric cambisol and preluvisol are weakly acid and luvisol is moderately acid in Ao and El and weakly acid in Bt. All soils are eubasic. 3. Chernozem and phaeozem have a very high cationic exchange capacity, while preluvisol, luvisol and eutric cambisol are registering high cationic exchange capacities.

4. Chernozem is normally supplied with nitrogen, while phaeozem, preluvisol, luvisol and eutric cambisol are well supplied with nitrogen.

5. Phaeozem and luvisol are intensely humiferous soils, while chernozem, preluvisol and eutric cambisol are moderately humiferous soils.

6. The differences between chernozem and phaeozem are consisting of larger value of the soil's reaction and base saturation degree for chernozem in all the horizons. Similarly, preluvisol is a more alkaline soil than luvisol, whose base saturation degree decreases in the El horizon due to the eluviation process.

REFERENCES

1. Bireescu G., Bireescu L., Breabăn I., Stan O.C., 2006 - *Ecological and enzymathical study upon soil resources from forest ecosystems in middle Prut river course.* Factori şi Procese Pedogenetice din Zona Temperată 5 S. nouă 93-104.

2. Crişan V.E., Enescu Raluca, Dincă Maria, 2016 - *Descrierea solurilor din cadrul Direcțiilor Silvice Giurgiu şi Maramureş*, Revista de Silvicultură şi Cinegetică Braşov nr. 39:85-89.

3. Curea D., 2016 - *Caracteristicile fizico-chimice ale solurilor din bazinul mijlociu al Prutului între Țuțora şi Gorban (Fața Prutului).* Simpozionul Factori şi procese pedogenetice din zona temperată, Iaşi.

4. Dincă L., Lucaci Dora, Iacoban Carmen, Ionescu Monica, 2012 - *Metode de analiză a proprietăților şi soluției solurilor*. Editura Tehnică Silvică, 173 p.

5. Dincă L., Spârchez G., Dincă Maria, Blujdea V., 2012 - *Organic carbon concentrations and stocks in Romanian mineral forest soils*. Annals of Forest Research, 55 (2): 229-241.

6. Dincă L., Spârchez G., Dincă Maria, 2014 - *Romanian's forest soil GIS map and database and their ecological implications*. Carpathian Journal of Earth and Environmental Sciences, 9 (2):133-142.

7. Niculiță M., Rusu C., 2010 - *The soil and geomorphological limits between the Copou hillslope and Cacaina floodplain (Iassy area*). Factori şi Procese Pedogenetice din Zona Temperată 9 S. nouă, 31-41.

8. Patriche C.V., Pîrnău R., Roşca B., Vasiliniuc I., 2011 - *Preliminary results regarding the application of statistical methods for spatial prediction of soil parameters in Dobrovăț basin (Central Moldavian Plateau).* Factori şi Procese Pedogenetice din Zona Temperată 10 S. nouă, 95-102.

9. Spârchez Gh., Târziu D., Dincă L., 2011 - *Pedologie*. Editura Lux Libris, Braşov, 293 p.

10. Târziu D., Spârchez Gh., Dincă L., 2004 - *Pedologie cu elemente de Geologie*. Editura Silvodel, Braşov, 343 p.

11. Todosi C., Niculiță M., 2015 - *Assessing soil erodability factor for Rusle2 in Bahluieț catchment, eastern Romania*. Soil Forming Factors and Processes from the Temperate Zone XX (2015) 31-44.

12. www.rosilva.ro

13. www.insse.ro

14. *** Amenajamentele Ocoalelor Silvice Ciurea (2008), Dobrovăț (2008), Grajduri (1989, 2009), Hârlău (2004, 2015), Iaşi (2006), Paşcani (2004, 2014), Podu Iloaiei (2007), Răducăneni (1997, 2007).

INFLUENCE OF VERMICOMPOST ON SEEDLINGS' OBTAINING AT SOME SPECIES OF ORNAMENTAL GRASSES

INFLUENŢA VERMICOMPOSTULUI ASUPRA PRODUCERII RĂSADURILOR LA UNELE SPECII DE IERBURI ORNAMNETALE

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Abstract. *The best alternative of nowadays environmental degradation is to realise a proper use of the available unutilized organic biodegradable wastes for converting them into compost within a short period. Vermicompost could be used as an excellent soil amendment for main fields and nursery beds and has been reported to be useful in raising nursery species plants. The current paper presents the influence of vermicompost on producing of seedlings at ornamental gramineous species Pennisetum setaceum. Vermicompost was utilized in doses of 10% (V1), 20% (V2), 30% (V3) and 0% (V4). For all variants the basic utilised substrate was formed by 2 parts peat and 1 part garden soil. All variants at which vermicompost was utilised provided a more vigorous seedling in comparison with control variant (V4) and the best results were recorded at V3. Vermicompost could promote early and vigorous growth of seedlings. Vermicompost has found to be effectively enhanced for root formation, elongation of stem and production of biomass.*
Key words: ornamental grasses, seedlings, vermicompost

Rezumat. *Cea mai bună alternativă actuală în ceea ce priveşte degradarea mediului înconjurător este realizarea unei utilizări corecte a deşeurilor organice biodegradabile neutilizate pentru transformarea acestora în compost într-o perioada scurtă de timp. Vermicompostul poate fi utilizat ca un excelent amendament al solului atât pe câmp cât şi în pepiniere unde s-a constat efectul lui benefic asupra diferitelor specii de plante. Lucrarea de faţă prezintă influenţa vermicompostului asupra producerii răsadului la specia de graminee ornamentală Pennisetum setaceum. Vermicompostul s-a utilizat în proporţie de 10% (V1), 20% (V2), 30% (V3) şi 0% (V4). Pentru toate variantele experimentale, ca substrat de bază s-a utilizat un amestec format din 2 părţi turbă şi 1 parte pământ de grădină. Toate variantele în care s-a utilizat vermicompost au dus la obţinerea unui răsad mai viguros comparativ cu varianta martor (V4), cele mai bune rezultate înregistrându-se la V3. Vermicompostul poate provoca creşterea timpurie şi viguroasă a răsadurilor. Vermicompostul s-a dovedit a fi eficient în formarea rădăcinilor, în creşterea tulpinilor şi a producţiei de biomasă.*
Cuvinte cheie: ierburi ornamentale, răsad, vermicompost

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INTRODUCTION

Vermicompost is a humus type material, obtained through vermicompostation which represent the process where earthworms are utilised to convert organic materials with different provenance. Vermicompost could contain coprolite, an undecomposed organic material and earthworms' cocoons and which could be, in general, superior to the conventional produced compost (Munroe, 2015).

Due to physical, biological and nutritional properties, vermicompost determine the improvement of seeds' germination percent and seedlings' quality. It could be considered as a source of bioactive molecules and microbial populations. Those bioactive compounds and microorganisms intensify the adsorption of nutrients, the initial development of roots and seedlings' development capacity (Atiyeh *et al*., 2000; McGinnis *et al*., 2004; Kalra *et al.,* 2010; Singh *et al.*, 2011; Mathivanan *et al*., 2012; Absar *et al.,* 2016).

The beneficial effect of vermicompost on seeds' germination and seedlings' quality was studied also at other plant species such as: petunia (Arancon *et al*., 2008), basil (McGinnis *et al*., 2004; Jelačić *et al.,* 2005; Absar *et al.,* 2016), *Arachis hypogaea* L. - ground nut (Mathivanan *et al.*, 2012), different legumes species (Suthar *et al.*, 2005, Singh *et al.*, 2011).

The current paper aimed to show the influence of vermicompost on producing of seedlings at *Pennisetum setaceum* species.

MATERIAL AND METHOD

As study material was used *Pennisetum setaceum* ornamental species. Pennisetum genus belongs to Poaceae botanical family and presents numerous taxons with ornamental value, also known under the name of ornamental grasses (Chelariu, 2013)

Experiences were organized in four experimental variants, represented by the rate in which vermicompost participated into substrate, respectively 10% (V1), 20% (V2), 30% (V3) and 0% (V4) (tab. 1). For all experimental variants as basic substrate was utilised a mixture formed by 2 parts peat and 1 part garden soil. To establish the experiments were used seeds from Tenerife, Spain. Research were carried out in the greenhouses belonged to Floriculture discipline from UASVM Iaşi, Romania

The used vermicompost in our experiments is a substrate obtained with earthworms at SC SUPERPĂMÂNT SRL, Iaşi Romania. As feed for earthworms was used a pre-composed mix, formed by manure from cattle, horses, swine, cereal straws, alfalfa, vegetal remains from trimming the lawn, water.

Table 1

Experimental design

Species	Biological material	Variant	Substrate for sowing
Pennisetum setaceum	seeds	V1	basic substrate + 10% vermicompost
		V2	basic substrate + 20% vermicompost
		V3	basic substrate + 30% vermicompost
		V4	basic substrate + 0% vermicompost (control)

Research were carried out during March-May 2017, period in which were made observations regarding seeds' germination rate, necessary period from sowing till emergence ends, seedlings' growing dynamics and seedlings' characterization before crops' establishing. The obtained results were statistically analysed.

RESULTS AND DISCUSSIONS

Vermicompost influenced germination of *Pennisetum setaceum* seeds function of the rate in which is founded in crop substrate. So, at variant V1 (10% vermicompost) germination rate was 82%, at V2 (20% vermicompost) percent was 91%, and at V3 (30% vermicompost) rate was 99%. At control variant V4, germination percentage was 74% (fig. 1).

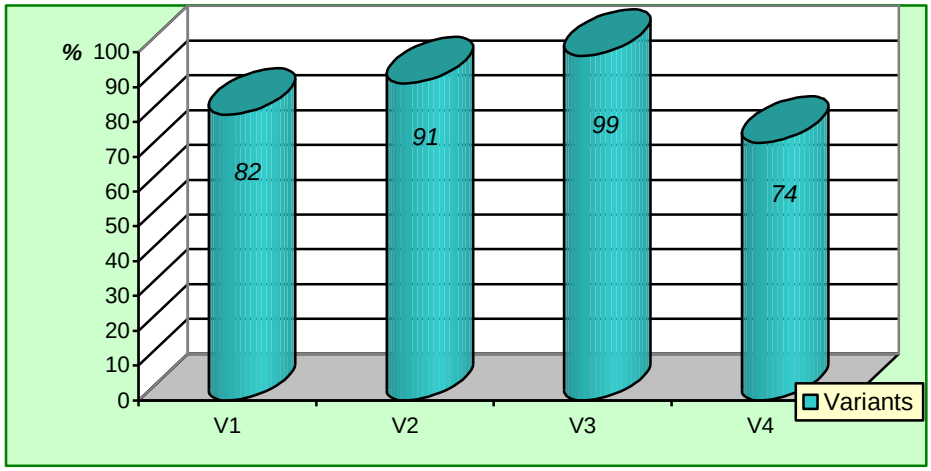

Fig. 1 Germination percentage (%)

Vermicompost influenced the moment of emergence start and determined the decreasing of seeds' germination period. So, at variant V3 (30% vermicompost) emergence started after 7 days from sowing, at variants V1 (10% vermicompost) and V2 (20% vermicompost) emergence started after 8 days from sowing and at variant V4 after 13 days (fig. 2). For a complete emergence, the necessary time calculated from sowing was 27 days for variant V3, 30 days for variant V2 and 31 days at variant V1. In case of variant V4 were necessary 41 days from sowing till emergence ended (fig. 2).

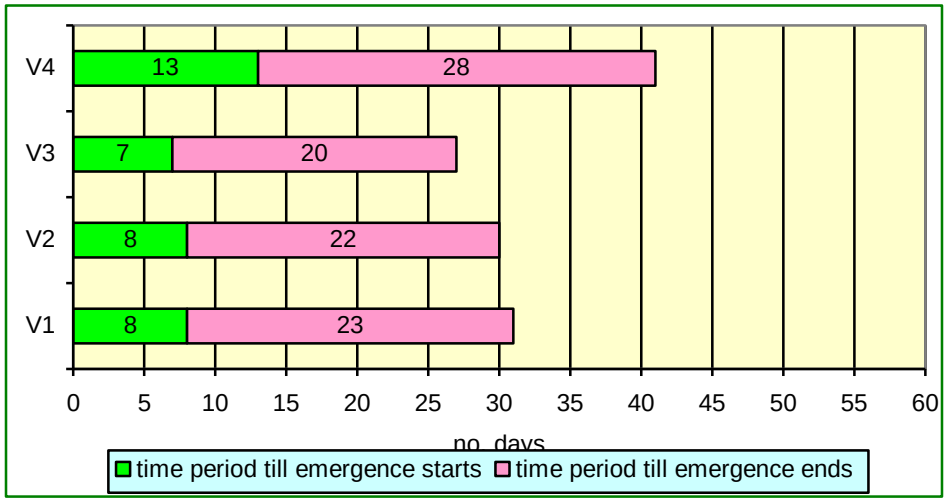

Fig. 2 Duration of germination (number of days from sowing)

Under the influence of vermicompost seedlings had a differential growing, function of its participation in the basic substrate. During research period was observed that seedlings from variants with vermicompost recorded growth of plants from 1.6 cm to 39.8 cm at V1, from 1.9 cm to 43.2 cm at V2 and from 2 cm to 47.7cm at V3. For the control variant V4 seedlings' growth was from 1.4 cm to 28.2 cm (fig. 3).

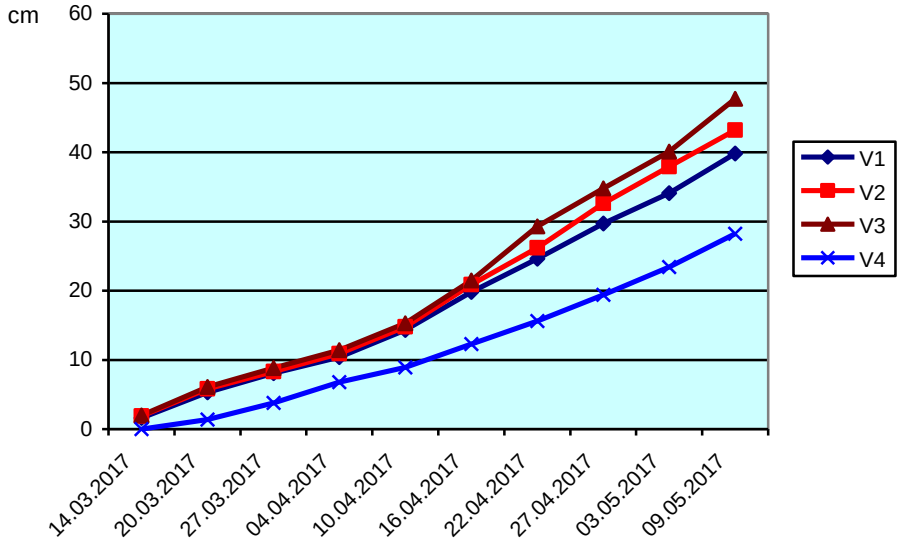

Fig. 3 Dynamics of seedlings' growing at *Pennisetum setaceum*

Table 2

Influence of vermicompost on morphological features of seedling at sowing in field

Variant	Mean height (cm)	Mean number of leaves per plant (pieces)	Mean number of roots per plant (pieces)
V_1	39.8***	10.8***	11.3***
V_2	43.2***	11.9***	12.5***
V_3	47.7***	12.7***	15.3***
V_4	28.2	8.6	8.3
	LSD 5% = 0.5 cm LSD 1% = 0.8 cm LSD 0.1% = 1.2 cm	LSD 5% = 0.2 pieces LSD 1% = 0.2 pieces LSD 0.1% = 0.4 pieces	LSD 5% = 0.3 pieces LSD 1% = 0.4 pieces LSD 0.1% = 0.7 pieces

Due to nutritional and biological value, vermicompost improve the quality of *Pennisetum setaceum* seedlings (tab. 2). Vermicompost (V1, V2, V3) determined the increasing of mean number of roots per plant, aspect which lead to formation of a higher mean number of leaves per plant than at control variant (V4) and implicitly to increase the seedlings' vigour.

CONCLUSIONS

At species *Pennisetum setaceum*, vermicompost determined the increasing of germination percentage and shortening of seeds' germination period.

Presence of vermicompost in substrate influenced the characteristics of *Pennisetum setaceum* seedlings, determining the increasing of main roots number per plant which also leaded to an increasing of leaves number and seedling height.

Seedlings with the best quality were obtained when in substrate exist a share of 30% vermicompost (V3).

REFERENCES

1. **Absar N., Kaur P., Singh A.K., Khan N., Singh S., 2016** – *Optimization of seed rate and seedling establishment technique for raising the nursery of French basil (Ocimum basilicum L.).* Industrial Crops and Products, vol. 85:190-197.
2. **Arancon Q.N., Edwards A.C., Babenko A., Cannon J., Galvis Paola, Metzger D.J., 2008** – *Influences of vermicomposts, produced by earthworms and microorganisms from cattle manure, food waste and paper waste, on germination, growth and flowering of petunias in the greenhouse*. Applied Soil Ecology, vol. 39:91-99.
3. **Atiyeh R.M., Subler S., Edwards C.A., Bachman G., Metzher J.D., Shuster W., 2000** – *Effects of vermicomposts an plant growth in horticultural container media and soil.* Pedobiologia, vol. 44:579-590.
4. **Chelariu Elena Liliana, 2013** – *Evaluation and completing the ornamental grasses assortment from the N-E area of Romania*. Research project in the project number POSDRU/I.89/1.5/S62371 "Postdoctoral School in Agriculture and Veterinary Medicine area", UASVM Iaşi, Romania.
5. **Jelačić Slavica, Lakić Nada, Beatović D., Vujošević Ana, 2005** – *Efects of different substrates on basil seedlings quality (Ocimum basilicum L.)*. Journal of Agricultural Sciences, vol. 50(2):107-115.

6. Kalra A., Chandra M., Awasthi A., Singh A.K., Khanuja S.P.S., 2010 – *Natural compounds enhancing growth and survival of rhizobial inoculants in vermicompost-based formulations*. Biol. Fertil. Soils, vol. 46:521–524.

7. Mathivanan S., Chidambaram Al Sundaramoorthy A.P., Kalaikandhan R., 2012 – *Effect of vermicompost on germination and biochemical constituents of ground nut (Arachis hypogaea L.) seedling*. Int. J. Res. Biol. Sci., vol. 2(2):54–59.

8. McGinnis M.S., Warren L., Bilderback T.E, 2004 – *Effect of vermicompost amended pine bark on basil production,* Container-Grown Plant Production, Sna Research Conference, vol. 49:120-123.

9. Munroe G., 2015 – *Manual de compostare şi vermicultură*, Centrul pentru Agricultură Organică din Canada, Traduceri Ecologice Independente, ediția I.

10. Singh B.K., Pathak K.A., Verma A.K., Verma V.K, Deka B.C., 2011 – *Effects of vermicompost, fertilizer and mulch on plant growth, nodulation and pod yield of French bean* (*Phaseolus vulgaris* L.). Vegetable Crops Research Bulletin, vol. 74:153-165.

11. Suthar S., Choyal R., Singh S., Sudesh R., 2005 – *Stimulatory effect of earthworm bodyfluid (vermiwash) on seed germination and seedling growth of two legumes*. J. Phytol. Res., vol. 18(2):219–222.

PROBLEMS OF PEDESTRIAN TRAILS IN URBAN SPACES

PROBLEME ALE TRASEELOR PIETONALE DIN SPAŢIILE URBANE

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Abstract. *Many urban spaces are mostly revealed by walking along or inside them. In this context, pedestrian trails play a special role because it mediates perception, establishes the speed, succession and character of landscape experiences. The walkways represent a means of directing or controlling landscaping. Trails development can be dotted with elements or points of interest, consisting of vegetation or constructed objects. Along alleys, the trails can be dilated in some places, becoming areas of ambient interest with resting places, water games and vegetal compositions. Navigating or scrolling along a trail, the landscape's perception must be controlled and dosed so that we do not reveal too much at once, nor do we strain the viewer by preventing it from penetrating in depth. The relaxation places must offer physical and psycho-emotional comfort, both through the elements of their design and by the aesthetic arrangement of the adjacent frame.*
Key words: walkways, landscaping, alleys, design

Rezumat. *Majoritatea spaţiilor urbane sunt dezvăluite mai ales prin parcurgerea lor. În acest context circulaţiile pietonale au un rol special deoarece mijlocesc percepţia, stabilesc viteza, succesiunea şi caracterul experienţelor peisagistice. Un mijloc de a direcţiona sau controla parcurgerile în peisagistică îl constituie traseele. Desfăşurarea traseelor poate fi ambientată cu elemente sau puncte de interes, prin vegetaţie sau obiecte construite. De-a lungul unor alei, traseele se pot dilata în mod studiat în anumite locuri, devenind zone de interes ambientate cu locuri de odihnă, jocuri de apă şi compoziţii vegetale. În parcurgerea unui traseu, percepţia peisajului trebuie controlată şi dozată astfel încât să nu dezvăluim prea mult dintr-o dată, dar nici să nu tensionăm privitorul împiedicând-ul să pătrundă vizual în profunzime. Locurile de odihnă oferite trebuie să dăruie confort fizic şi psiho-emoţional, atât prin elementele design-ului lor, cât şi prin ambientarea estetică a cadrului adiacent.*
Cuvinte cheie: trasee, peisagistică, alei, design

INTRODUCTION

Trying to introduce us into the spatial landscape universe, the great theoretician of modern landscaping John Ormsbee Simonds confessed that "many aspects of the art and science of design are revealed to the landscape artist when he realizes for the first time that he does not deal with surfaces but with spaces and volumes." (Simonds, 1967). A way of directing or controlling landscaping is

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the circulation called trail. "Nobody ever noticed a place except in a certain time, or a time at a certain place" (Trişcu, 1985). Following a route, the landscape's perception must be controlled and dosed in such a way that we do not reveal too much at once, nor do we strain the viewer by preventing it from penetrating in depth. That's why Simonds drew the attention of specialists involved in landscaping: "Do not design schemes that are meaningless or cold shapes. Better design a human experience" (Simonds, 1967).

MATERIAL AND METHOD

The paper analyses how the outdoor activities are influenced by the perception of the environment. Most urban spaces are mostly revealed by walking through them. In this context, pedestrian circulations play a special role because it mediates perception, determines the speed, succession and character of landscape experiences. Ignoring the principles of environmental psychology can lead to disturbing effects that cause environmental and mental discomfort. The successful combination of the effects of ambient elements creates the attractiveness attributes of places. Consequently, depending on landscape elements introduced in the city space, residents, as users of the environment, can benefit from the possibility of socialization, recreation and improvement of the quality of life. Individuals can experience feelings of physical and psychological welfare, the feeling that a space is well designed and built.

RESULTS AND DISCUSSIONS

COMPOSITION OF TRAILS is supported by the science of using the axes and the arrangement of interest points and areas of interest along them. Also, controlling the pauses and rhythms of positioning/organizing of the landscape morphological elements along the route and adjacent areas can lead or guide, can reveal or conceal mysteriously, encouraging, attracting, and prolonging walks in a particular direction, or certain favorable outlook. In navigating a route, the perception of the landscape must be controlled and dosed so that we do not reveal too much at once, nor do we tense the viewer, preventing our eyes from penetrating into the depths of the alleys. Trail development can be surrounded by elements of interest, called points of interest, consisting of vegetation or built objects. Routes along alleys or axes can dilate from place to place, and they become areas of ambient interest with resting places, water games and vegetal compositions. In landscaping, the trails are mainly created via pedestrian circulation routes. Alongside these, non-circulating visual axes can be opened during the course that leads the perspectives to important points or centers of interest (Dascălu and Cojocariu, 2016).

DISTINGUISHING AND MASKING of the interest points or areas of interest become the means by which the arrangements can be put to good use, stirring curiosity and focusing more attention. The composition technique consists of placing landscape objects or vegetation so as to reveal or mask certain objects.

Also, filtering images through vegetation groups or among various objects (statues, fountains, pergolas, etc.), using the principle of the perception gradation, contributes to the viewer's pleasure in discovering certain details. The images below illustrate some possibilities of perception (fig. 1, fig. 2).

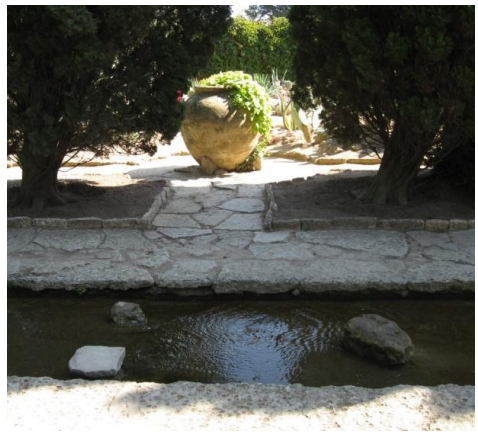

Fig. 1 Filtered perception through vegetation to an interest point
Balcik, Bulgaria - photo Dascălu D.M.

Fig. 2 Filtered perception through neogothic pillars
Balcik, Bulgaria-photo Dascălu D.M.

SUCCESSION, GRADATION AND RHYTHM are basic elements in trails design. Simonds draws attention to the importance of succession: "Deliberately designed, succession can be a highly effective compositional process. Succession expresses in the landscape composition a set of perceptions or experiences that have a continuity. Successions have no meaning unless and until they are lived by man" (Simonds, 1967). Succession involves targeting. Grading involves accumulation. Rhythm involves repetition, alternation between accents and

breaks. The rhythm can be static-linear or dynamic-directed. It can be obtained using volumes, surfaces, colors, light, functions, either by condensation or by dilution of interest (fig. 3).

Fig. 3 Succession and rhythm of interest points (palms and benches) along the promenade in Kuweit – photo Dascălu D.M.

Succession, gradation and rhythm have as effect the perception of time, involving movement, scrolling, searching for interesting details to be admired. The movement adds to the three-dimensional perception of the landscape the perception of the fourth dimension, means the time. It is important to understand that time can be perceived, capitalized and controlled by dosing, creating rhythms and breaks in landscape compositions (Dascălu and Cojocariu, 2016).

THE OBJECTS AND THE PLACES FOR RELAXATION ALONG TRAILS have become, in the last hundred years, indispensable for the quality of life, playing an important role in urban comfort (Dascălu and Dascălu, 2016). Urban public spaces, squares, gardens and parks need resting places. Inspired by indoor furniture, recreational items are presented in a wide variety of types of benches, seats and modules, fixed or movable, with or without a backrest, some of which are multifunctional.

Relaxation places can be classified according to several criteria: by the nature of the materials used, by color, shape and volume, by style, etc. If we take into account the number of people who can use them simultaneously, we can distinguish between individual, semi-collective and collective places. The role of resting places in any type of public space is of particular importance. If they are given opportunities to sit down, people spend more time in landscaping, if the places are of poor quality, the pedestrians will not stay but continue their journey. The existence of favorable conditions for sitting down stimulates the activities (reading, resting, conversation etc.) that are vital for the quality of public spaces and the public environment (Jurov, 2006).

In order for the sitting place to be attractive, they must meet a number of requirements as for climate, space, location, configuration, but also for material, shape, texture, color and volume. In urban spaces and beyond, there are many examples of situations where objects are placed either mistakenly or randomly in a non-functional manner that causes discomfort (fig. 4, fig. 5).

Fig. 4 Non-functional manner of placing benches in the green spaces-photo Dascălu D. M.

Fig. 5 Non-functional and non-aesthetical way of placing benches - photo Dascălu D. M.

The location of the resting places will determine the choice of those objects that are positioned offering protection and privacy, a good microclimate, the view of the surroundings/belle view, with the back protected.

The type of furniture is a criterion that differentiates users by age and temperament. Young people can easily sit down in multiple ways without using specially designed objects: straight on lawns or dredges, on various edges / borders, on the statue's trunk, on the jardinières, etc. For older people, adequate furniture is nevertheless an essential prerequisite for resting. A well-equipped public space should have comfortable arrangements for urban rest: benches, seats, chairs, decorative frames with resting seats.

Urban furniture must be adapted to some sustainable contemporary requirements such as: the use of multifunctional and removable furniture to allow multiple uses and locations so that urban space can be efficiently used without shocking it or leaving it unfurnished; integrating vegetation into furniture should be carefully designed, not to limit its use by discomfort; the fitting of the furniture and its positioning should favor both socialization and intimacy and protection; the furnished areas with adequate resting places should be present in as many urban spaces as possible, adapted to the areas and avoiding crowding; the textures of the materials must be judiciously adapted to the site, preferably natural and ecological, but also time-resistant; the color scheme harmonized with the environment should be suitably chosen for the functions; the stylistic adaptation of the furniture to the characteristics of the urban framework is necessary in order to avoid stylistic aggression.

CONCLUSIONS

Public pedestrian trails open to all must possess attractive features and, implicitly, provide a rich experience of meanings and well-being. Applying the principles of environmental psychology to landscaping can activate the elements of ambient attractiveness and contributes to the creation of spaces that will generate beauty and balance. The relaxation places offered in this way must give physical and psycho-emotional comfort, both by their location and design details, as well as by the aesthetic surrounding of the adjacent frame.

REFERENCES

1. Dascălu Doina Mira, Cojocariu Mirela, 2016 – *Design Peisagistic*, Ed. Ion Ionescu de la Brad, Iaşi, p.36-47.
2. Dascălu Doina Mira, Dascălu V., 2016 -*Trends in sustainable landscaping over the last decades,* Lucrări ştiințifice, Anul LIX, Vol 59, nr.2, seria Horticultură, Ed. USAMV Iaşi, pag. 283-289
3. Jurov C., 2006 - *Arhitectura ambianțelor*, Ed. Capitel, Bucureşti, p. 64-69.
4. Simonds J. O., 1967 - *Arhitectura peisajului*, Ed.Tehnică, Bucureşti, p. 32.
5. Trişcu A., 1985 - *Spații urbane pietonale*, Ed. Tehnică, Bucureşti, p. 72-75.

URBAN LANDSCAPE BETWEEN UTOPIA AND REALITY

PEISAJUL URBAN ÎNTRE UTOPIE ŞI REALITATE

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Abstract. *The paper investigates the theme of urban landscape and its proximity to the descriptions offered in literary utopias. Clarifying the "visionary"/"utopian" opposition requires the revaluation and the reappraisal of the original significations of utopian space, as they are fixed by the literary texts centered on the idea of social and urban reform, beginning with Morus' Utopia. Architects have always faced utopia, having to choose between accepting or refuting its ideological dimensions (economic, political, social). This confrontation led to interpretative speculations that encouraged a new architectural discourse, and in the 1970s many theorists declared the death of modernity and the end of utopia, causing a retheorization of utopia, especially at the end of the 20th century, marked by the fall of communism and its architectural projects. Today's urban and landscape design cannot ignore the retheorization of utopia, especially in postcommunist countries, modeled by social and urban engineering of communist ideology (utopia).*
Key words: *architecture, urban space, landscape, urban design, utopia, the history of utopia*

Rezumat. *Lucrarea investighează tema peisajului urban şi apropierea acestuia de descrierile oferite în utopiile literaturii universale. Clarificarea opoziției „vizionar"/„utopic" necesită recuperarea şi reconsiderarea sensurilor originare ale spațiului utopic, aşa cum sunt ele fixate de textile literare sau programatice, centrate pe ideea de reformă socială şi urbană, începând chiar cu Utopia lui Morus. Arhitecții s-au confruntat mereu cu utopia, având de ales între a accepta sau nu forțele ideologice ale ei (economice, politice, sociale). Această confruntare a dus la speculații interpretative care au catalizat discursul arhitectonic şi designul, în anii '70 existând numeroşi teoreticieni care au declarat moartea modernității şi sfârşitul utopiei, provocând o re-teoretizare a utopiei, în special la sfârşitul secolului XX, marcat de căderea comunismului şi a proiectelor sale arhitectonice. Proiectarea urbană şi peisagistica secolului XIX nu poate face abstracție de re-teoretizarea utopiei, mai ales în țările postcomuniste, modelate de ingineria socială şi urbanistică a ideologiei (utopiei) comuniste.*
Cuvinte cheie: *arhitectură, spațiu urban, peisagistic, proiectare urbană, utopie, istoria utopiei*

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INTRODUCTION

Following the most present-day utopian studies it is difficult to find an author who wrote positively on Utopia in contemporary urban literature. As far as "utopia" is defined and understood as a code word synonymous with totalitarianism or Communism (or Stalinism, according to Fredric Jameson), the contemporary approaches on relations between urban landscape and utopian thinking are highly negatively influenced. Today's urban and landscape design cannot ignore the retheorization of utopia.

For Manfredo Tafuri, the main challenge of the contemporary architecture is the return "to *pure architecture*, to form without utopia" (Tafuri, 1976). Tafuri observed that "The decline of the social utopia sanctioned ideology's surrender to the *politics of things* brought about by the laws of profit. Architectural, artistic and urban ideology was left with the utopia of form as a way of recovering the human totality of through an ideal synthesis, as a way of embracing disorder through order" (Tafuri, 1976).

Also Fredric Jameson pointed out very clearly: "What we have perhaps not yet sufficiently emphasized is the relationship of this seemingly political crisis of Utopia (generally attributed to the fall of the communist parties and their substitution by the new social movements and anarchist currents) to a more general crisis of representation attributed to the advent of postmodernity" (Jameson, 2005). It is a perspective we largely met: the broad consensus is that all utopian experiments generally failed, and today there is no place to discover. The crisis of utopia could be interpreted as the result of the evolution of time. Still, as Antoine Picon observed, the relevance of a utopian approach for urban planning is valid, because the contribution of a utopian project is the identification of social meanings that possess imaginary potential.

According to Françoise Choay, the city was replaced by *the idea of city*, following three main paradigms: progressive, cultural and naturalist. All these models were "rationalistic and utopian, having a corrosive influence upon the urban structures" (Choay, 2002). The urban landscape promoted by this kind of approach was impossible to be generalised, as they were incapable to get with touch in the elementary functions of people. But urbanism should abandon models and functionalism, as "the new urbanist language would lose its specific for acceding a new and superior plan of generality; it would draw the whole society, indirectly implying it by referring to the framework of all significant systems" (Choay, 2002)

Also, Choay's idea should be adopted in reassessing the role of the utopian writings in the urban planning: utopians were the first planners and urban planning is derived from the work of utopians. The return to utopia is necessary in discussing the new functions of utopia in contemporary urban landscapedesign.

MATERIALS AND METHODS

Projects of ideal cities are to be found in the entire history of architecture. Some projects have been materialized, but most of them belong to an imagined reality. For this reason, ideal cities were considered perfect examples of failures or models of unsuccessful planning. Mentioning some examples reveals that the concept of ideal city goes beyond utopia.

Sforzinda is an ideal city designed between 1457-1464 by Filarete (Antonio di Pietro Averlino) that was never built, and was named after Francesco Sforza, the duke of Milan. The shape is iconographic, having a basic layout of eight equidistant points, created by overlaying two squares. All the avenues from the gates and towers converged in a central square. Actually, three squares were imagined: one for the prince's palace, one for the cathedral, and one for the market. Specifically designed was also the House of Vices and Virtues, as a materialisation of one favourite theme of treatises on moral in that time. *Sforzinda* embodies the absolutist social ideal in which one ruler holds all the power.

Octagon City near Humboldt (Kansas, USA) was designed by Henry Clubb in 1856, on the expenses of The Vegetarian Kansas Emigration Company for vegetarian people. Clubb, a vegetarian activist himself, imagined that eight roads would lead away from a central octagonal town square. From there, the city would be made up of four octagon villages, complete with octagon farmhouses, town squares, and public buildings. The settlers who stayed faced a multitude of problems, including lack of water when the local spring dried up and diseases. Nothing remained of the town today, but Clubb's legacy lives on in several octagon houses that remain in the US and Canada.

Garden City was invented by Ebeneezer Howard in 1902, when he proposed in his treatise *Garden Cities of To-Morrow* a (new) vision of urban development. Howard imagined a series of ideal towns, planned on a concentric model and combining nature with society, as 'Town and Country must be married, and out of this joyous union will spring a new hope, a new life, a new civilization'. (Howard, 2003) Ebenezer Howard has initiated with this proposal the *garden city movement* in England, as a method of urban planning that imagined garden cities, that were intended to be planned, self-contained communities surrounded by "greenbelts", containing proportionate areas of residences, industry, and agriculture. Ebenezer Howard intended to found this community with a special feature regarding the property: every inhabitant was to be a shareholder.

Broadacre City was presented by Frank Lloyd Wright in 1932 (Wright, 1932).The model was initially displayed at an Industrial Arts Exposition in the Forum at the Rockefeller Center starting on April 15, 1935. Wright's imagined ideal community was a complete rejection of the American cities of the first half of the 20th century. According to him, cities would no longer be centralized and no longer beholden to the pedestrian or the central business district. *Broadacre City* was a thought experiment and also a serious proposal – one where the automobile would reign supreme. The key to Wright's "utopia" represented the tremendous technological advances made at the beginning of the 20th century, especially the automobile.

Le Corbusier's plan for *Ville Radieuse*was presented in 1930s, but the city has never become a reality. Le Corbusier's proposal unifies his visions of modern town construction and elements of residential building. Hence, the architect dealt with architectural and construction as well as with social image of the city. "If the city were

to become a human city, it would be a city without classes", affirmed Le Corbusier and for that reason he designed a pyramid of natural social orders (Le Corbusier, 1964)

Ville Radieuse alters the idea of the city-as-body: the city map still consists of a classical body with its head (business centre) and its heart (cultural centre). Though, the central axes are not bilaterally symmetrically applied. The plainness of the complex is seen as a biological development - like the roots of a tree. As a result, the city only consists of one central axe.

Many of its principles went on to influence modern planning: Le Corbusier himself designed Chandigarh in India (1949) and Lucio Costa, Oscar Niemeyer designed together with landscape designer Roberto Burle Marx between 1956-1960 Brasilia, the new Capital of Brazil.

Communism is characterised by a mixture of architectural tendencies: *Constructivist architecture*, a form of modern architecture (1920s-1930s) with Communist social purpose and *Stalinist architecture* or *Socialist classicism* (1933-1955), which was a part of the Soviet policy of rationalization of the country. All cities were built to a general development plan. Each was divided into districts, with allotments based on the city's geography. Projects would be designed for whole districts, visibly transforming a city's architectural image.

The post-Stalinist architecture lasted until the last years of Communism, and its legacy can be traced in all former Eastern Communist countries. Being connected with the idea of a society without classes, which remains a utopian concept, the architecture of the Communist age is often defined also as utopian.

RESULTS AND DISCUSSIONS

Differentiating between "utopian" and "visionary" should lead us to underline the main feature of utopian: "utopian" is a particular form of "ideal". In the history of utopian thinking and also in the history of architecture the alternative and synonymic usage of these two terms is relevantly frequent, as suggest a confusion. "Utopian" is originally related to utopian thinking and writing, directly derived from Thomas Morus' *Utopia* (1516): the place of nowhere – not necessary in future, but in present. "Visionary" is related to an imagined future, to projections of the future – could be one of the features of social utopias. The negativity of utopia in present-day discourses is influenced by the idea of the impossibility of the ideal city: "An ideal city doesn't exist" (Governour, 2011). The rejection of utopia is a consequence of the rejection of the idea of ideal city – linked with that of ideal society (Plato's original idea of ideal society is stated in *Republic*, 380 BC). As the concept of ideal city precedes utopian writings, we may affirm that "utopian" is a version of "ideal". Urban planning and landscape architecture should not be imagined without reflecting to the imaginary environments of a wide variety of fascinating and often controversial movements and figures, including Plato, Filarete, Leonardo da Vinci, Thomas More, Thomas Jefferson, Claude-Nicolas Ledoux, Charles Fourier, Etienne Cabet, Robert Owen, William Morris, Ebenezer Howard, Bruno Taut, Le Corbusier, Frank Lloyd Wright, the European Situationists, the Japanese Metabolists, Archigram, Superstudio and many more, as Ruth Eaton claims. Althoughthe ideal city belongs for the most part in the virtual domain of ideas,

Eaton explores the ability of ideal cities to stimulate reflection and change, and suggests under what conditions they might continue to exercise their vital function in relation to the urban environment of the future. The main suggestion is to recall together what Oscar Wilde affirmed: "a map of the world that does not include Utopia is not worth even glancing at" (Wilde, 1900)

The original source of the inspiration in what history named utopian architecture and urban development is undoubtedly Thomas More's *Utopia*. Here we are not interested necessarily in the political project, but in urban project derived from the political idea of an ideal, perfect landscape planning. This political idea implied a society without private property, equality and conformism: "Long unbroken rows of houses face each other down the whole block. The housefronts along each block are separated by a street twenty feet wide. Behind the houses a large garden - as long on each side as the block itself is hemmed in all sides by the backs of the houses. Every house has a front door to the street and a back door to the garden. The double doors, which is open easily with a push of the hand and close again automatically, let anyone come in - so there is nothing private anywhere. […] The Utopians are very fond of these gardens of theirs. They keep interested in gardening, partly because they delight in it, and also because of the competition among the blocks, which challenge one another to produce the best gardens. […] And from the fact it appears that the city's founder must have made such gardens a primary object of this consideration." (More, 2002)

"Utopian" and "ideal" may be opposed to reality, outside of reality, but they still provide suggestions and solutions in urban planning and landscape architecture.

As Tafuri observed, the crisis of modern architecture begins when the large industrial capital (its natural receiver) goes beyond ideology: the architectural ideology has no any purpose. This diagnosis could be extrapolated to the Post-Communist societies, after the fall of the Communist regimes, where there are no more coherent ideas of urban planning. It must be admitted that modern urbanism, including the Communist developments, has not been able to realize its models, in spite of being related with a utopian attempt to preserve a form for the city or to preserve a principle of form within the dynamics of urban structures.

We may affirm that only a return to architectural and super-technological "utopianism" could revive the new urban ideology. Technology and nature would play the role of the main catalyst, as they provide a synthesis of new languages and urban reality.

CONCLUSIONS

Having in mind this controversial history of the urban planning and urban landscape, strongly connected with utopian projections – and for this reason rejected after the fall of Communism –, we should take into account some fundamental clarifications made by Ruth Levitas. In *The Concept of Utopia*

(1990) and *Utopia as Method* (2013), Levitas insisted that the function of utopia is not escape, compensation or a description of a plan for the future. Utopia should be understood as a method for the imaginary reconstruction of society, as it is mostly an explanatory and educative tool. In order to have a contextual understanding, we should keep in mind that utopia is a framework for utopias.

As the ideal cities and utopian cities were considered situated in no-place, nowhere in reality, they were refuted, and their convergence with the future evolution of the urban planning is truly limited. But the return to the original sources of utopian representation of the ideal city is legitimate, as provides clarifications and delimitations that are very useful for the present-day architecture.

REFERENCES

1. Biciuțcă F., 2002 - *Uitatul în cetate*. Bucureşti: Paideia.

2. Choay F., 2002 - *Urbanismul. Utopii şi realități*. Translated by Kázmér Kovács. Bucureşti: Paideia.

3. Coleman N., 2013 - *Building in Empty Spaces': is Architecture a 'Degenerate Utopia'?.* The Journal of Architecture, 18: 2, pp. 135-166.

4. Coleman N., 2014 - *The Problematic of Architecture and Utopia*. Utopian Studies, Vol. 25, No. 1, pp. 1-22.

5. Eaton R., 2002 - *Ideal Cities: Utopianism and the (Un)built Environment*. London: Thames and Hudson.

6. Ganjavie A., 2012 - *Role of Utopia for Design of Future Cities: Utopia in Urban Planning Literature.* Studies in Literature and Language. Vol. 5, No. 3, pp. 10-19

7. Howard E., 1898/2003 -*To-Morrow. A Peaceful Path to Social Reform*. Original ed. with new commentary by Peter Hall, Dennis Hardy & Colin Ward, London: Taylor and Francis.

8. Jameson F., 2005 - *Archeologies of Future: The Desire Called Utopia and Other Science Fictions*. London and New York: Verso Books.

9. Le Corbusier, **1964** - *Radiant City*. New York: Orion Press

10. Levitas R., 2013 - *Utopia as Method. The Imaginary Reconstitution of Society*. Basingstoke: Palgrave Macmillan.

11. More T., 2002 - *Utopia*. Edited by George M. Logan and Robert M. Adams. Translated from Latin by Robert M. Adams. Cambridge: Cambridge University Press.

12. Rosenau H., **2007 (1959)** -*The Ideal City in its Architectural Evolution*. London and New York: Methuen & Co. Ltd.

13. Wilde O., 1900 – *The Soul of the Man*. London: Arthur L. Humpreys

14. Wright F. L., 1932 - *The Disspearing City*, William Farquhar Payson, New York

15. Tafuri M., 1976 - *Architecture and Utopia. Design and Capitalist Development.* Translated by Barbara Luigia la Penta. Cambridge, MA: The MIT Press.

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