BIOACCUMULATION OF HEAVY METAL IN OAT PLANT UNDER POLYPHENOLIC COMPOUNDS TREATMENT

BIOACUMULAREA METALELOR GRELE ÎN PLANTELE DE OVĂZ ÎN PREZENŢA UNOR EXTRACTE POLIFENOLICE

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Abstract. *Contamination of cereal crops by heavy metals is a major problem, as long as there is permanently a major risk to be ingested by humans. Secondly, the plant sensitiveness response to different pollutants action could be properly used in monitoring several pollution processes. The aim of this paper is to analyze the morpho- physiological response of Avena sativa plant in the presence of copper and cadmium ions through germination test experiments. The influence of spruce bark aqueous extract, in different concentrations (total ployphenols content 130, 191, 190 mg/L extract) on bioaccumulation of heavy metals ions in different parts of oat plantlet was evidenced by spectrophotometrically atomic absorption method. The biometric measurements were performed, along with quantitative determination of biomass accumulation and assimilatory pigments concentrations (chlorophyll **a** and **b**). Spruce bark polyphenolic extracts, block the bioaccumulation of heavy metal ions in oat plant, depending on extracts concentration applied in the experiment.*

Key words: copper, cadmium, chlorophyll **a** and **b**, atomic absorption spectrophotometry

Rezumat. *Contaminarea culturilor de cereale cu metale grele este o problemă majoră, atât timp cât există riscul ca acestea să fie utilizate în alimentaţia umană. Pe de altă parte, sensibilitatea unor plante la acţiunea poluanţilor poate fi folosită pentru biomonitorizarea unor procese de poluare. Lucrarea îşi propune o analiza a răspunsului morfo- fiziologic al plantulelor de ovăz în prezenţa ionilor de cupru şi cadmiu prin folosirea unor teste de germinare. Influenţa extractului apos din coaja de molid, în diferite concentraţii (conţinut total de polifenoli 130, 191, 190 mg/L extract) asupra bioacumulării ionilor metalici la nivelul diferitelor parţi componente ale plantulelor de ovăz a fost evidenţiată prin spectrofotometria de absorbţie atomică. De asemenea, au fost efectuate măsurători biometrice, determinări cantitative privind biomasa acumulată alături de concentraţia pigmenţilor asimilatori (clorofila **a, b**). Extractele obţinute din coaja de molid, în funcţie de concentraţia aplicată, blochează bioacumularea ionilor metalici la nivelul radicular al plantulelor.*

Cuvinte cheie: cupru, cadmiu, clorofila **a** şi **b**, spectrometria de absorţie atomică

INTRODUCTION

The main sources of heavy metal contamination are represented by ferrous platforms, ore processing industry, sludge from wastewater treatment stations, wastewater irrigation, fertilization and soil amendment. Crop species present different charactheristics depending on their sensitivity to micronutrients

deficiency or toxicity. Metal phytotoxicity is defined by the excessive concentration in essential or unessential metals. Cultivated plants that tolerate high concentrations of heavy metals represent a greater risk for consumer's health than those who are more sensitive and show different symptoms of toxicity.

Tannins are polyphenolic compounds with multiple hydroxyl groups and show a special affinity for heavy metals, especially uranium. Araújo do Nascimento (2006) noticed that citric, oxalic, vanillic and gallic acids, applied at 10 or 20 mmol/kg, solubilized significant amounts of Zn, Ni, and Cd from soil.

In this context the aim of this work is to underline the polyphenolic compounds role in copper and cadmium bioaccumulation process to different parts of oat plant.

MATERIAL AND METHOD

Obtain and characterization of aqueous polyphenolic extract. The polyphenolic extract was obtained through successive extraction in 125mL distilled water of 5, 10 and 20 g dry and milled spruce bark, raw material on a water bath at 80-90°C for 45 min. The aqueous polyphenolic extract was characterized in terms of dry matter content, organic matter content and total polyphenolic content. The total polyphenolic content was determined by using Folin Ciocalteau method and the concentrations were expressed in mg gallic acid/L extract (Bao et al., 2005).

Germination Tests. Germination tests were carried out in Petri disches. The Petri dishes were incubated in a thermostatic chamber at 25-27 °C for seven days. After 168h, oat seedling were exposed to day light for 48 h to promote chlorophyll pigments bioshynthesis. At the end of the experiment biometric measurements and quantitative determination were done. We tested three different concentrations of copper and cadmium ions (5, 12.5 and 25 µg/mL $CuSO_4$/$CdCl_2$) with and without spruce bark aqueous extract addition. The tested solutions were suggestively noted as: Me-5, Me-12.5, Me-25 for copper and cadmium salts and SB5Me-5, SB5Me-12.5, SB5Me-25 ; SB10Me-5, SB10Me-12.5, SB10Me-25 ; SB20Me-5, SB20Me-12.5, SB20Me-25, in the case of spruce bark extract (SB) addition. The experiment was carried out in triplicate. The experimental results were statistically processed.

Pigments assimilation assay. The chlorophyll was extracted in 80% acetone and spectrophotometrically determinated by reading the absorbances at fixed wavelength of 470, 646, 663 nm. The concentrations of chlorophyll pigments were calculated by using the specific coefficients suggested by Lichtenthaler and Wellburn (1983). The results were expressed in µg/g green biomass.

Heavy metal concentration evaluation. Copper and cadmium ions concentrations were spectrophotometrically determined by using a GBC Avanta atomic absortion spectrophotometer. Plantlet dry samples were mineralizated in nitric acid (65%) and H_2O_2 (30%) on a hot plate at 120°C,for at least five hours (Smith et al., 2008; Stingu et al., 2009).

Bioaccumulation coefficient = metal ions concentration into the plant / metal concentration into the growth medium (Stingu et al., 2009).

Translocation factor = metal ions concentrations into the shoots / metal ions concentration into the root (Sun et al., 2008).

RESULTS AND DISCUSSIONS

The spruce bark aqueous extract present the following characteristics (table 1). Dry matter content, organic matter content and total polyphenolic content increased proportionally with increasing the amount of extracted spruce bark raw material.

The presence of spruce bark polyphenolic extracts in a copper contaminated environment stimulates the rootlets and hypocotyls elongation and oat hypocotyls green biomass accumulation but inhibit the cotyledons growth and development, compared with the reference (fig.1- fig.3).

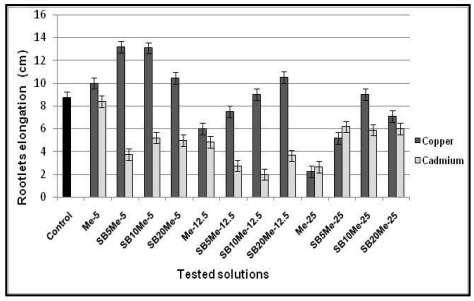

Fig. 1. Oat rootlets elongation under heavy metal stress and polyphenolic extracts treatments

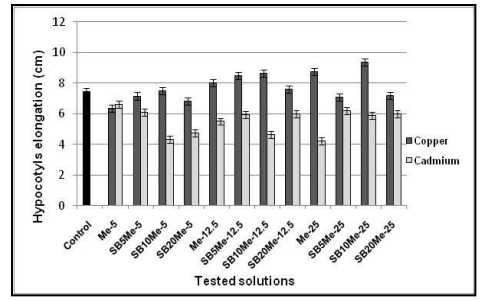

Fig. 2. Oat hypocotyls elongation under heavy metal stress and polyphenolic extracts treatments

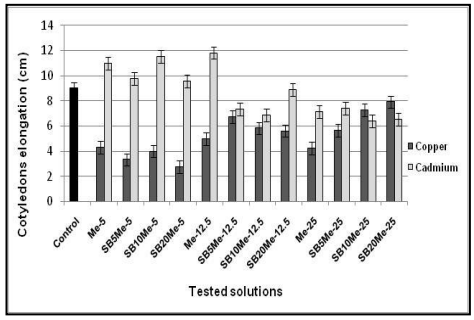

Fig. 3. Oat cotyledons elongation under heavy metal stress and polyphenolic extracts treatments

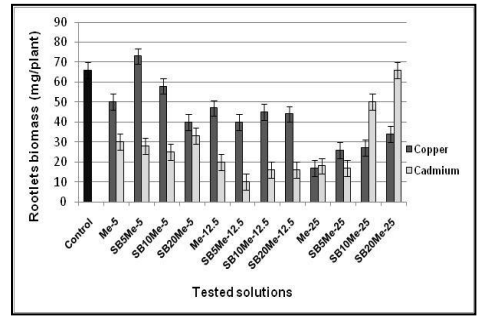

Fig. 4. Rootlets biomass variation under heavy metal stress and polyphenolic extracts treatments

Even if the spruce bark aqueous extracts treatment do not shows major stimulatory effects compared with the control, it was noticeable a decreasing of copper inhibitory action on plant growth and development in the presence of polyphenolic extracts.

Table 1

Spruce bark aqueous extract characterization

Extracted spruce bark, (g)	Dry matter content (g/L extract)	Organic matter content (g/L extract)	Total polyphenolic content (mg/L extract)
5	0.580	0.483	130
10	1.004	0.964	191
20	1.912	1.917	190

When the environment was contaminated with cadmium ions it could be observed that natural bioactive compounds from aqueous extract stimulate both green biomass accumulation and cotyledons elongation (fig.4 – fig.6). In the absence of natural extracts, the harmful effects of metal ions on *Brassica napus* plant growth and development would be much more noticeable.

Chlorophyll a biosynthesis was stimulated in heavy metal contaminated environment (5µg/mL $CuSO_4$/ $CdCl_2$) under spruce bark polyphenolic extract (20g) treatment. The polypeholic extracts in every concentration, in a copper contaminated growth medium, stimulate Chl **a** biosynthesis.

Not the same effects could be observed when the medium is contaminated with cadmium ions (table 2). Spruce bark extracts (0.58g/L, 1.91g/L) promote Chl **b** assimilation both in copper and cadmium (5µg/mL) stress conditions. Not any significant effects were registered for the other level of heavy metal contamination, comparing with control.

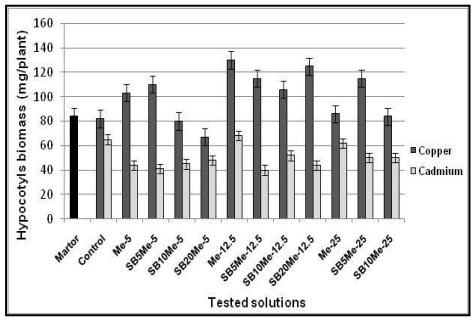

Fig. 5. Hypocotyls green biomass variation under heavy metal stress and polyphenolic extracts treatments

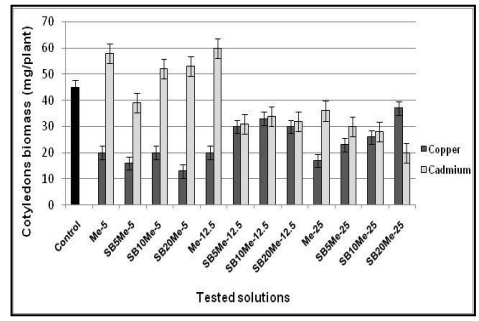

Fig. 6. Cotyledons green biomass variation under heavy metal stress and polyphenolic extracts treatments

Bioaccumulation coefficient registered minor values for copper contamination than for cadmium pollution. It could be observed a decreasing of copper bioaccumulation in oat plantlet under spruce bark aqueous extracts treatment.

Applying the spruce bark extract (1.912g/L) in oat plant growth medium, block the access of copper ions into the roots level (fig.7, fig. 8).

Applying polyphenolic extract in less concentrated solutions (0.85 ; 1.004 mg/L) translocation factor values increased and copper ions were transported to the upper parts of the plantlet.

The presence of polyphenolic extracts in a cadmium contaminated environment promote the bioaccumulation with increasing metal concentration level but reduce the translocation of the metal to the aerial parts of the plant.

These effects could be correlated with the possibilities of certain polyphenolic compounds from spruce bark aqueous extract to complex with heavy metal. All these interactions depends upon metal ions concentrations, total polyphenolic compounds concentrations and growth medium conditions.

Table 2

Assimilatory pigments content (µg/g)

Tested solutions		Chl a	Chl b	Chl a+b	Chl a/b
Martor		275.79	92.69	368.48	2.97
Copper	Me-5	565.12	83.84	648.97	6.74
	SB5Me-5	357.72	98.35	456.08	3.63
	SB10Me-5	497.71	81.86	579.57	6.07
	SB20Me-5	594.24	99.94	694.18	5.94
	Me-12.5	516.10	89.56	605.67	5.76
	SB5Me-12.5	348.88	39.71	388.59	8.78
	SB10Me-12.5	470.13	92.27	562.40	5.09
	SB20Me-12.5	446.23	75.44	521.68	5.91
	Me-25	119.17	6.87	126.05	17.37
	SB5Me-25	279.84	13.87	293.71	20.17
	SB10Me-25	384.41	69.55	453.96	5.52
	SB20Me-25	238.29	67.31	305.61	3.54
Cadmium	Me-5	291.95	170.83	462.81	1.70
	SB5Me-5	265.22	210.34	475.57	1.26
	SB10Me-5	269.63	166.63	436.26	1.61
	SB20Me-5	476.76	200.04	676.81	2.38
	Me-12.5	400.32	207.59	607.91	1.92
	SB5Me-12.5	540.68	193.82	734.50	2.78
	SB10Me-12.5	293.63	193.82	734.50	2.78
	SB20Me-12.5	362.68	210.17	572.86	1.72
	Me-25	516.91	251.36	768.27	2.05
	SB5Me-25	511.93	225.14	737.07	2.27
	SB10Me-25	442.16	230.44	672.61	1.91
	SB20Me-25	405.28	225.93	631.22	1.79

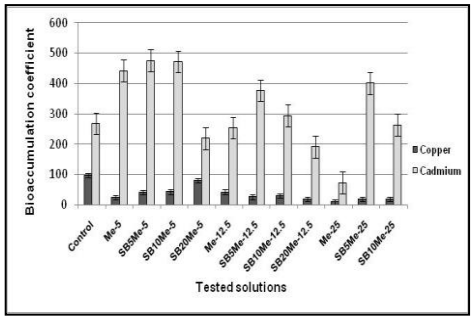

Fig. 7. The influence of polyphenolic extracts treatment on heavy metal bioaccumulation

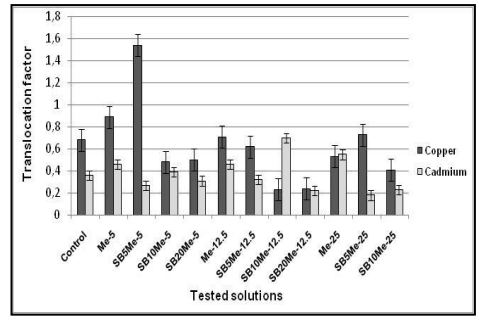

Fig. 8. The influence of polyphenolic extracts treatment on translocation factor variation

CONCLUSIONS

1. Oat plant shows a special affinity for cadmium ions bioaccumulation than for copper. The total polyphenolic compounds identified in aqueous extract (130mg/L extract) promote a decreasing trend in copper bioaccumulation process. On the other side, spruce bark extract (191mg/L) stimulate cadmium bioaccumulation in oat plant and blocked them into the roots level, protecting in that way the potential consumers.

2. Spruce bark aqueous extract could be properly used in amelioration of harmfully effects caused by the presence of heavy metals in the environment. Polyhenolic extract could be considered natural amendments with large spectrum of utilisation in phytostabilisation and phytoextraction.

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