Carpathian Journal of Earth and Environmental Sciences, February 2017, Vol. 12, No. 1, p. 141 - 152

THE EVALUATION OF HEAVY METALS POLLUTION IMPACT THROUGH SOME BIOCHEMICAL, PHYSIOLOGICAL AND HISTOANATOMICAL ASPECTS AT WOODY SPECIES FROM MINING AREAS AT SUCEAVA'S COUNTY, ROMANIA

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Abstract: The present study had the aim to evaluate the mining plant activities impact on forest ecosystems taking a common sense view of determination, at foliar level, of photosynthetic pigments content, of catalase's and peroxydase's activity - as enzymes involved in the defense against the reactive species of oxygen, the Krebs cycle dehydrogenases' activity - as a main metabolic path generative of energy, but also of the microbial dehydrogenases' activity from soil as a ecologic biomarker with key role in maintaining the soil's quality. In parallel with the mentioned biochemical indicators were performed a series of histo-anatomic investigations. Were taken in study samples from the foliar tissue from two angiosperms species (*Populus tremula* L. and *Salix caprea* L.) from areas of Suceava's County namely Rădăuţi (the reference area) and Crucea-Botuşana (area of uranium mining), but also samples of soil derived from different profiles of deepness (0-15 cm and 15-30 cm). The toxicity impact resulted as a consequence of anthropic activity of uranium holding in the Crucea area it is translated through an inferior content of a and b chlorophyll, the photosynthesis process being largely inhibited, in contrast with the superior net catalase's, peroxidase's, but also of foliar dehydrogenases' activity, these enzymes laying out values even four times higher comparatively with the reference lot. The structural differences evidenced especially at the foliar limb level of the two species could be due to the anthropic pollution resulted as a consequence of the mining activity.

Keywords: mining pollution; oxidative stress; plant; soil

1. INTRODUCTION

The mining represents one of the human activities with the most negative effect on the environment's quality (Donkor et al., 2005; Wang et al., 2014), with distructive effects at natural ecosystems' level which can lead to the apparition of some glitches at the soil's level translated through negative influences on the plants, the animals and the human (Cooke & Johnson, 2002).

The impact of mining activities on the environment is accompanied by the habitats' destruction through the reduction of biodiversity's resources (Getaneh & Alemayehu, 2006; Abdul-Wahab & Marikar, 2012), representing a permanent problem for the human health (Aslibekian & Moles, 2003; Patel et al., 2005; Franco-Hernández et al., 2010; Santos-Jallath et al., 2012) and depends, in the same time, of a large number of variables among which, more relevant would be the type of minereum, the mineralogy of waste, waste management in the exploatations operations and after the scrapping, by climatic conditions at the area etc. (Razo et al., 2004).

The contamination of the environment with radionuclides, especially through the uranium intermedium and of its degradation products, it's a serious problem at worldwide level, the development of science and of the nuclear technology leading to the increase of the nuclear wastes' content which contains uranium dispersed in the environment (Gavrilescu et al., 2009).

Also the mining plant of the uranium, in particular, cause enormous damages of the environment through the wastes accumulation and the inadequate utilization of radioactive material, having negative biological effects extremely negative on some important group of organisms of the food chain from the soil and, consequently, on the human's health (Gongalsky, 2003; Choy et al., 2006).

The aim of this study was to determine the physiological and biochemical answer of two *Salicaceae* species (*Populus tremula* and *Salix caprea*), also the evaluation of the microbial dehydrogenases' activity from soil samples harvested from two areas of the Suceava's County namely Rădăuţi (the reference area) and Crucea-Botuşana (uranium mining area).

2. MATERIAL AND METHODS

2.1. Sample collection

The investigations were realized, on a hand, on foliar material derived from two species of angiosperms (*Populus tremula* L. and *Salix caprea* L.) harvested in the second decade of May 2014 from two areas of Suceava's County - Crucea (uranium mining area) and Rădăuţi (considerate reference area), and on the other hand, on soil samples portioned from the same areas, from different profiles of deepness (0-15 cm and 15-30 cm). The vegetal material collected was identified and maintained in conditions of low temperature until the moment of the biochemical determinations. For the histo-anatomical study, the plant material was preserved in 70% v/v ethyl alcohol. Voucher specimens were deposited in the Biochemistry Laboratory of the same Department.

2.2. Biochemical and physiological investigations

Catalase (CAT, EC 1.11.1.6) activity was determined using the method of Sinha, 1972, quoted by Artenie et al., (2008). The method is based on colorimetric determination of chromic acetate obtained through reduction of potassium dichromate in acid medium by the hydrogen peroxide remained after enzyme inactivation.

Peroxidase (POX, EC 1.11.1.X.) activity was measured according to the method of Gudkova and Degtiari, 1968 (Artenie et al., 2008), on the basis of reaction between o-dianisidine and hydrogen peroxide (λ = 540 nm).

The total soluble protein was determined using Bradford's method (Artenie et al., 2008).

The microbial *dehydrogenases' activity* from soil was determined through the spectrophotometric evaluation (at the 540 nm wavelength) of trifenil-formazane formed after the reaction gived by the 2,3,5 clorure – trifenil-tetrazolium, after incubation at 30°C (Kiss & Boaru, 1965; Casida et al., 1964).

The content of assimilator pigments was determined through the spectrophotometric methode, at 645, 472 and 663 nm wavelengths, after the extraction with acetone (Căpraru & Băra, 2007).

2.3. Statistical analysis

All the investigations were made in triplicate. The results were expressed as means ± standard deviation. The differences between control and polluted area were compared with the Student t-test using standard statistical packages (Microsoft Excel). The results were considered significant if the P value was less than 0.05.

2.4. Histo-anatomical investigations

Cross-sections were made from the leaves, with the help of a botanical razor. The sections were later stained with iodine green and carmine red. Preparations thus obtained were analyzed with a Holland Novex microscope and subsequently photographed with a Sony Cyber-shot DSC-W730 camera. The reagents used for all determinations were of analytical purity.

3. RESULTS AND DISCUSSIONS

3.1. Biochemical investigations

The pollution generates undesirable changes in what concerns the physical, chemical and biological characteristics of the environment which affect drastically, ulterior, the life conditions of the human being and of the plants (Selvi & Sharavanan, 2013). Through the different types of pollutants, the pollution of the waters as a consequence of massively industrial discharges (Hussain et al., 1982), but also the one due to the mining exploatations (Schützendübel & Polle, 2002; Navarri-Izzoand & Rascio, 2010; Wahsha et al., 2011) occupies a place with distinctive impact on agro-ecosystems, causing negative effects at the soil's level, at the water's, the agriculture's, the flora's and fauna's level, namely due to the high remanence of these chemicals at the soil's level (Selvi et al., 2012). Although the industrialization represents a main tool in the development of a nation, the different types of human activity generate drastically changes on the environment through the impact on the soil, the water and on the air (Banupriya & Gowrie, 2012; Madhu et al., 2014). Besides, the pollutants exert phytotoxic effects affecting a series of metabolic functions like the sweat, the transpiration and the photosynthesis of the plants (Malhotra & Hockings, 1976).

From the graphical representation of the assimilatory pigments content at the species studied by us (Fig. 1), comes out that the samples took from the Crucea mining exploatation area lay out values net inferior to those from the control area (Rădăuţi), both in what concerns the chlorophyll *a* as well as the chlorophyll *b*. Thus, at *Populus tremula*, the chlorophyll *a* concentration varies between 18.657±0.581 mg.100 g^{-1} vegetal tissue at the exemplars derived from Rădăuţi area and 11.025±0.557 mg.100 g^{-1} vegetal tissue, at those harvested from the proximity of Crucea-Botusana uranium mining exploatation. From comparison, *Salix caprea* elevates values of 10.401±0.887 mg.100 g^{-1} vegetal tissue and 7.362±0.878 mg.100 g^{-1} vegetal tissue, the difference of concentration between the two zones being something less significant ($0.001<p<0.005$). The content of chlorophyll *b* follows the same trend, with maximal values at both harvested species from the reference area (11.545±0.519 mg.100 g^{-1} vegetal tissue at *Populus tremula*, respectively 5.685±0.503 mg.100 g^{-1} vegetal tissue at *Salix caprea*) and of 2-3 times lower at the samples derived from the Crucea area (5.442±0.266 mg.100 g^{-1} vegetal tissue at *Populus tremula* and 2.419±0.483 mg.100 g^{-1} vegetal tissue at *Salix caprea*). To explain the results we should take into account the fact that, as it show Kadam et al., (2013), the content of chlorophyll *a*, *b* and carotenoids in the leaves of some medical species (*Sesbania rostrata*, *Sesbania exaltata* and *Sesbania sesban*) vary seasonally, existing a markant difference between the concentration of chlorophyll *a* and *b*, on the one hand, and a significant seasonal variation of these ones on the other hand.

The specialty literature shows also the fact that, the decrease of chlorophyll content is given by the plants' answer to stress conditions, fact that leads to the reduction of the light's absorption (Bradel et al., 2000; Munné-Bosch & Alegre, 2000; Li et al., 2011).

It is known the fact that to may realize the photosynthesis process, the green plants are based on the interactive cooperation of two photosystems (Ohashi et al., 1989; Toma & Niţă, 2000), for a maximum efficiency, each of these ones must absorbing an equal quantity of light (Kargul & Barber, 2008; Tutu et al., 2011; Nakajima et al., 2012), the chlorophyll *a*/chlorophyll *b* rapport characterizing the distribution of the pigments between the reaction centers and the responsable complexes for the light's absorption (Bassi & Caffari, 2000).

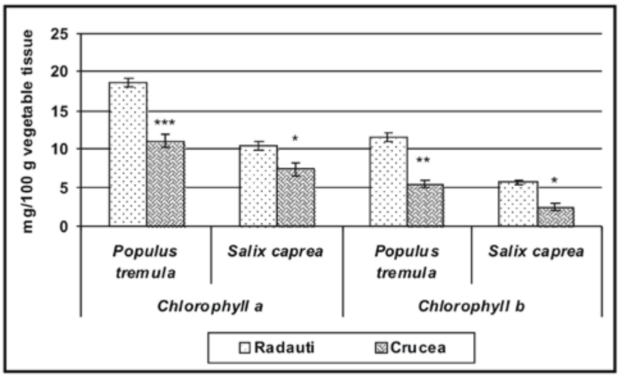

Figure 1. The content of chlorophyll at the *Populus tremula* and *Salix caprea* harvested from areas of Suceava's County. ***$p < 0.001$ (very significant); ** $0.001<p<0.005$ (significant); * $0.01<p<0.05$ (less significant); $0.05 <p<0.5$ (not significant)

The chlorophyll *a*/chlorophyll *b* rapport is supraunitary in the case of the both species taken into study, in the Rădăuţi area the concentration of chlorophyll *a* being of approximately 1.5 few times higher than that of the chlorophyll *b*, while, in Crucea area, the value of this rapport is of 2:1 at *Populus tremula*, respectively 3:1 at *Salix caprea*. The decrease of the assimilatory pigments content in the polluted area can be put on the view of the existence of the oxygen reactive species, phenomena associated with the diminution of the photochemical conversion efficiency. The inhibition of the photosynthesis process is associated with the reduction of the stomatal conductant and of the sweat resulting photoinhibitory conditions with negative effect on the photosystem's II activity (Osmond et al., 1999). The literature on the field signalizes, furthermore, the strong stressor effect of the heavy metals on the plants, metals that inhibit the photosynthesis and affect the chlorophyll's fluorescence and the stomatal resistance (Monni et al., 2001; Fayiga et al., 2004; Fayiga & Ma, 2005; Fayiga et al., 2007; Abdul-Wahab & Marikar, 2012).

It is known the fact that the mining is one of the human activities which could have a strong negative impact on the environment, after the mining activities resulting big quantities of sterile which is accumulated in the soil and from here in the tissues and in the plants' organs (Hellström, 2004; Donkor et al., 2005; Abdul-Wahab & Marikar, 2012) with direct role in the apparition of the perturbations of the ordinary metabolism, defined, among others, by the free oxygen radicals' production. The increase and the development of the plants are processes very sensitive at the action of the stressor factors from the environment (Lakshminarayan et al., 2006). The specialty literature signalizes the fact that the heavy metals - industrial contaminants which don't degrade, are accumulated in the water, soil, deep sediments and, from here, in the living organisms (Yabanli et al., 2014).

The toxicity impact of heavy metals at the level of the woody plants is due to the generation of the reactive oxygen species (ROS) and to the induction of the so called oxidative stress (Schützendübel & Polle, 2002), the counter-measuring of these ones being realized by different enzymatic and non-enzymatic antioxidant systems (Smirnoff, 1995; Navarri-Izzoand & Rascio, 2010; Gill & Tuteja, 2010), the debut of the stress in plants imposing a reorganization of the cellular metabolism in its assembly (Mohasseli et al., 2016), for an acclimatization of these ones at stress.

The evaluation of the oxidative stress' level at the *Populus tremula* and *Salix caprea* species was realized through the determination of the CAT and POX activity, bicomponent enzymes with haemoproteinic structure implied in the removal of the ROS.

At poplar (Fig. 2), the CAT activity reached a medium value of 14.261±0.942 UC/µg protein at the exemplars harvested from the witness area and 50.997±2.425 UC/µg protein at those emerged from the proximity of the uranium mine from Crucea. And at the *Salix caprea* samples derived from the Crucea area, it is observed an extremely high level of the oxidative stress translated through the catalasic activity of nearly 4 times higher comparatively with the reference sample (59.198±2.997 UC/µg protein, respectively 16.049±1.245 UC/µg protein).

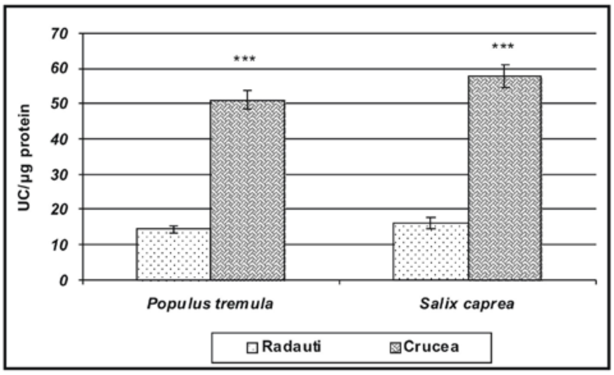

Figure 2. The CAT activity at the *Populus tremula* and *Salix caprea* harvested from areas of Suceava's County. ***p < 0.001 (very significant); ** 0.001<p<0.005 (significant);* 0.01<p<0.05 (less significant); 0.05 <p<0.5 (not significant)

The apply of the Student statistical significance test shows the existence of some strongly significant differences (p < 0.001) between the areas studied both at the poplar as well as at the willow. Our results concords with those from the specialty literature which show, on the one hand, the strongly stressor effect of the heavy metals and of the mining wastes on the plants through the producing of some high quantity of ROS (Tutu et al., 2009, 2011; Vanhoudt et al., 2009; Saenen et al., 2013), and on the other hand the fact that the enzymes of the antioxidant system (catalase, peroxidase, superoxid-dismutase etc.) are recognized like having a decisive role in the adaptability of phytoremediant species at the environment pollution, through the removal of some anomalies induced by the pollution at foliar level (Richardson et al., 1990; Ciornea et al., 2015).

Although the heavy metals are naturally presented in the soil, the geologic and anthropic activities as well as the extraction and the fusion of the metals, the burning of fossil fuels, the use of pesticides and fungicides, the removal of industrial and municipal wastes etc. (Alloway, 1990; Shen et al., 2002), increase the concentration of these elements at level that are harmful both for plants as well as for animals, the modifications of physiologic and biologic processes which appear at the plants grew on polluted soils determining perturbations of their growth and their development (Chatterjee & Chatterjee, 2000; Chibuike & Obiora, 2014; Cobb et al., 2010; Duray et al., 2015).

The high concentrations of heavy metals determine direct toxic effects like the massive accumulation of hydrogen peroxide and other free radicals – mobilized by the antioxidant enzymes, but, sometimes, they determine the inhibition of citoplasmatic enzymes and even the damage of the cellular structures due to the strong oxidative stress exerted (Jadia & Fulekar, 2009).

In the case of the samples taken by us in study (Fig. 3), the medium activity of the POX, at the exemplars from the witness area, varied between 1.784±0.111 UP/µg protein (*Populus tremula*) and 1.974±0.101 UP/µg protein (*Salix caprea*), respectively 2.121±0.092 UP/µg protein (poplar) and 3.087±0.203 UP/µg protein (willow) at those derived from the Crucea-Botuşana area, the Student test indicating the existence of some significant differences of activity (at *Populus tremula*) and moderately significant (at *Salix caprea*) between the two areas.

In interpreting the experimental results it must be taken into account the fact that it is possible that in the stressogenes conditions, in the leaves of the powerless resistant species at the anthropic pollution to exist a disparty of the balance between the production of the ROS and the enzymatic activity of antioxidative defensive, this proposal basing on the fact that the production itself of the antioxidants is affected by the stressogene conditions (Bowler et al., 1992).

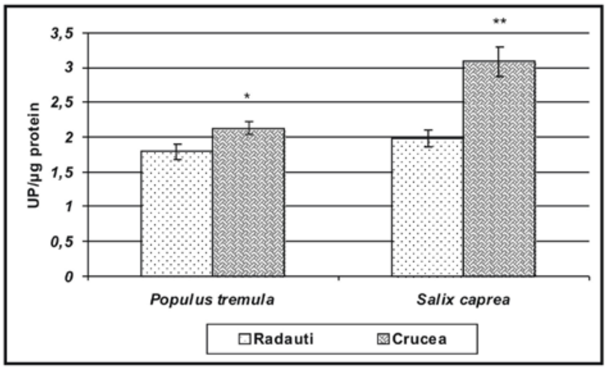

Figure 3. The POX activity at the *Populus tremula* and *Salix caprea* harvested from areas of Suceava's County. ***p < 0.001 (very significant); **0.001<p<0.005(significant);* 0.01<p<0.05 (less significant); 0.05 <p<0.5 (not significant)

Our results chime in with those from the specialty literature which indicate an increase of the peroxidasic activity at the plants subjected to a stress due to the pollution of the ecosystem (Kshirsagar & Aery, 2007).

Additional to tests related to the antioxidative defense, was opted for the determining of the dehydrogenase's activity of Krebs cycle, knowing the fact that an acclimatization of the plants to stress is associated with deep changes in the composition of the cellular proteome, the proteins being implied indirectly in the plants' resistance to the induced stress by the metalifer excess (Oniciuc et al., 2013).

In what concerns the activity of the main dehydrogenases of the tricarboxilic acids' cycle at the species derived from the area considered like being unpolluted (Rădăuţi), it can be ascertained that the intensity of the intermediary metabolism and of the respiratory processes is less decreased comparatively with the one decelated in the polluted area (Fig. 4). Thus, at *Populus tremula* the izocitrat-dehydrogenases' (IDH) activity reach the medium valuable level of 7.604±0.946 µg formazan/g vegetable tissue at the exemplars harvested from the Rădăuţi area and 35.935±4.719 µg formazan/g vegetable tissue at those from nearby the uranium mining from Crucea. At *Salix caprea*, the rate of mobilization of the izocitric acide through oxidative decarboxylation under the action of IDH is lower, in the foliar tissues harvested from Rădăuţi the enzyme reaching the minimum level of activity (4.798±0.953 µg formazan/g vegetable tissue), while, at those from Crucea area, the decelated activity was of approximately 3 times higher (14.835±1.402 µg formazan/g vegetable tissue).

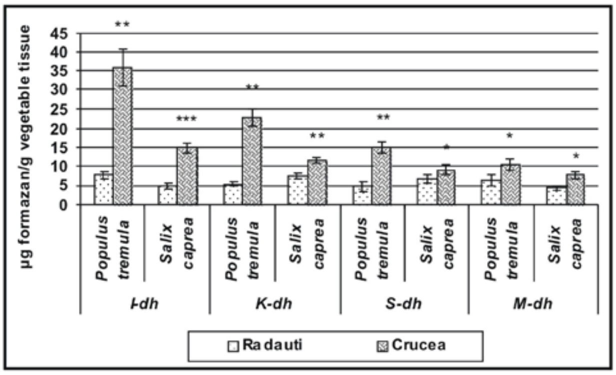

Figure 4. The Krebs cycle dehydrogenases activity in foliar tissue at *Populus tremula* and *Salix caprea* species harvested from areas of Suceava's County. ***p < 0.001 (very significant); ** 0.001<p<0.005 (significant); * 0.01<p<0.05 (less significant); 0.05 <p<0.5 (not significant)

The α-Cetoglutarat-dehydrogenase (KDH) follows the same trend, with higher values of the activities at the samples derived from the polluted area (22.872±2.343 µg formazan/g vegetable tissue - *Populus tremula* and 11.563±0.705 µg formazan/g vegetable tissue - *Salix caprea*) and significantly lower at those of reference (5.422±0.615 µg formazan/g vegetable tissue - *Populus tremula* and 7.416±0.927 µg formazan/g vegetable tissue – *Salix caprea*).

The significant difference and strongly significant between the decelated activity in the two areas (p<0.001 -IDH at *Salix caprea*; 0.001<p<0.005 - IDH at *Populus tremula* and KDH at the both species) may be put on the view of the fact that, in the case of the exposure at the heavy metals mining wastes, it is signalized a bioaccumulation phenomenon of the radionuclides produced through the dispersion of the uranium in tissues, process that has as consequence the stimulation ROS forming, but also the apparition of some lethal and lesslethal effects on the organisms of an ecosystem, whereas the autoizotops become part of the flux from the interior of the system and between systems (Hossner et al., 1998; Auerbach, 2006). Furthermore, data from the literature on the field appreciate that the lesslethal effects of the uranium on the plants can manifest at radicular level, these ones stopping from the growth, at foliar level through the leaves' death, lesions of the system and of the petiole, but also an abnormal number of chromosomes, reflected through the increasing of this one in the cells' nucleus (Ripley et al., 1996).

At a carefully analyze of the experimental results (Fig. 4) it can be observed a balanced deployment of the different stages of Krebs cycle, the conversion speed of the succinatum and of the malatum in fumarat and oxalo-acetat being more moderate. Thus, at *Populus tremula* from Crucea area the succinat-dehydrogenase (SDH) reaches the medium value of 14.877±1.64 µg formazan/g vegetable tissue, and the malat-dehydrogenase (MDH) of 10.423±1.328 µg formazan/g vegetable tissue, while *Salix caprea* lay out lower medium values of activity (9.002±1.196 µg formazan/g vegetable tissue - SDH and 7.668±1.077 µg formazan/g vegetable tissue - MDH). At the exemplars harvested from the witness area, the activity of this enzyme varies between 4.179±0.626 µg formazan/g vegetable tissue and 6.717±0.983 µg formazan/g vegetable tissue at *Salix caprea*, respectively 4.697±1.252 µg formazan/g vegetable tissue and 6.122±1.33 µg formazan/g vegetable tissue at poplar.

The statistical analyze of the obtained results shows, between the two areas, differences less significantly (0.01<p<0.05) of the foliar SDH and MDH activity both at the poplar as well as at the willow.

Worthily to keep in mind is the fact that the mining operations create multiple types of radioactive wastes in the Crucea–Botuşana area (Petrescu et al., 2010; Petrescu & Bilal, 2007) fact that leads to the Crucea area to have the highest radioactivity of the environment, the more so as the series of uranium radionuclides were found in very high quantities in the river foots and in the surface waters.

Given the fact that the mining plant operations lead to massive contamination of the soil, a last aspect of this study was the determination of the microbial dehydrogenases' activity in soil, the enzymatic activity of the soil being considerate an efficient indicator of the soil's quality change, due to the natural stress conditions, but also to anthropic perturbations (Dick, 1997; Kumar et al., 2013).

The microbial biomass from soil plays an important role in the mediation of nutrients cycle and of energy circuit and actions as an ecologic marker due to its active involvement in the nutrients freeing, the microbial enzymatic activity having a key role in the maintaining the soil's quality (Schloter et al., 2003; Abbas et al., 2014).

The specialty literature mentions the fact that the dehydrogenasic activity of the soil is influenced by a series of biotic and abiotic factors like the type of soil, the degree of soil's aeration, its pH, the humidity, the presence of different heavy metals, but also the deepness profile of the soil (Stępniewski et al., 2000; Agnelli et al., 2004; Levyk et al., 2007; Xie et al., 2009; Ciornea et al., 2014).

In the samples soil analyzed by us (Fig. 5), the potential dehydrogenase's activity varies, in the surface stratum (0-15 cm), from 10.729±1.031µg formazan/g soil at Rădăuţi at 20.717±1.841µg formazan/g soil at Crucea, while, in the profundity substratum (15-30 cm), are reached medium valoric levels of activity a lot decreased that are comprised between 2.109±0.219 µg formazan/g soil (Rădăuţi) at 5.491±0.574`µg formazan/g soil (the mining area Crucea). Furthermore, the apply of the Student statistical signification test demonstrate the existence of some significant differences between the two areas taken into study, the value of statistical probability (*p*) being between 0.001 and 0.005.

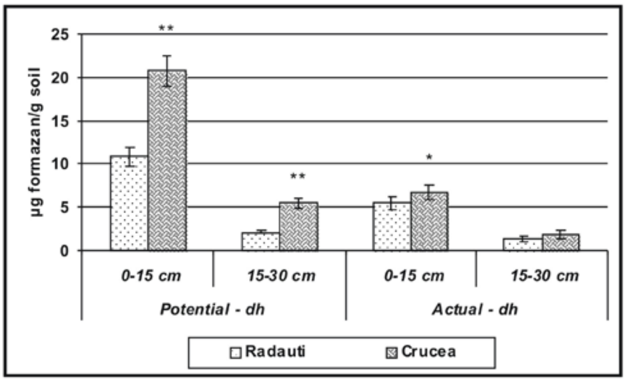

Figure 5. The dehydrogenasic activity in soil samples harvested from areas of Suceava's County.
***p < 0.001 (very significant); ** 0.001<p<0.005 (significant); * 0.01<p<0.05 (less significant); 0.05 <p<0.5 (not significant)

The actual dehydrogenasic activity evidences differences less significant (0.01<p<0.05) or even insignificant (0.05<p<0.5) between the samples soil derived from the witness area comparatively with those from the nearness of the uranium mining exploatation from Crucea-Botuşana. Thus, if in the superficial stratum, the dehydrogenasic activity oscillates between 5.475±0.735µg formazan/g soil at the reference samples and 6.684±0.812µg formazan/g soil at those from the polluted area, in the 15-30 cm portion of the soil, the medium enzymatic activity is of approximately 4 times lower, varying between 1.34±0.347µg formazan/g soil in the reference area and 1.78±0.519µg formazan/g soil at Crucea.

Our results concords with those from the literature data which evidence the fact that the deepness of the soil is one of the most known and popular factors that reduce the dehydrogenases' level from the soil, the biggest abundance of the microorganisms being at its surface (Agnelli et al., 2004; Levyk et al., 2007; Wolińska, 2010; Ciornea et al., 2014).

The accounting of the rapport between the potential dehydrogenase and the actual dehydrogenase emphasizes differences of 1.56 (15-30 cm), respectively 1.95 (0-15 cm) times highest in favor of the potential dehydrogenase at the samples from the witness area and of 3 times highest (no matter the prelevation location) at the samples from Crucea area, the dehydrogenation potential of the soil from this area being net superior.

3.2. Histo-anatomical investigations

To realize a more complex study were comparatively analyzed both the petiole as well the foliar limb from the species taken into study.

The cross-section contour through *Salix caprea* petiole is approximately quadratic, with the latero-adaxial flanks prominent (Fig. 6a). The epidermis is covered by a thick hide, and here and there it can be observed unicellular tectors hairs, more frequent between the latero-adaxial flanks. In the middle of the fundamental parenchyma there are 2 free-woody leading fascicules, situated with the wood face to face. Around this complex of leading tissues there is a discontinuous ring of sclerificated elements with the walls moderately callous, but intense lignificated. Both the liber and the wood have a secondary structure, in the liber observing much more big cells of parenchyma, and in the wood predominates the mechanic fibers between the vessels string (Fig. 6a; b).

At the foliar limb's level the median nervure stand out strongly at the adaxial face and a little at that abaxial. The epidermic cells are izodiametric and covered by cuticle. Here and there are noticeable short tector hairs, rare, localized mainly at the abaxial face of the median nervure (Fig. 6c).

At the taxon collected from Rădăuți the mesophyll is differenced in the tristratified palisadic tissue at the adaxial face, occupying 70% from the thickness of the assimilatory parenchyma and lacunos bi- or tristratified tissue at the abaxial face, formed from quadratic cells or even shortly rectangular, with very little aeration spaces between them. Here and there, the parenchymatic tissue appears to us formed of 4 cells stratum, and the lacunos one from a single cells stratum tangentially elongated (Fig. 6d). At the taxon collected from Crucea the mesophyll is differenced in 2 palisadic cells stratum, at the adaxial face and 3-4 stratum of lacunos tissue at the abaxial face. In the vicinity of the median nervure the palisadic tissue is tristratified, and the one lacunos with a disposition very regulated, with meatures extremely small between them (Fig. 6e). Khalili et al., (2010) evidenced the presence of bilayered palisade parenchyma in different species of the genus *Salix*.

At the *Populus tremula* taxon collected from Rădăuți the contour of cross-section through petiole is elliptic, of different thickness at the 2 poles, by comparison with the taxon from Crucea where the cross-section through petiole is cuneiform, with both poles rounded.

The epidermis has cells with all the walls thick, the external one being covered by an extremely delicate cuticle. Under epidermis there is an area of colenchymatic tissue, more thick at one of the faces. This tissue is more wicks developed at the taxon collected from Crucea. Koranda & Robison (1978) signalize the fact that the properties of epidermis and the foliar cuticle's structure would be directly implied on the radionuclides absorption. Other data from the specialty literature (Petrescu & Bilal, 2006) indicated heavy metal pollution in the area of the Crucea-Botușana mine, suggesting that the histo-anatomical changes at leaf level may not be due to radionuclides, but rather to heavy metals. Furthermore, Barceló & Poschenrieder (2004), Greger (2004), Günthardt-Goerg & Vollenweider (2007) point out the fact that the macro- and microscopic symptoms of the stress due to heavy metals are very complexes, the toxicity depending of species, ecotype, ontogenetic stage, as well as of edaphic and climatic factors.

The fundamental parenchyma at the poplar is of meatic type, here and there observing also aeration lacunos under the hypodermic collenchyma area; some cells contain ursins of calcium oxalate. In the fundamental parenchyma there are numerous free-woody leading fascicles, of collateral type, grouped in 4 centers at the collected taxon from Rădăuți (Fig. 7a). At the taxon collected from Crucea we observe also the formation of the 5th center, of much smaller dimension (Fig. 7b). All the fascicles, of different sizes, present each a thick cordon of sclerenchymatic fibers at the liber periphery. The most fascicles are separated between them by parenchymatic raies uni- or pluristratified. In the middle of each center of leading fascicles there is a parenchymatic tissue with cells extremely small, some of it having the walls easy callous and lignified. The most fascicles present both primary structures (strings of vessels separated by cellulosic woody parenchyma), as well as secondary structure (irregular dispersed vessels and separated by libriform fibers).

The literature data (Sebastiani et al., 2014) shows that the plants' answer at the stress produced by heavy metals varies from a species to another and depends of the metal type, implying complexes modifications at the physiologic and biochemical processes' level, at genic expression, as well as at the histo-anatomic level. On the other hand, Hermle et al., 2007, studying the effect of heavy metals on the *Populus tremula* leaves, evidence the presence of palisadic cells with whittled cellular walls, vacuolar cytoplasm, chloroplasts with reduced dimensions, as well as an increase frequency of the starch granules.

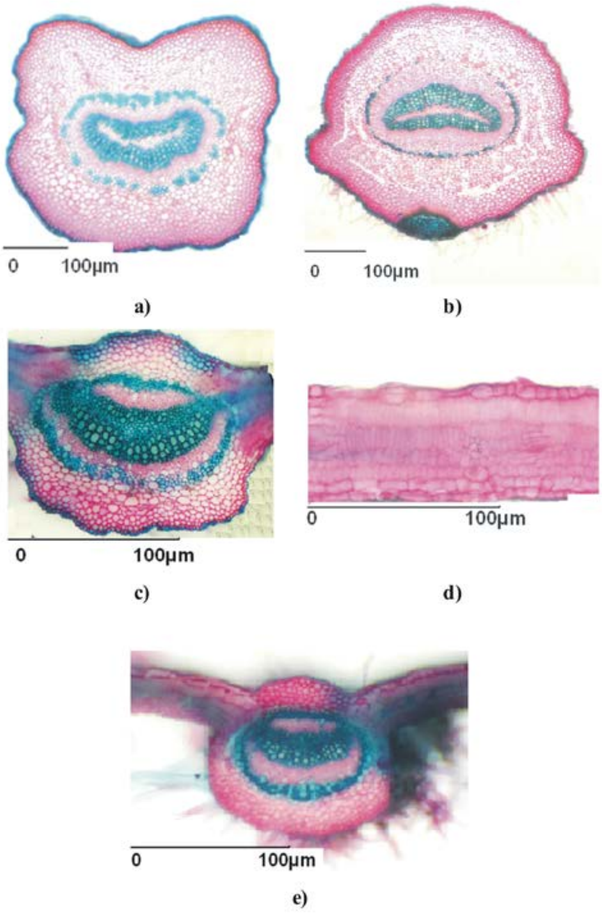

Figure 6. Cross-sections at *Salix caprea* species: a) petiole - Rădăuţi; b) petiole – Crucea; c) principal nervure of leaf lamina – Rădăuţi; d) zone between the nervures of leaf lamina – Rădăuţi; e) principal nervure of leaf lamina – Crucea

At the sample collected from Rădăuți, it is remarked at the foliar limb's level, that the median nervure points out at both the two faces of the limb, comprehending 3 free-woody fascicles of different sizes, 2 with the wood orientate to adaxial face and one very little, opposite them, with the wood oriented to abaxial face. All the leading fascicles present perifloemic sclerenchyma very well developed, which surround in fact all leading fascicles group from the median nervure (Fig. 7c). The mesophyll is differenced in bistratified palisadic tissue with very high cells, at the adaxial face and pluristratified lacunos tissue at the abaxial face, at both taxons (Fig. 7d; f). At the sample collected from Crucea in the median nervure's thickness there is a single leading fascicle with secondary structure, having each one a thick cordon of sclerenchyma at both poles (Fig. 7e).

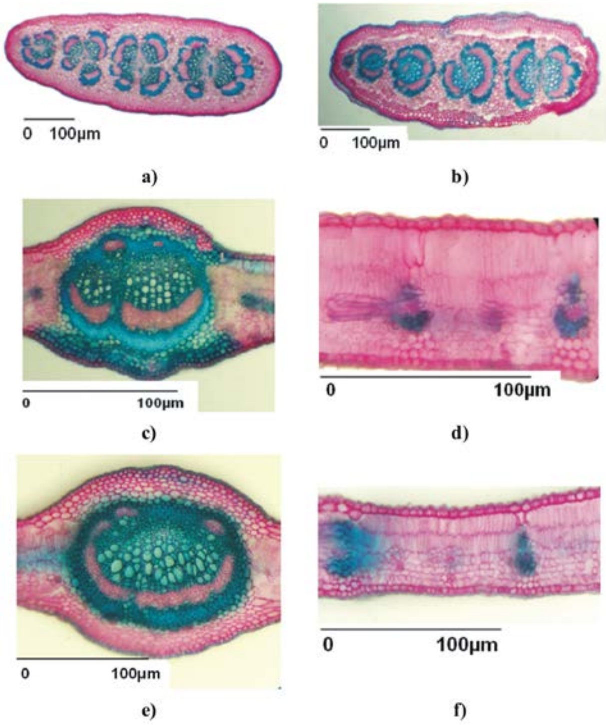

Figure 7. Cross-sections at *Populus tremula* species: a) petiole - Rădăuţi; b) petiole – Crucea; c) principal nervure of leaf lamina – Rădăuţi; d) zone between the nervures of leaf lamina – Rădăuţi; e) principal nervure of leaf lamina – Crucea; f) zone between the nervures of leaf lamina - Crucea

4. CONCLUSIONS

The content of assimilatory pigments was net inferior in the biologic material of the two species harvested from the Crucea mining area, in strong connection with the strong stressor effect determined by the presence of radioactive wastes in this area, effect that leads to generation of some enhanced quantities of free radicals of oxygen and, implicitly, at the photosynthesis process' inhibition through the reduction of light's absorption.

The toxicity of the woody plants' level, due to anthropic activities of uranium exploatation, is counteracted by a series of enzymatic and non-enzymatic antioxidant systems, the CAT, POX and dehydrogenases activity of Krebs cycle being net superior in the samples derived from the vicinity of the mine of uranium exploatation from Crucea-

Botuşana, these enzymes being known as having decisive role in the adaptability of phytoremediant species to environment's pollution conditions, through the „reparatory" action of some anomalies induced by the pollutants factors at foliar level. Our results demonstrate that the species *Populus tremula* and *Salix caprea* have a significantly tolerance to high levels of heavy metals in soil and can be used as species with potential of phytoremediation, them having a key role in the pathogenesis induced by the oxidative stress produced by heavy metals at cellular level.

The analyze of experimental results regarding the dehydrogenases activity shows the existence of some significant differences in behavior manner of these oxidoreductases, values net superior registering in the case of soil samples derived from Crucea area comparatively with Rădăuţi, on the one hand and in the light stratum (0-15 cm) towards the one inferior (15-30 cm) on the other hand, in strong connection with the aeration degree of the soil, the availability of organic materia, the influence of mining activities, but also the type of microbial colonies which predominate, the microbial biomass playing a key role in the mediation of nutrients cycles and of energy circuit.

At the *Salix caprea* taxons taken into study it was seen that, there weren't registered significant differences, at the petiole's level. In exchange at the foliar limb's level are seen differences in the formation of the mesophyll, these differences consisting in the number of cells stratum that form the palisadic tissue, respectively lacunos.

The realized sections at the petiole's level, at the *Populus tremula* taxons taken into study, evidence a structure relatively similar, with the mention that at the taxon collected from Crucea, appears a 5^{th} center of leading fascicles of smaller dimensions. At the foliar limb's level it is remarked that the leading fascicles differ as number at the 2 taxons. At the taxon collected from Rădăuţi we have 3 leading fascicles, while at the taxon harvested from Crucea we have a single big fascicle, central.

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Received at: 21. 02. 2016
Revised at: 06. 09. 2016
Accepted for publication at: 17. 10. 2016
Published online at: 28. 10. 2016