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THE GROWTH DYNAMICS OF SELECTED TREES IN SITES POLLUTED WITH HEAVY METALS NEIGHBORING BAIA MARE (ROMANIA)

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SYNOPSIS

Based on biometric descriptors for cuttings, we aim to indirectly investigate the relationship, if any, between micro – biota in soil (micorrhyza included) and the capacity of various species of cormophytes to adapt to the particular edaphyc conditions of tailing ponds. It is highly probable that soil in tailing ponds hosts species of fungi enabling the generation of new types of micorrhyza together with the seedlings planted there. In this respect, we have planted in soil samples from the tailing pond seedlings with sterilized roots. We summarize our observations on birch and poplar cuttings that we have planted, after the sterilization of roots, in the soil (not sterilized) of the tailing pond and that have witnessed significant growth. We have planted on one hand seedlings of *Quercus petraea, Populus tremula, Betula verrucosa, Salix caprea* in different locations and contexts within the tailing pond ("in situ") and, on the other hand, we have planted "ex situ" witness samples of the same species. Our aim was to profile the growth pattern of the stem and of cuttings.

INTRODUCTION

The environmental problems regarding the decantation ponds are complex (chemical, biological, technological, social) because of the high load of harmful substances, but especially because of the imminent danger that this represents for the environment and health. Their settlement is possible only through the co-working of specialists from different domains, who suggest innovative technologies and services, as the classic ones that have been used up to now have proven to be insufficient. Only the joint work of experts in various fields, proposing innovative technologies and services, could enable for a solution to be reached, as the classic approaches considered up to now proved to be far from enough.

Research studies reveal that remedial and restoration of vegetation in areas polluted with heavy metals, areas to which tailing ponds belong, could be enabled by a clever selection of tolerant species of plants as well as by selecting tolerant mycorrhizic fungi [3]. The advantages of the ecological rectification made through methods that improve the structure of the microbiot from the defaced soils, are as follows:

- the rectification of the quality of the soil by using mycelium in the decomposing of toxic waste and several other categories of pollutant substances;
- it contributes to the decomposing of organic matter and to the forming of soil on these areas with extreme ecological conditions;
- it contributes to the aeration, decomposing and transportation of organic an inorganic matter, without the outflow of silt;
- it determines the decrease of soil erosion protecting the useful fauna;
- it promotes the re-establishing of the indigene microbiot (mycorrhiza and saprophyte) having a role in soil formation;
- it facilitates the cultivation of ligneous species;
- it facilitates the installation of a larger spectrum of grassy species through the improvement of the soil's structure and composition due to the mycological activity;
- it supports the development of the phytocoenotic compartment of the future ecosystem, simulating the progressive succession process of the vegetation from the defaced areas;

MATERIAL AND METHODS

The on-site measurements conducted during April – July, 2008 stand as key source of our data regarding the dynamics of trees' growth in the particular ecologic circumstances in the tailing pond in Bozanta Mare. This experiment is part of a larger research initiative covering the use of micro biota in the overall regeneration of tailing ponds. Within this framework we monitor the role microorganisms (as well as fungi) could play in terms of supporting superior species to grow and to improve their rate of development under the poor environmental circumstances in tailing ponds. In order to spot possible correlations between the micro-biota of soil and cormophitic flora, and also in order to single out the species of trees that would be most effective for the ecologic remediation of tailing ponds, we have initiated a number of experiments that include a number of 460 individual samples to be monitored. They belong to four native species of trees, to be tested in a variety of ground layers. We have selected the following trees: *Quercus petraea, Populus tremula, Betula verrucosa, Salix caprea.* We have choosen these particular species as they are native to the area, belonging to the regional flora, and also because we have identified such species of trees germinated and spontaneously developed in the tailing pond. We have selected the following varieties of soil in order to conduct the experiments: 1 - tailing pond soil (B – value); 2 – sterilised tailing pond soil on which we have planted seedlings with sterilised roots (in order to remove the effect of the micro biota) (DS – value); 3 – tailing pond soil in which we have planted seedlings with sterilised roots (in order to detect to what extent and at which rate the micro biota specific to the soil in the tailing pond will „colonise” the roots (L – value); 4 – common forest soil, the witness sample (F – value). We have planted directly „in situ” seedlings without sterilised roots, in different locations within the tailing pond, in order to be able to define the influence of locational factors (such as coordinates, slope of the tailing pond, etc.) on the dynamics of growth. We have procured and planted seedlings already 1 – 2 years old sourced from natural forest conditions. We have measured before planting the main root and the secondary roots, while we have observed the extent to which mycorrhiza have colonised the roots, as we have also measured the main stem. In order to profile the initial stage of growth, our focus went on the measurement of the main stem (the terminal burgeon), and of three cuttings respectively. Our measurements took place 35, 40 and 70 days after plantation time. We have observed the survival rate of the seedlings planted in the tailing pond at different periods of time during the first season of vegetation as well as of different species of trees.

We have decided for variance analysis (one – way and two-way ANOVA) as relevant statistical tool and we have applied the SPSS, version 10, as application software of choice.

RESULTS AND DISCUSSIONS

The set-up of alien anthropic formations, such as dumps and tailing ponds accumulating the waste materials generated during the separation process of heavy metals by flotation are explained by an intensive mining activities during a few centuries in the county of Maramures. The results of these activities are larges pollutes areas, in most cases hot spots both in ecologic terms and in terms of landscape, as the presence of residuals of heavy metals in combination with water deficit, intense oxidation activity and acidity make – up together strong constraining factors in the normal rejuvenation of generations of plants. All attempts so far for ecologic remediation have allowed for improvement only in part and with unsatisfactory outcomes. Within the tailing pond in Bozanta Mare for instance, that makes our focus, even 20 years after the so – called “tailing pond remediation”, the background soil is in sight, fully exposed to direct rain and wind erosion. The construction of the tailing pond in Bozanta Mare has taken place back in 1977 on a 1,050,000 square meters area, at a 30 meters height and featuring 18–20O slopes. Hydro-cyclonic activity has allowed the transportation of flotation waters out which fine particles were deposited. This alien formation has about 150,000,000 cubic meters in volume. The sublayer of tailing pond is an acidic sandy clay loam with a very low content of organic matters. The grading curve of mud-setting pond sublayer is presented in figure 1. The aggregate grading corresponds to a sandy sublayer.

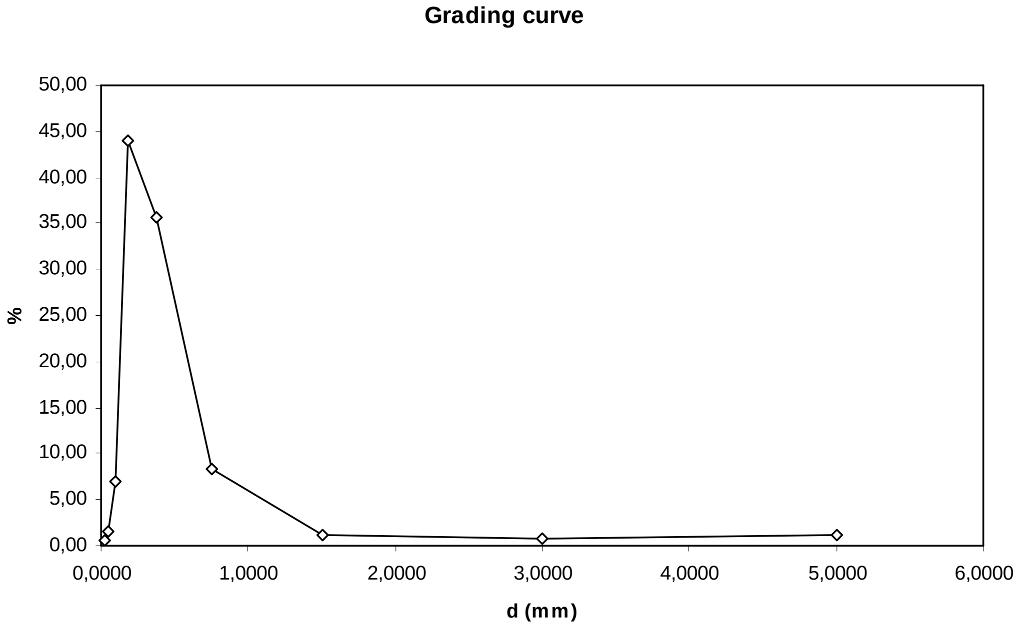


Figure No. 1. The grading curve of mud-setting pond sublayer

Twenty years ago, the area of the tailing pond was stage for “greening” attempts, consisting in the plantation of such trees as *Pinus nigra, Robinia pseudacacia* and *Betula verrucosa*, but effective outcomes failed to materialize. The phytoceno-genesis process proved to be so tedious that currently talking about vegetal associations is out of question. Depending on location and land slope, the coverage with vegetation layers does not exceed 1 – 25% of the total, and includes species coming from the neighboring ecosystems. The species of trees include *Pinus nigra, Betula verrucosa, Populus tremula, Robinia pseudacacia*, with rare presence for *Quercus petraea, Quercus robur, Salix caprea*. Such species as *Frangula alnus, Rubus hirtus, Prunus serotina* belong among the bushes in the area. Relative diversity defines the layer of grass, that includes *Carex sp. Juncus sp., Hieracium pilosella, Erophila verna, Tussilago farfara, Viola arvensis, Festuca pratensis, Holcus lanatus, Calamagrostis epigeios, Rumex acetosella, Setaria glauca, Agrostis capillaris* as representative species.

We consider that the phytoceno-genesis process is running, since the observations on the field point out a naturalization of the species set in the pond, many of these fructifying and generating seedlings by means of dissemination. This is an obvious phenomenon in *Pinus nigra, Prunus serotina, Rubus hirtus, Rhamnus frangula.* Numerous species present the tendency to occupy land by vegetative propagation *Hieracium pilosella, Rubus hirtus, Robinia pseudacacia, Populus tremula etc.* Even under such conditions, phytocoeno-genesis is just in the state of colonization even after more than 20 years.

Given the integrative weakness of the vegetal layer, winds displace with easiness fine particles toward the neighboring areas where agricultural land and human communities lie.

The study of ecologic relationships between species belonging to different ecologic groups within the biotopic particular conditions could conduce to better outcomes as regards the ecologic remediation of the tailing ponds [5]. The professional literature in the field brings evidence that simbiotic associations of their roots with micorrhyzic mushrooms, as well as with micro-organisms in rhizosphera, allow to trees species enhanced growth parameters, higher resilience to hydro stress and against high concentrations of heavy metals [6]. Ectomycorrhiza profile the root systems of many species of forest trees, and this is particularly the case for the *Pinaceae, Betulaceae* and *Fagaceae* families.

Research undertaken on *Pinus* and *Quercus* species has demonstrated their higher survival chances and better resilience in degraded and ameliorated soil or in soil richer in organic content if they display roots with mycorrhiza [4]. The number of mycorrhizic fungi is higher, and subsequently the adaptation capacity is superior in areas with indigenous species of trees. This explains our preference to experiment with native species, and not such alien species as *Pinus nigra, Prunus serotina, Quercus rubra* etc, previously selected for the attempts of ecologic remediation of degraded soils or of tailing ponds.

Little knowledge is available about conditions for ectomicorrhyza to form. The characteristics of soil impact first and foremost the activity of the two symbiotics. For a good breed, most of the fungi need aerated frequently and acidic soil, in spite of the exceptions in which there is a preference for neutral or slightly alkaline soil. Observations on forest soils have revealed an abundance in micorrhyza in soils with low nutrients content (N and P especially), in which plants display higher “acceptance” of micorrhyza to colonize their roots in order to get the minerals they need. Based on such observations, the soil in tailing ponds is “suggested” as being the ideal “candidate” to be populated with mycorrhiza.

It was generally demonstrated that micorrhyza support a rich bacteria rhizosphere, some bacteria living on the external layers of mycorrhized roots, in contact with hyphe, while others find a location in the gel surrounding the micorrhyza.

Nevertheless, many observations bring evidence that in sandy soils (in whose category tailing ponds would belong) reaching a symbiotic equilibrium is difficult, fungi behaving rather as parasitic presence. On the other hand, a growth in concentration of available inorganic ions could inhibit or even break the process of formation and elimination of mycorrhiza.

The present paper traces the dynamic of seedling breeding of different species of trees in the special conditions of a site polluted by heavy metals, where the sublayer is extremely acid and which cannot be considered genuine soil. Tracing the dynamic of breeding was correlated with the presence of fungicide and microbial flora of the sublayer in order to detect a possible probiotic relation between these categories of organisms.

Studies in the bibliography researching the species of ectomycorrhizic fungi in native forests and plantations reveal that artificial plantations enable the new associations between trees and new taxons of ectomycorrhizic fungi [2]. Therefore, we consider that chances are for seedlings planted in tailing ponds to meet in the soil species of fungi together with which will create new types of micorrhyza. Just for this reason, in one of the experiments undertaken for this study we use soil from the tailing pond in which we have planted seedlings with sterilized roots. In this respect, we provide our observations on seedlings of birch tree and poplar, whose cuttings have displayed significant growth, after having the roots sterilized and after being planted in tailing pond soil not sterilized.

Although numerous studies have been conducted worldwide about the symbiotic relationship within micorrhyza and their impact on the growth of different species of cormophites, such research in Romania is missing on tailing ponds formed because of the mining activities.

Shedding light on the symbiotic relationships between cormophites and fungi from the early growth stages of the vegetative development of trees was from the very beginning one of our working priorities in the present research paper. The literature in the field includes investigations regarding the correlations between the gradient of vegetation starting from the young seedling - stage and the presence of ectomicorrhyza on roots. The results thereof have revealed the correlation between the qualitative and quantitative composition of ectomicorrhyza and the age of the cormophite plants. The number of ectomycorrhizic fungi on roots grow in proportion with the age of trees and display a small decline during the climax stage. Also, the composition of ectomycorrhiza on mature trees and the deposits of ectomycorrhiza in soil demonstrate the presence of a succession gradient parallel to the secondary vegetation stage, but in the presence of many common morphos–types in different development stages of the vegetation.

The preliminary quantitative research on seedlings of *Salix caprea*, *Populus tremula*, evergreen oak and birch trees planted in the tailing pond in Bozanta Mare as well as on sterilized soil, demonstrates that even in incipient development stages, at 1 – 2 years old, ectomicorrhyza have colonized the roots of the seedlings. We have observed their presence in macroscopic and microscopic way and have indirectly substantiated the support the micorrhyza confer to trees in order for their cuttings to grow. Growth is considerably smaller for individuals with sterilized roots, and to trees cultivated on sterilized soil.

Macroscopic and microscopic observations on the roots of a number of *Populus tremula* and *Betula verrucosa* trees respectively, at ages between 5 and 15 years old, grown–up in the tailing pond, has revealed different degrees of colonization. The older is the tree, the higher the number of mycorrhized roots.

We have analyzed and compared in this context the bio-metric characteristics of seedlings (length and growth of cuttings, growth variations between different species of trees in the primary growth stage, on soils with high content of heavy metals, in the presence, and in the absence respectively, of microbiota, represented by microorganisms and fungi (ectomycorrhiza included).

Analyzing the differences in length of the seedlings (DELTAL) recorded at 35, 40, and 70 days, respectively, for the four species of trees, we notice the following situation (Figures No. 2, 3, 4).

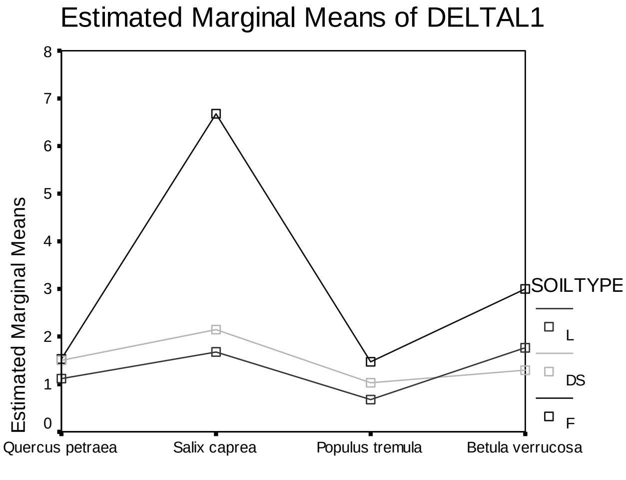
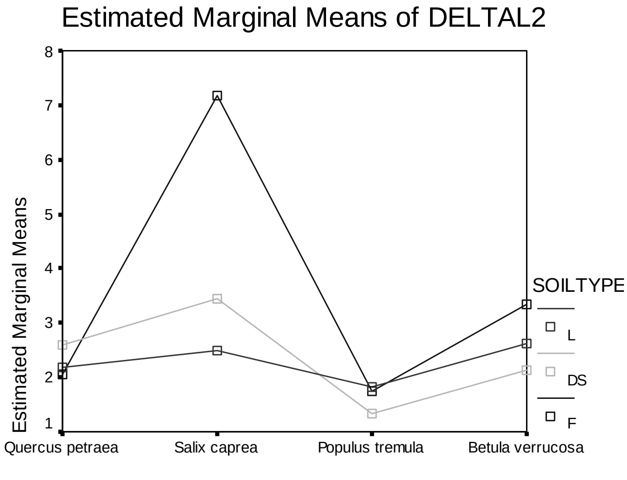


Figure No. 2



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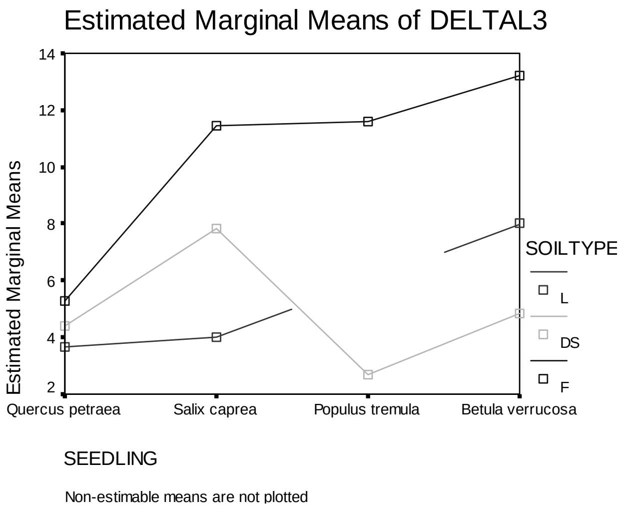

Figure No. 4

In the incipient stage of breeding, in the first 35 and then 45 days from planting, the breeding in *Quercus petraea* is not influenced by the presence/absence of microbiosis in the sublayer, nor by the nature of the sublayer, both in the witness forest soil and in the sterilized and unsterilized sublayer of the pond, the growth being considerable. Just during the third period of time, after 70 days, there is a noticeable difference in the degree of seedling length, taking into account the nature of the sublayer. After 70 days, the seedlings bred in witness conditions in forest soil have a real progress in what concerns growth, whereas, the seedlings having sterilized roots and bred on a sterile, unsterile respectively, pond do not present significant differences in length.

This observation discloses the fact that in the tailing pond's sublayer there are no fungi, that is the soil microorganisms compatible with the *Quercus petraea* species which, by establishing symbiotic relations would contribute to the maintenance of breeding under such circumstances.

Breeding of the *Populus tremula* over time, after 35, 40, 70 days since planting is firstly, dependent on the nature of the sublayer, on the forest soil, the individuals having differences of breeding significantly larger than those in the tailing pond's sublayer, but the presence or absence of the microbiosis from the polluted soil does not condition breeding. Sterilized/unsterilized sublayer parameter is not significant to the breeding of individuals, since there are no differences between the seedlings of the two experimental variants.

For the *Betula verrucosa* species the breeding dynamic of seedlings has followed closely the work hypothesis. Since the first stage of measuring breeding differences, the three experimental lots have clearly differentiated themselves. The witness seedlings grown in the forest soil record the fastest breeding rate. The seedlings grown in a pond sublayer have a different dynamic, according to the presence/absence of the microbiosis in this sublayer. The seedlings with sterilized roots grown in the pond's native sublayer have bred significantly more than those with sterilized roots grown in a sterilized pond sublayer. Accordingly, the in- time-propagated microbiosis in the tailing pond's sublayer show affinity with the birch, maintaining its breeding. This experiment is also sustained by the "in situ" observations in the tailing ponds around Baia Mare, where the birch has the highest natural colonization rate.

In the case of *Salix caprea*, significant differences are important just between the witness lot bred in forest soil and the one in pond sublayer. Breeding differences between the sterilized and unsterilized tailing pond sublayer do not reach the level of the analysis. Accordingly, the microorganisms and the fungi from the tailing pond's sublayer do not interact symbiotically with the willow root, thus not influencing significantly their breeding up to 70 days from planting.

Micro climatic particularities due to locational conditions drive significantly the growth dinamycs of vegetation. Within tailing ponds, besides the soil that obviously stands as strong constraining factor to growth, different locational particularities are specific to different areas, both because of the different concentrations of pollutants in soil and because of the way of exposure to sun light and the slope of the site that impacts the hydrologic stance of soil and the degree of coverage with soil of the background surface. We have planted the seedlings in a variety of locational conditions:

- on plateau no 1 at the smallest height, of about 15 meters above the tailing pond bottom, in flat land, behind a relatively well established curtain of trees, heading to the North – East; we have planted on that plateau exclusively evergreen oak seedlings (the locational conditions there are closest to the natural biotop of the evergreen oak);

- on plateau no 2 at about 25 meters in height above the tailing pond bottom, in flat land with stronger exposure to sun light because of the faint crowner of the few trees, we have planted willows and poplars;

- on plateau no 3 at about 30 meters above the tailing pond bottom, in flat land and very strong exposure to sun light, we have planted seedlings of poplars, birch trees, evergreen oaks and willows;

- on the top plateau „bordering" the tailing pond, in flat land, with high humidity, given the temporary stagnation of rain water and the mix of strongly oxidated and quasi leak-proof minerals, with no natural vegetation, with maximum exposure to sun light; we have planted here birch trees (basically because we have remarked a few surviving trees of this species from a previous plantation);

- on the East slope between plateau no 1 and no 2, with about 20O in slope, no vegetal layer, strong exposure to sun light, accentuated erosion by rain water; we have planted here birch trees;

- on the North - East slope between plateau no 2 and no 3, with about 20O in slope, no vegetal layer, average exposure to sun light; we have planted here birch trees;

The analysis of growth rates of the cuttings for the four species of trees planted only „in situ" in the tailing pond, but in differentiated locational circumstances, based on different ways of exposure to sun and on slope, has revealed as follows:

a) For the Evergreen oak growth was stronger both in terms of apical bud eye growth and in terms of cuttings (see Figures No. 5-6) on plateau No.1 (T1 – value) in protective locational conditions, with smaller exposure to sun light. Compared to all the other species tested, the *Quercus petraea* has displayed the highest growth rate of the apical bud eye because this type of growth is characteristic in the first year of seedling.

b) For the Poplar both the cuttings (T2l2 and T3l2 – values) and the apical bud eye growth (DELTA1 and DELTA2 – values) was stronger on plateau No 2 (T2 – value) as compared to plateau No 3 (T3 – value), thus demonstrating that growth evolves in inverse relationship with the degree of exposure to sun light.

c) As the birch tree was planted in five out of the six biotopic environments, results as regards the growth dynamics of seedlings planted in different locational situations proved to be the most significant. Locational factors inpact both the growth of cuttings and of the apical bud eye. Trees on flat land on plateau No 2 (T2 – value) and No 3 (T3 – value) have grown up the most. In spite of the graph suggesting the top plateau (Plat – value) as the area of highest growth, such conclusion lacks validity, as on the top plateau a high number of birch trees (~75%) have died during the time interval between the first and the second measurements, being subsequently excluded from the database for statistical analysis. The ample number of deaths for those trees is the consequence of severly threatening locational conditions.

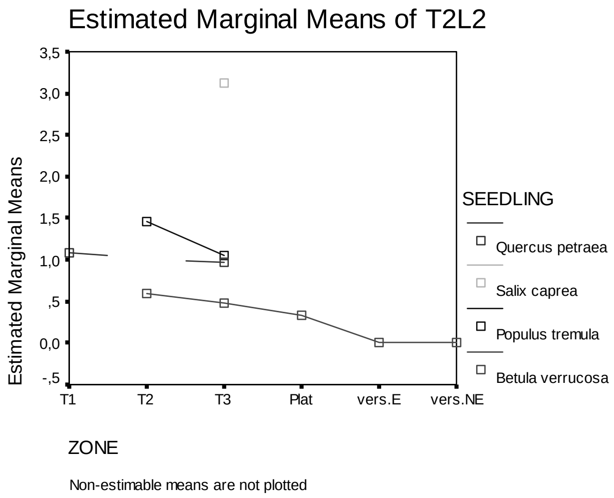

Figure No. 5

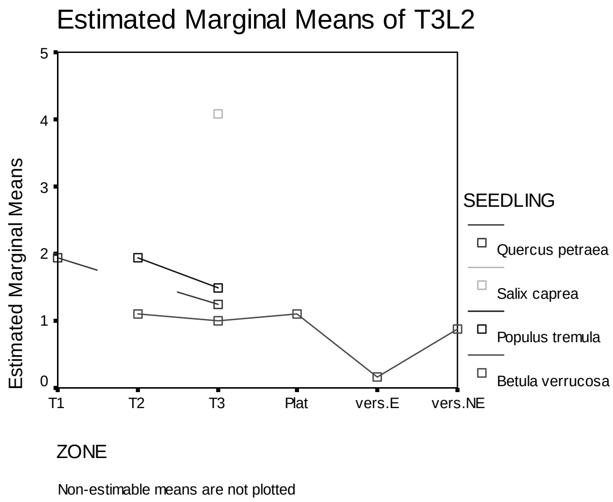

Figure No. 6

Growth goes in inverse relationship with the degree of exposure to sun light for seedlings planted on slopes. Conditions for growth are poorer on the East slope (vers.E - value), with stronger exposure to sun light, as compared to the North – East slope (vers.NE – value).

In the followed tables (No.1-4) are presented the percents of healthy, necrosis, dried up and died seedling for each analyzed species. The observed survival rate of the seedlings planted in the tailing pond at 35, 40 and 70 days after plantation shows that all four species have a good vitality. The death rate at 70 days is less than 30% even in such polluted area and is graphically presented in Figures No. 7-10.

DAYS35

		Frequency	Percent
Valid	healthy	70	93,3
	necrosis	5	6,7
	Total	75	100,0

DAYS40

		Frequency	Percent
Valid	healthy	70	93,3
	necrosis	5	6,7
	Total	75	100,0

DAYS70

		Frequency	Percent
Valid	healthy	64	85,3
	died	11	14,7
	Total	75	100,0

Table No. 1. *Quercus petraea*

DAYS35

		Frequency	Percent
Valid	healthy	20	100,0

DAYS40

		Frequency	Percent
Valid	healthy	20	100,0

DAYS70

		Frequency	Percent
Valid	healthy	20	100,0

Table No. 2. *Salix caprea*

DAYS35

		Frequency	Percent
Valid	healthy	44	88,0
	necrosis	3	6,0
	died	3	6,0
	Total	50	100,0

DAYS40

		Frequency	Percent
Valid	healthy	41	82,0
	necrosis	3	6,0
	died	6	12,0
	Total	50	100,0

DAYS70

		Frequency	Percent
Valid	healthy	34	68,0
	necrosis	1	2,0
	died	15	30,0
	Total	50	100,0

Table No. 3. *Populus tremula*

DAYS35

		Frequency	Percent
Valid	healthy	131	86,8
	necrosis	5	3,3
	dried up	10	6,6
	died	5	3,3
	Total	151	100,0

DAYS40

		Frequency	Percent
Valid	healthy	136	90,1
	necrosis	6	4,0
	dried up	4	2,6
	died	5	3,3
	Total	151	100,0

DAYS70

		Frequency	Percent
Valid	healthy	128	84,8
	necrosis	6	4,0
	dried up	2	1,3
	died	15	9,9
	Total	151	100,0

Table No. 4. *Betula verrucosa*

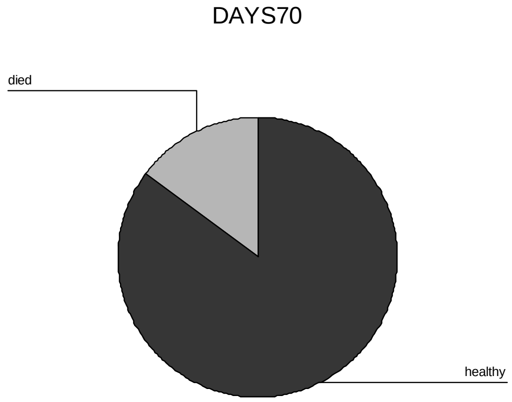

Figure No. 7. The survival rate for *Quercus petraea*

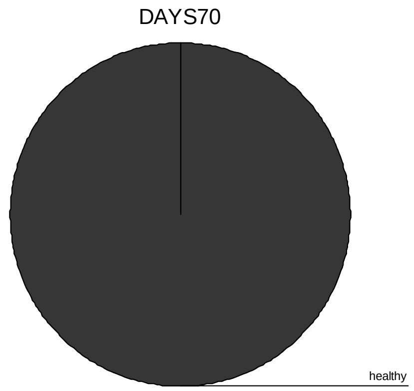

Figure No. 8. The survival rate for *Salix caprea*

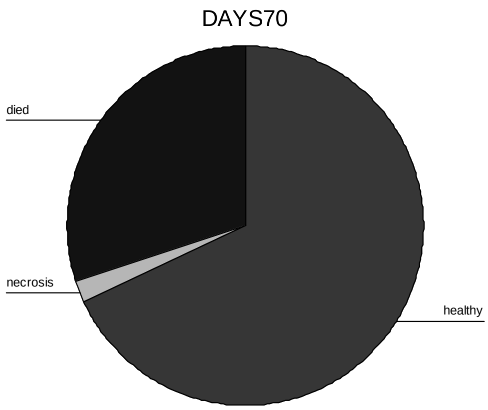

Figure No. 9. The survival rate for *Populus tremula*

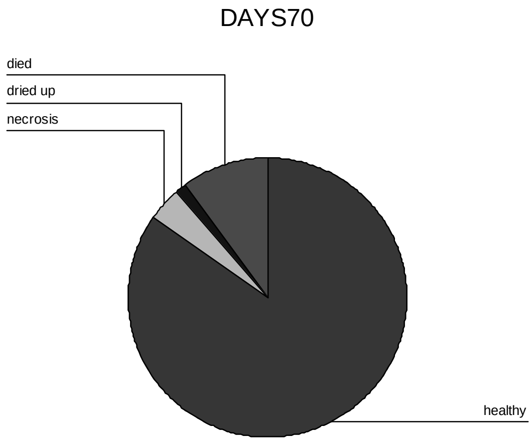

Figure No. 10. The survival rate for *Betula verrucosa*

CONCLUSIONS

The ecologic rehabilitation of tailing ponds is a complex process, depending on many variables whose sharper definition would require interdisciplinary correlations as regards the physical–chemical particularities of the soil, orographycs factors, and especially micro soil factors that impact the changes of micro climatic parameters, systematic studies, physiologic and ecologic studies on the various groups of organisms.

Resorting to the native species of plants, specific to the geographic area, is a must during the process of ecologic improvement of tailing ponds, as such plants establish multiple correlations and in shorter time with the micro biota in soil.

Planting degraded land with species characteristics to the area could contribute to the establishment of new symbiotic relationships among different species and the species of micro biota of soil adapted to the particular conditions of tailing ponds.

The extent to which micro biota in soil supports the species of trees and the way they adapt to the ecologic context of tailing ponds can be indirectly appreciated by means of bio metric parameters even during the incipient stages of growth.

Out of the species scrutinized, the *Betula verrucosa* has demonstrated a strong correlation with the micro biota of soil even during early stages of development.

For all the other species of trees, in spite of results suggesting a relationship between the degree of growth and the extent to which micro organisms and fungi are present in the soil and in roots, no direct relationship between these factors could be established during the early growth stage.

The survival rate and the growth rate of seedlings planted in tailing ponds depends on the nature of soil, on water sufficiency, on the size of the slope and especially on the degreee of exposure to sun light and on the erosion of soil.

Ensuring a grass layer able to protect the seedlings during the early stages of growth is beneficial for the seedlings' protection when planted in tailing ponds.

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