MONITORING OF FOLIAR LIMB STRUCTURE ASPECT OF TWO SPONTANEOUS PLANT SPECIES FROM THE CRISUL NEGRU RIVER, NW ROMANIA, FOR IDENTIFY THE HEAVY METAL INFLUENCE

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Abstract

We monitoring – in two years – the structure of the foliar limbs of nettle (Urtica dioica L.) and blackberry (Rubus fruticosus L.), taken from four sites from the Crişul Negru River course, from its spring (Băiţa Plai area) and all the way to its exit from the country (Ant area), into a sites heavily mined of outcrop rocks, for identifying the possible pollutions with heavy metals (Cu, Cd, Ni, Co, Zn), comparatively with the ones identified at the control batch, located on the course of the Crişul Repede River, towards Bulz area. The resulted data have been correlated with the chemical indexes for monitoring the heavy metal pollution by - atomic absorption spectrometry - found in the same locations. At the end of study we concluded that, even the first two sites, Băiţa and Fânaţe, situated in the vicinity of the mining area displayed the higher concentrations in terms of the chemical species, taken into consideration in the present study, these concentration not affect the structure of foliar limb of nettle or blackberry.

Key words: leaf structure, Crisul Negru, nettle, blackberry, heavy metal

INTRODUCTION

The foliar limb's structure is the result of the action of a group of internal and external factors, during plants phylogeny but also ontogeny (Toma C. and Rugină R., 1998). There are edaphic, biotic and anthropic factors which influence the plants' organism (Botnariuc N. and Vădineanu A., 1982). Along with factors independent from the human activity, the anthropic factors, among which pollution, have drastic consequences over the structure and implicitly over the vegetal organs functioning, respectively vegetal organisms, dependent on its nature (Mudd J.B. and Kozlowski T.T, 1975; Günthardt-Goerg M.S. and Vollenweider P., 2007).

Pollution with heavy metals is among the most dangerous pollutions, its removal being a difficult issue, implying very expensive and long term actions. High concentrations of heavy metals can generate sometimes, lethal effects on plants **(**Mendelssohn I.A. et al, 2001).

In the period 2004-2006, the surface water chemistry measurements suggest high values for copper in the upstream sections as a direct influence of mining activities, with mean values of 10.2 μgg-1 (Josan N. et al, 2003) upstream Site Băita Plai and with mean values between 20-30 µgg-1 for downstream sites of tailing ponds at Băita Plai or with values in the range of 50-60 µgg-1 at Fânate (Filip C.C. et al, 2009). For these metals the concentration at downstream sites is much lower indicating a possible dilution and eventually their uptake by other ecological sectors of the river through a biotic processes, by the benthic and hyporheic sediments, as it has been suggested for the river Aries (Marin C. et al, 2010) (like other species of vertebrates or invertebrates, sediments etc) then in the upstream sites (Josan N. et al, 2003). Similar studies were made on the accumulation of heavy metals (Cu and Cd) in *Phaseolus vulgaris* (St□ngu A. et al, 2009) and Cd and Pb in various medicinal plants (Diaconu D. et al, 2009). Da Silva L.C. and colleagues (2005), at *Eugenia uniflora* and *Clusia hilariana* seedlings maintained for 40 days under acid rain damage have identified the morphological, anatomical and ultrastructural lesions. Structural changes indicated a greater sensitivity of the species *E. uniflora* to acid rain harmful action, compared with *C. hilariana*, probably because the latter species analysed had epidermis with thicker cuticle layer and the existence of three cell layers hypodermic and a leaf mesophyll thicker and more compact.

The present research is part of a comprehensive project about monitoring of the heavy metals content the main environment factors and their influence over the ecosystems from the Crișul Negru River route, in 2008-2009 periods. Analysing the histoanatomical data was made in correlation with the morphologic (Petruș-Vancea A. et al, 2010), physiologic changes (assimilating pigments determination) in same plants, with the quantitative values of the heavy metals identified in the water, vegetation and soil (Fodor et al, 2011 a and b) or in fish which living in these water, namely the common minnow (*Phoxinus phoxinus)* and the carnivorous European chub (*Leuciscus cephalus)* (Petrovici M. and Pacioglu O., 2010), from the same location made in the area. The aim proposed for this project is very topical and regional and has national importance, given that in the Băița area is still processing mining activities.

MATERIAL AND METHOD

At sampling the foliar limb of *Urtica dioica* L. and *Rubus fruticosus* L. used for the accomplishment of the vegetal biology studies we respected the standard rules for collecting the vegetal material **(**Andrei M. and Paraschivoiu R.M., 2003), establishing fixed points for sampling the assays from the Crişul Negru River (western Romania), using the Global Positioning System (GPS) (Table 1), in the areas: Băița Plai (upstream), Fânațe (near tailing pond see Filip C.C. et al., 2009), Borz (site ROSCI0061 Nature 2000 in Crişul Negru narrow path) (http://www.apm-bihor.ro/SituriNatura2000/Situri.Nat.2000.htm), Ant (downstream, at the river's exit from Romania, towards Hungary), and as control we chose a site in Bulz area, placed on the banks of the Crişul Repede, considered as unpolluted (site Natura 2000, ROSCI0062 Crişului Repede narrow path - Pădurea Craiului).

We have chosen this two species, a herbaceous one and a ligneous one also from ecological reasons, because, Rijmenams J. (1983), studying the distribution between *Urtica dioica* L. and *Rubus fruticosus* L. in some areas in Belgium, observed that, the two plant species behave, obviously, antagonistically, in matters of reactions towards the ecologic factors. Generally, identifying numerous nettle specimens in a certain perimeter represents an indicator of the presence in that soil of the nitrates or phosphates, resulted as animal or human residues, but this was not the object of our study. The increase of the microelements in plants tissues is conditioned by some characteristics of plants as: plant species, vegetative organs development with the greatest capacity to accumulate heavy metals compounds and age (Luduşan N., 1983).

Table 1

GPS coordinates at sampling sites.

Coordinates	*Site 1* **Bulz (*control*)**	*Site 2* **Băiţa Plai**	*Site 3* **Fânaţe**	*Site 4* **Borz**	*Site 5* **Ant**
Latitude (N)	46°28'55"	46°28'55"	46°30'16"	46°40'22"	46°39'45"
Longitude (E)	22°40'28"	22°36'12"	22°32'16"	22°11'32"	21°28'8"
Altitude (m)	366.8	483.7	355.6	210.7	98.5

The number of sampled assays for the studies regarding the foliar limb histoanatomy was 30 from each sampling site, meaning a total of 150 assays per sampling. The sampling was made in every month, in the August – December 2008 and Mars – July 2009 period.

Foliar limb anatomical structure was studied in transverse plane sections applied manually by plant material preserved in alcohol 70%. Cutting leaves taken from the two studied species (blackberry and nettle) was performed as described by Andrei M. and Paraschivoiu R.M. (2003). Microscopic preparation was examined at MOTIC Model: BA 200, a microscopic with digital camera CCD for MOTIC 480 added, 1/3 CCD sensor, 712*582 pixels and digital and analogue output.

RESULT AND DISCUSSION

Result concerning nettle foliar limb histoanatomy

Hipostomatice foliar limb presents upper (Fig. 1 A) and lower (Fig. 1 D) epidermis unistratified, with isodiametrical cell whose walls are thin. Leaf mesophyll is composed of a palisade parenchyma, unistratified, just below the upper epidermis, which is rich in chloroplasts, with uniform rectangular oblong cells, arranged with the short edge perpendicular to the epidermis cell wall, the cells being closely linked together and from a spongy parenchyma, located towards the bottom of the foliar limb (bifacial dorsiventral structure) multistratified, with irregular size and shape cell, generally oval - oblong, with fewer chloroplasts than palisade parenchyma, showing lacunas in the main nervure.

At the level of main nervure, in some cases, we noted the presence of hypodermic collenchyma above xylem, because of additional thickening with cellulose of cell walls (Fig. 1 A). Vascular tissue has composed of a single vascular bundle, with xylem oriented to the adaxial side and with phloem to the abaxial side (Fig. 1 B). In some cases, have been reported sclerenchyma springs under the phloem (Fig. 1 D).

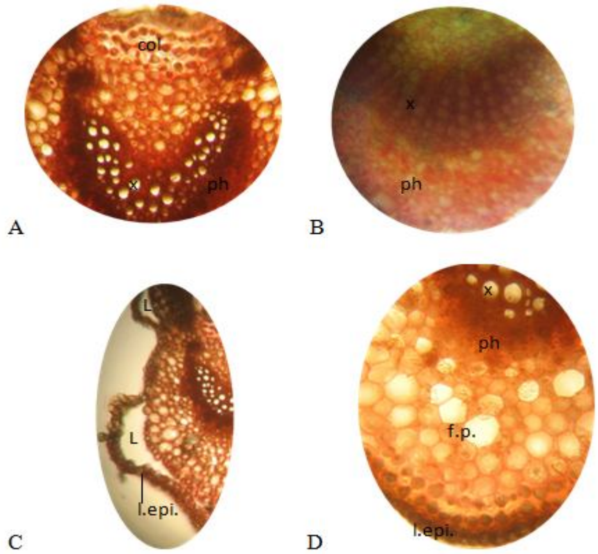

Fig. 1 Foliar limb structure of nettle *(Urtica dioica* L.*)*: A – upper epidermis and colenchyma (400X); B – vascular bundle from main nervure level (400X); C – lower epidermis from abaxial side, with lacunas (200X); D – lower epidermis and adjacent tissue (400X) (col – collenchyma; f.p. – fundamental parenchyma; L – lacuna; l.epi. – lower epidermis; ph – phloem; x - xylem).

At the level of foliar limb of nettle leaves taken from Baita Plai (from first sampling), the cross sectional area of the upper epidermis notes the presence of a cuticle layer with a thickness of 9 - 10 mm, coating well not show (in transversal section) to other foliar limbs, taken from other points (Bulz - control, Fanate, Borz or Ant). Cuticle, like a protective layer is formed on the outside of organ epidermis, with an important role both in reducing evapotranspiration, and defense against attacks from the environment (wind, pollutants, UV radiation, parasites) (Viskari E.L., 2000), but the leaf surface, generally, is not protected from inorganic gases such as NH3 from atmosphere (Kolattukudy P.E., 1996).

To median nervure, in early September, the number of collenchyma layers above the vascular bundle was higher than the control, at the specimens taken from the same period, this mechanical tissue represented by five layers in October, to only three were reported in control specimen, harvested from the same sampling.

In fundamental parenchyma of median nervure, underlying vascular bundle, which was composed from a similar number of layers, as in nettle leaves harvested from the Bulz (control), in the same calendar period, it can sow lacunas (Fig. 1 C), more numerous than in controls and calcium oxalate crystals. Xylem vessels highlight higher lumen, namely metaxylem, since September, while the control they have smaller lumen, even in October. If the first samples (August-September), were not highlighted elements of sclerenchyma, but to the specimens taken in December from this point, there was sclerenchyma islands in the immediate vicinity of the phloem.

Reported in the anatomical aspects of foliar limb of nettle (*Urtica dioica* L.) specimens taken from the Crisul Negru River, during the months August to December 2008, compared with those observed in leaves collected from the Bulz (control) over the same period , showed no major differences, except that the leaves was taken from point Baita Plai, the foliar limb had a thicker cuticle layer, and lacunas in the spongy parenchyma, which were more numerous and larger, to those identified in the control leafs, but also belonging leafs to specimens taken from other collection points on the shore of Crisul Negru River (Fânațe, Borz or Ant).

In the project, Fodor A. and collaborators (2011 a) found that even when the values of heavy metals concentration in vegetation during the studied period were fluctuating, there was no significant difference in the content of heavy metal in the samples taken from Crişul Negru area compared to those registered in the control area (Bulz-Crişul Repede).

But the presence or absence of mechanical tissue type or collenchyma or sclerenchyma springs, the moment of they occur around the vascular bundle or the degree of their development are items strictly related by ontogenetic stage of the plants were, depending on altitude and climate area.

Result concerning blackberry foliar limb histoanatomy

In both epidermises, upper and lower, tector hairs are observed more frequently in the lower epidermis, secretory hairs and emerging spin (Fig. 2 A, B). The palisade parenchyma cells and in the parenchymal cells are observed fundamental rib or ursine single crystal of calcium oxalate (Fig. 2 C). Since 1887, Fritsch K. reported the presence of calcium oxalate crystals, cellular inclusions and later described by Fell K.R. and Rowson J. M. (1957) in leaf mesophyll of leaves of *Rubus fruticosus* L. as druses or as single crystals. Leaf mesophyll is differentiated into palisade parenchyma, unistratified, disposed immediately below the upper epidermis with its cells being perpendicular on this, and a spongy parenchyma (dorsiventral bifacial structure), in the rib area is still a central fundamental parenchyma, above which there is only one vascular bundle which is collateral type (Fig. 2 D).

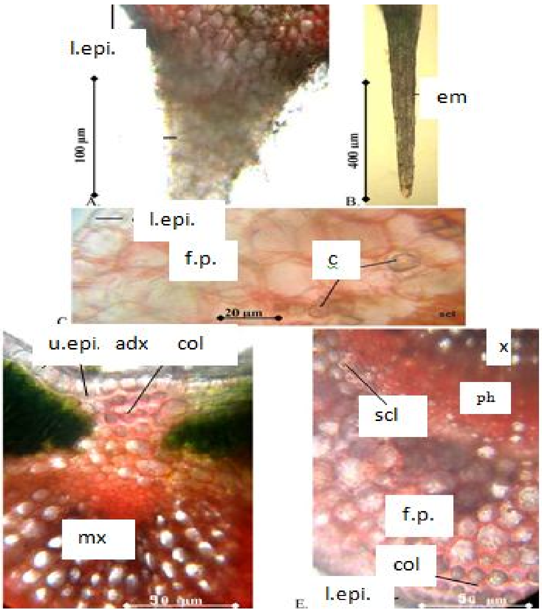

Fig. 2 *Rubus fruticosus* L.: structure of epidermal emergences formed on inferior part of main nervure (A – emergence base and B – emergence tip); calcium oxalate crystals from fundamental parenchyma of main nervure (C); superior epidermis, hypodermic collenchyma and vascular bundle (D); arrangement of layers in the inferior epidermis (adx. – adaxial side; col - collenchyma; cr – calcium oxalate crystal; em - emergence; f.p. – fundamental parenchyma; l.epi.-lower epidermis; mx - metaxylem; ph –phloem; scl – sclerenchyma; u.epi. – upper epidermis; x - xylem).

The xylem is composed of vessels with large lumen, arranged in rows (Fig. 2 D), and phloem possess, on periphery, sclerenchyma springs (Fig. 2 E). At leaves from Baita Plai, at sampling, was observed a lighter green of their, and appearance of spots and holes in the leaf, even in March, from the youngest leaves (so these symptoms were caused by entry leaves in senescence, as we assumed in autumn 2008), however the causes of these symptoms may be multiple.

However, leaves from Bulz had the largest and most numerous lacunas (unlike the nettle that the lacuns were small and few at Bulz and at Baita Plai many and large, this histology demonstrating once again, with aspects of foliar limb morphology (Petruş-Vancea A. et al, 2010), antagonistic behaviour of two species of plants). The fundamental parenchyma of blackberry leaves from Baita Plai and Fânațe were reported most numerous calcium oxalate crystals, no evidence of elements of sclerenchyma in spring, but in July they were obvious.

Similar conclusions was found in the research regarding the exterior aspect of the leaf's foliar limb epidermis, made at the nettle or blackberry, Petruș – Vancea A. and collaborators (2010) concluded that, the differences regarding the epidermal morphology are due to the ontogenetic phase in which the specimens were in at the sampling moment or the different climate conditions, according to the area where they grew (mountain - plain). The authors did not meet at the studied plants' leafs morphological aspects, characteristic for some polluted areas.

CONCLUSION

Foliar limb of blackberry and nettle growing on the bank of Crisul Negru (Băita Plai, Fânațe, Borz and Ant), compared with those identified in the control group (Bulz) present normal aspects depending on plant age, season and climate region in which they grew. The nettle and blackberry leaves studied showed no relevant histoanatomical changes, which suggest that the harmful action of polluting factors.

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