EVALUATION OF THE PHYTOREMEDIATION POTENTIAL OF THE *SALIX CAPREA* IN TAILING PONDS

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Abstract. The goat willow (*Salix caprea*) belongs to the typical pioneer vegetation at former ore mining sites in East and Middle Europe as a result of its high metal-tolerance. Ectomycorrhizal (EM) fungi are known to contribute essentially to the tolerance of plants against abiotic stress. We have determined the absorption/ accretion potential of the goat willow related to heavy metals, as well as the growth rhythm of this species in conditions offered by polluted sites, correlated to the presence/ absence of the microbiota in the soil attached to the root. The research unravels how the goat willow (*Salix caprea*), as a native species can be used in remediation actions of polluted sites based on its high adaptive potential, as well as on its capacity to absorb and accumulate heavy metals. In terms of survival strategy, mycorhrised fungi attached to the polluted soil play a significant part, since they induce plants a certain resistance by diminishing the absorption of heavy metals by the host plant. We suppose that the majority of EM associations of *S. caprea*, in combination with high portions of metal-chelating organic acids containing fungal partners, contributes essentially to the high tolerance of this species against high metal concentrations in the soil.

Keywords: *Salix caprea*, fungi, heavy metals, phytoremediation, mycorrhizae

INTRODUCTION

Willows (*Salix spp.*) belong to the typical pioneer vegetation on devastated areas. *Salix caprea* is a shrubby tree and has naturally adapted to colonise derelict, spoiled and polluted land [14, 23]. Plant roots in the majority of plant species are associated with mycorrhizal fungi, which can modify the sensitivity of roots to metal stress [24, 35, 23]. *Salix spp.* associate with arbuscular mycorrhizal and ectomycorrhizal (EM) fungi. EM colonisation can reach more than 80% of the fine roots of willows, whereas the colonisation with arbuscular fungi is with 5% lower of the fine root length [22, 36, 23]. EM fungi can promote the establishment of plant species in mining soils by immobilizing heavy metals in the soil, thereby reducing the availability of metals to plants [11, 23]. EM fungi contribute directly to the establishment of woody plants in soils polluted with heavy metals by binding metals to fungal hyphae in roots or in the rhizosphere [19]. The plants colonized with these fungi show a higher degree of tolerance to toxic metal concentrations [8, 23]. Different species of fungi allow for a variable degree of efficiency in protecting plants, in the presence of different toxic metals [28]. A wide range of fungi from all major taxonomic groups are reported in metal-polluted habitats and are capable of surviving and growing in the presence of toxic concentrations of heavy metals [19, 40]. Toxic metals affect fungal populations by reducing their abundance, the diversity of species and the available selection of tolerant fungi populations [13]. Strong differences can occur in terms of tolerating metals even between EM plants of the same family, but the reasons for particularly successful protection of the host plant by the EM community are still unknown [1]. *S. caprea* should be a host of an EM community with fungal partners of high tolerance to heavy metals. To the best of our knowledge, no research focusing on the behavioral pattern of a diverse EM community interacting with species of *S. Caprea*, highly tolerant to heavy metals, has been published so far.

The importance of the willow is currently emerging in a wide array of practical applications to restore damaged ecosystems. Willows are increasingly used for conservation and ecosystem restoration, for biomass production, and for phytoremediation and bioengineering aiming to minimize the negative impact on the environment [27] However, willows can accumulate significant quantities of metal in roots and shoots.

Plants are considered as monitors of the areas contaminated with heavy metals because of their capacity to act efficiently as interceptors of chemical pollutants [3, 16, 39] and because phytoremediation is considered to be a friendly technology through which life conditions may be remediated in polluted areas. The selection of such plants plays an important part in developing methods of decontamination, stabilization and reintegration in the landscape of the polluted areas [18]. In the area researched we identified: non-native and fast growing species, such as *Pinus nigra, Robinia pseudacacia, Prunus serotina* etc., planted in the area, and some species belonging to the regional flora, like *Salix caprea, Quercus petrea, Populus tremula, Betula pendula*, spontaneously settled. Taking into account the two categories of species, the goal of our research is, among others, to highlight the absorption capacity of the goat willow, as a native species, of heavy metals in the ground layer, its resistance to extreme ecological conditions and to evaluate the possibility of introducing this species in the re-vegetation schemes in affected areas. At the same time, we analyze the concentration of heavy metals in the organs of *Salix caprea* in correlation with the microflora in soil so as to detect the symbiotic relations that sustain survival in the conditions offered by the tailing ponds. The presence of an efficient and adequate microflora in soil increases the chances of superior plants to survive, supporting efficiently in time the creation of a well formed vegetal layer. It is proved that the microflora in soil, but especially the mycorrhized fungi stimulate the density of roots in herbaceous and ligneous plants, thus making them more efficient in stabilizing and consolidating the ground layer [38]. An increased level of symbiosis may improve the survival potential of plants in polluted sites by increasing the amount of water supplied, by improved feeding with nutrient substances, resistant to pathogens, improving the soil structure and producing phytohormons [26]. Moreover, both soil micro-organisms and fungi attached to roots, in the case of mycorrhizae, change the solubility and mobility of heavy metals in the ground layer, as well as their accessibility/ penetration in plants and, sometimes, even the proportion in which these metals accumulate in different organs of the cormophyte plant. The idea that mycorrhizae can act in the sense of reducing metal deposits/ translocations in the host plant is controversial [29, 32]. The relevant literature in the field quotes cases when ectomycorrhizae reduce the absorption of metals in plants, ensuring a better survival on contaminated ground [44], while other studies show that ectomycorrhizal fungi protect the plant from toxicity, binding the metal in the cellular wall or in the hyphae [20, 41]. For example, arbuscular mycorrhiza can diminish zinc absorption in the host plant [29]. We notice a certain growth of accumulations of heavy metals, especially zinc, in some species in the presence of mycorrhizae [29], but in other cases they seem to have no effect whatsoever. The response of mycorrhizal plants to heavy metals cannot be generalized [37] and the causal relationship metal/ fungi/ vegetal species also depends on the pH of soil and on the concentration of metals [24]. That is why, in order to develop a proper remediation program [4], research reinforces the importance of using in such sites plants and fungi adapted to the climatic and soil conditions in that particular site [15].

Bibliographic sources covering the composition of species of ectomycorrhizae fungi in native forests and plantations, suggest that in artificial plantations new associations between trees and new taxa from ectomycorrhizae fungi are favored [5]. We consider that the young plants on tailing ponds are susceptible to find in the edaphic ground layer species of fungi with which to create new types of mycorrhizae. The present research aims to review the role of native species in the remediation of polluted areas, by enabling the development of plants similar to the original vegetation in the area, thus avoiding unwise attempts with species just because they are "well known" as phytoremediators, but that most often are not native to the area of concern. We consider that this step is important, as non-native species are more and more considered to offer doubtful support to ecosystems. Introducing them in these perimeters may lead to the creation of some non-native species reservoirs for the surrounding ecosystems, since these species, once integrated, spread in the neighbouring lands. The danger of invasion is thus an issue, as such species are more competitive as eurybia than the native species. Using some species of native trees when trying to restore vegetation generates a grassy layer easily colonized with the existent native microflora. Such settlement system for vegetation following a more or less natural succession is cheaper, and by spreading species both through seeds and vegetative means the erosion of soil is thus slowed over a short time horizon [38]. The extent to which such remediation initiative has turned into a long-term success becomes apparent after only a few vegetal seasons entailing follow-up and monitoring activities.

MATERIALS AND METHODS

1. Site description. For the interests of our case study we have selected the tailing pond in Bozanta Mare, built in 1977 and covering 1,050,000 m^2. It has 30 m in depth and an embankment of 18-20°. The *Remin Tailing pond* is the result of sedimentation of water filled with sterile from the flotation of ore. This pond, now in preservation, is partly covered with vegetation either inherited from the previous attempts to ameliorate the area or from the spontaneous settlement of some species, due to a primary succession. Winds easily transport the fine particles in the dam. There were a few attempts to consolidate the pond by planting trees, of which we mention the bur (*Cirsium lanceolatum*) and acacia (*Robinia pseudaccacia*). For the above mentioned *Preserved Tailing Pond*, Table 1 includes the physical characteristics of the underlying soil.

Twenty years ago, on the pond dam black pine (*Pinus nigra*) and acacia (*Robinia pseudacacia*) have been planted. The recent analysis effort on the area indicates that only a small part of those species rezisted and growed (about 10-20%), only a few have 5-10 m in height, and they did not cover more than 10% of the

Table 1. Characteristics of soil

Nr.	Parameter	Value	Nr.	Parameter	Value
1	Texture	Sandy clay loam	8	Particle composition, % > 0.2 mm 0.2 ÷ 0.2 mm 0.1 ÷ 0.05 mm 0.05 ÷ 0.02 mm 0.02 ÷ 0.01 mm 0.01 ÷ 0.015 mm < 0.005 mm	 3 4 18 10 15 11 39
2	Type	Alluvial			
3	Organic matter, g· kg^{-1}	0.57			
4	Organic carbon, g· kg^{-1}	0.38			
5	Water holding capacity, mm/cm depth of soil	38.7			
6	Cationic exchange capacity (CEC), cmol· kg^{-1}	12.6			
7	Mineralogic composition: Quartzite (sand) Clay Feldspar Sulphides Sericite, Carbonates,	 40-45 20-25 10-15 7 – 8 23 – 7	9	Parameters of sandy clay loam Natural humidity, % Plasticity, % Porosity, % Cohesivity factor, Kpa Specific weigh, KN· m^{-3}	 27-37 56-61 60-17 22.46 24.13

pond's surface. In such situation we cannot speak about a unitary crowning as it would be expected for such plantation. On the E mountainside of the pond there are no significant differences in height as compared to the reference height of vegetation. Some higher *Pinus nigra and Robinia pseudacacia* (5-10 m in height) can be spotted, but the shadow of the crown on soil is not larger than 5%. Moreover, there are some specimens of *Salix caprea, Populus tremula, Betula pendula, Quercus petrea* spontaneously settled on the tailing pond, being of different ages and sizes, but without serious coverage.

2. Vegetation experiment. The following data referring to the settlement of *Salix caprea* on the tailing pond have been taken into consideration: the capacity to absorb heavy metals (Cu, Zn, Pb, Cr, Ni, Co, Mn, Fe, Cd) in the ground layer (expressed in terms of concentration of these metals in the roots and leaves of the plants), the content of Na and K of individual plants of the same species, the rate of growth of the *Salix caprea* young plants, the role of the microbiota in soil in the survival potential of the plant. The research relies on the analysis of the following experimental samples: 1 - young plants grown in pots in normal soil, considered as "witness samples"; 2 - *Salix caprea* individuals planted „in situ"; 3 - young plants with sterilized roots grown in the ground layer of the pond; 4 - individuals with sterilized roots (to remove mycorrhizae fungi) grown in vegetation pots in soil from the pond but sterilized in order to remove the microbiota. We have ensured the removal of the mycorrhizae fungi by applying a fungicide an soil sterilization under air-pressure at 121 degrees Celsius for 20 minutes. In each case we have considered three replicas with eight plants.

3. Laboratory procedure. In each of these experiments we have determined the content of heavy metals stocked in plants, in roots as well as in leaves, so as to assess the phytoremediation potential of this species, along with the role of the microbiota in the ground layer as regards the adaptability potential of each species. The vegetal samples consisting in roots and leaves were taken at the beginning of autumn so as to leave time for stocking heavy metals throughout the vegetal season. The soil and vegetal samples were taken from every experimental variant, were washed with deionized water and dried at 60°C for 72 hours, and were finally converted into powder. 100 grams of powder were taken from each sample subjected to mineralization and then converted into solution. The samples were diluted, filtrated in compliance with ISO11466 and analyzed in terms of heavy metals content (Cu, Zn, Mn, Cd, Pb, Co, Fe, Cr and Ni) through atomic spectrophotometry. We have selected a computer – assisted AAS800 Analyser. Flamephotometry by using a Flame Photometer 410 Sherwood has allowed for the analysis of Na and K. A witness sample without vegetal material was also analyzed.

4. Plant growth dynamics. We also conducted measurements throughout the vegetal season so as to detect the growth dynamics of the reference species in conditions of extremely polluted soil. The young plants were between 1 and 2 years old and came from natural forest ecosystems. When planted, we measured the main root, minor roots, and we noticed the degree of colonization with mycorrhizae of the roots, as well as of the main branch. Measurements regarding the dynamics of growth in the initial stage were done on the main stalk (the final bud) for three scions, respectively. Measurements were taken at 35, 40 and 70 days after planting.

5. Data analysis. The SPSS – version 10 software was the statistics analysis instrument of choice in order to conduct the variance analysis (mainly consisting in, but without being limited to, one-way and two-ways ANNOVA tests).

RESULTS

We determined the concentration of heavy metal in roots and leaves, as well as the proportion of metal stock in these organs. Following these measurements we compared the concentrations of heavy metals in soil/ ground layer with those in plants, for different experimental variants. The results emphasized the survival capacity of *Salix caprea* on polluted land, its capacity to stock heavy metals, in some cases func-

Table 2. The content of heavy metals in the ground layer and in the *Salix caprea* at different experimental variants.

Heavymetals	Experimantal	Total content mg/kg plant (DW)			Heavymetals	Experimantal	Total content mg/kg plant (DW)		
		substratum	roots	leaves			substratum	roots	leaves
Cu	1	14.61	13.37	12.75	Cu	1	11952.67	19733.62	5783.38
	2	142.96	47.86	10.69		2	13490	213.64	6819.61
	3	142.96	151.24	18.22		3	13490	19499.38	2784.84
	4	142.96	95.37	16.92		4	13490	499.4	2913.92
Pb	1	40.13	19.93	25.17	Pb	1	66.85	80.37	33.73
	2	386.75	21.87	22.05		2	75.99	59.02	36.25
	3	386.75	64.39	10.7		3	75.99	98.4	12.06
	4	386.75	48.92	12.16		4	75.99	84.27	17.64
Ni	1	0.09	26.55	19.65	Ni	1	895.36	592.37	1059.85
	2	0.07	3.44	5.13		2	260.93	280.78	189.77
	3	0.07	65.73	9.66		3	260.93	103.18	185.38
	4	0.07	59.82	8.84		4	260.93	118.33	510.96
Zn	1	40.41	9.33	146.13	Zn	1	244.28	541.19	216.19
	2	112.9	30.27	141.42		2	518.14	196.41	86.9
	3	112.9	64.87	39.37		3	518.14	586.51	71.27
	4	112.9	58.66	70.33		4	518.14	913.51	101.15
Co	1	17.39	18.3	13.16	Co	1	2063.89	7874.8	8087.85
	2	2.98	10.66	10.37		2	656.66	5650.04	3304.15
	3	2.98	19.87	6.55		3	656.66	2846.01	2922.91
	4	2.98	17.14	6.92		4	656.66	3357.5	4023.21

tioning as a hyper-accumulator, as well as involving the microbiota in the ground layer and mycorrhizal fungi in the variations of concentration of heavy metals in this species.

Data in Table 2 refers to the mean values as established based on three tests. The experimental variants are: 1 - witness sample with individuals grown on soil; 2 - sample “in situ” with young plants grown on the tailing pond; 3 - experimental sample in which the mycorrhizal fungi were removed by fungicidal wash; 4 - experimental sample in which roots were sterilized by fungi, and the ground layer of microbiota removed through sterilization.

DISCUSSIONS

1. Stocking heavy metals and Na and K in Salix caprea specimens.

Getting a thorough understanding about what sort of trace elements the plants uptake essentially depends on our detailed knowledge about the composition of soil. The content in trace elements in soil is a descriptor of the concentration of heavy metals available to plants for absorbtion [18]. The concentration of trace elements in soil is variable depending on the type of soil, on time, on the vegetation cover, on the activity of microorganisms, on the regime of waters and on the heterogeneity of soil. Rainfall, evaporation or plant transpiration can change the concentration of trace elements in soil.

Such heavy metals as Cu, Zn, Pb, Cr, Mn, Ni, Co determine general changes in cells since they act as enzymatic inhibitors or they precipitate important metabolites [21]. Heavy metals connect sulphydryl and protein groups involved in chatalytic functions or in the structural identity of proteins [33, 2].

We have conducted our analysis on *Salix caprea* specimens after a season of vegetation both on soil and in the polluted ground layer of the tailing pond. Our examination was extended on plants both in the presence of microbiota and of mycorrhizal fungi and in their absence. With few exceptions of initial necrosis, no toxicity symptoms could be spotted on plants. We determined the concentration of heavy metals in roots and leaves, as well as the proportion of metal stock in these organs. Following these measurements we compared the concentrations of heavy metals in the soil/ground layer with those in plants, for different experimental variants. The results have confirmed the survival capacity of *Salix caprea* on polluted land, its capacity to stock heavy metals, in some cases functioning as a hyper-accumulator, as well as involving the microbiota in the ground layer and mycorrhizal fungi.

Table 2 suggests that the goat willow is a Ni gatherer both on witness soil and on the sterile in the pond. The quantity absorbed exceeds a number of times the level of concentration in the ground layer. Plants lacking mycorrhizal fungi record the highest concentration of Ni in roots, followed by plants with sterilized roots grown on a ground layer without microbiota (Fig. 1).

Both cases prove that, for the goat willow, mycorrhizal fungi act as shield which diminishes the absorption and stock of Ni in roots. In the variants with intact mycorrhizae (1-witness and 2- in situ) Ni is found in a larger quantity in leaves, thus proving their influence in moving the Ni from roots towards the leaves. The same was also true for other heavy metals and other species of trees [29].

Graphs in Figure 2 depict the capacity of the goat willow to absorb cobalt. The quantity of cobalt absorbed by plants grown both in the witness soil and in the soil of the tailing pond is in direct relationship to the concentration of cobalt in soil, while slightly higher in the roots. While comparing plants with roots without mychorrizic fungi (3) with plants grown in soil without microbiota (4), we can conclude that mychorrizic fungi stand routinely as barrier against the radicular absorbtion of cobalt. In the absence of fungi, the concentration in roots went up to double over the concentration in plants not sterilized. Sample 3 compared to sample 4 reveals that microbiota in soil has a small contribution in the accumulation of small quantities of cobalt by solubilization.

The behavior of the goat willow against iron depends to a large extent on the availability of micro-organisms attached and adapted to the underlying soil, as those micro-organisms have oxyde-reduction impact of different intensities. Even the genuine forest soil displays high concentrations of iron. By comparing the witness sample (1) with the sample „in-situ” (2) different concentrations of iron in the roots can be noticed, that are not in proportion to the concentration in soil. Iron absorbtion is strongly correlated to the availability of iron in soil, which depends in turn on the soil’s pH, its redox potential, as well as on a couple of particular circumstances such as the concentration of macro-nutrients and the proportion in which other heavy metals are present [10, 6, 9, 25, 31].

In the environment of the tailing pond, plants with the roots that have colonised mychorriza „in-situ” have absorbed a small quantity of iron that penetrated the plant up to sprouts. By comparing the samples 3 and 4

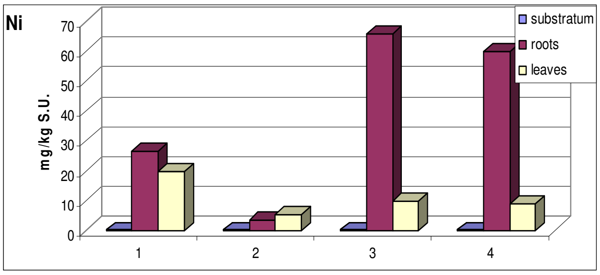

Figure 1. Nichel concentration in substratum, roots and leaves.

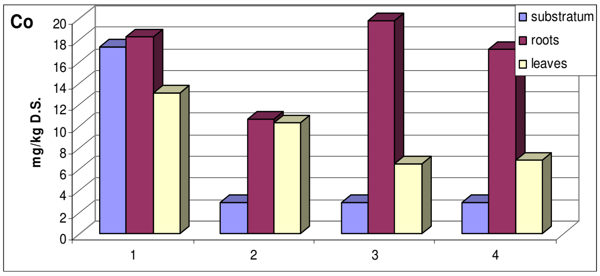

Figure 2. Cobalt concentration in substratum, roots and leaves.

we can clarify the role michorryzic fungi and of the microbiota in soil play. Sample 3 gives evidence that microbiota in soil in the tailing pond make soluble high quantities of iron making it available to plants, on one hand, and that the mychorrizic fungi have a breaking effect, reducing the radicular absorbtion of iron, on the other hand. Therefore, the concentration in iron is highest in sample 3, with sterilized roots. The concentration in iron (Fig. 3) in plants is lowest in sample 4, missing any impact of micro-organisms (fungi and bacteria).

The absorbtion, namely the accumulation of lead by the willow is foresseable (Fig. 4), in tune with what the professional literature depicts. Plants will barely absorb lead, as it is highly insoluble. Even on the layer of the tailing pond that contains lead in high quantity, the concentration in the root of the plants under analysis was not significantly superior than in the witness sample of soil. The role of mycorrhizal fungi in protecting the plant against lead in high concentrations comes out, however, when comparing the sample 2, „in situ”, with sample 3, without mycoorrhizal fungi. The mycorrhizal fungi stand as radicular barrier against a high metal penetration in the plant. The role of the fungi in preventing the access of lead to the leaves comes also out when comparing the samples with mychorriza (1 and 2) with those without (samples 3 and 4). Even under high lead absorbtion conditions, in plants without michorriza lead was concentrated in roots, the concentration in leaves being inferior to the concentration in plants that were not sterilized. We think that even if no improvement occurrs in terms of the capacity to absorb lead, the presence of mycorrhiza confers higher resistance to the plant in the environment of a polluted site, and contributes to the consolidation of the vegetal layer and to diminished pollution in neighboring areas, by discontinuing the wind and pluvial erosion.

Copper, a part in the structure of selected enzymes, is one of the microelements needed for the nutrition of plants. As revealed in a number of bibliographic sources, it is one of the most mobile as well. The concentration of copper in plants is in direct relationship with its concentration in the ground (Fig. 5). Waste in tail ponds is very rich in copper, and the roots of plants display particularly high concentrations. The benchmarking of all samples based in waste soil from the tailing pond reveals that the roots of goat willow display a higher concentration of copper than any other sample. It becomes therefore apparent that the goat willow stores a higher quantity of copper in roots than in sprouts, a mechanism that other equivalent studies confirm [30, 12, 28]. As described in other experiments [30, 7], because copper can be substitute to the manganese in the structure of clorophyll, altering and degrading its structure, the plant has developed mechanims by which as little copper as possible will reach up to leaves. Our experiment reveals the importance of mychorriza as part of the survival mechanisms of the goat willow within the context of a site polluted with copper. Mychorrizic fungi make the radicular absorbtion of copper to decline, while micro-organisms in soil eliminate additional quantities of copper following their oxydative activities. However, the plant has its own mechanisms that prevent copper to reach up to the sprouts, and even in the context of a very high absorbtion, the concentration in leaves was only slightly higher.

Zinc displays one of the highest mobilities and leaves are where it is particularly found (Fig. 6). The concentration of zinc is higher in leaves than in the roots, as confirmed in all experiments we have conducted as well as in literature [43]. Without being a bio-accumulation element, the goat willow is very resilient in sites polluted with zinc because of the association with mychorrizal fungi. These diminish the absorbtion of zinc in the ground and prevent its radicular accumulation. In sample 4, with both micro-organisms and mychorriza absent, the concentration of zinc in plants is higher than in sample 3, with only fungi missing, which reveals that microorganisms

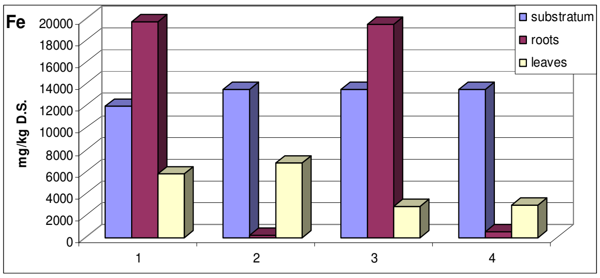

Figure 3. Iron concentration in substratum, roots and leaves.

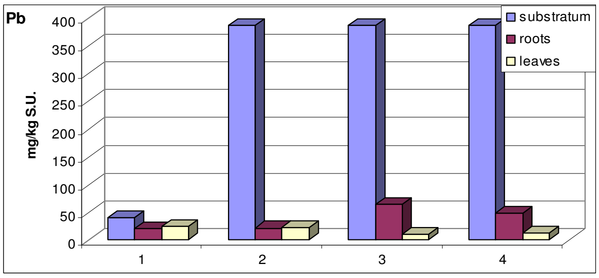

Figure 4. Lead concentration in substratum, roots and leaves.

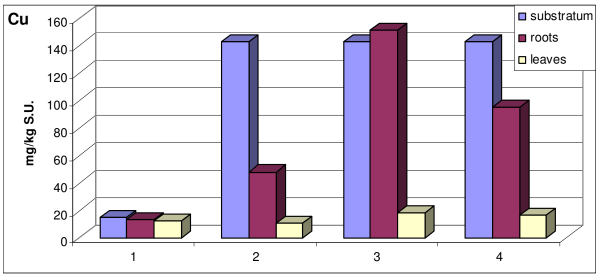

Figure 5. Copper concentration in substratum, roots and leaves.

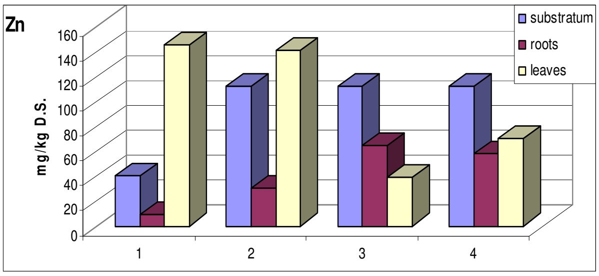

Figure 6. Zinc concentration in substratum, roots and leaves.

routinely retain part of the zinc in the ground layer. Micro-organisms retain variuos heavy metals in capsule and in walls, as quoted in a few cases.

The behavior of the goat willow in the presence of chromium depends on the nature of soil and certainly on the subsequent species of fungi, adapted to each type of ground layer. In spite of the concentration of chromium being smaller in the witness sample than in the soil in the tail pond, the concentration of chromium in the roots of plants in the witness soil is higher than in the roots of plants in the tailing pond (Fig. 7). The goat willow in the witness sample of soil tends to accumulate a higher quantity of Chromium than plants growing in the ground of the tailing pond. The high level of the other heavy metals in the tailing pond certainly determines a diminished absorbtion of chromium, and could impact the behavior of fungi in the tailing pond. The fact is that fungi are a radicular barrier against chromium, akin to their role against the other heavy metals, which comes out as explanation when we compare with our experiments no. 2, 3 and 4. The mychorrizic fungi in the tailing pond enhance the mobility of chromium towards the sprouts; in the absence of fungi, the roots display the highest concentration in chromium. Micro-organisms in the soil have little impact on chromium to get into the plant, as they only increase its solubility.

The concentration of manganese, a microelement with nutrition role particularly in the context of the tailing pond, is smaller as compared to the concentration in the witness soil. The goat willow tends to accumulate manganese as revealed in all our experiments, the cumulative concentration in roots and sprouts being superior to the concentration in soil (Fig. 8). While the mychorrizic fungi act as a barrier against the absorbtion of such heavy metals as zinc, copper, chromium or cobalt, those fungi enhance the absorbtion of the manganese; therefore, sample 2 displays a higher concentration of manganese both in the roots and in the leaves as compared to sample 3. The concentration of manganese is higher in sterilized soil, as comes out by comparing sample 3 with sample 4, which gives evidence that micro-organisms in soil retain important quantities of manganese and render it more insoluble.

Sodium stands as „competitor" to heavy metals, as far as the absorbtion capacity for sodium of the goat willow is concerned (Fig. 9). A high content of heavy metals concentrated in the soil will translate into low „apetite" of the goat willow for sodium. In spite of the concentration of sodium being about double over the concentration in the witness sample, the concentration of sodium in plants in the tailing pond is in general lower, except sample 4. The mychorrizic fungi make the radicular absorbtion of sodium to decline, as comes out of all of the 4 samples considered, while micro-organisms in soil retain a portion of the sodium. Subsequently, sodium appears in the highest concentration in plants grown on sterilized tail pond soil. The „barrier effect" that mychorriza play against sodium confers resilience to the willows against salty environments.

Potassium is a macroelement that plants need to meet their nutritional requirements of minerals. Therefore, all samples display massive absorbtion of potassium, whose concentration in plants is substantially superior to the concentration in soil. Plants develop active potassium - absorbtion mechanisms, in reverse relationship with the concentration in soil and based on exchange with other ions, such as sodium. Figure 10 reveals that michorriza improve the nutritional stance of the host plant, including as enabling factor the higher radicular absorbtion of potassium. Micro-organisms in soil also make use of significant quantities of potassium; this is why plants with sterilized roots and cultivated on sterilized soil display higher concentration in potassium than plants in which micro-biota is intact.

To conclude, the goat willow develops symbiotic relationships with fungi recruited from soil, both in the witness soil and in the soil of the tailing pond. The differences in behavior of the fungi in the soil of the

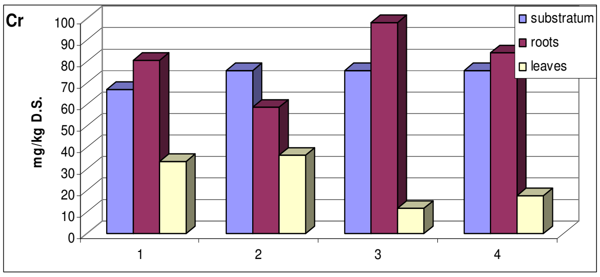

Figure 7. Chromium concentration in substratum, roots and leaves.

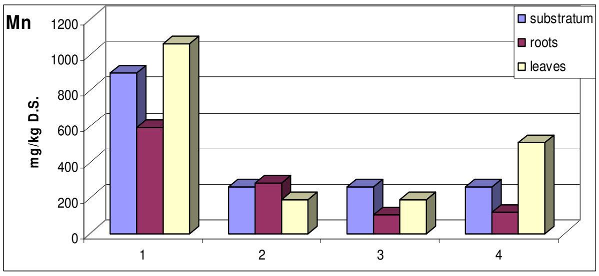

Figure 8. Manganese concentration in substratum, roots and leaves.

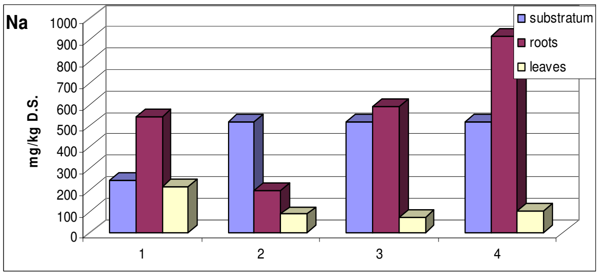

Figure 9. Sodium concentration in substratum, roots and leaves.

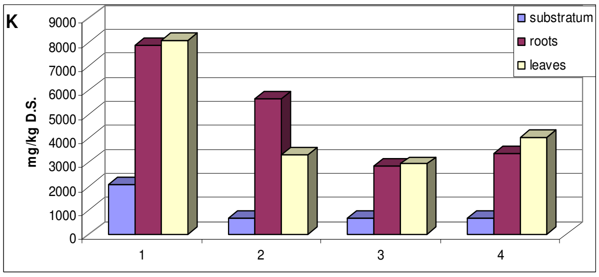

Figure 10. Potassium concentration in substratum, roots and leaves.

tailing pond and in the witness soil, as well as the analysis of sporiferous items identified in both cases give us full certitude that in both cases different species of fungi colonise the roots. In all circumstances, mychorrizic fungi act as enabling factor for plant survival under the stressful environment that important concentrations of heavy metals generate. The supporting mechanism consists in the diminished radicular absorbtion of heavy metals in soil, while making potasium more easily available to the plant.

2. Growth dynamics in Salix caprea. One of the hypotheses presented in this paper was to make prominent the involvement of the symbiotic relations between cormophytes and fungi from the very first stages of vegetal development in trees. Such research is also presented in the specialized literature relating the correlations between the vegetal gradient starting from the stage of a young plant and the presence of ectomycorrhizal fungi on the roots. Results emphasized the correlation between the qualitative composition and the quantity of ectomycorrhizae and the age of the individuals of cormophyte plants. The number of ectomycorrhizal fungi on the roots grows with the age of the trees, recording a slight decrease at its climax stage. Also, the composition of the groups of ectomycorrhizae on mature trees and the proliferated deposits of ectomycorrhizal in the soil are proves of the existence of a successive gradient parallel with the stage of secondary vegetation, but with the presence of numerous common morphotypes at different stages of vegetation development [42].

Preliminary quantitative research on young willows, trembling poplar, durmast and birch planted on the tailing pond in Bozanta Mare, as well as the experimental variants on sterilized ground layer show that from primary vegetative stages, at very young ages (1 or 2 years) the plants already have ectomycorrhizae colonized roots. Their presence was noted micro-scopically and macroscopically and indirectly pointed out through the support these mycorrhizae give to tree species in their development. On individuals whose roots are sterilized, or on those grown on sterile ground layer, the growth is considerably smaller.

The macroscopic and microscopic observations done on the roots of some specimens of *Quercus petrea, Populus tremula* and *Betula verrucosa* grown on the pond and having between 5 and 15 years in age, different stages of colonization. The older the tree, the higher the number of mycorrhizae.

In such a context, biometric characteristics of the young plants were analysed (length and growth of grafts), growth variations at different species of trees, from the primary stage of youth, on substratum with high concentrations of heavy metals, in the presence or in the absence of soil microbiota, represented by micro-organisms and fungi (including ectomycorrhizae).

We have conducted our research on the following categories of samples:

- sterilized tail pond soil, in which we have planted seedlings with sterilized roots – in order to counteract the effect of microbiota (labeled/ marked DS in the graphs)
- tail pond soil in which we have planted seedlings with sterilized roots – in order to detect the degree and the speed with which microbiota specific to the soil in the tailing pond „colonizes" the roots (labeled/marked L in the graphs)
- normal forest soil as witness sample (labeled/marked F in the graphs).

We have performed measurements about the dynamics of growth on three sprouts, selected among the longest available, for each type of seedling under scrutiny.

Our measurements took effectively place 35, 40 and 70 days after plantation.

Figure 11 displays the differences in length of seedlings (DELTAL) recorded at 35 (Fig. 11a), 40 (Fig. 11b) and 70 (Fig. 11c) days for all four tree species.

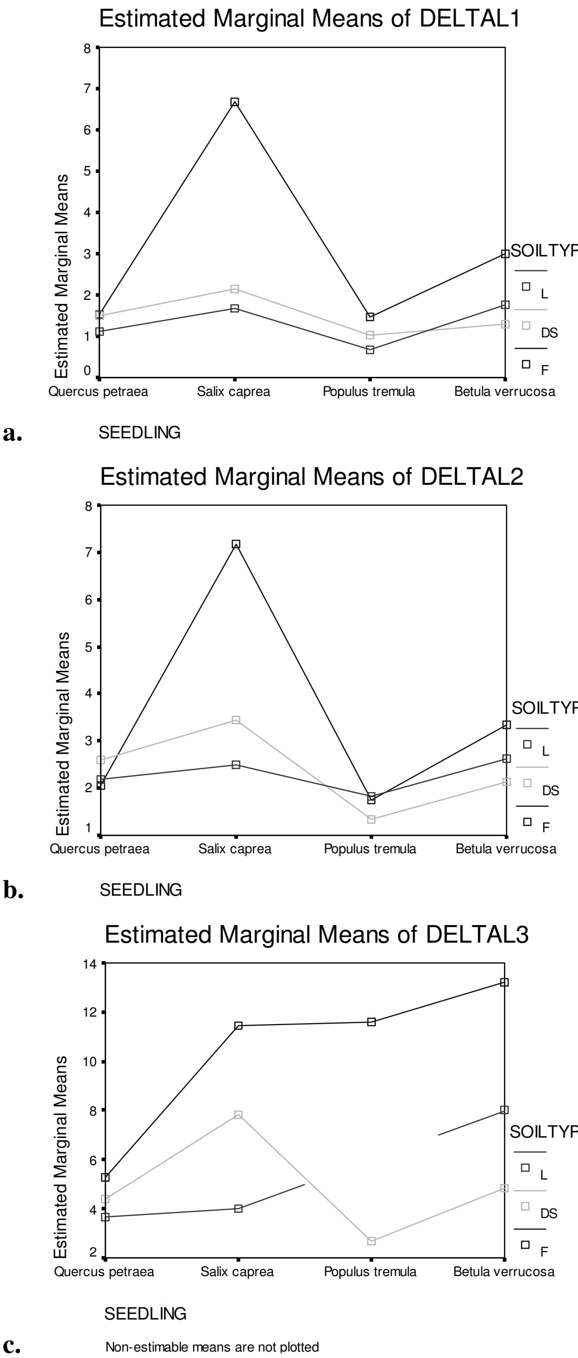

Figure 11. The differences in lengh of seedlings recorded at 35, 40 and 70 days

We have extended our examination work on *Salix caprea* for all of the three experimental samples to a total of 32 plants (seedlings). The goat willow displays significant differences in growth patterns only between the witness sample hosted by forest soil and the sample in the soil of the tailing pond. Growth differences between the sterilized tailing pond soil and the soil that was not sterilized are out of the significance threshold of our analysis.

Therefore, up to 70 days after plantation no simbiotic interference occurs between the micro-organisms and fungi in the soil of the tailing pond and the roots of the goat willow, whose growth pattern is not significantly influenced. Differences have appeared because of other factors such as the different innate heritage of the various plants we have used.

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