ECOPHYSIOLOGICAL BEHAVIOUR OF HALOPHYTES COMMUNITIES IN RELATION WITH NATURAL HABITAT CHARACTERISTICS FROM RAZELM LAKE VICINITY (DANUBE DELTA BIOSPHERE RESERVE)

Ligia ACATRINEI[1], Mariana HUŢANU[2], Feodor FILIPOV[2]

e-mail: ligia.acatrinei@icbiasi.ro

Abstract

The investigations were carried at the low, flat and flooded area near Razelm, Enisala, Beibugeac (Plopu) and Saraturii lakes (Murighiol) in Danube Delta Biosphere Reserve in different areas: 1310 - communities of *Salicornia* and other annuals colonising mud and sand; 1530* Pannonic salt steppes and salt marshes. The ecophysiological analyses were performed in representative plants belonging to the three identified plant associations (communities): *Salicornietum prostratae* Soó 1964, *Astero tripoli – Juncetum gerardii* Šmarda 1953 and *Obionetum verruciferae* Ţopa 1939. Investigated soils, Gleyc Solonceak and Sali-sodic Gleysoils, have medium or coarse-medium texture and electrical conductivity values varied from 1019 µS/cm up to 5427 µS/cm. The chlorophylls biosynthesis showed a higher amount of chlorophyll *a* in species of *Poaceae* and *Juncaceae* than in the *Chenopodiaceae* family. In *Aeluropus littoralis* species of the C4 - photosynthetic type, photo-assimilatory pigments varied slightly during the vegetation period. Among the carbohydrate fractions, the disaccharides, important in maintaining cell osmotic pressure, are increased and as well as polysaccharides.

Key words: halophytes, photosynthetic pigments, carbohydrates, RBDD

According to Natura 2000 Habitats (Annex I), the 1310 habitat type- communities with *Salicornia* and other annuals colonizing mud and sand predominantly consist of annual species, particularly of *Chenopodiaceae* (*Salicornia* sp.) or grass species which colonizes the muddy or sandy areas, periodically flooded of interior salty swamps, while the 1530* habitat type - Pannonic salt steppes and salt marshes formed of steppes, depressions, superficial lakes of Ponto-Sarmatic salt marshes are areas with extreme temperatures and the soil enrichment with salts due fresh water evaporation and summer aridity. These types of habitats partially have natural origin and are distinctly influenced by bovine grazing (Doroftei, Anastasiu 2014; Ciocârlan *et al*, 2013). Species exposed to saline/drought stress responded with an increasing in osmotic adjustment, changes in the cell wall elasticity, decreasing in biomass and other adaptations (succulence, ions movement, antioxidant systems and salt concentration or excretion which allow halophytes to have the most appropriate water conservation strategies (Motos *et al*, 2017 Hasanuzzaman *et al*, 2014). Salt tolerant plants may belong to the photosynthetic type C3 or C4. Most species of the C4 photosynthetic species were found in the *Chenopodiaceae* and *Poaceae* families. Annual species are mostly C3 type and most perennial plants are C4 species. Vegetative bioremediation of salt-affected soils, economic solutions that have proved the potential of halophytic plants to accumulate or excrete salt quantities depends on the capacity of their above or below ground biomass (Hasanuzzaman *et al*, 2014).

The aims of present paper are to evaluate the ecophysiological adaptations of representative species from three plant communities: *Salicornietum prostratae* Soó 1964, *Astero tripoli – Juncetum gerardii* Šmarda 1953 and *Obionetum verruciferae* Ţopa 1939 identified in studied area Danube Delta Biosphere Reserve (DDBR), in order to find out new resources for phytoremediation of saline soils.

MATERIAL AND METHOD

Study sites

Four representative natural salt marshes and salt steppes from Danube Delta Biosphere Reserve were selected from the geographical area:

1. Murighiol (45°01´41.02”N, 29°09´31.99”E) a natural mesophilic salt marsh on the Saraturi Lake shore with *Puccinellia limosa, P. distans, Juncus*

[1] NIRDBS-Institute of Biological Research, Iasi

[2] „Ion Ionescu de la Brad” University of Agricultural Sciences and Veterinary Medicine, Iasi

gerardii, Hordeum marinum, Salicornia europaea etc. as a representative species on Sali-sodic Gleysoils.

2. Sarinasuf (45°0.5´50.59”N, 29°04´53,34”E), a natural salt marsh and steppe grazing on the Razelm Lake shore with *Juncus gerardii, Halimione verucifera, Salicornia europaea, Suaeda maritima, Spergularia rubra* etc. as a representative species on Gleyc Solonceak Soil.

3. Plopu (45°0.1´23.47”N, 29°06´33,63”E), a natural salt marsh with *Salicornia europaea* on Sali-sodic Gleysoils

4. Enisala (44°54´33.51”N, 28°49´56,28”E) a natural salt steppe with *Limonium gmelinii, Halimione verrucifera, Salicornia europaea* as a representative species on Gleyc Solonceak soil etc.

Sampling and extraction

Based on field research, floristic list was elaborated which permitted flora analysis and ecological indices calculation. The vegetation analysis was made according to Braun-Blanquet method, being noted four surveys included in three plant associations. The scientific names of the cormophytes followed Ciocârlan (2009). Halophyte vegetation consists of plant communities in dry salty depressions and steppes, wet salty meadows and annual plant communities in salty lakes periodically flooded.

The spectrophotometric determination of assimilating pigments was by fresh leaves solvation in 85% acetone (Meyer-Berthrand modified by Ştirban, 1985). The results were expressed in mg/g fr.w. as fresh weight. The analyzed sugars indicators content from leaves (mono-, di- and polysaccharides) were analyzed by using Bertrand method combined with method Borel in dried plant material. Results were expressed as g % of dry matter). Leaf water content and dry substance and as well as soil moisture was investigated by gravimetric method at constant weight.

RESULTS AND DISSCUSIONS

In our research we were identified three plant associations (communities):

***1. Salicornietum prostratae* Soó 1964.** The association's phytocoenoses was observed at Murighiol, Plopu and Enisala. The phytocoenosis is characteristic for the soils with high salinity and excess moisture, at least in the spring. The dominant and characteristic species is *Salicornia europaea*, accompanied by a number of halophiles species. Most of the bioforms are hemicryptophytes and therophytes, with 77.7%, so besides a nucleus of annual species there are also many perennial species. Among the geoelements, Eurasian, European and continental species prevail (55.5%), followed by cosmopolitan species (22.2%). The association is characterized by the predominance of heliophile plants, generally of the eurythermic which prefer wet soils. The soil reaction (soil pH) is marked by strong halophilous species, euriphic and eury-nitrophilous plants.

2. *Astero tripoli - Juncetum gerardii* Šmarda 1953. The association's phytocoenosis was observed at Murighiol (more humid) and Sarinasuf and is dominated by *Juncus gerardii, Puccinellia limosa* and *Aster tripolium*, and is found on moist, poorly-sown soil. In the case of bioforms, the therophytes (21%) are less numerous in both plant association no. 1 (27.7%) and no. 3 (33.3%); the hemicrypthophytes (about 58%) are the best represented from all 3 plant associations. In this phytocoenosis as geoelements, Eurasian species (47.3%) and cosmopolitan species (15.7%) are best represented. The plant association is characterized by the predominance of helophilous plants, eury-thermophilous and thermophilous plant species and moderately moist (moderate-wet) to moist soils. Also, in the phytocoenosis of this association are eurionic plants and those which prefer slightly (weakly) alkaline soils, as well as nitrophilous plants. In terms of salinity, there are mainly plant species adapted to saline to hypersaline soils.

3. *Obionetum verruciferae* Topa 1939. The association was identified at Enisala plot. It is also found on high salinized soils, the dominant and characteristic species being *Halimione verrucifera*, alongside other plants of salt steppe. In this association, among bioforms, the annual therophytes (33.3%) are more than in plant associations 1 or 2, and the hemicryptophytes (46.6%) are fewer in comparison with previous associations. The geoelements, Eurasian and continental species are the most numerous (46.6%) and European species are missing. The association is characterized by the predominance of sunny plants and that prefer open-areas and well-drained soils. Most species are eurynitrophilous and prefer slightly alkaline soils, with a medium-to-high content of soluble salts (*table 1*).

During our research in this area (RBDD) we have identified two rare species in Romania (Dihoru et Negrean, 2009):

1. *Zygophyllum fabago* L., vulnerable (V), found at Jurilovca, in sandy places and *Polypogon monspeliensis* (L.) Desf., vulnerable (V), at Sarinasuf, to the border of Razelm lake being a new place for this species.

The investigated soils, Gleyc Solonceak and Sali-Sodic Gleysoil have medium or coarse-medium texture and electrical conductivity values of aqueous extract (1:5, g/ml) varied from 1019 µS/cm up to 5427 µS/cm. In August, the soil moisture ranged in depth from 25.67% at soil

surface (crust) to 57% in depth in Murighiol plot (more humid) and also, from 4% - 7% (crust) to 14 % (in depth) at Plopu and respectively, Enisala, both dryer and with salt crusts at soil surface (*table 2*).

Table 1

Distribution of bioformes and geoelements (ecological indices) of studied plant communities

Bioforms and geoelements	Plant association **1** (%)	Plant association **2** (%)	Plant association 3 (%)
T; T-H; T-Ht	27,7	21	33,3
H; Ht	50	57,9	46,6
Ph	5,5	5,2	6,6
G; G (HH)	16,6	15,7	13,3
Euras.	33,3	47,3	33,3
Eur.	11,1	-	-
Cont.	11,1	10,5	13,3
Cosm.	22,2	15,7	13,3
Pont.- Pan.	11,1	10,5	13,3
Atl.-med.	5,5	5,2	6,6
Adv.	5,5	5,2	6,6
Circ.	-	5,2	6,6
Submedit.	-	-	6,6

The driest soil at surface was observed at Plopu followed by Enisala with a small difference, and salt crusts on large surfaces were noted in both plots. In the deeper layers of soil, on both plots, soil moisture registered close values (14%).

Table 2

Soil humidity and dry substance at investigated habitats from RBDD

(in 9-th August 2017)

Plots	Soil depth	humidity %	dry substance %
Murighiol	crust	25.67	74.33
	10-20 cm	57.42	42.58
Plopu	crust	4.69	95.31
	10-20 cm	14.76	85.24
Sarinasuf	crust	14,58	85.42
	10-20 cm	17.04	82.96
Enisala	crust	7.52	92.48
	10-20 cm	14.29	85.71

Regarding the eco-physiological analyses, the chlorophylls biosynthesis showed a decreasing during June to August, especially from chlorophyll a. In June, higher amount of chlorophyll a registered in species of the *Poaceae (Hordeum marinum)* and also, of *Juncaceae (Juncus gerardii*) than in the *Chenopodiaceae* family, among them, the obligate halophiles, *Salicornia europaea* showed a small variation of the photo-assimilatory pigments during the vegetation stage. In *Aeluropus littoralis* species with the C4 - photosynthetic type, photo-assimilatory pigments varied slightly during the vegetation period (*figure 1*). The degradation of the carotenoid pigments, an integral part of the tilakoid membrane which also acts as a photo-oxidative protection of the chlorophylls, decreasing once with chlorophylls due to the lower soil moisture and the salts increasing in Na + and Cl-.

The chlorophyll a / chlorophyll b ratio declined on average from 3.04 (June) to 2.64 (August), due in particular to the decreasing in the total quantity of assimilating pigments (*table 1*). The largest decreasing occurred at chlorophyll a, by 1.62 times lower in August compared with June, and followed by a decreasing in total carotenoid pigments until 1.5 times, on average. According to the literature, chlorophyll a / chlorophyll b ratio is a stress indicator in the case of increasing the N content in the soil respective of the increase of the solar radiation. Lower values below that average were found in June at *Puccinellia limosa* (1.5), *Salicornia europaea* (2.42) and *Suaeda maritima* (1.7), in August at all species from Enisala plot, dry and with nitrate salts. The chlorophylls / carotenoid ratio, an indicator of senescence and chlorophyll degradation, recorded a slight decrease over the studied period. Thus, this ratio was 4.2 in June and dropped around 4 in August (*table 1*).

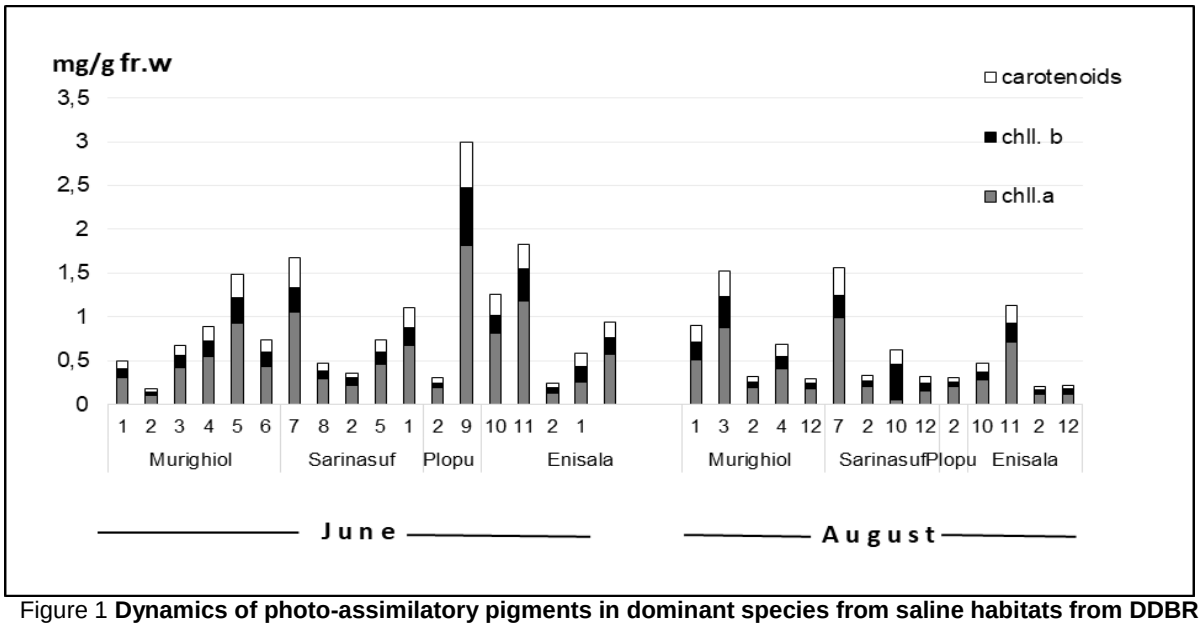

Figure 1 **Dynamics of photo-assimilatory pigments in dominant species from saline habitats from DDBR**

Legend : 1-*Puccinellia limosa,* 2 - *Salicornia europaea,* 3 - *Puccinelia distans,* 4 - *Juncus gerardii,* 5 - *Hordeum marinum,* 6 - *Juncus bufonius,* 7 - *Aeluropus littoralis,* 8 – *Spergularia rubra,* 9 - *Bassia hirsuta,* 10 - *Halimione verrucifera,* 11 - *Limonium gmelinii,* 12- *Suaeda maritima*

Physiological studies at *Juncus* have shown that soluble carbohydrates (glucose, fructose, sucrose or fructans) and soil moisture contribute significantly to mechanisms of saline stress adaptation and osmoregulation (Gill *et al*, 2011; Slama *et al*, 2017).

Among the carbohydrate fractions, the disaccharides, important in maintaining cell osmotic pressure, are increased (*Puccinelli*a sp. with content between 6-11% among plots) but a also, the total polysaccharides content (in *Hordeum marinum* - 30 %). At the beginning of the summer season, monosaccharides recorded the lowest values for euhalophytes, ranging from 0.46 % in *Salicornia europaea* (Murighiol) and about 0.37 % in *Halimione verrucifera* (Enisala) which are in concordance with lower chlorophylls biosynthesis (*figure 1*). Polysaccharides represented, in this period, the highest amount of leaf sugars, on average 65% of their total. Insoluble polysaccharides (cellulose, starch, etc.) have a structural role in cell wall structure and in maintaining homeostasis, but they also have importance along with disaccharides in maintaining the osmotic concentration of the vacuolar sap and determine the salinity resistance. Thus, the insoluble polysaccharides accumulate amounts ranging from 17 g% to *Hordeum marinum* (development of fruit phenophase) from Murighiol and decreases to 4 g% at *Salicornia europaea* in Plopu. It is also observed that fractions registered higher values over 8.5 g% in halophile species from mesophilous steppe of Murighiol (*Juncus bufonius* and *J. gerardii*) and Sarinasuf (all investigated species including *Salicornia europaea*). Leaf soluble polysaccharides, including mucilages, hemicelluloses, pectins, have been found to play an active role in salting plants in salinity resistance by water and ions absorption (Slama *et al*, 2014). The variation of soluble polysaccharides recorded the subunit values (*Haliminone verrucifera* and *Limonium gmelinii* from Enisala but also *Bassia hirsuta* from Plopu) to values over 8 g% (*Salicornia europaea* and *Puccinellia limosa* from Sarinasuf) (*figure 2*).

Table 3

Ecophysiological parameters in studied halophile plant communities

Association	Chll. a	Chll. b	Caro-tenoids	Total pigments	Mono-sacch.	Dissach.	Insolub. Polysacc.	Solubl. Polysacc.	Total carbohydr.	Insolub/ Solub ratio
As. 1	0.18	0.06	0.06	0.29	1.62	5.13	6.37	4.46	17.58	2.07
As. 2	0.50	0.19	0.17	0.85	1.34	6.87	8.90	4.20	21.31	1.68
As. 3	0.51	0.23	0.16	0.74	1.38	2.98	8.19	0	12.55	2.89

A comparative view among investigated plant communities showed that association no. 2 (*Astero tripoli - Juncetum gerardii*) registered highest amount of photo-assimilatory pigments and total carbohydrates than association no. 1 (*Salicornietum prostratae*) and respectively, association 3 (*Obionetum verruciferae*) (*table 3*). Association no. 1 and association no. 3 with obligate euhalophytes registered variable values of sugars metabolism and phtosynthetic pigments.

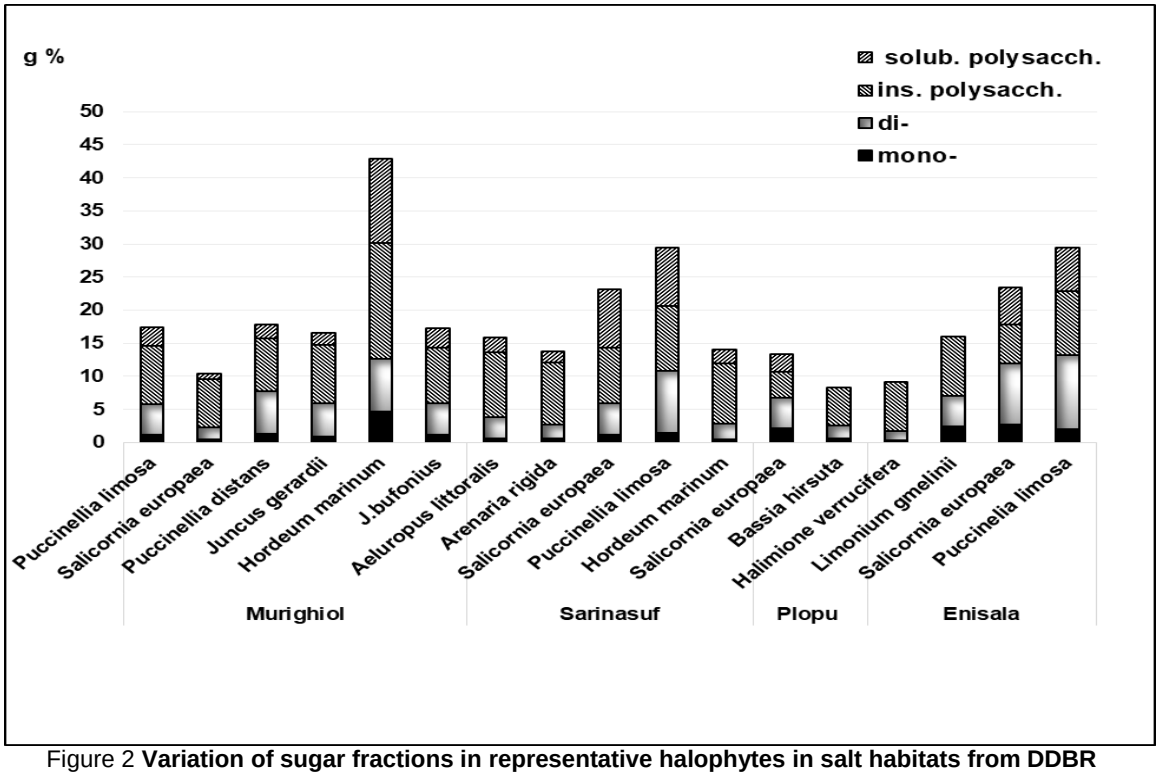

Figure 2 **Variation of sugar fractions in representative halophytes in salt habitats from DDBR**

Association no. 3 showed higher chlorophyll a and chlorophyll b amount, a lower dissacharides content and total carbohydrates but highest insoluble/soluble catbohydrates ratio of 2.89 (*table 3*). That higher value of insoluble content soluble carbohydrates is linked with biomass accumulation and that community are well adjusted to eco-pedological conditions. In that community which included *Halimione verrucifera* and *Limonium gmelinii* the excreting salt euhalophytes registered lowest soluble amount of carbohydrates, including dissacharides and water-soluble polysaccharides that are responsible with osmoregulation and salt stress from Enisala

Association no. 1 represented by communities with *Salicornia,* concentrating salt, showed the lowest value of chlorophyll *a* and chlorophyll *b* and also carotenoids and an increasing soluble saccharides (disaccharides and water-soluble polysaccharides) (*table 3*). Association 2 have all carbohydrates fractions well represented and higher photosynthetic capacity but a relatively small insoluble/soluble carbohydrates ratio which are linked with the catabolism of carbohydrates at an increasing salt soil concentration during summer condition.

CONCLUSIONS

The three plant associations presented are characterized by higher amount of salinity and moisture in the low plain and meadow areas. These phytocoenoses, are dominated by hemichryptophytes and therophytes (among life forms), and the Eurasian, continental and cosmopolitan species (as geoelements). From the ecological point of view, most of the species are specific to open and flat places, so they prefer higher temperatures and an increased light intensity. There are plants that have a great tolerance to the soil salts and a rather alkaline soil reaction.

During the studied period, carbohydrate metabolism is generally characterized by the increased accumulations of disaccharides and insoluble polysaccharides fractions. Monosaccharides showed a quite small quantitatively during this period. Species such as *Limonium gmelinii, Salicornia europaea* and *Puccinellia limosa* have values close to 2 g % in Enisala - dryer habitat with salt crust on soil surface.

Chlorophyll biosynthesis showed higher values at the beginning of the summer

(characterized by higher humidity) in *Poaceae* and *Juncaceae* than those in *Chenopodiaceae* family.

Although chlorophyll decreased in August compared to June, succulents recorded the smaller variation of photosynthetic pigments (*Salicornia europaea, Suaeda maritima*) followed by salt-excreting (*Halimione verrucifera, Limonium gmelinii*). In *Aeluropus littoralis* species with the C4 - photosynthetic type, photo-assimilatory pigments varied slightly during the vegetation period.

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