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PhD THESIS SUMMARY

PERSPECTIVES ON THE MINE TAILINGS RECLAMATION BY BIOLOGICAL METHODS – CASE STUDY: BOZANTA TAILINGS STORAGE FACILITY (MARAMURES)

Keywords: tailings storage facility, flotation tailings, acid rock drainage, acid mine water, a. ferrooxidans, a. thiooxidans, phytotoxicity, amendaments

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INTRODUCTION

The object of the present research is the tailings storage facility (TSFs) Bozânta (Maramureș), a field pond, without a base waterproofing system, placed on a layer of sandy clay with relatively high permeability. During its operation, 1976–2006, 44.5 million tons of tailings were deposited on the pond.

The surface of the pond was conventionally divided into 11 sectors: on S1–S4, tailings from the Central Flotation were deposited, and on S5–S11, tailings from the Săsar Preparation Plant. In terrace T1, it has been stored sterile since 1977; the most recent tailings, from terrace T4, date from 2005–2006.

After the completion of each terrace, plantations were made with acacia (Robinia pseudoacacia) or red acacia (*Robinia hispida*): a) on the S4 sector, with tailings from the processing of the Cu–Pb–Zn complex ore (T1-T2 – acacia in pits with borrow soil and cover the surfaces of the terraces with soil for the purpose of grassing; T4 - no plantings were carried out on the S5 sector, with tailings from the processing of Au- Ag, (T1 – acacia in soil pits, without surface covering; T2 (western area) – red acacia in soil pits and surface covering; T2 (eastern area) – acacia in soil pits, without surface covering; T3 – acacia in tailings pits, without soil; T4 – no plantings were carried out).

In 1995, on terrace T1 of sector S5, the first signs of drying of acacias, especially those planted on T1 and T2, were reported. And limestone was scattered. Just simply spreading limestone on the surface of the pond did not have the intended effect.

It is currently known that the phenomenon of oxidation of iron and sulfur, contained in sulfides from rocks deposited in tailing ponds (for example, pyrite) is biocatalyzed by iron- and sulfur-oxidizing bacteria that live naturally in these materials; at the time the trees were planted on the Bozânta pond, 1975, only 30 years had passed since the American researchers Temple & Colmer (1951a), in the laboratories, isolated, described and named the microorganism involved in acid mine drainage (acid mine drainage - AMD), or acid rock drainage (eng. acid rock drainage - ARD): the bacterium Acidithiobacillus ferrooxidans

Under the influence of alkaline materials, the iron- and sulfur-oxidizing bacteria Acidithiobacillus ferrooxidans (Bond et al., 2000a), acidophiles that live at an optimal pH between 1.5 and 2.8, should decrease their activity. However, the phenomenon of acid drainage of rocks is not maintained exclusively by *A. ferrooxidans*: *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, *Starkeya novella*, *Thiobacillus thioparus*, *Thiomonas intermedia*, *Acidianus brierleyi* are just some of the bacterial species whose association leads to the creation of a biodiversity of oxidative microflora with the potential to live in the most extreme environments.

Following the observations made on the Bozânta pond, as part of the activities for the completion of the undergraduate thesis "*Biodiversity of iron- and sulfur-oxidizing bacteria in the Bozânta tailings settling pond - Maramureș*" (Faculty of Biology and Geology, Biology Specialization, Babeş-Bolyai University, Cluj-Napoca), under the guidance of Prof. Dr. Vasile Muntean, in the period 2013–2015, over 40 years after the planting of the first acacias on the pond and 20 years after the first indication of the drying of the vegetation, we found the extent of the phenomenon and we started researching the causes: the acid drainage of the rocks, in an advanced stage of manifestation (Jelea, 2014; 2015).

After the analysis of the consortia of oxidative bacteria installed in the tailings from the pond terraces, after identifying the effects of the acid drainage of the rocks on the tailings in the pond and the vegetation (planted or spontaneously installed), but also the polluting effects exerted on environmental factors and human communities (Jelea, 2015), the next stage had to include solutions.

First were the studies carried out in the laboratory, with synthetic solutions, to highlight the effects of heavy metals on plants known as bioaccumulators:
Efectele fitotoxice ale cuprului asupra germinării semințelor și creșterii biomasei la *Triticum aestivum L* și *Lactuca sativa L* (Jelea et al., 2016);

- - Phytotoxic effects of copper on growth, morphology and anatomy of vegetative organs of Triticum aestivum L plants (Jelea et al., 2017).

During the Master's studies, 2015-2017, Specialization in Environmental Management and Protection within the Faculty of Environmental Science and Engineering, Babeş-Bolyai University, Cluj-Napoca, in the research for the elaboration of the dissertation "*Study of the effects of heavy metals contained in the flotation tailings from the Bozânta Lake on the plants used in revegetation works*", under the guidance of Mr. Prof. Dr. Călin Baciu and Ms. Associate Professor Dr. Dana Malschi, we moved from the studies carried out with synthetic monometallic solutions to a new stage:

- The study of the effects of heavy metals on the germination of seeds and the growth of vegetative organs and the biomass of plants used in revegetation works, using industrial eluate (soil solution) obtained from tailings taken from the Bozânta pond.

The research was carried out with complex solutions, containing iron, copper, lead, zinc, cadmium, nickel, etc., obtained from the waste from the pond.

The activities in the field, with the observations regarding the damage to the terraces, the condition of the plants on the terraces and on the lake shore, the tailings sampling activities, represented an opportunity to deepen knowledge, but also to raise awareness of the accelerated pace of transformations arising from the dynamics acid drainage processes in the tailings stored on the terraces of the pond.

The activities in the laboratory, with the preparation of the solutions necessary for the experiments, the monitoring of seed germination and the effects on the growth of vegetative organs, the processing of waste samples, the physical, physico-chemical, chemical analyses, the creation of waste mixtures with amendments, the study of plant growth on mixtures, the interpretation of the results , represented an enrichment of knowledge and a higher level understanding of the phenomena.

The doctoral thesis, entitled "*Perspectives regarding the rehabilitation of flotation tailings deposits by biological methods - Case study: Bozânta Lake (Maramureș)*" is a natural continuation of the activities started in 2013.

The aim of the research was to test some species of herbaceous plants regarding growth in the conditions offered by the flotation tailings from the Bozânta pond and to develop some mixtures with amendments in order to create a vegetal carpet with a role in increasing the stability of the surfaces, reducing the scattering of tailings in the neighboring areas, stopping /decreasing the processes of acid drainage of the rocks.

In the first chapter, *Acid drainage of rocks - causes and effects*, the mechanisms of the degradation of the tailings resulting from the exploitation and processing of non-ferrous metal ores are described, in the process of acid drainage of rocks, under the catalytic influence of iron- and sulfur-oxidizing bacteria, with the formation of water mine acids loaded with heavy metals.

In the second chapter, *Biodiversity of iron- and sulfur-oxidizing bacteria in mine tailings deposits*, the evolution of research on the bacteria involved in the biosolubilization of metals from sulfides and the mechanisms of biooxidation processes is presented.

Chapter Three, *Transfer Pathways of Heavy Metals in Plants. Phytoremediation and revegetation*, presents the transfer mechanisms of soluble heavy metals, from the soil/sterile to the plant, their transport and accumulation in the vegetative organs. The effective plant species in the phytoextraction of metals and phytostabilization processes, the types of amendments that can be used to regulate pH, or reduce the mobility of heavy metals in polluted soils are presented.

The fourth chapter, *The effects of acid rock drainage on the Bozânta flotation tailings settling pond*, presents the field observations and the results of laboratory analyzes carried out during the last years, in the period 2020–2023, regarding the evolution of the state of vegetation and the degree of alteration of the tailings from the terraces and the beach of the pond.

Through the results of laboratory, physical, physico-chemical, chemical and microbiological analyses, the different stage of manifestation of the phenomenon of acid drainage of the rocks in the tailings from the pond terraces and the effects on the degree of alteration of the vegetation were highlighted. A model was proposed to estimate the stage and evolution of the acid drainage process of the rocks in the tailings of the pond terraces, based on the physico-chemical properties, the identified bacterial species and their numerical ratio.

Considering that the terraces of the pond are found in different stages of manifestation of the phenomena of acid drainage of the rocks, research was carried out to identify the appropriate measures to stop / reduce the degradative phenomena and their differentiated application on the terraces of the pond, depending on the degree of alteration of the rocks, estimating the evolution of the degradative processes and the consequences regarding the installed vegetation. These researches are the subject of the fifth chapter Study of the effects of heavy metals on plants used in revegetation works.

Two types of tests were carried out:

- tests to highlight the effects of the solutions in the pond on the germination indicators and the growth of the vegetative organs of the plants;

- emergence and growth tests on substrates with pond tailings and amendments.

On the tailings from the pond terraces, the plants were tested in 11 working variants, in order to apply the most effective substrates in the field according to the particular conditions identified on the pond terraces.

Herbaceous plants from the species: *Lolium perenne* L., *Agrostis capillaris* L. Sibth., *Sinapis alba* L. and *Trifolium pratense* L. were tested.

Following the analyzes regarding the growth of plants on the substrates, the mixtures that will be proposed for application in the field, for each terrace, were selected.

Novelty contributions

- The development of a complex, multidisciplinary research methodology of the acid drainage processes of the rocks in the tailings flotation pond, was defining for obtaining the results and the higher level interpretation of the obtained data.

- An important contribution is the development of a model for estimating the stage and evolution of the acid drainage process of the rocks, based on the physico-chemical properties of the tailings samples, the identified bacterial species, their numerical ratio and the interspecific relationships in the tailings pond. settling. The model is based on the observations and results obtained during the 10 years of research and on the deepening of the knowledge regarding the mechanisms of the acid drainage processes of the rocks, in the framework of the doctoral studies which represented an intense period of concentration of attention on details.

- The entire research process was developed in order to create appropriate revegetation solutions, to be applied differently, depending on the particularities of the tailings in each terrace of the pond.

- Through the way of carrying out the research in this doctoral thesis, a new perspective is proposed regarding the approach in a complex and complete way to the studies that are carried out for the revegetation of flotation tailings deposits.

ACID DRAINAGE OF ROCKS - CAUSES AND EFFECTS

Deposits, natural accumulations of useful elements, which, together with the rocks they contain, form ores, are unevenly distributed on a planetary level in the earth's crust, both in terms of mineralization types and contents. Concentrations of useful elements in ores of non-metallic mineral substances can vary from 25% to 90%; in non-ferrous and rare ores, the useful elements can represent between 0.3–0.6%, the concentrations being higher for ferrous ores (Lăzărescu, 1983).

Regardless of the extractive technology used to exploit the deposits, underground (mine) or on the surface (quarry), in addition to ores with useful elements in extractable concentrations, there are also rocks without mineralization or with concentrations below the technological level of extraction, considered mine tailings. They do not enter the technological extraction circuit and are deposited near mines or quarries, constituting the so-called mine tailings dumps, deposits with different granulometry, with variable contents of useful elements.

In the non-ferrous metals industry, the extraction of useful elements from ores involves the application of grinding operations and chemical treatment of fine granular material, a technology known as preparation by flotation; after processing, the useful mineral substance and flotation tailings - waste from the extractive industry, which are stored in flotation tailings settling ponds (tailings storage facility - TSF) result.

Although called tailings, thousands of tons of useful mineral elements are stored in mine dumps and tailings ponds; for example, in 1,000 tons of tailings from the mining of a non-ferrous metal ore containing 0.1% Cu (below the workable concentration, which is above 0.6%), a quantity of one ton of copper is stored.

From the perspective of sustainable mining, useful substances left in tailings, not recovered at the time of production, represent potential resources for future reprocessing. In 1994, 2% of total global copper production came from reworked tailings (Alcalde et al., 2018). In Chile, since the early 1990s, mining companies dedicated exclusively to the reprocessing of tailings for the recovery of copper and molybdenum have been established. The reprocessing of tailings from old deposits could lead to a higher production rate than the processing of primary ores, as classical recovery technologies have been ineffective (Edraki et al., 2014).

In tailings deposits, dumps and ponds, resulting from the exploitation and processing of non-ferrous metal ores, natural phenomena of rock alteration have been observed.

The natural phenomena of bacterially mediated rock oxidation are known under various names: biosolubilization of ores, bioleaching, bacterial leaching (eng. bioleaching; fr. biolixiviation), acid rock drainage (eng. acid rock drainage - ARD), or acid mine drainage (acid mine drainage (AMD) (Bond et al., 2000).

The acid drainage of rocks consists in the biocatalyzed oxidation of iron and sulphur, contained in the sulphides present in the rocks (eg: pyrite – FeS_2) with the formation of trivalent iron (Fe^{3+}) and sulfuric acid (H_2SO_4); other sulfides (e.g.: chalcozine – Cu_2S; coveline – CuS; sphalerite – ZnS; galena – PbS; arsenopyrite – FeAsS, etc.) release the ions of the component metals into solutions, forming elemental sulfur, So, which is then oxidized to sulfuric acid (Zarnea, 1984; Muntean, 2009). Sulfuric acid acts on oxidic minerals leading to the solubilization of the contained metals; the resulting solutions, with acidic pH and high metal concentrations, are acid mine waters (Bond et al., 2000). Acid rock drainage phenomena lead to multifactorial pollution and are present in all tailings and flotation deposits from non-ferrous ore processing as well as in active or abandoned mining operations (Ighalo et al., 2022).

Acidic solutions loaded with metals exfiltrate from tailings deposits, where rock biosolubilization processes take place, reaching surface waters and the groundwater (Yuan et al., 2022). The color of the exfiltrations suggests information about their composition: the reddish color indicates the presence of ferric iron ions, Fe^{3+}, the greenish color the presence of Cu^{2+} ions, and the bluish color the presence of ferrous iron ions, Fe^{2+} (Masindi et al., 2022).

The toxicity produced by heavy metals depends on their concentration and bioavailability in the soil. Depending on the bioavailability of heavy metals/metalloids in the soil, they can be classified into: easily bioavailable (Cd, Ni, Zn, As, Se, Cu); moderately bioavailable (Co, Mn, Fe); less bioavailable (Pb, Cr). Contaminated soil and acidic conditions cause the degradation of vegetation (Schippers et al., 2000; Jelea, 2015), and prevent the formation of a vegetal carpet, which amplifies erosion phenomena (Moreno-Jiménez et al., 2016). Fine tailings particles are dispersed by wind contaminating agricultural crops (Zine et al., 2020). Metals in contaminated soil affect the structure and good functionality of microbial communities and indirectly, the quantity and quality of production (Kafle et al., 2022; Thomas et al., 2022).

Among the methods of ecological remediation of tailings ponds, we mention artificial remediation and spontaneous restoration. Artificial ecological remediation includes vegetation restoration (Merino–Martín et al., 2017; Song et al., 2022) and substrate improvement (Song et al., 2022; Jelea & Baciu, 2023). Spontaneous restoration describes the use of plant species naturally installed on the surface of mining dumps, adapted to the composition of the substrate (Damian & Damian, 2006).

BIODIVERSITY OF IRON-AND SULFOXIDATING BACTERIA IN MINING STERILE DEPOSITS

Colmer & Hinkle (1947) describe the role of microorganisms in acid mine drainage and then isolate and describe bacteria involved in accelerating the oxidation of iron and sulfur, together with Temple (Colmer et al., 1950; Temple & Colmer, 1951a) and I call them *Thiobacillus ferrooxidans* (Temple & Colmer, 1951b).

Temple & Delchamps (1953) present evidence that *T. ferrooxidans*, in bituminous coal, is an autotrophic oxidizer of iron disulfide (pyrite), forming ferrous sulfate, ferric sulfate, and elemental sulfur, and T. thiooxidans is involved in the oxidation of the resulting sulfur following the oxidation of pyrite, with the formation of sulfuric acid.

Morphological or physiological differences, highlighted over time, determined the confirmation, reclassification or renaming of species and reorganization of genera: T. novellus (in Starkeya novella) (Kelly et al., 2000), T. ferrooxidans (in *Acidithiobacillus ferrooxidans*), *T. thiooxidans* (in *Acidithiobacillus thiooxidans*) (Bond et al., 2000).

Based on re-evaluations, other species in the genus *Acidithiobacillus* were recently recognized, namely: *A. ferrivorans* (Hallberg et al., 2010), *A. ferridurans* (Hedrich & Johnson, 2013) and A. ferriphilus (Falagán & Johnson, 2016; Nuñez et al., 2017).

The species of the genus *Acidithiobacillus* are represented by flagellated, gram-negative bacilli, with a length of 1–2 μ and a diameter of 0.5–1 μ.

Most species of the genus grow in liquid media or on rocks with a pH between 0.5 and 5, the optimum for their development being below 3.0, while others, for example *A. thiooxidans*, have a spectrum of Very wide pH in which they can be active, from strongly acidic to neutral (Ko et al., 2013).

Among species of the genus *Acidithiobacillus*, *A. thiooxidans* and *A. ferrooxidans* are mesophilic, with optimum temperatures around 28–30 °C (Falagán et al., 2019; Kelly & Wood, 2000).

Acidithiobacillus sp. they are autotrophic, chemosynthesizing bacteria, the source of energy being the chemical energy resulting from the oxidation of various inorganic substances (Robertson & Kuenen, 2006; Rzhepishevska, 2008; Li et al., 2023), not having the ability to decompose organic substances, their presence negatively influencing the development their. The source of carbon for the synthesis of its own substances is carbon dioxide from the liquid medium (Muntean, 2009).

The essential characteristic of the genus *Acidithiobacillus* is the ability to oxidize sulfur and/or iron. Based on the differences in energy substrates, the species of the genus Acidithiobacillus can be divided into two groups (Wang et al., 2019):

- the group of sulfur-oxidizing species *A. thiooxidans*, *A. caldus* and *A. albertensis*;

- the sulfur- and iron-oxidizing species group, which includes *A. ferrooxidans*, *A. ferrivorans*, *A. ferriphilus* and *A. ferridurans*.

Acidithiobacillus thiooxidans sunt bacterii sulfuroase nefilamentoase, chemolitoautotrofe, obligat aerobe (Yang et al., 2019).

A. thiooxidans oxidizes elemental sulfur, sulfite, thiosulfate and tetrathionate, having a role in the solubilization of metals by producing sulfuric acid; they do not oxidize sulfides and cannot oxidize iron (Rohwerder & Sand, 2003; Ko et al., 2013; Yang et al., 2019; Saavedra et al., 2020).

They live in environments with a wide pH spectrum, between 1 and 7, with an optimum between 2 and 4 (Ko et al., 2013); they are tolerant to very high acidity, up to pH 0.5 (Robertson & Kuenen, 2006). The optimum temperature range is 28–30 ^{o}C. There are also studies that indicate an optimal temperature of 37 ^{o}C (Ko et al., 2013).

They are widespread in soils and ores containing sulfur and mineral sulphides, in pyritic coal and in acid or sulphurous mine waters.

Acidithiobacillus ferrooxidans are non-pigmented, non-filamentous, chemolithoautotrophic, aerobic and non-sporulating sulfur bacteria (Bosecker, 1997; Saavedra et al., 2020; Li et al., 2023).

The species *A. ferrooxidans*, described by Temple & Colmer since 1951, provides its energy by oxidizing natural sulfides containing S^{2-}, elemental sulfur (S^{o}) and other sulfur compounds, such as thiosulfate ($S_2O_3^{2-}$) and tetrathionate ($S_4O_6^{2-}$), to sulfuric acid and ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) (Silverman & Lundgren, 1959; Valdés et al., 2008; Li et al., 2023).

They are acidophilic bacteria, with an optimal pH between 2.0–2.5, but they also tolerate lower values (Rohwerder et al., 2003). The optimal temperature for development is between 25–35 ^{o}C (Smith et al., 1988; Nemati & Webb, 1997; Li, 2016).

In adverse geochemical environments, e.g. pH >4, in the absence of readily available energy sources (pyrite) or lack of oxygen, *A. ferrooxidans* can survive using ferric iron as an alternative electron acceptor for sulfur reduction under anaerobic conditions (Sugio et al., 1985; Pronk et al., 1992; Ohmura et al., 2002).

They are widespread in acid mine waters, in natural or industrial waters with iron and sulfur content (Zarnea, 1994), in soils and ores with sulfide content, in sulfur and iron ores (Li et al., 2023) and in those of pyrite coal.

In the same environment, or in similar environments, live other bacterial genera with a role in the biosolubilization of metals: *Starkeya* (Santer et al., 1959); *Thiomonas* (Milde et al., 1983); *Sulfolobus* (Pivovarova & Golovacheva, 1985); *Thiobacillus* (Karavaiko, 1985; Kelly & Wood, 2000); *Leptospirillum* (Hippe, 2000; Coram & Rawlings, 2002); *Acidiphilium* (Rohwerder & Sand, 2003) etc.

Leptospirillum ferrooxidans is an autotrophic, coiled bacterium with polar flagella and vibratory movements (Hutchins et al., 1986).

It aerobically oxidizes ferrous iron as the sole source of energy at temperatures between 28–30 °C and a pH between 1.5 and 3.0 (Gadd, 2009; Nicolova et al., 2017; Bleeze et al., 2018) and does not oxidize sulfur or mineral sulfides. It can produce oxidation of pyrite and chalcopyrite if co-cultured with the sulfur-oxidizing bacteria *Acidiphilium acidophilum* or *A. thiooxidans* (Karavaiko, 1985).

The iron-oxidizing activity of leptospirilla accelerates at a temperature above 40 °C and at high acidity of the environment (pH of 0.7–1.0), under conditions of adequate aeration, energy source (ferrous ions) and carbon (Rojas-Chapana & Tributsch, 2004). Pyrite dissolution is faster in environments where both leptospirillae and iron-oxidizing thiobacilli of the genus *A. ferrooxidans* are found and continues at acidic pH values, inhibitory for *A. ferrooxidans* (Hutchins et al., 1986).

Starkeya novella is a gram-negative, nonflagellate, facultative sulfur-oxidizing coccobacillus that has the ability to live as a facultative chemolithoautotroph (Santer et al., 1959), heterotroph, or methylotroph (Kelly et al., 2000; Kappler et al., 2012). It can oxidize sulfides, sulfite and thiosulfate to sulfate (Hein et al., 2023).

The temperature range for growth is 10–37 °C with an optimum of 25–30 °C and a pH range of 5.7–9.0 with an optimum at pH 7.0 (Kelly et al., 2000). They grow on thiosulfate and tetrathionate media under aerobic conditions, but not on sulfur or thiocyanate (Kappler et al., 2012). Ammonium salts, nitrates, urea and glutamate can serve as nitrogen sources. Several studies supporting heterotrophic growth on substrates including glucose, formate, methanol, oxalate have been published (Kappler et al., 2012).

It lives at an optimum pH between 4.5–7.0. It is a bacterium tolerant to high acidity, being found in soils with a pH of 2.2, in ores and mine waters, having metabolic activity even at a pH lower than 0.6 (Müller, 1968).

Thiomonas intermedia is a gram-negative, moderately acidophilic, facultatively heterotrophic bacterium. It has the ability to utilize tetrathionate under oxic and anoxic conditions. It can utilize glucose, yeast extract and sulfate from the environment (Wentzien & Sand, 1999; 2004).

TRANSFER PATHWAYS OF HEAVY METALS IN PLANTS. PHYTOREMEDIATION AND REVEGETATION

High concentrations of heavy metals in ore mining areas pose a risk to nearby soils, rivers, vegetation and population. If heavy metals are found in soluble forms, they are transferred from the soil to the plant. Soil concentration of metals and their bioavailability depend on a number of factors such as particle size fraction, organic matter content, cation exchange capacity, nutrient content, structure and chemical composition of metal exchange sites in organic and inorganic soil matrices and affinity towards anionic ligands in the soil solution (Kabata–Pendias & Pendias, 2001). The transfer depends on the chemical nature of the metal, the plant type, the pH and Eh of the soil solution, the soil texture, the hydrological regime, the climatic conditions, the clay content (Mihali et al., 2013).

Plants take up metals from the soil solution in ionic form or in chelated complex form. The uptake mechanisms of metals from the soil are specific. Lead and nickel are absorbed at root level passively, and copper, molybdenum and zinc actively. Metals are translocated through xylem to shoots. The transport process is followed by metabolism, detoxification and storage of metals. In aerial organs metals are accumulated in vacuoles. By sequestering metals in vacuoles, excess metal is removed from the cell cytoplasm and their involvement in physiological processes is reduced (Patra et al., 2021).

3.1 Types of phytoremediation

Phytoremediation includes *phytoextraction*, *phytovolatilization*, *rhizofiltration*, and *phytostabilization*.

Phytoextraction describes the use of plants for the translocation and accumulation of heavy metals in vegetative organs. This technique includes the mobilization of contaminants in the rhizosphere, the absorption of metal cations with the help of plant roots, the formation of the metal-ligand complex, the translocation of the complexes in the leaves and in their vacuoles where they are stored (Kafle et al., 2022).

Plants used for phytoextraction must have fast growth, high biomass production (>3 tons s.u/ha/year), be easy to harvest, accumulate high metal concentrations in biomass (>1000 mg/kg biomass), provide economic benefits, well-developed root system and not to be consumed by herbivorous animals (Kafle et al., 2022). After phytoextraction, heavy metals may partially remain in the soil or in plant roots.

Different plant species are used for phytoextraction: *Brassica juncea* (for Pb), *Lativa sativa* and *Lolium perenne* (for Ni, Co, Fe), *Nicotiana tabacum* (for Cd), *Salix sp.* (for Cd), *Solanum nigrum* (for Cu, Zn, Cd), *Heliantus annuus* (for Cu, Zn, Pb, Hg, As, Cd, Ni) (Kafle et al., 2022).

Phytostabilization is also called *in-situ inactivation* or *phytoimmobilization*. Plants tolerant to heavy metals are used for phytostabilization. The success of phytostabilization depends on the ability of the plant to tolerate high concentrations of heavy metals in the soil and extreme pH values. Constantinescu et al. (2019), recommends the species *Agrostis capillaris* for the phytostabilization of soils contaminated with Cd, Cu and Zn.

Phytovolatilization involves the transformation of some metals absorbed by plants and their translocation in shoots in a less toxic and volatile form. Through phytovolatilization the contaminants are not completely removed and are transformed into another phase (Zulkernain et al., 2023).

Rhizofiltration involves a mechanism specific to aquatic and terrestrial plants that favors the precipitation of heavy metals (Cd, Cr, Cu, Pb, Ni, Zn) at the root surface or the absorption by the root of soluble pollutants existing in the soil solution, which surrounds the root zone (rhizosphere). Rhizofiltration is a method of treating surface and groundwater containing trace metals.

The principle of phytoremediation is based on the ability of some plants to uniquely tolerate environmental pollutants. Depending on this capacity, plants are accumulators, *hyperaccumulators*, *excluders* or *indicators* (Burges et al., 2018).

Accumulator plants are indigenous to a certain type of soil and are geobotanical indicators of the formed deposits. An accumulator actively takes up heavy metals in their aboveground biomass, and a hyperaccumulator takes up metals above 1% of their dry weight (Baker & Brooks, 1989).

Hyperaccumulating plants are plants that, when exposed to high concentrations of heavy metals, are able to accumulate them in the aerial parts without symptoms of phytotoxicity (Suman et al., 2018).

Exclusion plants tolerate heavy metals and are able to survive in soils containing high concentrations of heavy metals. These plants have a low metal extraction potential and are used to prevent and/or stabilize the erosion of metal-polluted soils.

Indicator plants take up enough metal to reach equilibrium with its concentration in the rhizosphere (Lasat, 2000; Burges et al., 2018).

Remediation and revegetation of polluted areas

Plants have been used in recent years as a means of stabilizing or removing heavy metals from contaminated soils. In soil, heavy metals can be a) associated with the soil solution (metal ions and soluble metal complexes); b) adsorbed by the inorganic constituents of the soil at the ion exchange sites; c) related to the organic matter in the soil; d) precipitates in the form of oxides, hydroxides or carbonates; e) incorporated in the structure of silicate minerals (Lasat, 2000).

In the short term, plants prevent the dispersion of metal particles by airborne and water erosion and reduce the mobility of metals. In the long term, plants improve the structure and quality of contaminated soil (increase the content of organic matter, nutrients and cation exchange capacity) and soil biodiversity (Arienzo et al., 2004). Plants used for remediation must develop resistance to diseases, pests and environmental stressors, allow association with other species and adapt to agroecological conditions.

Grasses are important for remediation because of their increased biomass and ability to grow evenly on less fertilized soils and because of their potential in soil conservation and erosion control. The Poaceae family includes 780 genera and 12000 plant species. Poaceae (monocotyledons) have a high pollutant tolerance compared to dicotyledons.

Table 3.1 shows the perennial herbaceous plants used for remediation

Table 3.1 Perennial grasses representing potential genetic resources for remediation (after Pandey & Maiti, 2020)

Scientific name	Popular name	Contaminate	Bibliography
Agrostis alba	iarba-câmpului	Ti, Ni, Mo, Cr, Cu, Sn	Elekes & Busuioc (2011)
Agrostis tenuis	iarba vântului	Cu, Cr, Sn	Houben & Sonnet (2015); Sharma et al. (2018)
Cynodon dactylon	iarba Bermudelor	Cr, Cu, Mo, Ni, Pb, Sn	Maiti & Prasad (2017)
Festuca arundinacea	păiușul înalt	Ni, Pb, Cd, As	Sun et al. (2011)
Festuca rubra	păiuș roșu	Cd, Cu, Zn, Ni	Langella et al. (2014); Gołda & Korzeniowska (2016)
Festuca pratensis	păiuș de livadă	Cd	Soleimani et al. (2010 a, b)
Poa pratensis	schinduf	Cd, Ni, Pb	Huang et al. (2004); Mahmoudzadeh et al. (2016)
Lolium perenne	raigrasul peren	Cd, Pb, Cr, Cu, Sn	Gołda and Korzeniowska (2016); Cui et al. (2018)
Stipa capillata	năgară	Sn	Elekes & Busuioc (2011)
Triticum aestivum	grâu	Se, Cd, Cu, Ni, Pb, Zn, Cr, As, Hg	Suchkova et al. (2010); Yasin et al. (2015)
Vetiveria zizanioides	Vetiver	As, Cd, Cr, Pb, Cu, Zn, Fe, Mn	Ghosh et al. (2015); Verma et al. (2014)

Monocultures or pluricultures (compatible) can be used for phytoremediation. Frérot et al. (2006) used legumes mixed with herbaceous species. Legumes grow and survive in nutrient-poor environments, being important because they provide organic nitrogen to a developing soil (with the help of *Rhizobium* bacteria). When leguminous species are mixed with non-leguminous species, the biomass of the latter increases (Goris, 2012) and thus stable and persistent plant communities can be created.

Woody species (*Salix sp., Populus sp.)* are used for phytoextraction because they produce a large amount of biomass in a relatively short time. Their above-ground biomass is high compared to that of hyperaccumulating grasses, because of this a large amount of heavy metals accumulate in the aerial parts. Metals can be continuously removed from the site by harvesting leaf biomass. Woody species through their deep root system reduce soil erosion and prevent dust dispersion (Suman et al., 2018).

For the decontamination of a site (Lommel in Belgium with Cd and Zn content) species of *Populus sp*. and *Salix sp*. Based on the recorded data, it was determined that it would take between 12.5 and 25 years to restore the contaminated area with a Cd content of 1 mg·kg-1 soil (Vangronsveld et al., 2009).

Phytomanagement of degraded sites encourages the selection of native species, which in the long term will form self-sustaining plant communities. Native species carry out the natural revegetation of degraded lands (Maiti, 2013). Studying native plants on mine tailings provides information on their degree of tolerance. For the restoration of mining deposits Maiti (2013) and Gajić et al. (2016) proposed the creation of a self-sustaining vegetation with mixtures of herbaceous plants, legumes and trees.

For soils containing low concentrations of metals, the phytoextraction technique is used, and for mine tailings dumps and settling ponds, phytostabilization is recommended. The phytostabilization technique aims to limit the mobilization and bioavailability of heavy metals by correcting the pH and using amendments (Neagoe & Iordache, 2021).

As for the organic amendments that can be used, they can be: fertile soil enriched with organic matter; chopped legumes (Constantinescu et al., 2019); manure (Tejada et al., 2008); compost from the food industry (Tejada et al., 2008); wood products (Hattab et al., 2015); compost and sewage sludge (Neagoe & Iordache, 2021); plant compost (Neagoe & Iordache, 2021). Amendments with fertile soil and clay are important because fertile soil contributes to the formation of communities, and clay allows the absorption of heavy metals on its surface, reducing their mobility from the soil and uptake by plants (Neagoe & Iordache, 2021).

Experiments with organo-zeolites have shown that they retain metals in soil (Lee et al., 2019), increase soil pH (Prisa, 2020), improve soil quality (Damian et al., 2018; Damian et al., 2019; Lee et al., 2019) and increase the cation exchange capacity for NH^{4+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} (Langella et al., 2000).

THE EFFECTS OF ACID ROCK DRAINAGE ON THE BOZANTA TAILINGS STORAGE FACILITY

Near the city of Baia Mare, only 4 km away, between the villages of Săsar and Bozânta Mare, north of the confluence of the Săsar and Lăpuş rivers (fig. 4.1), millions of tons of flotation tailings are stored in the Transgold, Săsar and Bozânta settling ponds (Modoi et al., 2010; Jelea, 2014; 2015).

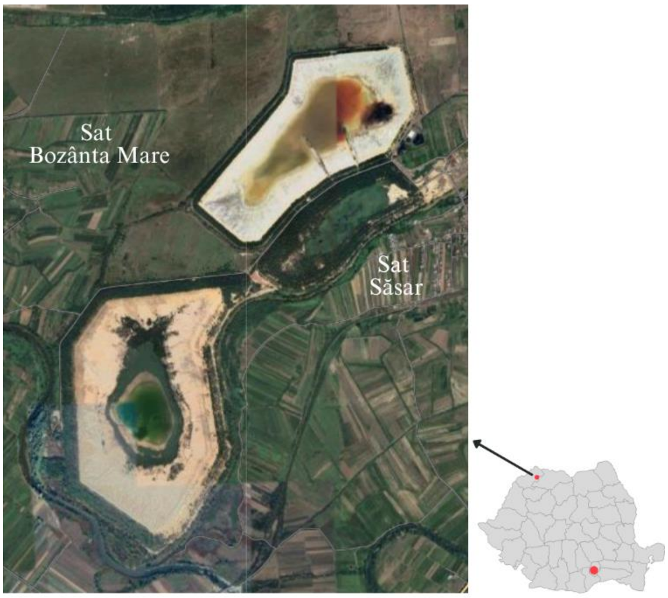

Fig. 4.1 Bozânta, Săsar and Transgold settling ponds (Google maps)

a

b

Fig. 4.2 a) View of Baia Mare municipality from the Bozânta pond;
b) The village of Bozânta Mare, view from the Bozânta pond

The Bozânta settling pond has been in operation since 1976; it is currently under conservation as a result of the stoppage of the exploitation of gold ores in 2001 and of complex Cu–Pb–Zn in 2007 (fig. 4.2).

The pond, together with the annexes, occupies an area of 120 ha; the actual pond occupies an area of 82 ha and was simultaneously used for the storage of tailings from the Central Flotation Preparation Plant (processing of complex ores) and from the Săsar Preparation Plant (processing of gold-silver ores).

The surface of the pond was divided, depending on the type of tailings deposited, into 11 sectors, for an easier monitoring of technological activities:

- Sectors S_1–S_4 are located on the N, NW, W and SW sides, they contain the tailings resulting from the processing of complex ores containing Cu, Pb and Zn, originating from the Central Flotation Preparation Plant and deposited for 31 years, in the period 1976–2007;
- Sectors S_5–S_{11}, are located on the S, SE and E sides and contain tailings resulting from the cyanide processing of the gold ores extracted from the Săsar Mine. Tailings from the Săsar Flotation Preparation Plant were deposited on these sectors for 25 years, in the period 1976–2001, and tailings from the Central Flotation Preparation Plant, deposited in the period 2001–2007 on the upper terrace T4, as a result of stopping the extraction and processing of gold ores from the Săsar Mine.

The purpose of the research

The purpose of the research was to characterize the effects of the phenomena of acid drainage of rocks on the terraces of the settling pond and to identify the pressures exerted on the environment.

Methods of investigation

Between 14.09.2020-30.08.2023, 11 field campaigns were carried out, during which the following activities were carried out:

- observations regarding the state of the terraces on the pond sectors, the rain and wind transport of tailings to the base of the terraces, the formation of ravines, landslides, the scattering of tailings, the exfiltration of solutions from the pond to the surface of the terraces;
- an assessment in the field of the degree of alteration of the tailings was carried out based on the appearance of the samples, according to the color of the tailings; gray - unaltered or an early stage of alteration, yellow - oxidation of sulfur and the formation of sulfuric acid, reddish yellow - the beginning of the oxidation processes of iron from pyrite with the formation of ferric iron, Fe^{3+}, deep red - very active iron oxidation;
- observations on the effects of flotation tailings on watercourses, lands and vegetation in the adjacent areas, the discharge of tailings and acid mine waters in the stream, wind mobilization of the tailings and transport in the adjacent areas (agricultural crops and localities);

- identification activities of plant species spontaneously installed on the pond sectors;
- observations regarding the state of the vegetation on the pond sectors, the drying of the vegetation on the terraces affected by the biooxidation of the tailings;
- activities to identify/determine the fauna of wild mammals that move on the surface of the pond;
- taking samples of tailings in order to carry out microbiological and physico-chemical analyses;
- collection of tailings samples for carrying out germination experiments and those for the cultivation of grass species;
- harvesting plants to obtain the necessary seed material for revegetation experiments.

4.1 State of vegetation and the degree of alteration of the tailings on the terraces of the Bozânta pond

Field trips for the identification of plant species and making observations regarding the condition of the plants were carried out during the 4 years.

The inventory of plant species on S4 and S5 was carried out by applying the stationary inventory method and for the rest of the sectors the route inventory method was applied (tab. 4.1).

The floristic inventory was made using different specialized works (Clinovschi, 2005; Ciocârlan, 2009; Tomescu, 2020).

4.1.1 State of vegetation and degree of alteration of the tailings on the terraces of the S_4 sector

On terraces T_1 and T_2 at the base of sector S_4, an acacia (*Robinia pseudoacacia*) plantation was created in pits with borrowed topsoil between 1976 and 1980; the surfaces of the two terraces were covered with a 5 cm layer of soil.

On terrace T_3, the planting of acacias was also done in pits with borrowed soil, but the layer of topsoil was not deposited on the surface of the terrace.

The T_4 terrace was not subjected to any planting or earth deposition intervention.

As a result of the different way of maintaining the terraces in this sector, different states of evolution of the vegetation and also of the terrace surfaces were observed.

Terrace T_1

In addition to the planted species, *Robinia pseudoacacia*, 3 species of woody and 2 herbaceous plants that appeared spontaneously were also identified on the terrace.

Terrace T_2

On the T_2 terrace, along with the dominant species, *Robinia pseudoacacia*, 6 woody species and 18 herbaceous species were identified.

Terrace T_3

On terrace T3, the planted species, *Robinia pseudoacacia*, and a subshrub species were identified: stubble mulberry (*Rubus caesius*) – subshrub.

Terrace T_4

On terrace T4, specimens of acacia and a grassy species, which appeared spontaneously, were identified.

Table 4.1 The plant species inventoried on the terraces of the Bozânta pond, the sectors S_4 and S_5

No. crt.	Scientific name	Popular name	S_4				S_5			
			T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
1	*Acer tataricum* L.	glădiş, arţar tătăresc	-	+	-	-	-	+	-	-
2	*Achillea millefolium* L.	coada şoricelului	-	+	-	-	-	+	-	-
3	*Agropyron repens* (L.)P.Beauv	pir târâtor	-	+	-	-	-	+	+	+
4	*Agrostis capillaris* L.	păiuş	-	-	-	-	-	+	-	-
5	*Alopecurus pratensis* L.	coada vulpii	-	-	-	-	-	-	+	-
6	*Amorpha fruticosa* L.	salcâm-mic	-	-	-	-	+	+	+	+
7	*Arctium lappa* L.	brusture, lipan	-	-	-	-	-	+	-	-
8	*Artemisia vulgaris* L.	pelinariţă	-	+	-	-	-	-	-	-
9	*Aspera spica-venti* (L.)P.Beauv	iarba-vântului	-	-	-	-	-	-	-	+
10	*Betula pendula* Roth	mesteacăn	-	-	-	-	+	-	-	-
11	*Centaurea cyanus* L.	vineţea, albăstriţă	-	+	-	-	-	-	-	-
12	*Centaurea micranthos* L.	vineţele, albăstriţă	-	-	-	-	-	+	+	-
13	*Chamaenerion angustifolium* L.	zburătoare, răscoage	-	+	-	-	-	-	-	-
14	*Chelidonium majus* L.	rostopască	-	+	-	-	-	-	-	-
15	*Chenopodium glaucum* L.	lobodă sălbatică	-	-	-	-	-	-	-	+
16	*Cirsium vulgare* (Savi.) Ten.	pălămidă	-	+	-	-	-	-	-	-
17	*Conium maculatum* L.	cucută	-	-	-	-	-	+	-	-
18	*Digitalis grandiflora* Mill.	degetărelul galben	-	-	-	-	-	+	-	-
19	*Echium vulgare* L.	iarba şarpelui	-	+	-	-	-	-	-	-
20	*Erigeron canadensis* L.	bătrâniş	-	+	-	-	-	+	-	-
21	*Fallopia japonica* Houtt.	iulişca	+	+	-	-	-	-	-	-
22	*Festuca pratensis* Huds.	păiuş de livadă	-	-	-	-	+	+	-	-
23	*Glyceria plicata* Fr.	mana apei subţire	-	+	-	-	-	+	-	-
24	*Humulus lupulus* L.	hamei	-	+	-	-	-	-	-	-
25	*Lolium perenne* L.	iarbă de gazon	-	-	-	-	-	+	-	-
26	*Phragmites communis* Trin.	stuf	-	-	-	+	-	+	-	-
27	*Plantago major* L.	pătlăgina mare	-	+	-	-	-	-	-	-
28	*Populus alba* L.	plop alb	-	-	-	-	+	-	-	-
29	*Populus nigra* L.	plopul american	-	+	-	-	+	-	-	-
30	*Prunus serotina* Ehrh.	cireş negru	+	+	-	-	-	-	-	-
31	*Quercus cerris* L.	cer	+	-	-	-	+	+	-	-
32	*Quercus robur* L.	stejar	-	+	-	-	+	-	-	-
33	*Robinia pseudoacacia* L	salcâm	+	+	+	+	+	+	+	-
34	*Robinia hispida* L.	salcâm roşu	-	-	-	-	-	+	-	-
35	*Rosa canina* L.	măceş	+	+	-	-	-	-	-	-
36	*Rubus caesius* L.	mur de mirişte	+	+	+	-	-	-	-	-
37	*Rumex acetosella* L.	măcriş mărunt	-	-	-	-	+	+	+	+
38	*Salix pentandra* L.	salcie	-	+	-	-	-	-	+	-
39	*Salvia nemorosa* L.	năduf	-	+	-	-	-	-	-	-
40	*Saponaria officinale* L.	săpunariţă, odogaci	-	+	-	-	-	-	-	-
41	*Silene alba* Mill.	guşa porumbelului	-	+	-	-	-	+	-	-
42	*Trapogon orientalis* L.	barba caprei	-	+	-	-	-	-	-	-
43	*Urtica dioica* L.	urzica mare	+	-	-	-	-	-	-	-
44	*Verbascum nigrum* L.	luminiţa neagră	-	+	-	-	-	-	-	-

4.1.2 State of vegetation and degree of alteration of the tailings on the terrace of the S_5 sector

The terraces of the S_5 sector were treated differently from a technical point of view, in terms of the way of planting acacias: on terrace T_1, with the oldest tailings, deposited in the period 1975-1980, acacias (*Robinia pseudoacacia*) were planted in pits with borrowed topsoil, without a covering of the terrace surface for the purpose of weeding; on the T_2 terrace, in 1985, a shrub species of ornamental acacia, red acacia (*Robinia hispida*), was planted in the western area of the sector and common acacia (*R. pseudoacacia*) in the eastern area. The surface of the sector, in the area of ornamental acacias, was covered with a thin layer of topsoil; on terrace T_3, acacias of the *R. pseudoacacia* species were planted directly in the waste, without borrowed soil, and no topsoil was applied on the surface of the terrace; no vegetation was planted on the upper terrace, T_4.

Terrace T_1: In addition to *R. pseudoacacia*, five tree species, one shrub species and two herbaceous plant species were identified on this terrace.

Terrace T_2: On terrace T_2, in addition to the two planted species, *R. pseudoacacia* and *R. hispida*, the following plant species were identified: two species of trees, one shrub and 14 species of herbaceous plants, all on the slope of the area with R. *hispida*.

Terrace T_3: Two shrub species and four herbaceous species were identified on terrace T_3.

Terrace T_4: One species of shrub and four species of herbaceous plants were identified on terrace T_4.

4.1.3 Vegetation condition and degree of tailings alteration on the tailings lake beach

The stoppage of tailings discharge as a result of the Bozânta pond being conserved has determined the drying up of the tailings lake and its retreat towards the center. The sterile, which during the immersion period remained unaltered, is now subjected to intense bacterial oxidation phenomena. On the surface of the former settling lake, 6 tree species, 4 shrub species and 30 herbaceous species were identified.

4.2 Biodiversity of iron and sulfur-oxidizing bacteria, producing rock trainage, in the tailings of Bozânta pond

In order to carry out the physical, chemical and microbiological analyzes necessary to assess the degree of oxidation of the flotation tailings from the Bozânta pond, 55 tailings samples were taken (fig. 4.3): 40 samples, representing 5 average samples from each terrace of the S_4 sectors and S_5; 10 samples from the crown of the pond, from the perimeter of sectors S_4 and S_5; 5 samples from the beach, in the perimeter of the S_5 sector, at a crowning distance of 25 m inside the beach.


a


b

Fig. 4.3 Sterile with varying degrees of alteration
a - barren on the crown of the S_5 sector; b - tailings samples from the terraces of the S_5 sector

4.2.1 Physical and chemical analyzes of tailings samples

The equipment used to carry out the physico-chemical analyzes of the samples were the HACH HQ40d digital multimeter and the Palintest multiparameter, and the chemical analysis was carried out using an atomic absorption spectrometer (AAS) – Perkin-Elmer 3110.

Sector S_4

It is observed that the effects of the biooxidation processes are different, depending on the age of the tailings, the sampling depth and the arrangement of the terrace surfaces (tab. 4.2-4.4).

Table 4.2 Physico-chemical analysis, of tailings samples S_4T_1

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S_4/T_1	0-5	7,6	4,86	130,2	108,3	77,1	50,8
	5-10	6,2	5,17	111,0	92,8	66,6	44,6
	10-15	4,0	6,36	47,0	63,2	45,1	32,8
	15-20	3,5	6,30	46,7	66,2	47,0	34,3
	20-25	3,5	5,81	76,5	79,8	57,3	39,7

Table 4.3 Physico-chemical analysis, of tailings samples, S_4T_2

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S4/T2	0-5	1,3	5,09	115,1	110,5	78,7	51,5
	5-10	1,2	5,80	77,7	85,3	51,2	41,8
	10-15	2,0	6,80	22,5	69,7	49,6	35,5
	15-20	2,2	6,82	21,5	96,5	68,5	46,0
	20-25	3,8	7,19	2,9	119,5	85,0	55,2

Table 4.4 Physico-chemical analysis, of tailings samples, S_4T_3

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S4/T3	0-5	2,0	3,46	202,2	463,0	328,0	201,0
	5-10	5,3	4,62	140,0	201,0	143,0	88,5
	10-15	3,3	5,68	83,6	76,0	54,0	37,8
	15-20	2,9	5,88	72,7	77,2	55,0	38,5
	20-25	2,8	6,75	26,5	94,5	67,1	45,1

In terraces T_1 and T_2, the tailings are older. The layer of earth applied to the surface of terraces T_1 and T_2 and the plant biomass from the vegetation had the effect of reducing the oxidative bacterial processes, by reducing the amount of oxygen. In the tailings from the terraces not covered with soil, the pH was more acidic, 3.5–6.7 in T_3 and 2.5–3.1 in T_4, compared to the values from the terraces covered with soil and plant biomass, 4, 8–6.4 in T_1 and 5.1–7.2 in T_2. Although the tailings from the T_4 terrace are the least old, here the most obvious oxidative processes are manifested (tab. 4.5-4.6).

Table 4.5 Physico-chemical analysis, of tailings samples, S_4T_4

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S_4/T_4	0-5	1,5	3,10	222,1	1310,0	925,0	580,0
	5-10	3,0	2,56	249,9	1905,0	1,35	863,0
	10-15	4,4	2,66	244,5	1992,0	1,42	907,0
	15-20	6,5	2,68	243,8	1993,0	1,42	901,0
	20-25	7,8	2,67	244,1	1984,0	1,41	895,0

Table 4.6 Physico-chemical analysis, of tailings samples, S_4C

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S_4/C*	0-5	4,2	3,50	200,5	163,2	116,0	72,9
	5-10	9,0	3,65	193,2	138,0	97,6	62,6
	10-15	10,0	3,78	185,5	132,5	93,5	60,2
	15-20	12,3	3,95	176,2	160,2	114,0	71,6
	20-25	11,1	3,62	194,0	159,7	110,0	70,9

S_4C* - coronament, sectorul S_4

Maintaining a pH suitable for plant growth, between 5.0 and 7.2, allowed the installation in the T_2 terrace. In the tailings in the upper part of the sector, on the crown, where the humidity is kept higher than in the terraces, the bacterial processes of acid drainage are very active, in all samples the pH value is between 3.5–3.9. Here the oxidation processes are more obvious.

Sector S_5

The absence of a layer of earth in this sector led to the triggering of the biooxidation phenomena of the tailings shortly after being deposited on the pond: the first signals of the drying of the vegetation were made as early as 1992, just 7 years after the planting (1985) of the two acacia species, *Robinia hispida* and *Robinia pseudoacacia*, on the T_2 terrace and 15 years after depositing the tailings in the pond (tab. 4.7-4.12).

Table 4.7 Physico-chemical analysis, of tailings samples, S_5T_1

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S_5/T_1	0-5	1,8	4,13	166,0	77,4	55,2	37,7
	5-10	5,3	4,55	99,5	67,6	48,0	34,9
	10-15	6,1	5,07	127,5	127,1	90,6	56,9
	15-20	6,7	4,72	146,4	103,9	73,1	47,2
	20-25	6,5	4,36	139,0	86,0	61,8	42,4

Currently, the pH of the tailings in all the terraces of this sector has values below the limit that the vegetation can tolerate: 4.1–5.1 in T_1; 3.8–4.2 in T_2; 3.7–4.2 in T_3 and 2.2–3.9 in T_4.

On the crown, high humidity, 5.4–10.4%, sustains the sustained activity of acid mine rock drainage by iron- and sulfur-oxidizing bacteria.

Table 4.8 Physico-chemical analysis, of tailings samples, S_5T_2

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S_5/T_2	0-5	4,1	3,8	188,0	86,2	61,1	41,4
	5-10	5,2	3,83	191,0	98,4	70,0	45,5
	10-15	6,6	3,96	185,7	103,8	73,7	47,7
	15-20	7,1	4,15	174,5	113,4	80,5	51,6
	20-25	9,4	4,10	176,7	100	71,6	46,0

Table 4.9 Physico-chemical analysis, of tailings samples, S_5T_3

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S_5/T_3	0-5	4,3	3,5	204,0	130,4	92,7	59,0
	5-10	7,2	3,65	202,0	116,7	82,8	53,4
	10-15	7,6	3,69	195,0	105,1	75,0	49,0
	15-20	7,5	4,14	170,0	78,1	54,5	38,0
	20-25	7,7	4,24	166,0	63,7	45,2	32,5

Table 4.10 Physico-chemical analysis, of tailings samples, S_5T_4

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S_5/T_4	0-5	1,0	2,13	279,0	1480	1,66	665
	5-10	4,4	2,22	273,0	2,05	1,45	910
	10-15	5,1	2,74	243,4	1870	1,33	845
	15-20	6,6	2,63	250,0	2,03	1,44	887
	20-25	5,8	3,93	178,0	1610	1,14	724

Table 4.11 Physico-chemical analysis, of tailings samples, S_5C

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S_5/C*	0-5	5,4	2,78	244,4	443	3,15	192
	5-10	8,3	2,85	240,0	1525	1,08	680
	10-15	8,6	2,91	234,4	1800	1,28	813
	15-20	9,5	2,88	234,0	1780	1,29	819
	20-25	10,4	2,75	244,0	1798	1,28	813

S_5/C* - coronament, sectorul S_5

Table 4.12 Physico-chemical analysis, of tailings samples, S_5P

Proba	Depth (cm)	Humidity (%)	pH	Eh (mV)	Cond (µS/cm)	TSD (ppm)	Salinity (ppm)
S_5/L*	0-5	3,2	2,97	234,0	3	213	130
	5-10	6,4	2,75	246,0	424	300	184
	10-15	32,0	2,55	257,0	1730	1,22	779
	15-20	28,4	3,81	189,0	1531	1,09	680
	20-25	25,9	3,97	180,0	1452	1,03	647

S_5P* - plaja lacului, sector S_5

The phenomena are just as intense on the beach of the lake, where the humidity in the layers deeper than 10 cm exceeds 25%, ensuring optimal conditions for bacteria to live.

The results of the chemical analyzes of the average tailings samples from the T_2 terraces of the S4 and S5 sectors are presented in tables 4.13–4.16.

The iron and sulfur content in the samples from both sectors (mostly in the form of pyrite) indicates the still quite high potential to sustain acid drainage processes. The reserve of iron and sulfur is even higher in the tailings from sector S_4, while part of the iron and sulfur from sector S_5 has already entered the biogeochemical cycle generated by the action of iron- and sulfur-oxidizing bacteria.

The concentrations of heavy metals in the solutions, especially those in the tailings from the S_5 sector, represent a phytotoxic potential that explains the drying of the vegetation on the T_1 and T_2 terraces in this sector, together with the very acidic pH.

Table 4.13 Chemical analysis, of tailings samples, S_4T_2

Elements	The content in heavy metals and other elements (%)					
	Sampling depth (cm)					
	0-5	5-10	10-15	15-20	20-25	0-25
Cu	0,18	0,15	0,11	0,10	0,08	0,14
Pb	0,11	0,10	0,09	0,09	0,10	0,10
Zn	0,81	0,72	0,48	0,53	0,42	0,65
Fe	6,89	5,04	5,20	5,34	5,87	6,07
S	1,68	1,55	1,66	1,33	1,56	1,57
Ca	1,06	0,89	0,65	0,76	1,23	0,79
Mg	0,74	0,66	0,67	0,63	0,69	0,64
SiO_2	62,65	68,76	73,16	67,58	77,16	68,20

Table 4.14 Chemical analysis of the eluate 1:5 from the tailingssamples, S_4T_2

Elements	The content in heavy metals and other elements (ppm)					
	Sampling depth (cm)					
	0-5	5-10	10-15	15-20	20-25	0-25
Cu	1,42	1,12	0,78	0,43	<0,05	0,84
Pb	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Zn	0,06	0,06	<0,05	<0,05	<0,05	<0,05
Fe	0,146	0,068	0,06	0,06	0,06	0,092
SO_4	784	686	720	1480	1440	882
CaO	58,2	66,82	128,4	164,4	208,6	98,48
Mg	12,6	14,6	18,4	17,0	16,5	16,12
pH	7,14	7,28	7,22	7,36	7,40	7,26
Eh (mV)	-4	-8	-7	-14	-18	-9

Table 4.15 Chemical analysis, of tailings samples, S_5T_2

Elements	The content in heavy metals and other elements (%)					
	Sampling depth (cm)					
	0-5	5-10	10-15	15-20	20-25	0-25
Cu	0,02	0,02	0,03	0,03	0,07	0,03
Pb	0,08	0,09	0,09	0,04	0,04	0,08
Zn	0,58	0,66	1,07	1,14	1,11	0,82
Fe	2,94	2,76	2,43	2,68	3,08	2,77
S	1,14	0,96	0,88	1,43	2,21	0,97
Ca	0,14	0,48	0,54	0,92	0,87	0,46
Mg	0,28	0,14	0,22	0,48	0,44	0,36
SiO_2	74,82	75,05	75,49	74,44	75,63	75,18

Table 4.16 Chemical analysis of the eluate 1:5 from the tailingssamples, S_5T_2

Elements	The content in heavy metals and other elements (ppm)					
	Sampling depth (cm)					
	0-5	5-10	10-15	15-20	20-25	0-25
Cu	7,64	7,42	8,92	9,4	9,83	8,34
Pb	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Zn	52,46	76,20	96,43	104,68	167,3	78,45
Fe	1276,4	3870,0	676,4	573,6	29,61	1459,7
SO_4	4800	1745	1972	1620	2960	2243
CaO	827,3	842,4	294,8	496,7	754,3	635,2
Mg	68,0	124,8	65,4	44,2	38,8	71,5
pH	3,16	2,92	3,8	4,23	4,56	3,84
Eh (mV)	+227	+238	+185	+179	+158	+183

4.2.2 Microbiological anlyzes of tailings samples

The identification of bacterial species, the knowledge of their role in the stages of the acid drainage processes of rocks and their numerical ratio, are useful information, necessary, together with the physical and chemical characteristics of the materials subject to degradation, for the evaluation of the stage in which the process is found, the estimation of development trends , but also for the research and application of appropriate measures to stop/reduce the degrading effects. The microbiological characterization of tailings samples involved the isolation and numerical analysis of the iron- and sulfur-oxidizing species already identified on the Bozânta pond in previous studies (Jelea, 2014; Jelea, 2015; Jelea & Baciu, 2023): *Acidithiobacillus ferrooxidans*; *Leptospirillum ferrooxidans*; *Acidithiobacillus thiooxidans*; *Thiomonas intermedia* and *Starkeya novella*. Numerical analysis: by the method of determining the most probable number - NCMP (eng. Most Probable Number - MPN), on dilutions up to 10-7, with seeding in 3 test tubes in parallel. For each tailings sample, inoculations were made for the identification and numerical estimation of three groups of bacteria: a) the group of iron- and sulfur-oxidizing bacteria, which is analyzed together with the iron-oxidizing bacteria, represented by the species *A. ferrooxidans* (acidophilic, iron- and sulfur-oxidizing) and *L. ferrooxidans* (acidophilic, iron oxidizing); the two species are analyzed on the same nutrient medium; b) sulfur-oxidizing bacteria, *A. thiooxidans* (pH 1–7, does not oxidize iron, oxidizes elemental sulfur, thiosulfate and tetrathionate to sulfuric acid); c) the group of bacteria *T. intermedia* and *S. novella* (slightly acidic pH, facultatively heterotrophic); the two species are analyzed together on thiosulphate medium. For the identification and quantitative estimation of bacteria from the *A. ferrooxidans* and *L. ferrooxidans* group, Mackintosh medium (1978) was used. Hutchinson's medium (Hutchinson et al., 1965) was used for the identification and quantitative estimation of the sulfur-oxidizing bacterium *A. thiooxidans*. The identification and quantitative estimation of the group of *T. intermedia* and *S. novella* bacteria was carried out using the modified Matin and Rittenberg medium (1971).

Figure 4.4 shows the nutrient media after sterilization.

Fig. 4.4 Nutrient media after sterilisation

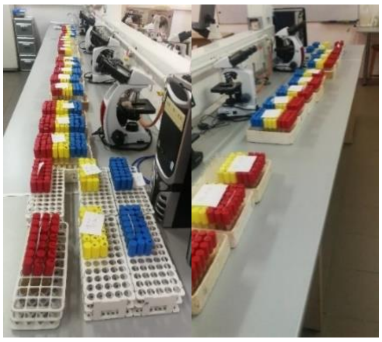

Fig. 4.5 Inoculated nutrient media

After seeding (fig. 4.5), the 3 series of 21 test tubes with media specific to each group of bacteria (in total 61 test tubes for each sample, 315 test tubes for each sampling point, 3355 test tubes for the samples from the terraces and the pond beach), were incubated for 28 days, in incubators with constant temperature, at 28ºC (fig. 4.6).

After the 28 days of incubation, the presence of bacteria was identified in the series of test tubes with the seeded media and the scoring of the positive test tubes:

- the identification of the presence of the group of iron-oxidizing bacteria *A. ferrooxidans* and *L. ferrooxidans* was made based on the coloring of the bacterial environment, from yellow-green to red-brown, as a result of the formation of iron hydroxide $Fe(OH)_3$ through the oxidation of ferrous sulfate $FeSO_4$ (fig. 4.7);
- the identification of the presence of the sulfur-oxidizing bacteria *A. thiooxidans* was achieved based on the decrease in pH in the environment, from 4.5 to pH <3.5, conditioned by the formation of sulfuric acid, as a result of the bacteria's oxidation of the sulfur added to the bacterial environment ; the pH in each tube was measured and positive tubes with pH <3.5 were noted;
- the identification of the presence of the group of bacteria *T. intermedia* and *S. novella* was achieved based on the increase in acidity in the environment, from 6.7 to pH <5.5, conditioned by the formation of sulfuric acid through the oxidation of sodium thiosulfate, $Na_2S_2O_3$; the pH in each tube was measured and positive tubes with pH <5.5 were noted.

The interpretation of the results, based on the test tubes marked as positive from the series of 21 test tubes with the nutrient medium seeded from the dilution series, is a statistical calculation made with the help of the McCrady table (McCrady, 1915; Swaroop, 1938).

- **Sector S_4**

On **terrace T1**, the species *A. ferrooxidans* and *L. ferrooxidans* were identified in all medium samples, from 103 bacteria/ g sterile, at a depth of 0–15 cm, to 36 bacteria/ g sterile at a depth of 25 cm; the number of bacteria in the tailings decreased with the increase in the pH value (from 4.8 to 6.4) and the numerical increase in the presence of the other species, *T. intermedia* and *S. novella*, whose density exceeds $1.1x10^9$ bacteria/ g sterile.

- The species *A. thiooxidans*, with a wide pH spectrum, is the premise of the acidification of the underlying layers, through the intense activity of transforming sulfur into sulfuric acid, which will cause the proliferation of the iron-oxidizing species *A. ferrooxidans* and *T. ferrooxidans* and the numerical decrease of the species less acidophilus, *T. intermedia* and *S. novella*. The soil layer deposited on the surface of the terrace at planting and the plant biomass accumulated over time kept at a moderate level the factors that were potentially phytotoxic for plants. The phenomena of seepage of solutions and transport of tailings from the beach of the pond and from the upper terraces, the exfiltrations from the bottom of the pond and from the level of the terrace have damaged the quality of the environmental factors, so there are prerequisites for the acceleration of the microbiological processes of acid drainage, which will leads to an increase in the phytotoxicity of the substrate in this terrace.

Fig. 4.6 Incubate for 28 days the test tubes with the seeded media

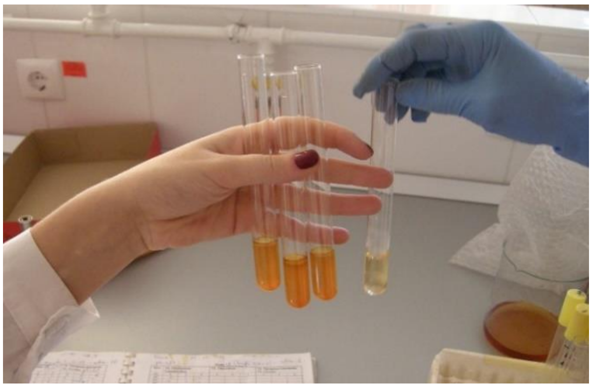

Fig. 4.7 Presence identification iron- and sulfur-oxidizing bacteria

In **terrace T_2,** you can see the layer of tailings colored in yellow, as a result of intense oxidative acidification activities, under the influence of the three sulfur-oxidizing bacterial species; The pH, still in the neutral and slightly acidic range (5.0–7.2), at the depth level of the roots of grass plants allows them to grow well, without visible manifestations of phytotoxic pressures; the numerical level of sulfur-oxidizing bacteria and the acidification of the surface layer, 0–10 cm, is a signal regarding the future trends of acceleration of degradative phenomena.

Terrace T_3, the acacia plantation in pits with borrowed soil, but without a covering layer of soil on the rest of the surface, is found in a similar stage to that of terrace T_2, in terms of the numerical values and the proportions between the bacterial species installed in sterile; it must be emphasized, however, that the pH of the tailings has a lower degree of acidity and the appearance of the surface much more damaged compared to the T_2 terrace. On this terrace, the tailings colored yellow as a result of the presence of sulfuric acid, the superficial layer where pyrite oxidation activities also take place under the influence of iron- and sulfur-oxidizing bacteria, is periodically entrained by the solution leaks from the upper terraces. As a result of the unfavorable conditions, the herbaceous plant species have already disappeared, while the acacia plantation presents the whole range of aspects regarding the deterioration of the vegetation.

The conditions on the **T_4 terrace** allowed the installation of the phenomena of acid drainage of the rocks at a rate that led to acidic pH values, between 2.6-3; the degree of alteration of the tailings and the erosive manifestations on the T4 terrace being an example of the speed of deterioration of the substrate under the influence of mining rock drainage processes.

In the tailings from this terrace, sulfur-oxidizing bacteria are present throughout the studied depth, while iron-oxidizing bacteria have an intense iron-oxidizing activity in the upper, more oxygenated part of the tailings column.

On the crown, **S_4C**, where the tailings are 15–17 years old, oxidation phenomena are very active under the influence especially of iron- and sulfur-oxidizing bacteria of the species *A. ferrooxidans.* The sulfur-oxidizing bacteria *A. thiooxidans* are active in the layer from 20–25 cm deep, which signifies the advancement in depth of the acid drainage phenomena of the rocks.

The damage of the 20–25 cm layer will lead to the intensification of the sliding phenomena of the tailings layers, between which areas with smaller grain and with a high capacity to retain water films arise.

Sector S_5

In the T_1 terrace, with tailings deposited in the period 1975–1980, the phenomena of acid drainage of the rocks were visible since the period 1992–1995: the drying of the acacia branches; drying of herbaceous plants; red coloring of the waste; erosion, rain and wind phenomena.

30 years after these observations, the processes of acid drainage of the rocks are accelerated by the large number of bacteria: >10^9/g sterile *A. ferrooxidans* and *L. ferrooxidans*; 106/ g sterile *A. thiooxidans* and 10^2–10^7/ g sterile *T. intermedia* and *S. novella*. Acidification of the tailings, pH 4.1–5.1 led to the almost total destruction of acacia specimens and spontaneously installed species. As a result of the chemical processes of oxidation, tailings particles and exchange of granulometric waters, the dimensions decrease, the erosion processes produced by rain or wind are much more intense, which leads to massive dislocations of tailings material.

As a result of the deposition of a layer of soil in the area of the ornamental acacia (*R. hispida*) plantation, terrace T_2 later came under the influence of acid drainage phenomena.

Numerous herbaceous and arboreal plant species have been installed on the surface of the T_2 terrace, with a role in maintaining stability. The results obtained highlighted the fact that the soil layer decreased the installation time of the acid drainage process. All the bacterial species that contribute to the degradation of the tailings were isolated from the analyzed samples, in proportions that highlight the acceleration of the degradative processes: 10^2-10^3/g of tailings *A. ferrooxidans* and *L. ferrooxidans*; 10^4/g of *A. thiooxidans* sterile and 10^7–10^9/g sterile of *T. intermedia* and *S. novella*.

On terrace T_3, the presence of the three physiological groups of bacteria is dominated by the thiosulphate-oxidizing bacteria, *T. intermedia* and *S. novella*, but the sulfur-oxidizing bacteria *A. thiooxidans* and the iron-oxidizing ones *A. ferrooxidans* and *L ferrooxidans* are also present in large numbers, pH- ul 35–4.2 being suitable for the specialty of all species present in the tailings.

Acidic pH values led to deteriorating conditions for all plant species, even those considered acidophilic. The waste is highly oxidized, and the surface of the terrace shows many pits, gullies and exfiltrations.

The T_4 terrace, on which no planting or earth protection works were carried out, shows a high degree of acid drainage phenomena of the rocks under the influence of all groups of bacteria, which are found in very large numbers: >10^9/g of sterile *A. ferrooxidans* and *L ferrooxidans*; 10^6/g of *A. thiooxidans* sterile and 10^8/g of *T. intermedia* and *S. novella* sterile.

The deposition of materials with a high pyrite content on this terrace and on the crown, shortly before the transition of the pond to conservation, is the cause of very active acid drainage processes, resulting in acidification at the extremely acidic pH level, 2.1-3.9. As a result of the position of these deposits on the highest surface of the pond, the acid tailings are continuously dispersed over long distances, in the adjacent inhabited areas.

The same situation is also valid for the crown of the pond in the area of sector five, S_5C, in the composition of which materials similar to those stored in terrace T4 are found.

The lake beach, S_5P, was made using the tailings transport and deposition technique by hydrocycling: the finer fraction of the tailings was deposited in the settling pond, together with the transport water, while the coarser fraction was deposited on the terraces. The beach of the lake, immersed during the period of operation of the pond, is made up of the finest particles deposited in the pond. They provide a large contact surface with iron- and sulfur-oxidizing microorganisms.

By removing the superficial layer of oxidized tailings, new material is brought to the surface with a high content of pyrite and other sulphides that enter the degradative processes of acid drainage; thus new surfaces become the site of action of iron- and sulfur-oxidizing bacteria.

I propose the following model to estimate the stage and evolution of the acid drainage process of the rocks in the tailings from the terraces of the settling pond under the influence of bacteria of the *A. ferrooxidans*; *L. ferrooxidans; A. thiooxidans; T. intermedia and S. novella* species:

1. Although all three groups of bacteria were constantly identified in the tailings samples, analyzing their numerical distribution according to the diversity of environmental conditions existing at a given moment, it is considered that there is a sequence of their installation, in which one physiological group "prepares" the necessary conditions for another physiological group that continues / amplifies the action of acid drainage of the rocks.

2. In the non-oxidized tailings from the processing of non-ferrous metal ores, with pyrite content and neutral pH, the acid drainage process of the rocks was triggered by the action of the sulfur-oxidizing bacteria *S. novella* and *T. intermedia*.

3. The ability of the species S. novella to live as a facultative chemolithoautotroph and methylotroph, at high pH values, gives it the advantage of being among the first to settle in an environment less accessible to other species. Through the metabolic activity of degrading a wide range of sulfur compounds, with the formation of thiosulfate and sulfate, the pH of the fresh tailings decreases, from neutral values 7.0–7.5, to slightly acidic, <6.5, to acid, 4.5.

4. The drop in pH below 6.5, the presence of thiosulfate and sulfate produced by *S. novella* are conditions that favor the installation of another species with requirements for a less acidic pH, such as *T. intermedia*. The ability of this bacterium to live as a facultative heterotroph, also using organic substances from the living environment (for example glucose and other substances that come from the degradation of plant biomass), gives it a great advantage for proliferation on vegetated terraces, where it accelerates the evolution of drainage phenomena acid of the waste.

5. The activity of the *A. thiooxidans* species can be carried out simultaneously with the thiooxidizing species in a wide pH spectrum, from neutral and weakly alkaline 7-8, to extremely acidic 1.0, the condition being the presence of elemental sulfur and its derivatives (sulfite , thiosulphate, tetrathionate) which they oxidize to sulfuric acid. It does not oxidize the sulphides or the iron in the pyrite existing in the tailings; however, by producing sulfuric acid, it indirectly solubilizes metals and other elements in the tailings and lowers the pH value below the survival limit of thiooxidants.

6. The <4 pH value, as a result of the activity of the *A. thiooxidans* species, allowed the establishment of the iron- and sulfur-oxidizing bacteria *A. ferrooxidans*. Their metabolic activity in tailings is particularly complex: they oxidize elemental sulfur S^o, all sulfides with S^{2-} (for example, pyrite, present in large quantities in tailings deposited in sectors S_5–S_{11}) and intermediate degradation compounds, to sulfuric acid; oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). Through this complex activity, it has a particular impact in increasing the speed of acid drainage processes in the tailings from the terraces.

7. Following the metabolic activities of the species *A. ferrooxidans*, through the oxidation of pyrite, ferrous iron (Fe^{2+}) results in the environment, the substrate required for the oxidation activity of the species *L. ferrooxidans*. Although the oxidation spectrum of *Leptospirillum* is limited only to the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), it is favored by a numerical increase and an accelerated iron-oxidizing activity at temperatures above 30ºC, which tailings in the tailings pond provides a large part of the year. However, high temperatures

decrease the iron-oxidizing activity of the species *A. ferrooxidans*. Together, these two species ensure a continuous oxidation of pyrite and the processing of intermediate compounds.

8. The metabolic activity of pyrite degradation by the species *A. ferrooxidans* also results in the release of sulfur. When the numerical decrease or the decrease in the metabolic activity of the species *A. ferrooxidans*, the continuation of the pyrite oxidation in the tailings takes place by means of the ferric iron (Fe^{3+}) provided by the oxidative processes produced by *L. ferrooxidans*; by the "indirect mechanism", of chemical oxidation of pyrite; sulfur leaves the crystal lattice, in the S^{2-} form, in the environment as elemental sulfur S^{o}.
9. Elemental sulfur, S^{o}, can accumulate in the environment or be oxidized by the sulfur-oxidizing bacteria, *A. thiooxidans*, or those of the *A. ferrooxidans* species, upon resumption of activity, continuing the acid drainage processes.

Of course, all this unfolds in a complex interrelationship, which conditions the dynamics of acid drainage processes in tailings in the settling pond.

STUDY OF THE EFFECTS OF HEAVY METALS ON PLANTS USED IN REVEGETATION WORKS

The purpose of the research

The purpose of the research was to test some species of herbaceous plants regarding their growth in the conditions offered by the flotation tailings from the Bozânta pond and to develop some mixtures with amendments in order to create a vegetal carpet.

Experimental activities

In order to test the growth of plant species under the conditions provided by the flotation tailings from the terraces of the settling pond, ecotoxicity tests were carried out regarding the germination of seeds and the growth of vegetative organs of plants. The experiments were carried out in accordance with the requirements of the ISO 11269–1:2012 and ISO 11269–2.2012 protocols.

During the research, the following types of activities were carried out:

1. Highlighting the effects of the industrial eluate on the seeds, by carrying out germination tests regarding the germination capacity (FG), germination energy (EG), germination percentage (PG) and germination index (GI);

2. Highlighting the effects of the industrial eluate on the growth of the vegetative organs of the plants (root system and aerial parts): evaluation of the average length of roots and stems; stress tolerance highlighting - stress tolerance index (IT%); evidence of root growth inhibition (ICR%); dry biomass.

3. The study of plant emergence and growth on the tailings from the pond and on the tailings mixed with the incorporated amendments in order to improve plant nutrition conditions.

5.1 Germination tests. Highlighting the effects of industrial eluate on germination indicators

5.1.1 Principile of metod

The germination test consists in establishing the germinal energy (EG) and the germinative faculty (FG), ensuring the seeds optimal conditions of humidity, temperature and aeration (Cenușă et al., 2015).

Taking into account the longer time required for seed germination and the slow growth of vegetative organs in one of the tested species (Agrostis capillaris) and in the absence of references from the specialized literature, in the present experiments we set for both grass species 7 days for energy germinative (EG_7), respectively 14 days for the germinative faculty (FG_{14}), in accordance with the experiments carried out on the tailings from the beach of the Bozânta pond (Jelea & Baciu, 2022). The germination process was monitored during 28 days.

The germination index (GI) is an indicator calculated based on the results obtained after the completion of the 14 days specific to the germinating faculties for the two grass species.

5.1.2 Materials and metods

The biologic material use

The germination tests were carried out for two species of monocotyledonous herbaceous plants, from the Poaceae family: *Lolium perenne* and *Agrostis capillaris*, due to the following considerations: they are perennial plants, they have fasciculate roots, pollination is anemophilic.

Both species are found in the list of terrestrial plants used for testing vegetative vigor when exposed to chemical products: *Lolium perenne* in Annex 2 List of species historically used in plant testing and *Agrostis capillaris* in Annex 3 Potential species for plant toxicity testing, from the OECD Guidline for the testing of chemicals (OEC, 2006).

L. perenne L. (ryegrass, lawn grass, sedge) is suitable for planting on soils contaminated with heavy metals. Studies have shown that it can rehabilitate soils contaminated with Cd and Pb (Arienzo et. al., 2004). It is not a metal hyperaccumulating plant, but it is tolerant to some heavy metals, resistant to adverse conditions and allows rapid soil coverage in the areas where it is planted (Casler & Undersander, 2018; Masotla et al., 2023).

Certified *Lolium perenne* seeds, produced and distributed by SC Everde SRL Satu Mare, lot EV0395-3SM01, were used in the experiments.

A. capillaris L., synonym *A. tenuis* Sibth. (field grass) is part of the native plants recommended for revegetation of lands contaminated with heavy metals (Wang & Delavar, 2023). It is found in meadows that populate gently or moderately sloping slopes on well-drained, poorly to moderately nutrient-rich, slightly acidic soils.

The seeds of *A. capillaris* used in the experiments were obtained from plants taken from the Bozânta pond.

Experimental conditions

Germination experiments were carried out in Linhard type ceramic vessels.

For each plant species there were 3 repetitions: 3 control lots (control) and 3 experimental lots (test). 100 seeds were used for each batch (fig. 5.1).

The experiments were carried out at a temperature of 20±2^{o}C, with variations between day and night periods. The average relative humidity was between 55–65%. The photoperiod was between 10–11 hours.

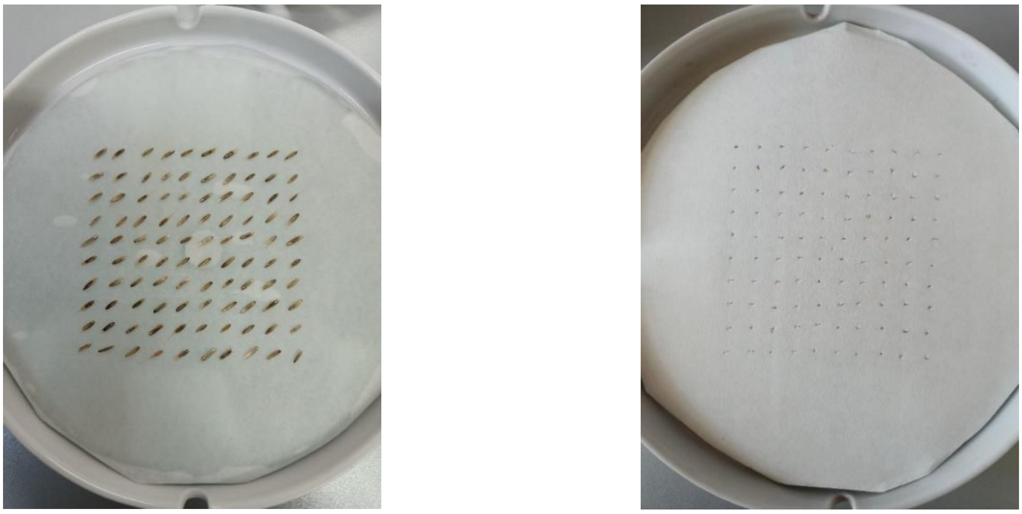

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Fig. 5.1 Linhardt germination pots with batches of 100 seeds
1. *Lolium perenne*; 2. *Agrostis capillaris*

Trace mineral water was used to moisten the seeds in the control batches.

The seeds from the test lots were moistened with an industrial eluate, obtained according to the soil extract method from the average tailings sample collected from the depth of 0–20 cm from the terrace T2 of the S5 sector. The chemical composition (mg/l) and physico-chemical parameters of the industrial eluate were as follows: Cu – 8.34, Pb <0.1, Zn – 78.45, Fe^{Total} – 1459.7, Ca^{2+} – 635.2, Mg^{2+} – 71.5, Na^{+}– 1.25, SO_{4}- – 2243, dissolved oxygen – 11.5, Cl^{-} – 2.30, ammonium (NH^{4+}) – 10.6, nitrate (NO^{3-}) – 36.5, total dissolved solids (TSD) – 174; oxygen saturation – 106%, redox potential (mV) – 263.5, conductivity (μS/cm) – 135.5, salts – 52.3, pH – 3.8.

The dynamics of seed germination was followed daily, during 28 days.

The results obtained after 7, 14 and 28 days were taken into account for the calculations regarding the indicators of the germination process.

5.1.3 Rsults and discussions

5.1.3.1 Determination of the faculty and the germinative energy for the grass species (*Lolium perenne*)

Figures 5.2 and 5.3 show the results obtained in the control and test experiments. The maximum values for EG_7 were reached after 6 days, both for the control variant and for the test, and those for FG_{14}, at 14 and 13 days, respectively. Germination percentage at 28 days, PG_{28}, increased insignificantly compared to FG_{14}, both for the control variant and for the test variant.

In the graphic presentation in figure 5.3, regarding the dynamics of the germination process, it is observed that in the case of the control variant, more than 50% of the seeds germinated in the first 3 days, and for the variant with industrial eluate, a similar percentage was reached one day later.

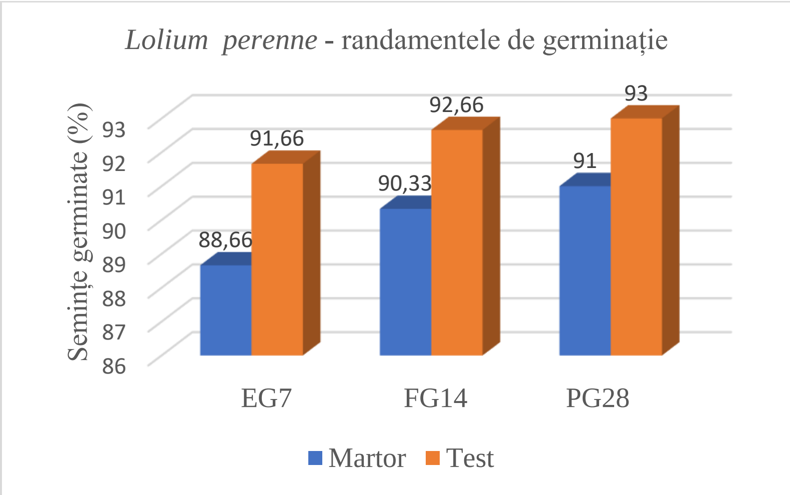

Fig. 5.2 Germination yields for *Lolium perenne*

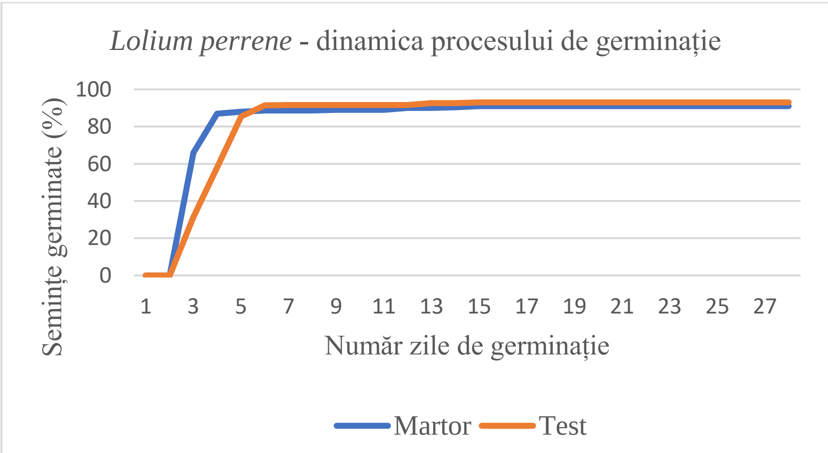

Fig. 5.3 The dynamics of the germination process for *Lolium perenne*

The differences between the germination percentages in the control and test variants were relatively small, 2-3%, but throughout the 28 days, in favor of the test variant.

The germination index, IG_{14} with a value of 102.6%, indicates an insignificant difference, between the control and the test, which leads to the conclusion that, for *L. perenne*, the presence of metals and the acidic pH of the industrial eluate did not have significant effects in the germinal process.

5.1.3.2 Determination of the faculty and the germinative energy for the grass species *Agrostis capillaris* (field grass)

The results obtained in the control and test experiments on the germination of field grass seeds are presented in figures 5.4 and 5.5. The FG_{14} germination capacity, after 14 days, was 27.33% for the control variant and 77% for the test, as a result of the germination of 82 and 231 seeds, respectively (fig. 5.4).

Throughout the monitoring period, the differences between the germination percentages of the control and test batches were significant: 10% for EG_7, 49.67% for FG_{14} and 28.34% for PG_{28}, all in favor of the test variant, with industrial eluate.

Regarding the dynamics of the germination process (fig. 5.5), in the case of the control variant the germination process started on the fourth day, one day after the test variant. In the test groups more than 50% of the seeds germinated after 10 days, while in the control groups it took 28 days to reach the same percentage.

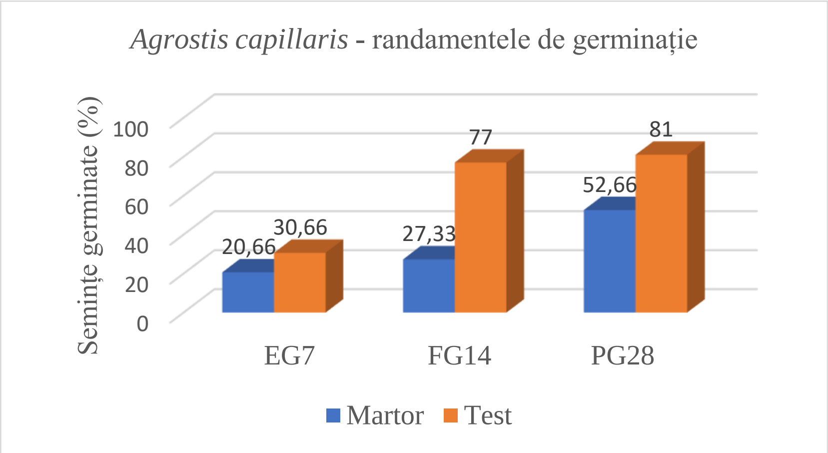

Fig. 5.4 Germination yields for *Agrostis capillaris*

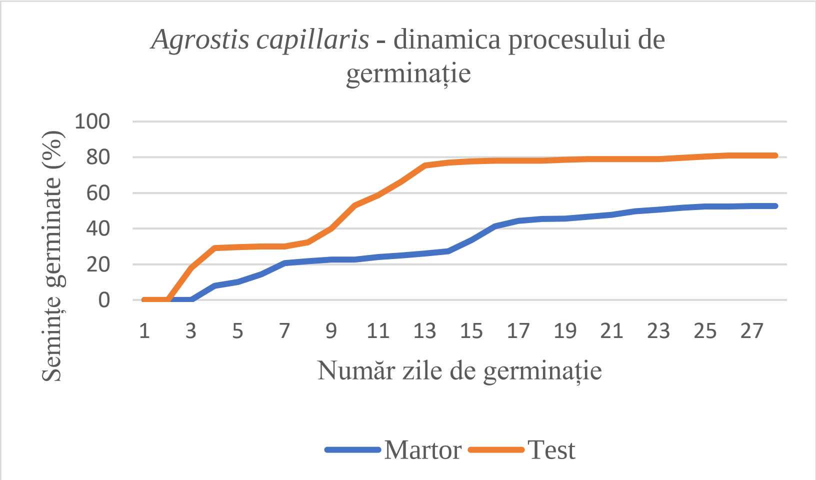

Fig. 5.5 The dynamics of the germination process for *Agrostis capillaris*

At the end of the monitoring period, a significant difference was observed between the percentage of germination at 14 days (27.33%), respectively at 28 days (52.66%) in the case of the control variant, which means a slower development of the germination process, unlike the test variant where growth during the period 14-28 days, was from 77% to 81%, of only 4%, most of the seeds germinating in the first 14 days.

For *A. capillaris*, the inhibitory effect of the alkaline pH in the trace mineral water was visibly manifested: in the control variant, the germination process was delayed by two weeks, and the percentage of germination at 28 days was much lower than that obtained in the experiments with acidic solution. The effect of alkaline pH was manifested by doubling the number of days required to germinate the maximum number of seeds in the control experiment. Also, the number of germinated seeds at 14 days was 49.67% lower compared to the test variant and remained at 28.34% at 28 days.

The germination index, IG_{14}, whose value was 281.7% for *A. capillaris*, shows that the species exhibits tolerance to heavy metal contents in the industrial eluate during the germination period, but was sensitive to alkaline values of pH- of trace mineral water.

For *A. capillaris* seeds harvested from the Bozânta pond terraces, where the pH conditions are acidic, the industrial eluate had a stimulating effect on germination.

The results justify the choice of monitoring periods for germination indicators and the need to follow the processes over a long period of time (eg PG at 28 days).

5.2 Growth testis. Highlighting the effects of industrial eluate on the growth of vegetative organs of plants

5.2.1 Principle of method

Growth tests of vegetative organs are often more sensitive indicators of seed quality than those of germination, the phytotoxic effects caused by heavy metals being much more visible (Barral & Paradelo, 2011). Measure the growth in length of roots and stems and weigh the biomass of stems and, if possible, the biomass of roots. The statistical estimation of the differences in the growth of the vegetative organs between the control and test variants was carried out by the t-Student test. Phytotoxic effects on the growth of vegetative organs were estimated with stress tolerance index (IT%) and root growth inhibition index (ICR%). The stress tolerance index (SI%) is a measure of a plant's ability to survive under stress conditions and is used to assess the ability of organisms to tolerate and accumulate heavy metals. It is an indicator of phytotoxic effects on root growth. The calculation of the root growth inhibition index (ICR%) was made based on the average values, following the measurements made in the control and test groups. If ICR% >0, this denotes an inhibitory effect, while an ICR% <0, means growth stimulation (Vaverková et al., 2020).

5.2.2 Materials and methods

To highlight the effects of the industrial eluate, measurements were made of the length of the vegetative organs at 7, 14 and 28 days, of the seedlings from the control and test groups.

The dry biomass of the whole seedlings (stems and roots) was weighed at 28 days. The plant material was brought to constant weight in the oven at a temperature of 105°C.

5.2.3 Rezults and discission

5.2.3.1 Evaluation of the main seed quality indices regarding growth and development capacity in the species *Lolium perenne*

Tables 5.1 and 5.2 present the average values and statistical estimates with reference to the length of vegetative organs of *L. perenne* seedlings at 7 days.

Tabelul 5.1 Influence of industrial eluate on growth in length of the roots of *Lolium perenne* at 7 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	42,03 ± 1,43	39,21 ± 0,85	40,61 ± 1,14	40,61
Test (mm)	34,72 ± 0,94	30,44 ± 0,80	39,00 ± 1,08	34,72
p**	<0,001	<0,001	<0,001	Difference: 5,9
D%***	–17,40	–22,37	–3,97	–14,5%

X ± Es* – average values ± statistical estimation; p** – statistical significance; D%*** - percent difference from control

Tabelul 5.2 Influence of industrial eluate on growth in length of *Lolium perenne* stems at 7 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	47,09 ± 1,38	37,27 ± 2,44	46,47 ± 0,91	43,61
Test (mm)	31,80 ± 1,25	28,91 ± 1,01	35,00 ± 1,08	31,9
p**	<0,001	<0,001	<0,001	Difference: 11,7
D%***	–22,47	–22,43	–24,68	–23,19%

Explanation to table 5.1

The growth of the vegetative organs during the first 7 days after the initiation of the experiments was slower in the seed batches of the test variant compared to the control batches.

Tables 5.3 and 5.4 show the average values and statistical estimates for the growth of vegetative organs of L. perenne seedlings 14 days after the start of the experiments.

Table 5.3 Influence of industrial eluate on growth in length of the roots of *Lolium perenne* at 14 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	72,23 ± 2,17	73,86 ± 2,65	71,44 ± 2,33	72,51
Test (mm)	68,66 ± 1,89	69,90 ± 2,20	61,21 ± 2,46	66,59
p**	NS****	NS****	<0,001	Difference: 5,9
D%***	–4,94	–5,36	–14,31	–8,2%

Explanation to table 5.1

Regarding the length of the radicles at 14 days (tab. 5.3), the differences compared to the control were insignificant -4.94% (batch 1), -5.36% (batch 2) and significant -14.31% (batch 3). The difference between the mean length in the control and test groups was 5.9 mm.

The aerial part of the seedlings (tab. 5.4) from the test variants did not exceed the average height of the control, in groups 1 and 3: the differences compared to the control were -3.20% (group 1) and -14.37% (batch 3).

Table 5.4 Influence of industrial eluate on growth in length of *Lolium perenne* stems at 14 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	98,10 ± 2,50	95,95 ± 2,59	101,08 ± 2,12	98,37
Test (mm)	94,96 ± 2,36	100,32 ± 1,92	86,55 ± 2,27	93,94
p**	NS****	NS****	<0,001	Difference: 4,4
D%***	-3,20	+4,45	-14,37	-4,4%

Explanation to table 5.1

Tables 5.5 and 5.6 present the average values and statistical estimates obtained after processing data on the growth of vegetative organs after 28 days.

Table 5.5 Influence of industrial eluate on growth in length of *Lolium perenne* stems at 28 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	78,08 ± 2,44	79,71 ± 2,01	80,15 ± 2,64	79,31
Test (mm)	71,22 ± 2,60	72,72 ± 2,00	73,13 ± 3,20	72,35
p**	<0,04	<0,04	<0,001	Difference: 6,96
D%***	–8,78	–8,76	–8,75	–8,77%

Explanation to table 5.1

Table 5.6 Influence of industrial eluate on growth in length of *Lolium perenne* stems at 128days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	104,34 ± 3,02	108,81 ± 2,52	102,84 ± 2,78	105,33
Test (mm)	107,38 ± 2,32	111,19 ± 1,20	104,89 ± 1,12	107,82
p**	NS****	NS****	NS****	Difference: -2,49
D%***	+2,91	+2,18	+1,99	+2,36%

Explanation to table 5.1

In the test variants, the results obtained were as follows: 71.22 ± 2.60 mm (group 1; $p < 0.04$), 72.72 ± 2.00 mm (group 2; $p < 0.04$), 73, 13 ± 3.20 mm (group 3; $p < 0.001$).

Regarding the strain, the percentage differences compared to the control were superior in the test groups with the following values: 2.91% (group 1); 2.18% (batch 2) and 1.99% (batch 3).

The stress tolerance index (IT%) for the L. perenne species was calculated for root growth at 7 days (85.49%), 14 days (91.85%) and 28 days (91.22%). Tolerance to stress caused by soluble phytotoxic elements, or other factors (eg pH) is lower the closer the value is to 100%. From the values of the tolerance index (IT%) it can be observed that the phytotoxic elements of the industrial eluate had a low impact on the slowing down of root growth and it was especially manifested in the first 7 days after the experiments were set up.

ICR% has values higher than 0 (7 days - 14.5%; 14 days - 8.16%; 28 days - 8.77%), which indicates the inhibitory effect on the root, manifested especially in the first days of germination .

For the dry biomass of the plants it was calculated at 28 days (fig. 5.6). The difference between the dry biomasses is 14.33% at the expense of the test group, although the average length of the stems in the test groups exceeded at 28 days the average length of the control groups.

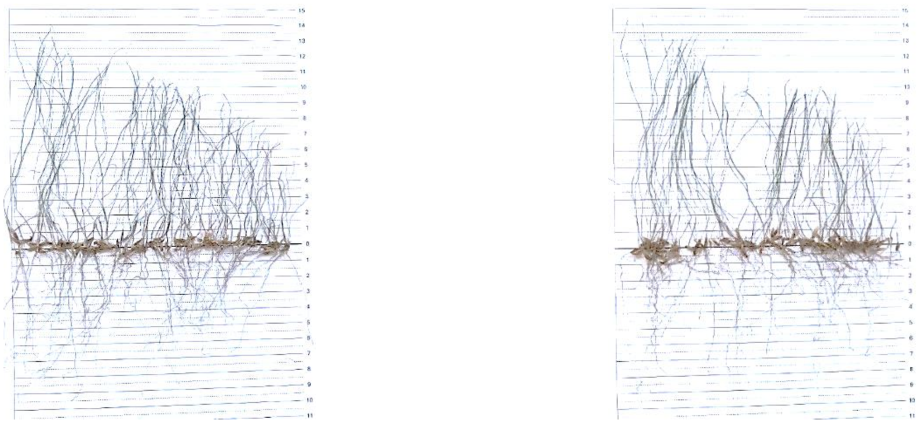

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Fig. 5.6 *Lolium perenne*, dry biomass: 1 – control; 2 – test

- Analyzing the effects of the industrial eluate on the germination of seeds and the growth of vegetative organs in the grass species *L. perenne*, the following can be stated:
- - in the test batches, the germination percentages were higher than those in the control batches;
- - the lower percentage and speed of germination in the control groups could be influenced by the alkaline pH 7.9 of the trace mineral water;
- - regarding the growth of vegetative organs, the influence of the eluate was observed especially at the beginning of the monitoring period;

- the decrease in the growth differences, starting from the second week, at the level of the roots also had an effect on the growth of the stems, the differences between the growth of the control and test plants decreasing significantly;
- - the dry biomass in the test variants was reduced compared to the control.

According to some authors (Masotla et al., 2023), the slower growth of roots in the presence of heavy metals is due to the reduction of meristematic cells at the root level and the reduced absorption of nutrients and water.

5.2.3.2 Evaluation of the main seed quality indices regarding growth and development capacity in the *Agrostis capillaris* species

Tables 5.7 and 5.8 present the average values and statistical estimates with reference to the growth of vegetative organs of *A. capillaris* seedlings at 7 days. The industrial eluate stimulated in the test variants, in the first 7 days, the increase in the length of the vegetative organs, as a result of a process of adaptation to the acidity of the environment.

Table 5.7 Influence of industrial eluate on growth in length of *Agrostis capillaris* roots at 7 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	2,53 ± 0,38	2,22 ± 0,28	2,16 ± 0,23	2,30
Test (mm)	2,42 ± 0,20	3,36 ± 0,31	3,54 ± 0,35	3,10
p**	NS	<0,02	<0,001	Difference: –0,8
D%***	–4,35	+52,25	+63,88	+34,78%

Explanation to table 5.1

Tabele 5.8 Influence of industrial eluate on growth in length of *Agrostis capillaris* stems at 7 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	2,90 ± 0,46	3,42 ± 0,54	3,23 ± 0,49	3,18
Test (mm)	4,48 ± 0,48	6,25 ± 0,66	6,62 ± 0,71	5,78
p**	<0,001	<0,01	<0,001	Difference: –2,6
D%***	+54,48	+82,74	+104,95	+81,76%

Explanation to table 5.1

Tables 5.9 and 5.10 show the average values and statistical estimates of the growth of vegetative organs (root, stem) for the species *A. capillaris* at 14 days.

The measurements made 14 days after the start of the experiment revealed the maintenance of the tendency of the rootlets from the test groups to exceed in growth the length of the rootlets from the control groups: the difference in the average lengths of the groups was 0.51 mm, the percentage difference being 13.67%.

Table 5.9 Influence of industrial eluate on growth in length of *Agrostis capillaris* roots at 14 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	3,58 ± 0,27	3,72 ± 0,07	3,89 ± 0,45	3,73
Test (mm)	4,84 ± 0,34	3,90 ± 0,20	4,00 ± 0,10	4,24
p**	<0,01	<0,01	<0,001	Difference: 0,51
D%***	+35,19	+4,83	+2,82	+13,67%

Explanation to table 5.1

Table 5.10 Influence of industrial eluate on growth in length of the strain of *Agrostis capillaris* at 14 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	12,10 ± 0,40	12,00 ± 0,20	11,50 ± 0,20	11,86
Test (mm)	15,74 ± 0,55	15,33 ± 0,69	14,73 ± 1,03	15,26
p**	<0,001	<0,001	<0,001	Difference: –3,4
D%***	+23,13	+21,73	+22,04	+22,3%

Explanation to table 5.1

Regarding the results regarding the average growth of the stems, a difference in the growth rate of the stems was observed in the test groups by 22.3% compared to the control; the difference between group means was 3.4 mm in favor of the test groups.

Table 5.11 shows the measurements obtained after 28 days for the growth in length of the roots, and table 5.12 shows the values for the stems.

Table 5.11 Influence of industrial eluate on growth in length of *Agrostis capillaris* roots at 28 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	4,97 ± 0,29	4,20 ± 0,50	4,30 ± 0,29	4,49
Test (mm)	5,28 ± 0,88	4,65 ± 0,50	4,87 ± 0,40	4,93
p**	<0,01	<0,01	<0,01	Difference: –0,44
D%***	+24,82	+9,68	+11,71	+5,4%

Explanation to table 5.1

Table 5.12 Influence of industrial eluate on growth in length of the strain of *Agrostis capillaris* at 28 days

The experimental variant	Lotul 1 X ± Es*	Lotul 2 X ± Es*	Lotul 3 X ± Es*	Average (mm)
Control (mm)	16,92 ± 1,31	17,18 ± 0,19	17,96 ± 0,60	17,35
Test (mm)	18,92 ± 0,41	19,18 ± 0,10	19,96 ± 0,20	19,35
p**	<0,25	<0,25	<0,25	Difference: –2,0
D%***	+11,82	+11,64	+11,13	+11,53

Explanation to table 5.1

Throughout the three weeks, the average length of the roots in the test groups was higher than the average of the control groups, the biggest difference being recorded in the first week. Regarding the growth dynamics of the strains, a significant growth difference was observed in favor of the test groups throughout the experiment.

Analyzing the values obtained from the calculation of stress tolerance (ST), based on root growth, it is observed that the effects of the industrial eluate were visible throughout the duration of the experiment (7 days – 134.78%; 14 days – 113.67%; 28 days – 109.8%), this causing an increase in the average lengths in the test groups compared to the control groups.

The results of calculations for assessing the effects of the industrial eluate on root growth (ICR%) indicate the stimulatory effect it had during the first 14 days (7 days - -34.78%; 14 days - -13.67%). At the end of the experiment, the root mean value greater than 0 indicates the inhibitory effect of trace mineral water on root growth in the test plots (fig. 5.7).

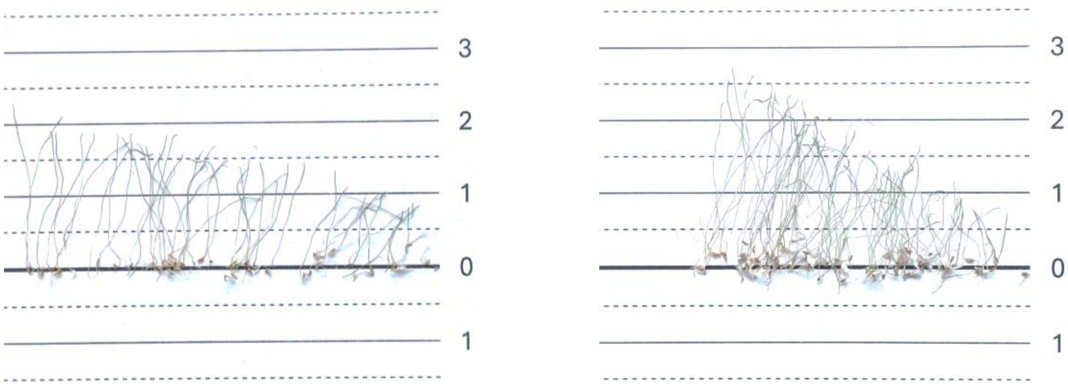

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Fig. 5.7 Agrostis capillaris, dry biomass: 1 – Control; 2 – Test

From the results obtained for the germination of seeds and the growth of vegetative organs in the species *A. capillaris*, the following emerges:

- the industrial eluate had no inhibitory effects regarding seed germination;
- the tolerance of the species to heavy metals and the preference for acidic pH, explains the lower seed germination yields in the control samples, with trace mineral water and alkaline pH, compared to the test lots, with industrial eluate containing heavy metals and pH acid;
- - correlating the results of the germination yields with those regarding the growth in the length of the roots and stems, in the species *A. capillaris* it is observed that the industrial eluate had no inhibitory effects on these processes;
- - the inhibitory effects of alkaline pH were also evident in terms of the slower growth of vegetative organs in control samples with trace mineral water;
- - the dry biomass in the test version was increased compared to the control.

5.3 Emergence and growth tests on pond tailings substrates and amendments

5.3.1 Metods

The tests were carried out in accordance with the OECD recommendations (developed in 1984 and revised in 2006) and ISO 11269-2:2012(E). The seeds were sown in pots of vegetation, placed under standard conditions for seed germination and plant growth. The days required for plant emergence were monitored.

5.3.2 Materials and method

The biological material used

For the growth tests on the substrates, seeds of *L. perenne* and *A. capillaris* from the same batches used for the germination tests were used.

Experimental conditions and variants

The experiments consisted in testing the emergence and growth of plants on substrates represented by the flotation tailings from the Bozânta pond and its mixtures with fertile soil and amendments necessary to improve the physical and nutritional qualities.

The mixtures used in the germination and plant growth tests were made from the following materials: fertile soil (P); flotation tailings (St); organo-zeolitic material (Oz); zeolite (Z); zeolite enriched with Ca and Mg (Z_{CaMg}); dolomite with Ca and Mg (D).

a) The fertile soil (P) used in the experiments was a universal flower soil
b) The flotation tailings (St) was represented by the average sample, collected from 0-20 cm from terrace 2, T_2, sector 5, S5, Bozânta pond (pH – 3.8).
c) The organo-zeolitic material (Oz) was obtained according to the Leggo method (2004). The mixture originates from volcanic tuff of Bârsana (Maramureș). The organo-zeolitic material was used by Damian et al. (2013) and by Jelea & Baciu (2023).
d) Zeolite (Z), natural supplement for plants (pH – 6.43).
e) Zeolite enriched with $CaCO_3$ and $MgCO_3$ (Z_{CaMg}) (pH – 7.4).
f) Dolomite (D), sedimentary rock containing calcium and magnesium double carbonate. (pH – 7.23). It is used as a source of calcium and magnesium and for pH regulation.

The experimental variants, with the ratio between the components and the parameters of the aqueous extract 1:5, are presented in table 5.13 and figures 5.8 and 5.9.

At the base, a drain layer with a height of 3 cm, consisting of sand with a grain size of <2 mm, was placed in each pot (fig. 5.10a). In variants 1 and 2, the seeding layers were made of fertile soil (P), respectively the flotation tailings (St), with a height of 10 cm. Test variants 3–11 were organized to simulate their application in the field, on the surface of the terraces.

In these variants, the layers of mixtures, with a height of 3.5 cm, made for the tests of seed germination and plant growth, were placed over two litter substrates:

- non-oxidized tailings substrate (St_{No}), average sample collected from the depth of 30–40 cm from terrace 2, T_2, of sector 5, S5 (pH – 6.56), with a height of 4.5 cm (fig. 5.10b);

- oxidized tailings substrate (St_{Ox}), average sample collected from the depth of 20–30 cm from terrace 2, T_2, of sector 5, S_5 (pH – 3.02), with a height of 2 cm (fig. 5.20c)

Table 5.13 The composition of the experimental variants

Variants	The composition of the experimental variants(%)						Parameters of the aqueous extract1:5			
	P	St	Oz	Z	Z_{CaMg}	D	pH mV	Cond. µS/cm	TSD ppm	Săruri ppm
1 P	100						7,27	160	1,13	714
2 St		100					3,8	135,5	174	52,3
3 StP	15	85					6,54	1262	895	558
4 StOz		85	15				6,9	785	559	343
5 StPOz	15	70	15				7,12	1414	1,0	628
6 StZ		85		15			4,5	143,7	102	63,3
7 StPZ	15	70		15			6,19	589	418	226
8 StZ_{CaMg}		85			15		7,87	665	472	288
9 $StPZ_{CaMg}$	15	70			15		7,79	820	581	357
10 StD		85				15	8,02	797	566	347
11 StPD	15	70				15	7,72	922	662	409

The mixtures prepared for seeding were moistened and left to rest for 48 hours. Distilled water with a pH of 6.0 was used to moisten the mixtures.

For each variety of substrates, 100 seeds of the two grass species were sown. The experiments were carried out in a temperature range of 20±2oC, with variations between day and night periods. The photoperiod was between 11–12 hours. Humidity was maintained between 50–60%.

Fig. 5.8 Materials used in mixtures: a) P; b) St; c) Oz; d) Z; e) Z_{CaMg}; f) D

Fig. 5.9 Experimental variants: 1 P; 2 St; 3 StP; 4 StOz; 5 StPOz; 6 StZ; 7 StPZ; 8 StZ_{CaMg}; 9 $StPZ_{CaMg}$; 10 StD; 11 StPD

Fig. 5.10 Substrates of sand and tailings: a) Sand; b) St_{No}; c) St_{Ox}

Observations were made on the dynamics of plant emergence at 7, 14, 28 and 35 days.

Five characters of plant growth were considered manifestations of the phytotoxicity effects of the tailings on plants: yellowing of leaves, drying of leaves, leaf limb narrower and thinner stems compared to the vegetative organs of the plants grown in the soil variant, the red-brown coloring of the leaf sheath at the base of the stem.

Stem and root length measurements and dry biomass analysis were carried out at the end of the experiment, 35 days after sowing.

5.3.3 Rezultats and discussions

5.3.3.1 Determination of emergence on the experimental layers for the grass species *Lolium perenne*

Table 5.14 Shows the number of sprouted plants during the course of the experiment. *Lolium perenne* (perennial ryegrass)

Variants	Number of emerged plants			
Days	7	14	28	35
1 P	81	81	91	91
2 St	63	71	85	85
3 StP	77	82	91	91
4 StOz	68	69	91	91
5 StPOz	75	82	92	92
6 StZ	72	75	84	84
7 StPZ	84	84	87	87
8 StZ_{CaMg}	81	84	91	91
9 $StPZ_{CaMg}$	79	83	94	94
10 StD	90	91	95	95
11 StPD	85	87	94	94

5.3.3.2 Manifestarea fitotoxicității sterilului asupra plantelor de *Lolium perenne*

Observations regarding the phytotoxicity manifestations of the tailings on plant growth, made 28 days after sowing, are presented in table 5.15.

Table 5.15 Observations on the condition of *Lolium perenne* plants (lawn grass) 28 days after planting

Remarks \ Variants	1 P	2 St	3 StP	4 StOz	5 StPOz	6 StZ	7 StPZ	8 StZ_{CaMg}	9 $StPZ_{CaMg}$	10 StD	11 StPD
Green leaves	X		X	X	X		X		X		X
Yellowed leaves		X				X		X		X	
Dry leaves		X				X					
Vigorous leaf	X		X	X	X		X		X		X
The narrower leaf		X				X		X		X	
Wider leaf				X	X						
Vigorous stems	X		X	X	X		X		X		X
Thinner stems		X				X		X		X	
Thicker stems				X	X						
Green base	X										
Red-brown base		X	X	X	X	X	X	X	X	X	X
Phytotoxicity	-	5	1	1	1	5	1	4	1	4	1

After 35 days of running the experiment, the plants were recovered from the vegetation pots and the lengths of the roots and stems were measured (tab. 5.16).

Comparing the way the roots grow, it was observed that in the variant with soil (1 P), the roots grew over the entire height of the substrate, while in the test variant with organo-zeolitic material (4 StOz) the roots grew in the layer represented by the mixture of waste with the amendment and in the oxidized tailings (St_{Ox}) layer. The obvious difference is in the way the roots develop, in the form of long and thin bundles, poorly branched, in the control variant with soil (1 P) and shorter, but very thick and branched, in the test variant with organo-zeolitic material.

Table 5.16 Mean root and stem lengths, IT and ICR, in *L. perenne* (perennial ryegrass) 35 days after planting

The experimental variant	Average root length (mm)	Average stem length (mm)	Stress tolerance index (IT%)	Growth inhibition index (ICR%)
1 P	130	250,23	-	-
2 St	3,71	61,0	2,85	97,14
3 StP	87,91	230,84	67,62	32,37
4 StOz	51,84	250,51	38,87	60,12
5 StPOz	75,41	238,6	68,0	41,99
6 StZ	24,2	124,39	18,77	81,38
7 StPZ	103,64	232,75	79,74	20,25
8 StZ_{CaMg}	120,14	174,06	92,41	7,58
9 $StPZ_{CaMg}$	108,53	226,75	83,48	16,51
10 StD	117,6	168,4	90,46	9,53
11 StPD	72,48	221,74	55,75	44,24

Table 5.17 shows the values obtained for the dry weight of roots and stems. The dry weight of the stems was superior to that of the control variant in all test variants with waste, amendments and soil and lower in the variants where the mixtures were represented only by waste and amendments, except for the variant with organo-zeolitic material.

Comparing with the values obtained for the average lengths, it is observed that the average weight of the stems is in direct correspondence with them.

Table 5.17 Root and stem dry weights in *Lolium perenne* (perennial ryegrass) 35 days after planting

The experimental variant	Greutatea uscată (g)			
	Sunrise plants		Recalculated 100 plants	
	Roots	Strains	Roots	Strains
1 P	0,316	0,920	0,347	1,01
2 St	0,105	0,134	0,123	0,154
3 StP	0,386	0,999	0,424	1,097
4 StOz	0,360	1,830	0,395	2,011
5 StPOz	0,296	1,824	0,321	1,982
6 StZ	0,245	0,416	0,291	0,495
7 StPZ	0,580	1,073	0,666	1,233
8 StZ_{CaMg}	0,835	0,485	0,917	0,533
9 $StPZ_{CaMg}$	0,781	1,042	0,830	1,108
10 StD	0,753	0,434	0,792	0,457
11 StPD	0,377	0,928	0,401	0,987

Compared to the control variant, the dry weight of the roots was higher in the test variants, with the exception of the variants with steril 2 St, steril and zeolite 6 StZ and steril, soil and organo-zeolitic material 5 StPOz.

5.3.3.3 Determination of emergence on the experimental layers for the grass species *Agrostis capillaris* (field grass)

Table 5.18 shows the number of plants that sprouted during the experiment.

After 35 days, at the end of the experiment, the highest germination percentages, 23% and 24%, were obtained in the variants with the zeolite with calcium and magnesium content, 8 StZ_{CaMg} and 9 $StPZ_{CaMg}$, variants in which the pH of the substrates was weak alkaline, 7.9; the lowest percentage of emergence, 7%, was obtained in variant 6 StZ, where the pH of the substrate was acidic, 4.5.

Of the total number of plants emerged for each variant by the end of the experiment, in six of the variants over 70% of the plants emerged in the first 7 days, over 85% in 8 variants after 14 days and 100% in variants 2–11 after 28 days.

Table 5.18 Shows the number of sprouted plants during the course of the experiment.
Agrostis capillaris (field grass)

Variants	Number of emerged plants			
Days	7	14	28	35
1 P	10	11	12	13
2 St	5	8	12	12
3 StP	9	15	16	16
4 StOz	3	10	18	18
5 StPOz	5	12	13	13
6 StZ	4	4	7	7
7 StPZ	15	18	19	19
8 StZ_{CaMg}	21	23	23	23
9 $StPZ_{CaMg}$	21	22	24	24
10 StD	10	14	14	14
11 StPD	12	16	16	16

5.3.3.4 Manifestation of the phytotoxicity of the tailings on *Agrostis capillaris* plants

Observations regarding the phytotoxicity manifestations of the tailings on the growth of *Agrostis capillaris* plants, made 28 days after sowing, are presented in table 5.19.

After 35 days, the lengths of roots and stems were measured (tab. 5.20). The conclusion is that the maximum 40–42 mm dimensions of the roots in the test variants in which growth inhibition phenomena did not occur were determined by the slow rate of plant growth, not by direct inhibition phenomena exerted by the underlying layer.

Since in all variants the number of sprouted plants was below 25% and the sizes of vegetative organs were very reduced, we considered that the values obtained for dry biomass are not significant and may introduce a high degree of error; consequently the values obtained for dry biomass were not taken into account.

Table 5.19 Observations on the condition of *Agrostis capillaris* (field grass) 28 days after planting

Remarks \ Variants	1 P	2 St	3 StP	4 StOz	5 StPOz	6 StZ	7 StPZ	8 StZ_{CaMg}	9 $StPZ_{CaMg}$	10 StD	11 StPD
Green leaves	X		X								
Yellowed leaves		X		X	X	X	X	X	X	X	X
Dry leaves		X		X	X	X	X	X	X	X	X
Green stems	X		X								
Short stems		X		X	X	X				X	X
Tulpini lungi	X		X				X	X	X		
Dry stems		X		X	X	X	X	X	X	X	X
Phytotoxicity	-	4	0	4	4	4	3	3	3	4	4

Table 5.20 Mean root and stem lengths, IT and ICR, in *Agrostis capillaris* (field grass) 35 days after planting

The experimental variant	Average root length (mm)	Average stem length (mm)	Stress tolerance index (IT%)	Growth inhibition index (ICR%)
1 P	26,08	42,5	-	-
2 St	0,16	16,5	0,61	99,39
3 StP	32,28	42,0	123,77	-23,77
4 StOz	6,55	23,77	25,11	74,88
5 StPOz	5,5	13,91	21,1	78,91
6 StZ	0,4	13,8	1,53	98,46
7 StPZ	24,57	43,43	94,21	5,79
8 StZ_{CaMg}	13,4	34,0	51,38	48,62
9 $StPZ_{CaMg}$	24,52	39,26	94,02	5,98
10 StD	9,14	27,57	35,05	64,95
11 StPD	9,66	25,44	37,04	62,96

Following the analysis of the results, the main characteristics of the substrate variants and their effects on the plants were stated

1. Layer of topsoil

The soil placed on the surface of the tailings can be a cultivation layer favorable for the germination, growth and development of plants. However, the variant has the following disadvantages: the accumulation and concentration in a short period of time of the heavy metals that reach the fertile soil layer through capillary ascent, will increase the level of phytotoxicity; acidification of the fertile soil layer by the solutions from the underlying oxidized layer; the phenomena of erosion and transport of the soil layer at the base of the pond by the rainwater during the periods necessary for the execution of the work of setting up and seeding the substrate; the need for a large volume of fertile land, which involves aspects regarding its availability, but also transport and commissioning costs.

2. Incorporation of topsoil into the tailings on the pond terrace surfaces

The soil incorporated in the tailings layer will have the following favorable effects: adjusting the pH of the substrate in the slightly acidic or neutral range; improving the granulometry of the substrate; improving the drainage/circulation of solutions through the substrate; efficient retention of rainwater and maintaining a moisture reserve in the substrate; quantitative and qualitative improvement in nutrients; reducing the oxygen content of the solutions that will percolate to the underlying tailings layer.

3. Incorporation of amendments into the tailings layer

Incorporation of different amendments into tailings will lead to physical and chemical changes: pH change depending on the content of alkaline substances and the mixing ratio with the tailings; improving the substrate with the specific substances contained in the applied amendment; the change in grain size properties according to the grain size fractions of the amendment.

4. Incorporation of soil mixtures and amendments into the tailings

Incorporation of soil mixtures and amendments into tailings brings together the favorable effects that these materials have on improving physical, chemical and nutritional qualities.

On the T_4 terraces, which were not grassed, from all sectors, S_1–S_{11}, as well as on other terraces without vegetation, according to the closure/greening project, waterproofing layers, anti-erosion mats and a layer of topsoil that will be grassed are provided; the project provides for covering the surfaces with earth for the rest of the terraces with vegetation.

Taking into account the transformations of the last 15 years, we propose the following solutions:

1) For the T_3 terraces in the S_4 sector, but also in the rest of the S_1–S_3 sectors, it is proposed to use amendments with dolomite and earth, 11 StPD, and mulching with *L. perenne*. The surface of these terraces is devoid of grassy vegetation, soil or organic material as a result of the phenomena of entrainment downstream by rainwater, seepage waters and the scattering of tailings from the upper terrace. After the covering of the upper terrace, T_4, the tailings spills and scattering will cease; a large part of the biomass will remain in place; the soil and plant biomass will decrease the oxygen intake in the tailings by decreasing the activity of aerobic chemolithotrophic iron- and sulfur-oxidizing bacteria; trees with undeveloped crowns will allow access of light for the installation of the vegetal carpet.
2) For the T_2 terraces in sectors S1-S4, with vegetation and pH 5.1–6.8, it is proposed to incorporate amendments with zeolite, 7 StPZ, which will maintain the pH of the surface within the current limits. The surface of the terrace is populated with almost 20 species of herbaceous plants and 6 species of trees, shrubs, subshrubs, which produce an appreciable amount of plant biomass. Zeolite amendments will have a beneficial, long-lasting effect on the composition of the substrate solutions. The increase in the amount of biomass, oxygen consumption and organic matter content will have an inhibitory effect on the development of bacteria, which will diminish the processes of acid drainage of the rocks

3) Terrace T_1, with tailings at the beginning of oxidation, with more advanced acid drainage than terrace T_2, more acidic pH, 4.8–6.4, has a low number of herbaceous species, but due to strong exfiltration, one of the species has invaded the surface over extensive areas: the juliška (*Fallopia japonica*). The terrace is shaded by acacias and sky specimens (*Quercus cerris*). On this terrace, with biomass that covers the terrace surface well, it is proposed to incorporate soil and zeolite with calcium and magnesium, 9 $StPZ_{CaMg}$. The amendment will improve the pH, bring a surplus of magnesium, necessary for photosynthesis processes. Fertilization with *L. perenne* is not absolutely necessary.
4) For the T_3 terraces in sectors S_5–S_{11}, approaches regarding the coverage method will be done differently. In sectors S_5 and S_6, partly also in S_7, the vegetation is almost completely destroyed. Unlike the T_3 terraces in sectors S_1–S_4, where the pH was extremely acidic in the first 10 cm, in these terraces the pH is 3.5–4.2 throughout the depth to which the roots of herbaceous plants can reach. The number of iron- and sulfur-oxidizing bacteria exceeds the value of 10^6/g sterile. The processes of acid drainage of rocks are very intense and the possibility of reducing them by incorporating amendments is less certain; that is why the cover is proposed, together with the T_4 terrace with the waterproofing layers.
5) The situation from the point of view of the acidity of the tailings in the T_2 terrace in the S_5 sector does not differ much from that in the T_3 terrace, but the pH is higher and the bacterial processes of acid drainage of the rocks, both iron oxidizers and sulfur oxidizers are with a orders of magnitude less. On this terrace and those in sectors S_6 and partially S_7, dolomite and soil, 11 StPD will be incorporated and *L. perenne* will be sown.
6) Although the T_1 terrace of sector S_5 still survives planted or spontaneously established old trees, the active processes of acid drainage from the surface layer have removed the herbaceous vegetation. On this terrace it is proposed to incorporate soil and zeolite with calcium and magnesium, 9 $StPZ_{CaMg}$. Considering the very sunny surface, due to the small number of remaining trees, it is proposed to cover with *L. perenne*, in mixed culture with *A. capillaris*.
7) A layer of earth will be deposited along the technical road to strengthen the already existing vegetal carpet, represented by clumps of grasses of the *A. capillaris* species.
8) *A. capillaris* appeared spontaneously in the wetlands, in the path of the trickling water that drains from the pond. In the form of a vegetal carpet or clumps, it has an important role in retaining part of the fine waste particles and reducing the volume of solid pollutants that reach the water. Erosional processes represented by wind blowing or those with tailings entrainment and low humidity prevent plant development.
9) *L. perenne* will create a vegetal carpet on the sloping surfaces of the pond. The erosion processes, represented by the transport of waste by rainwater, will be reduced as a result of the presence of aerial vegetative organs and strongly branched roots.

CONCLUSIONS

The object of the research, the Bozânta tailings storage facility, located north of the confluence of the Săsar and Lăpuș rivers, near the municipality of Baia Mare and the towns of Săsar, Recea, Lăpusel and Bozânta Mare, represents a major source of pollution for the neighboring areas.

As a result of the activities in the field, the following were found:

- the tailings, material with sub-millimeter grain size, acid pH and heavy metal content, is carried by the wind, from the pond beach and from the terraces devoid of vegetation, on the agricultural or grazing land, but also in the inhabited areas located a short distance away;

- the acidic waters, with the contained heavy metals, drained from the beach and the terraces of the pond, reach the two rivers, passing over the guard ditch and the technical road;

- from the upper terraces there were massive slides of tailings at the base of the pond and outside its perimeter;

- the surface of the terraces is furrowed by ravines that can exceed 200 cm;

- there are areas where the contour dike is damaged;

- the degradation to the point of disappearance of the plantations or the spontaneous vegetation on the terraces of the pond;

- the erosive, wind and rain phenomena are amplified by the acidic drainage processes of the rocks.

All these transformations can have consequences in terms of the stability and safety of the pond and endanger the health of the inhabitants of the adjacent areas.

The research carried out to establish the causes of vegetation degradation, the assessment of the stage and the ascertainment of the effects of the acid drainage processes of the rocks led to the following observations:

- the processes of acid drainage of the rocks are present, in different stages, in the tailings of all the terraces and in the beach of the pond;

- the origin of the tailings, the age of storage, the presence/absence of vegetation and the way of planting tree species were determining factors regarding the dynamics of the acid drainage processes of the rocks;

- bacterial, iron- and sulfoxidizing metabolic activity led to a decrease in pH, the formation of acidic solutions containing heavy metals in forms assimilable by plants, resulting in the alteration of living conditions and the partial or total drying of vegetation.

Following the analysis of the results, we proposed an original model for evaluating the stage and estimating the evolution of the acid drainage process of the rocks, based on the physico-chemical properties, the identified iron- and sulfur-oxidizing bacterial species and their numerical ratio in the tailings from the pond terraces. The application of the work methods and the proposed evaluation and estimation model can become a useful tool for collecting and comparing data, considering the sometimes long periods of time between the completion of the closure/greening projects and the commissioning of the works - in the case of the Bozânta pond, 14 years have already passed.

The results of the research carried out during the doctoral studies show that the situation described in the closure/greening project regarding the state of the vegetation on the terraces of the sectors has changed profoundly: for example, on the terraces on which the project specifies "it is considered that the vegetation is well developed, is preserved and will be supplemented with saplings", currently very active processes of acid drainage of rocks and partial or total drying of plants are noticeable.

Research was carried out to identify the appropriate measures to stop/reduce the degradative phenomena, in order to apply them differently on the terraces of the pond, depending on the degree of alteration of the rocks, to estimate the evolution of the microbiological processes and the consequences on the installed vegetation.

The results of the tests, carried out for the grass species *L. perenne* and *A. capillaris*, regarding the effects of pond solutions with acid pH and heavy metal content, on the indicators of germination and the growth of vegetative organs, showed that the two grass species are suitable for the conditions offered by pond solutions: both plants had higher germination and growth percentages than the control samples.

After testing the growth of plants on the tailings substrates with amendments, carried out in order to apply a vegetal carpet with a role in stopping/reducing the processes of acid drainage of the rocks, increasing the stability of the surfaces and reducing tailings scattering, the mixtures that will be proposed for application in the field, for each terrace, specifically, depending on the stage of the acid drainage processes of the rocks.

Novelty contributions

The way of organizing and carrying out the research in this doctoral thesis proposes a new perspective regarding the approach in a complex and complete way to the studies that are carried out to stop/reduce the processes of acid drainage of rocks and revegetation of mine waste deposits. Practically, a multidisciplinary research methodology of the processes of acid drainage of rocks in the flotation tailings settling pond was developed.

An original model was developed to assess the stage and estimate the evolution of the acid drainage process of the rocks, based on the physico-chemical properties, the identified iron- and sulfur-oxidizing bacterial species and their numerical ratio in the tailings from the pond terraces.

In order to revegetate the surfaces, different application variants were tested depending on the particularities specific to each terrace of the pond

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