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VARIANCE ANALYSIS ON DIFFERENT TREES SPECIES DEPENDING ON SOIL TYPE – UNCONTAMINATED AND HEAVY METALS CONTAMINATED ONES

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Abstract. This paper summarizes our research work regarding the dynamics of vegetation growth of miscellaneous species of trees planted and monitored in the particular environment of the tailing pond in Bozanta Mare (Maramures County). The structure of soil bearing high content of heavy metals and cyanides considerably impacts the ecologic conditions of tailing ponds. Aspects related to soil characteristics (such as structure, size of particles, porosity, texture, chemical composition) are included. Vegetal species that have accommodated within the tail pond are included as well. In the framework of our experiment we have planted seedlings belonging to four species of trees: *Quercus petraea, Populus tremula, Betula verrucosa, Salix caprea.* We have planted the seedlings in different location contexts in the tailing pond ("in situ"), as we have also planted "ex situ" witness trees. Our aim was to monitor the dynamics of growth of the stem and of cuttings. Our contribution, based on the outcomes of our research, consists in the formulation of functional correlations spotted between cormophites and micro biota, between the species of trees and their environmental underlying conditions, with the overarching goal to optimize the activities undertaken in order to alleviate the tailing ponds inherent to mining activities.

Key words: ecologic remediation, tailing ponds, dynamic of growth, main stem, cuttings, environmental underlying conditions.

INTRODUCTION

Tailing ponds entail highly – complex environment issues (of a chemical, biological, technological and social nature) stemming from the high content of harmful components but particularly because of the impending dangers that such components inflict on environment and health. 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 ecologic restoration of degraded soils relying on a combination of native cormophites and an improved micro biotic component brings advantages such as:

- soil remediation because of using mycelia in order to disaggregate toxic residuals and miscellaneous categories of pollutants
- the degradation of organic materials and the formation of soil in areas plagued by extreme ecologic conditions
- the aeration, decomposition and transport of organic and inorganic compounds, without the outflow of alluvial deposits
- the decline of soil erosion conferring more protection to beneficial fauna
- the revival of indigenous micro biota (mycorrhizic and saprophytic) with role in the regeneration of soil
- the easier cultivation works of wood species
- the improvement of soil in terms of structure and composition, because of the mycological activity, that allows for a larger spectrum of herbal species to develop
- the enlargement of the vegetal associations in the future ecosystem, boosting the progressive succession of vegetation on degraded soils

MATERIALS AND METHODS

The on-site measurements conducted during April – May, 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 days and 40 days respectively after plantation time. 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

Long – term and intensive mining activities up to about 10 years ago in the county of Maramures explain 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. The formations span along considerable areas and remain in the vast majority of 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 18–20^{O} 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. **Table 1** (Characteristics of soil) gives the structure of soil.

Table 1. Characteristics of soil

Nr. crt.	Parameter	Value
1.	Texture	Sandy clay loam
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.	Mineralogical composition:	
	Quartzite (sand)	40-45
	Clay	20-25
	Feldspar	10-15
	Sulphides	7 – 8
	Sericite, Carbonates,	23 – 7
8.	Particle composition, %	
	> 0.2 mm	3
	0.2 ÷ 0.2 mm	4
	0.1 ÷ 0.05 mm	18
	0.05 ÷ 0.02 mm	10
	0.02 ÷ 0.01 mm	15
	0.01 ÷ 0.015 mm	11
	< 0.005 mm	39
9.	Physico-mechanic parameters of sandy clay loam	
	Natural humidity, %	27-37
	Plasticity, %	56-61
	Porosity, %	60-17
	Coesivity factor, Kpa	22.46
	Specific weigh, KN· m^{-3}	24.13

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 *Betula verrucosa, Populus tremula, Robinia pseudacacia*, with rare presence for *Quercus petraea, Salix caprea*. Such species as *Frangula alnus, Rubus sp.,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. 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 ecto – micorrhyza 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.

This paper aims to demonstrate indirectly (by using biometric descriptors for cuttings) the impact of micro-biota in the soil, micorrhyza included, on the adaptation capacity of various species of cormophites to the particular edaphyc conditions of tailing ponds. 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 (**Graph 2-3**).

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.

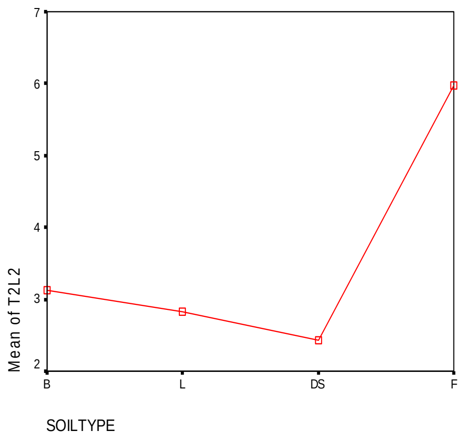
The preliminary quantitative research on seedlings of *Salix caprea*, *Populus tremula*, evergreen oak and birch trees planted in the tailing pond in Bozanta Mare as well as on sterilized soil, demonstrates that even in incipient development stages, at 1 – 2 years old, ectomicorrhyza have colonized the 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 micro – biota, represented by microorganisms and fungi (ectomycorrhiza included).

It is thus known that micorrhyzic relationships stimulate growth for the cuttings of *Salix fragilis* and for the bio mass in the circumstances of a soil contaminated with heavy metals, to a significant percentage more than for the reference trees [1]. Biometric measurements on *Salix caprea* in the tailing ponds (B – value), even during the primary stage of growth (T2l2 – value) taking place 35 and respectiely 40 days after plantation, have conduced to results similar with those cited in the literature. *Salix caprea* seedlings with sterilized roots (L and DS – values), namely in no association with fungic agents, have grown to a significantly smaller extent than the reference trees, consisting in seedlings not sterilized and planted on forest soil (F – value) (**Graph 1**) ($F_{(3,52)}$= 12,940, p= 0,000). Based on the values for this coeficient, growth is significantly different for the *Salix caprea* seedlings depending on the type of soil (different soil and also different chemical structure and the presence of microbiota).

Even more, concludent results can be reached if we compare the above – mentioned case with other types of trees such as willow seedlings not sterilized and planted on tailing pond soil not sterilized. The high growth of the later species stands proof for an improved growth dynamics because of the native rhizosphera with which seedlings are associated from the soil of origin. High growth stands in a similar manner proof for the fact that their roots could get associated with micro organisms/fungi from the soil of adoption. This observation could become the starting point for researching new possible associations between species of trees and new taxons in the micro biota of soil.



Graph 1

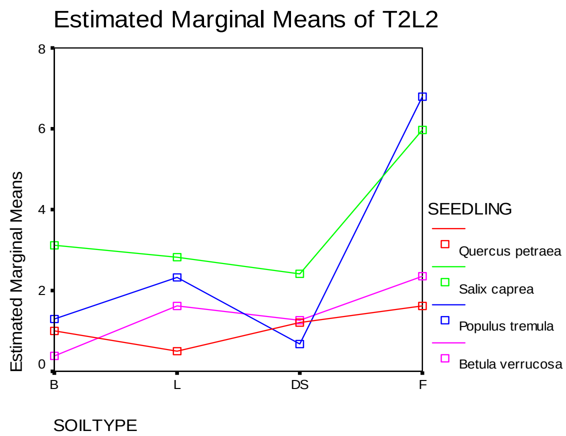
The analysis for all four species of trees of the bio metric measurements on cuttings (T2l2) conducted 35 days after plantation (interval in which diferences in growth could be spotted) has revealed the following situation (**Graph 2&3**):

a) *Salix caprea* has displayed the most predictible behavior as compared to the results included so far in the literature. For this species, the quality of soil and the micro biota affect considerably the growth of cuttings. Thus, seedlings with sterilized roots and planted in tailing pond soil also sterilized (any trace of micro biota was removed both in the soil where the trees were planted and in the soil from which they were sourced) have recorded the smallest growth.

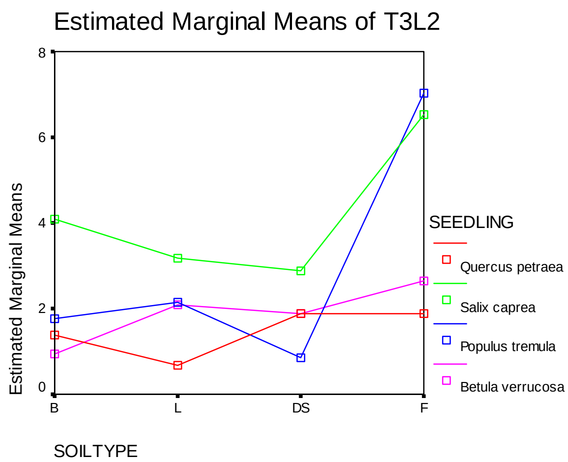
The small growth of cuttings of seedlings with sterilized roots planted in tailing pond soil demonstrates that the colonisation process of their roots with mcrobiota is a slow one.

High growth of *Salix caprea* cuttings was recorded in the tailing pond soil for seedlings whose roots were not sterilized, while the witness trees planted in common forest soil have grown–up to the highest extent, as proof for the fact that micro biota in soil has positive influence on the growth of willows even in early vegetative stages.

The same trend has continued also 45 days after plantation (T3l2), with the remark that on seedlings directly planted in the soil of the tailing pond the development of cuttings was more advanced, because of the inception and consolidation of a risosphera. This outcome suggests, in correlation with the initially slow growth of cuttings on sterilized seedlings in the tailing pond soil, that the native microbiota in the tailing pond colonises in a relatively slow process the roots of *Salix caprea.*



Graph 2



Graph 3

b) For the one year old seedlings of *Quercus petraea*, the growth of cuttings 35 days and 40 days respectively after plantation does not display significant differences depending neither on the micro biota of soil nor on its nature. No significant differences were spotted in terms of bio metric measurements on cuttings grown on normal and sterilized tailing pond soil as compared to the common forest soil. This means that no direct correlation applies between growth and the presence of microbiota in soil at this growth stage.

c) During the first stage of growth, the presence in soil of the micro biota has particular impact on *Populus tremula*. Thus, seedlings with sterilized roots and planted in sterilized tailing pod soil display a very small growth of cuttings (but also small growth of the main stem), even if such seedlings were isolated from the micro climatic perturbing effects generated because of location or because of the slope of soil. Witness seedlings grown up in common forest soil have recorded the highest growth rate. The differences spotted between the lot of poplars planted in natural conditions directly in the tailing pond (with intact micro flora on roots from the soil of origin and ensuing rizoshpera from the micro flora natie to the ailing pond) and the lot planted in tailing pond soil but with sterilized roots are not fully relevant because of the interference with the micro climatic factors that put the lot cultivated in natural conditions at a disadvantage.

d) The growth of cuttings within the two time intervals was comparable with that of the other species for *Betula verrucosa.* Growth was most defficient for seedlings relocated in tailing pond soil, in micro climatic conditions different from those in which the seedlings were staying before relocation. Small growth was the norm for seedlings with sterilized roots, planted in sterilized soil, a fact that substantiates without any doubt the role the presence in soil of microbiota could play on the rate of growth. Should such seedlings, grown up in pots, be exposed to the effects of micro - climatic differences, they would have displayed the smallest growth rate. Birch tree seedlings, whose terminal bud eye was intact in the vast majority of cases, have displayed the same gap in terms of growth as for the main stem.

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:

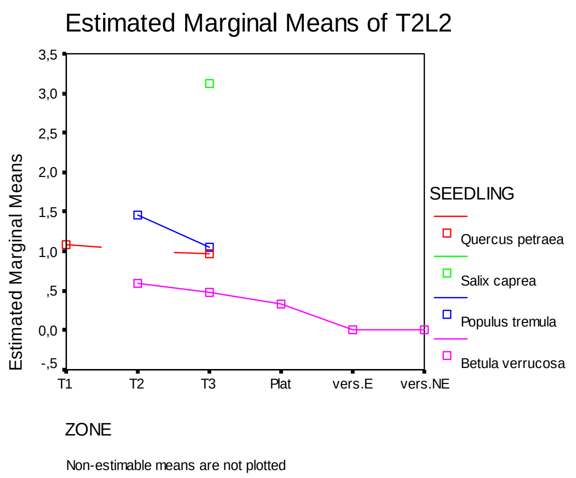
- on plateau no 1 at the smallest height, of about 15 meters above the tailing pond bottom, in flat land, behind a relatively well established curtain of trees, heading to the North – East; we have planted on that plateau exclusively evergreen oak seedlings (the locational conditions there are closest 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 20^{O} 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 20^{O} 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:

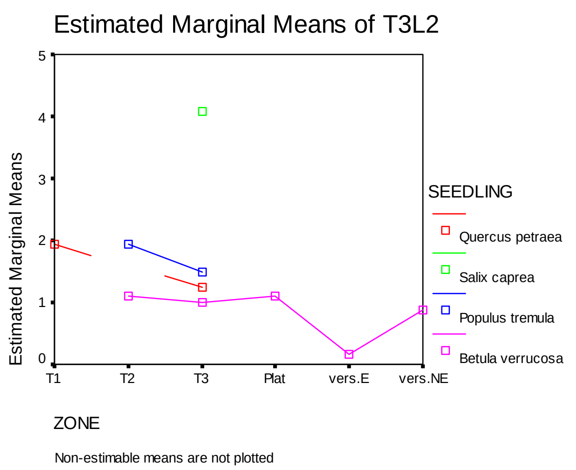
a) For the Evergreen oak growth was stronger both in terms of apical bud eye growth (**Graph 4-5**) and in terms of cuttings (**Graph 6-7**) on plateau no.1 (T1 – value) in protective locational conditions, with smaller exposure to sun light. Compared to all the other species tested, the *Quercus petraea* 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.



Graph 6



Graph 7

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).

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 *Salix caprea* 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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