

INTEGRATIVE ECOLOGICAL NOTES ON HALOPHYTES FROM “VALEA ILENEI” (IAȘI) NATURE RESERVE

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Halophytes distribution from Valea Ilenei (Iași) nature reserve is strictly correlated to environmental factors (soil salinity, humidity), reflecting the morphological and anatomical adaptations found in different species. The observed and analyzed species have different ecological spectra and occupy well delineated micro-habitats. Integrative ecology can easily and naturally provide operative adaptive profiles for each studied species.

Key words: halophytes, ecology, adaptation, ecosystem, integrative, salinity.

1. INTRODUCTION

Halophytes represent a polymorphous ecological group of plants; they include species with a complex set of anatomical features, allowing them to survive in high soil salinity conditions [21, and references therein]. Data regarding halophytes responses to salinity are generally abundant, but they are mainly related to experimental conditions [20, 26, 27]. Unfortunately, despite of this progress recorded in understanding plants responses to salinity conditions, the knowledge about halophytes-environmental factors interrelationships, especially in the terms of adaptations, is still scarce. While many of studies have been focused on biochemical and molecular aspects of salinity tolerance, the mechanisms of survival on ecosystem scale have been neglected for longtime. In Romania, apart from several classic works dealing with ecology of halophytes, the data about saline ecosystems are very limited and scattered. Within large contributions, written especially on the first half of the past century, it should mentioned those of Prodan [48 – an extensive study] and further in one book chapter [49]), Țopa [56], briefly revised in 1954 [57], Bucur [5, 6, 7], and Șerbănescu [54].

In addition to these extensive works and in order to integrate the morphological and anatomical adaptations of halophytes with environmental factors [26,

27, and references therein], a new approach in dealing with plants ecology has been proposed by Grigore, Toma and Boscaiu: plant integrative ecology [32]. This approach assumes that complete adaptive profile of halophytes can be obtained only by using morphological, anatomical, physiological, and biochemical information.

Salt areas from Valea Ilenei (Lețcani) nature reserve occupy a surface of about 10 ha; this nature reserve is located 4 km from Lețcani railway station, next to Iași-Dorohoi railways at the confluence of Valea Ilenei and Bahlui rivers (Fig. 1). Yet it is a small nature reserve, several species are included in the *Red Book* of Iași district, such as: *Lepidium crassifolium*, *Petrosimonia triandra*, *Plantago schwarzenbergiana* [44]. Although very interesting and attracting from botanical point of view, little has been done in respect of studying halophytes distribution and their ecology.

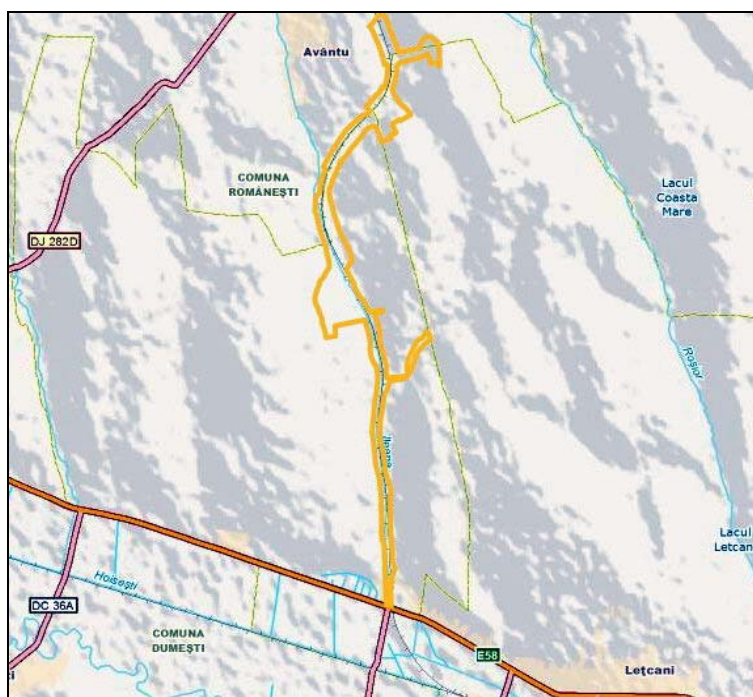


Fig. 1. Localization of Valea Ilenei (Lețcani) nature reserve [62].

Few botanical notes accidentally refer on species vegetating in this area [8, 9, 41, 42, 50, 51]. Burduja [8] particularly drew attention to Valea Ilenei saline environments, when describing *Lepidium crassifolium* Waldst. et Kit. for the first time in this location; he also described the ecology of this rare halophyte, giving interesting data about life conditions of the species. In 1987, Mititelu *et al.* [43] published the first monographic paper related to flora and vegetation of Valea Ilenei nature

reserve. As far as it is known, this is the single existing in the Romanian botanical literature, but this paper only enumerates the species and plant associations, without any ecological comments. Grigore and Toma [26] briefly described the ecology of several halophytes collected from Valea Ilenei nature reserve, when analyzing their anatomical structure in relation to environmental factors. Grigore *et al.* [36] discussed the ecological and evolutionary significance of C_4 photosynthesis in halophytes vegetating in salty areas from this nature reserve. Not in the last, Grigore and Toma [29] concisely made ecological observations in this area, emphasizing on the role of anatomical adaptations allowing to halophytes to cope with salinity and other environmental factors.

This paper aims for promote and deepen the plant integrative ecology approach, here adapted to halophytes, trying to highlight the importance of anatomical adaptations as elaborated mechanisms that perfectly define the relation of halophytes to ecological factors.

2. MATERIALS AND METHOD

Ecological observations included in this contribution have been recorded in June, 2011. Many of the halophyte species we refer in the present study were have been anatomically studied since 2006, when we started to make contact with this nature reserve and established correlations between halophytes adaptive strategies and environmental factors [26, 27, and the personal works here mentioned]. These observations have been interpreted in the manner of plant integrative ecology, as we previously described [32]. In addition, pH in water (1:2:5) was measured with a pH-meter and electrical conductivity (EC) was measured in a soil extract (1:5) using an electrical conductivity-meter; the soil samples were taken from the rhizosphere of several species.

3. RESULTS AND DISCUSSIONS

Salt areas from Valea Ilenei nature reserve do not have a uniform distribution; their zonality is strongly influenced by local environmental factors: soil humidity and salinity, rainfall and elevation of salt-affected surfaces.

Our ecological observations revealed a halophytic transition starting from the wettest and saline area to the elevated areas, where the soil humidity and salinity decreased.

Three micro-habitats were delimited and described (Fig. 2).

1. Wet micro-habitats, seasonally/often flooded (Fig. 3), delineated on side by Ileana River, and other side, by hilly areas; thus, a depression-surface is created, having a very high soil humidity also favored by water flowing from elevations

opposite to river. Interestingly, under prolonged drought conditions, the soil cracks taking a typical appearance (Fig. 4). In this segment of observations, of 10–15 meters wide, the plant distribution is clearly noticeable, according to soil humidity and salinity.



Fig. 2. Satellite image from Valea Ilenei Nature reserve and micro-habitats delineation [63].

Thus, the river bank is greatly dominated by *Phragmites australis*, which forms a large and compact belt, of about 2–3 meters wide; isolated individuals (but rather smaller) or confined in restricted patches may be also found on higher distances from the river.

As this belt with *Phragmites* is left in the right side, thus by going far away from the river, another heterogeneous belt of vegetation has been observed; this occupies a marshy, salinized area. It consists of *Bolboschoenus maritimus* (rare individuals), *Juncus gerardi* (grouped in dense patches), *Carex distans*, *C. vulpina*, and *Alopecurus arundinaceus* (discretely found among other species).



Fig. 3. Wet microhabitat from Valea Ilenei nature reserve (original).

These species were previously described as *amphibious* halophytes [26, 28, 31], especially taking into considerations the role and ecological significance of bulliform cells for these species (Figs. 5–8). They regularly vegetate in wet habitats, but sometimes these environments could be exposed to seasonally drought conditions (also see the Fig. 4); in this way, despite are obviously hygro-halophytes, sometimes they have to face drought – especially to physiological drought [30] and thus xeromorphic features may be involved in drought tolerance. The relation of bulliform cells with drought stress has been taken into discussion by many botanists [4, 14, 18, 37, 39, 60]. The bulliform cells were found to be more developed in desert ecotypes than in mesophytic ecotypes of some plants investigated [59], clearly suggesting that these cells are involved in plant adaptive response to water and salt stress.

For other authors, these cells were considered as water storage [15, 47, 58] and can participate in the young leaf expansion. Their implication in leaf rolling and/or folding of mature leaves was discussed by some researchers [38, 52]. According to Esau [17], during excessive water loss, the bulliform cells, together with or without colorless cells, became flaccid and enabled to leaf either to fold or to roll. Clayton and Renvoize [quoted in 1], opined that bulliform cells favoured the light entrance in the mesophyll cells. In some species, bulliform cells were not actively or specifically related to unfolding and hygrosopic leaf movement, since they accumulated large amounts of silicon and their outermost walls might thicken and cutinize, becoming stiff [16].

It was shown that in water stress conditions, the activity of these cells become more intensely. For instance, *Loudetiopsis chrysothrix* and *Tristachya leiostachya* showed leaf rolling of mature and young leaves during water stress [1]. According to Moulia (1994) [quoted in 1], the leaf rolling is a xeromorphic characteristic and

has adaptive value, reducing light interception, transpiration and protecting the leaf from dehydration and overheating. This it would be a mechanism to minimize light exposition and water transpiration, thus keeping the stomata in a microclimate with higher humidity, preventing drought conditions [12, 53].

As it can be emphasized, species nominated as *amphibious* halophytes and vegetating in this wet but sometimes temporarily dry micro-habitat represent a good example of adaptation to a continuum in variation of ecological factors; apart from salinity, a pivotal role is also played by soil humidity that directly influences soil salinity [26]. Actually, soil salinity is a versatile factor and has a great implication in defining and understanding halophytes definition and their subsequent adaptations. The measured values of electrical conductivity (EC) for several observed and investigated species show variations and implicitly, different ranges of affinity for soil salinity, in the case of several taxa (Table 1).

Table 1

Values of pH and EC for several halophytes vegetating in Valea Ilenei nature reserve (the underlined species and their values show important differences within multiple soil samples from the rhizosphere of the same species)

Species	pH	EC (dS/m)
<i>Halimione verrucifera</i>	8.58	1.54
<i>Suaeda maritima</i>	9.3	4.03
<u><i>Salicornia europaea</i></u>	<u>8.55–8.87</u>	<u>8–11.82</u>
<i>Atriplex littoralis</i>	9.2	2.49
<i>Limonium gmelinii</i>	7.92	2.29
<u><i>Lepidium crassifolium</i></u>	<u>9.04–9.78</u>	<u>4.54–10.56</u>
<i>Artemisia santonica</i>	8.01	0.57
<i>Aster linosyris</i>	8	0.35
<i>Bolboschoenus maritimus</i>	8.95	2.39
<i>Juncus gerardi</i>	9.05	4.92

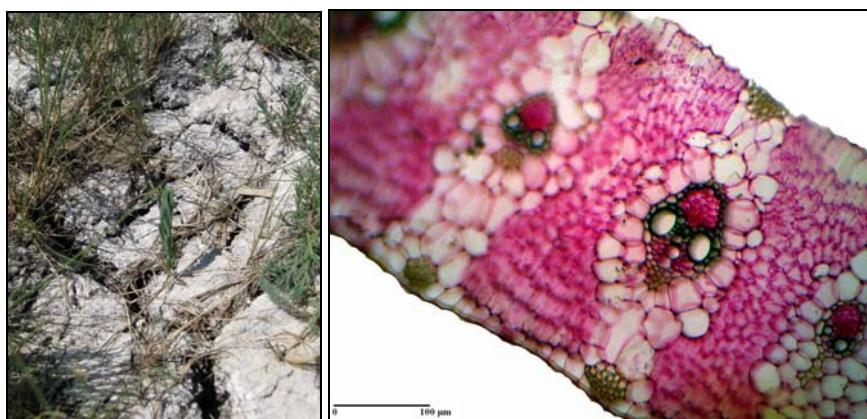


Fig. 4. Typical fissures on soil surface during drought periods (original).

Fig. 5. Cross section through the lamina of *Bolboschoenus maritimus* [31].

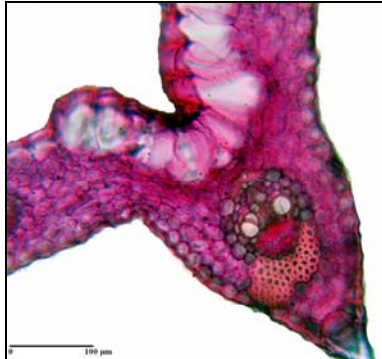


Fig. 6. Cross section through the lamina of *Carex distans* [31].

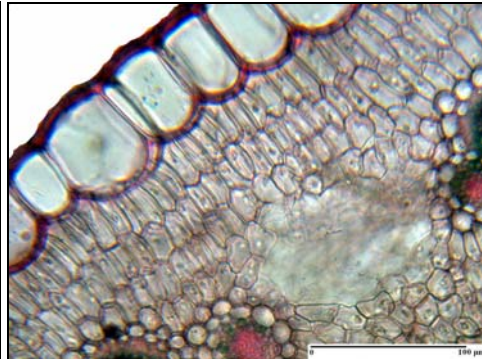


Fig. 7. Cross section through leaf sheath of *Juncus gerardi* [31].

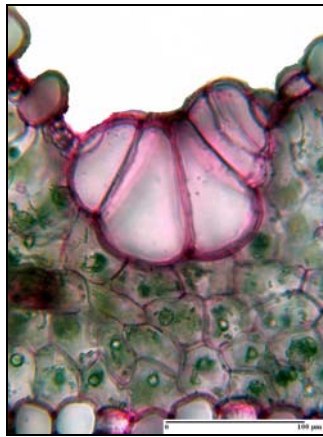


Fig. 8. Cross section through lamina of *Alopecurus arundinaceus* [31].

Bolboschoenus maritimus has been included in the second “category” of halophytes by Prodan [49] and has been considered as a preferential halophyte [57], neohalophyte [7]; mesohygrophilous and facultative halophyte [10] (for the used terminology and additional comments, see Grigore’s work [21]).

Juncus gerardi has been considered as a halophyte from the first category [49], as a preferential halophyte [2, 57], euhalophyte [6] and mesohygrophilous halophyte [10]. Bucur *et al.* [6] characterized it as perennial, common, hygrophilous, mesothermophile to subtermophile, heliophilous, from less to strongly halophytic; it develops on salinized humic gley soils, indicating a permanent wet saline soil.

Carex distans is a halophyte from the second category [49], preferential halophyte [57], mesohygrophilous, facultative halophyte [10].

Therefore, all these salty marsh species have an ecology strictly related to soil salinity and humidity; as it has been assumed by Toma and Grigore [23], multiple environmental factors such as the salinity and the hypoxic, anoxic and xerophytic

conditions are often convergent and involved in defining various stress types. The hypoxic/anoxic stress and the salt stress which includes the ionic and the dehydration stress are some of the multiple stress types. Under these circumstances the halophytes develop histo-anatomical features that represent a response to the corresponding environmental factor. As it has been previously mentioned, species from this micro-habitat can be also subjected to flooding, due to higher humidity derived from the proximity of the river or by water influx from hills drainage. In this way, for this component of environmental conditions, halophytes have evolved other well established adaptive mechanisms, such as aerenchyma, at the level of underground organs. It can be found in *Aster tripolium* and *Juncus gerardi* (Figs. 9, 10). The air stored by aerenchyma serves as air reserves during the waterlogging [22, 24, 26].



Fig. 9. Cross section through the rhizome of *Aster tripolium* [26].

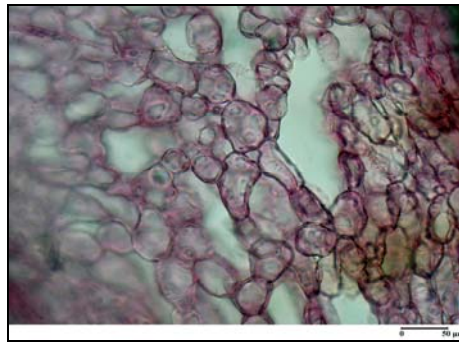


Fig.10. Cross section through the rhizome of *Juncus gerardi* [26].

On the border of this micro-habitat, *Salicornia europaea* and *Suaeda maritima* (*Chenopodiaceae*), as smaller individuals were also found; their life cycle is strictly related to soil humidity [26].

These two euhalophytes (strictly adapted to salinity) are succulent species; while *Salicornia* (Fig. 11) has an articulated (segmented) shoots, *Suaeda* has succulent leaves. Succulence is a very important structural strategy of halophytes (apart from salt secretion) that allow to these species to face the physiological drought induced by high salt concentrations in the soil [20, 26–28, 33–35]. Succulence is a quite common and largely discussed adaptive feature found in halophytes. Evidenced especially as a water storage tissue, succulence provides in hygrohalophytes (such as *Salicornia* and *Suaeda*, Figs. 12–14) the erect position and the possible dilution of salts; in addition, it can be induced and “maintained” by chloride ions, because it is known that the two species listed above were included in the group forming the “chloride” plant associations. In xerohalophytes, aqueous tissues represent in the same time reserves of water required during drought, and a mechanism for dilution of salts. It is well known that when the soil dries, salts are more concentrated [20].



Fig. 11. *Salicornia europaea* (original).

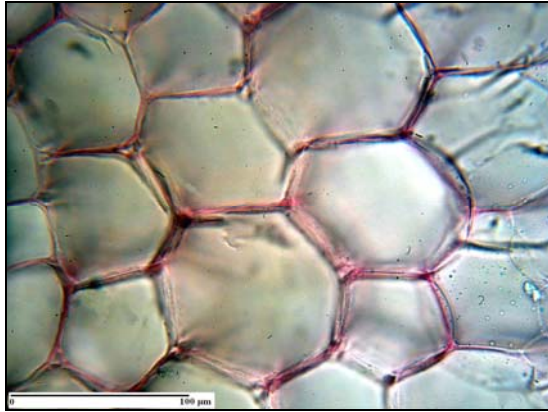


Fig. 12. Cross section through a big succulent segment of *Salicornia europaea* [26].



Fig. 13. Cross section through a small succulent segment of *Salicornia europaea* [26].

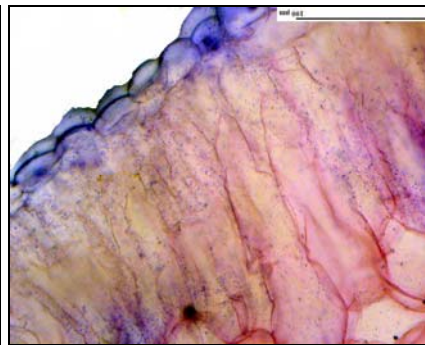


Fig. 14. Cross section through the lamina of *Suaeda maritima* [26].

Another interesting and intriguing anatomical structure found within fleshy tissues of *Salicornia* is represented by tracheoididioblasts (Fig. 13), whose functions are still a matter of debate. While several botanists assumed that they are included in the mechanical system of these species (indeed, with less developed mechanical tissues) [3, 13, 40, 55], others believed that they are water-storage structures [11, 19, 46, 60, 61].

Salicornia europaea is a hygrophalophyte, vegetating, just like *Suaeda maritima*, in wet sometimes waterlogged salt areas. The presence of succulence, beside the previously mentioned dilutive effect, also interferes in the maintenance of the cell turgor, which means another way in order to maintain the erect position of the plant, as it is well-known that the mechanical tissues are rudimentary and poorly developed in this species. “As soon as the turgor is no longer assured, due to the lack of water, the plant fatally dies out”, says Prodan [48], with reference to *Salicornia*. Therefore, the species is confined to intensely saline, chloride, but wet

environments; this is practically about a closed micro-ecological chain, in other words, the chloride salinity of the soil induces the succulence, playing the part of both diluting the toxic ions and of maintaining the osmotic pressure, the turgor, which allows the plant to uptake the sap on the one hand, as well as the erect position of the plant, on the other hand. As the soil lacks the water, the plant may die either because the salts are concentrating in cells (that would be lethal), or because of the loss, reversible or not, of the erect position. Therefore, the water balance, beside the balance of salts, must by no means be neglected.

Thus, *Salicornia europaea* is a hygrophilous species, from moderately to intensely halophilous, being developed on salinized, water meadows wet in the depth and less wet towards surface [6], having basically the same ecological requirements as *Suaeda maritima*. They are both species that vegetate in chloride associations [54]. Moreover, speaking about the importance of water for *Salicornia*, one must mention the fact that the seed germination begins, in most cases, under the precipitation water, when the salts are much diluted, as the same author stated.

Often associated to *Salicornia europaea*, *Suaeda maritima* is also a hygrophilous species, strict and very intensely alkaliphilous, from moderately to intensely and very intensely euhalophilous. It grows on wet saline meadows, slightly clogged or not; it is a plant with superficial root, with succulent stems and leaves [6].

Prodan [48] includes it, beside *Salicornia*, in the category of halophytes with “rudimentary mechanical tissues, which need to ensure cell turgor in order to secure their existence”.

Șerbănescu [54] includes it in the category of halophytes of very strong chloride associations, which are wet especially in spring, till the beginning of the summer.

Both *Salicornia* and *Suaeda* are C₃ species [26]; this is a subtle, but consistent data, since the photosynthetic pathway can play an important ecological role in habitats exposed to extreme environmental conditions, such as aridity and salinity [36].

The last two mentioned chenopods can be also found on the entire surface of this micro-habitat, but they rather prefer opened areas; when present isolated among other species, they seem smaller and less developed. Here and there, several rosettes of basal leaves from *Aster tripolium* have been noticed; this is a perennial, hygrophilous species that usually flowers later, in the autumn. However, a single individual has been identified in flowers; this was a small one, growing in an opened area of the micro-habitat. At the border of this micro-habitat (opposite to river), thus in less humid areas, *Atriplex prostrata* and *A. littoralis* were localized and identified.

2. A large, heterogeneous micro-habitat (Figs. 15, 16), connecting the previous area to the third one (the most elevated micro-habitat) that make transition to the access road. In this area, attention should be paid on *Lepidium crassifolium* (Fig. 17) that develops in large patches, especially in lower places, closed to

previous described microhabitat. This is the most halophilous species among *Lepidium* genus that is also represented here by two other less halophytic species: *L. perfoliatum* and *L. latifolium*, the lesser halophilous. *L. crassifolium* has a very interesting ecology: it vegetates almost always on surfaces where saline efflorescence is noticeable; although the soil seems dry, this species is closely related by soil humidity, since it has a marshy, clayed consistency – a fact easily noticed in the field.



Fig. 15. Micro-habitat from Valea Ilenei nature reserve (original).



Fig. 16. Micro-habitat from Valea Ilenei nature reserve (original).



Fig. 17. *Lepidium crassifolium* (original).

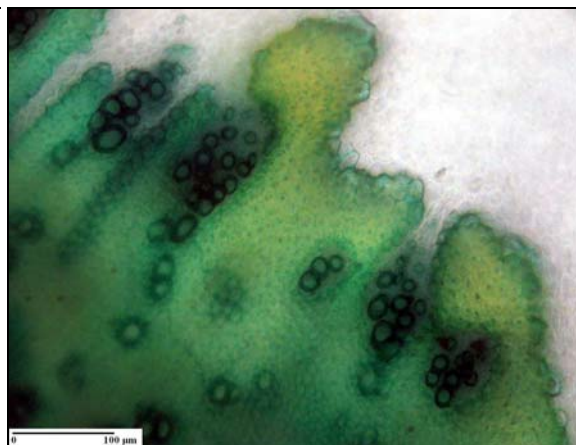


Fig. 18. Cross section through the rhizome of *Lepidium crassifolium* [25].

Actually, it is a perennial species (Fig. 18) [25], with well developed rhizome and root, a fact that may explain the capacity of penetrating deep layers in the soil; the explanations of Burduja [8] are also very valuable, referring on its ecology. Grigore and Toma [25] based on the anatomical structure of this species, emphasized that this species has a rhizome, thus clarifying several inconsistencies about the perennial character of it. In Romanian Flora [45] it is stated that is a perennial species, without any mention about the organ conferring perennial appearance.

Even when it vegetates on saline restricted surfaces, in elevated and apparently dry points, *L. crassifolium* is sometimes accompanied by *Phragmites australis* and *Juncus gerardi*, a simple clue that there is a water resource in the soil layers. In addition, this aspect is also easily observed since the drained water from surrounding hills may transform in small rivers that finally will flow in Ileana river or will have lost until making contact with Ileana river.

Lepidium crassifolium has been included in the first category by Prodan [49] and has been classified as obligatory halophyte [57], euhalophyte [6], mesophilous – mesohygrophilous, obligatory halophyte [10]. Bucur *et al.* [6] characterized it as perennial, mesophilous to hygrophilous, mesothermophile to megathermophilic, heliophilous, strongly alkaliphilous. It develops on salinized wet meadow soils, which dry on surface during the summer [6].

Halimione verrucifera (Fig. 19) has been also observed in large, compact, and free of vegetation patches, especially in dry surfaces. On the driest, elevated surfaces, *Aster linosyris*, *Limonium gmelinii* and *Artemisia santonica* have been found.

Halimione is a salt-secreting chenopod; it has been included in the first category of halophytes [49] and considered as an obligatory halophyte [57] and euhalophyte [6]. It is a rare species, mesophilous to xerophilous, mesothermophile -megathermophilic, heliophilous, strongly alkaliphilous, strongly

euhalophilous [6]. This C_3 species has salt hairs (bladders) on the surface of leaf epidermis (Fig. 20), having an important role in eliminating the excess of salts from the plant organs [27].



Fig. 19. *Halimione verrucifera* (original).

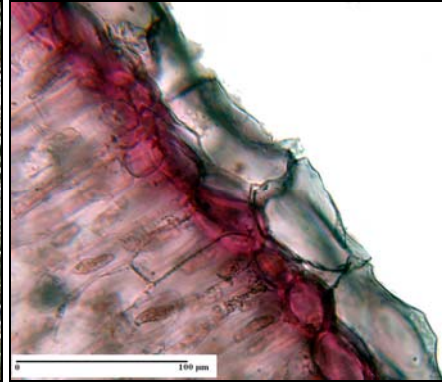


Fig. 20. Cross section through the lamina of *Halimione verrucifera* [27].

Limonium gmelinii has been included in the first category of halophytes [49] and considered as an obligatory halophyte [2, 57], euhalophyte [6], and mesohydrophilous halophyte [10]. Bucur *et al.* [6] described this species as a perennial one, very common in salty areas, mesohydrophilous (its tap root develops deeply in the soil thus exploiting the salinized water table); mesothermophile, from less to strongly alkaliphilous. It can develop on salinized water meadows, as well on dry slopes. This species has been found in dry, free of vegetation elevated areas; it has typical salt glands (Figs. 21, 22), common in *Plumbaginaceae* species [27].

Aster linosyris and *Artemisia santonica* also vegetate in dry, elevated points, having xeromorphic anatomical features [26]; *Aster* and *Artemisia* occur in large and dense patches in sun-lighted slopes (Fig. 23).



Fig. 21. Cross section through the lamina of *Limonium gmelinii* [27].

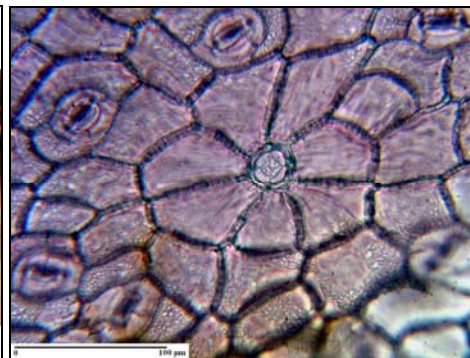


Fig. 22. Salt glands from lamina lower epidermis of *Limonium gmelinii* [27].



Fig. 23. Micro-habitat from Valea Ilenei nature reserve (original).

3. Meso-hygrophilous meadows, less or moderately salinized, extended on a large surface, where many species were observed, but their adaptations and ecology are not closely related to salinity factor: *Dianthus* species, *Allium vineale* and many *Fabaceae* species (Fig. 24).



Fig. 24. Micro-habitat from Valea Ilenei nature reserve (original).

4. CONCLUDING REMARKS

Halophytes distribution in this nature reserve is strictly related to environmental factors (soil salinity, humidity), reflecting the ecological adaptations found in different species. The observed and analyzed species have different degrees of salt tolerance and occupy restricted and well established micro-habitats. Therefore, due to some high specialized adaptations (succulence, salt secretion), euhalophytes – strictly adapted to high salinity – are able to cope with harsh environment conditions, while others, occupying less salinized areas, show transitional adaptations between euhalophytes and non-halophytic species.

Due to its restricted surface and vegetation distribution, this nature reserve could be useful as a model for complex investigations regarding halophytes biology.

This small and yet less studied nature reserve needs further attention, in order to get a complete picture of halophytes distribution, since the vegetation aspect is changing every year, due to climatic conditions, but also to the anthropic impact.

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