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MAŁGORZATA KRAWCZYŃSKA[1], BARBARA KOŁWZAN[2], KRZYSZTOF GEDIGA[3], ANDRZEJ M. DZIUBEK[1], KAZIMIERZ GRABAS[1], ELENA KARPENKO[4]

EVALUATION OF THE POSSIBILITY OF PHYTOSTABILIZATION OF POST-FLOTATION TAILING PONDS

The effect of organic fertilization and microbiological preparations for phytostabilization of copper ore tailings were examined. It has been shown that the introduction of the pulp, waste from the sugar industry, leads to the mobilization of trace metals. The copper content in aqueous extract of enriched waste was greater than 500-fold higher than in controls. It has also been stated an increase in the toxicity of extracts. While the use of biopreparations for seed treatment contributed to the stimulation of nutrients uptake by plants. In the biomass of plants from treated seeds with a suspension of *Azotobacter* strain EK, and biosurfactant was found more phosphorus and calcium than in plants from control.

1. INTRODUCTION

Lower Silesia is one of those Polish regions in which there is a wide variety of mineral wealth with copper ore as the most significant. Exploration activity related to copper caused a number of unfavorable effects in the environment, leading to anthropogenic changes of soil surface and devastation of the landscape. The above-mentioned effects are the result of the occupation of large areas by tailings generated during mining and processing of copper. The quantity of post-flotation tailings is disproportionately large relative to the amount of copper concentrate produced during the enrichment process. Flotation ponds appeared to be especially dangerous, which,

[1]Wrocław University of Technology, Faculty of Environmental Engineering, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland, corresponding auhor K. Grabas, e-mail: kazimierz.grabas@pwr.edu.pl

[2]Polish Sanitary Engineers' Association, ul. Piłsudskiego 74, 50-020 Wrocław, Poland.

[3]Wrocław University of Environmental and Life Sciences, Faculty of Life Sciences and Technology, pl. Grunwaldzki 24A, 50-363 Wrocław, Poland.

[4]National Academy of Sciences of Ukraine, Institute of Physical Organic Chemistry, Naukova St. 3a, Lviv, 79053, Ukraine.

moreover, have become a secondary source of pollutant emissions [1, 2]. Studies of ponds located in the area of Lower Silesia have shown that in majority they are not inhabited by living organisms. The reason explaining this phenomenon is probably not only the toxic effect of deposited trace elements or lack of nutrients, but also the granulometric composition of post-flotation tailings preventing plants anchor and their colonization by living organisms [3].

Unfavorable landscape transformation and secondary air pollution resulting from the lifting of waste particles into the atmosphere force to carry out remedial actions [2]. The natural way to protect against dust emission is to cover the surface of ponds by plants. Reclamation of waste, which has been carried out so far is, however, very expensive, long-lasting and do not provide high efficiency. Therefore, the use of phytostabilization was offered as a method reducing the toxicity of waste, allowing the stabilization of the surface and inhibiting the erosion process as well as ion metal leaching [1–3]. Phytostabilization is based on the use of plants in order to immobilize the contaminants in soil and reduce availability in the environment. The immobilized pollutants are then much less bioavailable and more difficult included in the food chain in the area of the contaminated ecosystem. During phytostabilization, absorption and accumulation of metals in plant roots, adsorption on the surface of roots and precipitation in the rhizosphere may occur [4]. In this process, the contaminated soil is protected against water and wind erosion, the plant cover is rebuilt and immobilized substances have reduced capability for further movement to other food chain links.

Phytostabilization is applicable both to elimination of organic and inorganic pollutants [5, 6]. This technology does not lead to the destruction of soil, removal of the organic soil matrix together with microorganisms, or changes of its texture. However, the development of phytostabilization technology must be adapted to the conditions of specific contamination. In any case, selection of suitable plant species, adaptation of the cultivation method, basic adjustment of fertilization are necessary to meet the needs of nutritional requirements of plants, taking into account the abundance of reclaimed ground, and also its specific properties, such as pH, the granulometric composition, sorption capacity etc. This process should include those procedures which are necessary to achieve optimal conditions for plant growth, e.g. pH adjustment, the enrichment with organic matter from cheap sources or complementation of nutritional deficiencies. Areas subjected to phytostabilization process must be constantly monitored to record unfavorable effects which may lead to the spread of contamination.

Phytostabilisation process may be assisted by the introduction of the organic substance additives in the form of biomass, compost or mineral additives (clay minerals, phosphates, carbonates), and by increasing pH as a result of liming. The best results are obtained on fine-grained soils with a high content of organic substances. The plants used in this process should have low coefficient of metal translocation into above-ground shoots, and should also be characterized by high production of biomass and high tolerance to soil contaminations. High efficiency of the regeneration of degraded areas can be provided through the use of plants suitable to the structure and chemical composition of soil, and by taking into account the qualitative and quantitative composition of microorganisms inhabiting the area [7]. A substantial buffer capacity of soils or surfaces of higher content of clay fraction as well as an increased amount of organic matter, generally promote phytostabilisation, however the key role is played by the selection of species for this process. These plants – especially in symbiosis with microorganisms – usually determine the success of the revitalization process [8, 9]. Bio-preparations used for the treatment of seeds increase their resistance to environmental factors and facilitate germination [10, 11]. Moreover, their introduction into the root zone stimulate co-operation of plants and microorganisms. The microorganisms utilizing organic substances secreted by the roots provide protection against parasites and toxicity of substances for the plants, causing their sequestration in the root zone and enabling the uptake of biogenic compounds [12].

In this study, one proposed to use the phytostabilization, as a method of surface stabilization, inhibiting the process of erosion and leaching of metal ions from post-flotation tailings. One also analyzed the possibility of the use of organic fertilization and application of bio-preparations for the stimulation of plant growth in the post-flotation tailings.

2. MATERIALS AND METHODS

The subject of the conducted study was the post-flotation pond "Wartowice" located in the region of Bolesławiec (51° 12′ 50″ N, 15° 40′ 30″ E). Waste flotation in an amount of 100 kg was collected from the surface of the post-flotation tailings (50 cm deep), and after carefully mixing it has been used as a plant growth substrate. In the studies considering the possibility of organic fertilization of post-flotation ponds, one used pressed pulps from the process of sugar production. The chemical composition of the pulp consisted of sugar (15.3 wt. %), protein (9.74 wt. %), pectin (9.03 wt. %), and fat (0.38 wt. %) [13]. The pulps were introduced into the post-flotation tailings (10 wt. %), and subjected to incubation for 7 days at 25 °C. Then, they were subjected to physicochemical and toxicological studies. Samples were prepared according to the procedure given by Dechema [14]. Physicochemical tests were performed according to the standard methods [15]. Toxicity tests were carried out using the Microtox (Strategic Diagnostics, Inc., Newark, USA) system, with *Vibrio fischeri* luminescent bacteria as a bioindicator. Tests were performed with an M 500 analyzer and lyophilized bacteria, according to the standard procedure specified by the manufacturer (SDI). The measure of toxicity was the decrease in luminescence after 30 min incubation of the test bacteria in the presence of the compound being examined [16]. The results were calculated using the manufacturer's software MicrotoxOmni. The results of analyses were expressed in terms of EC_{50} (sample concentration reducing luminescence by 50%).

For the treatment of seeds, one used biosurfactant and bacterial preparation. Biosurfactant, so-called biocomplex, was obtained from *Pseudomonas* sp. PS-17 culture liquid and contained rhamnolipids and exopolysaccharide. It was characterized by a low surface (29.5 mN/m) and interfacial (0.17 mN/m, *n*-heptane) tension, and ability for emulsification (emulsification index E_{24} = 85%) [17]. The biocomplex solution was used at the concentration of 0.05 g/dm^3.

The bacterial preparation was a suspension of bacterial strain *Azotobacter* EK, which bound atmospheric nitrogen. The bacteria came from the collection of the Ukrainian Academy of Sciences, the amount of bacteria in the suspension was 38–156 cfu/cm^3. However, in a mixed suspension, for *Azotobacter* EK strain suspension, also biosurfactant at a concentration of 0.05 g/dm^3 of medium was added.

Five species of plants were used in the studies: maize (*Zea mays*), lupine (*Lupinas lúteas*), peas (*Pisum sativum*), mustard (*Sinapis alba*) and oats (*Avena sativa*). The culture was carried out in a phytotron with a division to day (16 h) and night (8 h) at 20–22 °C. Plants were grown in flowerpots of the diameter of 14 cm. To each of them, 12 seeds were seeded, namely *Zea mays*, *Lupinas lúteas*, *Pisum sativum*, *Avena sativa*, and 25 seeds of *Sinapis alba*. At the bottom of each vase, a weighted amount (100 g) of coarse-grained gravel was placed followed by the use of a drain. Vases were filled thoroughly by mixing 475 g of post-flotation sludge and 25 g of non-contaminated sand. In terms of monocots, one applied 10 mg of N as NH_4NO_3, 20 mg of P as K_2HPO_4 (50 mg K) and 10 mg of Mg as $MgS0_4 \times 7H_2O$. The dose of nitrogen for legumes was 2.5 mg N per 0.5 kg of culture medium. Pots were filled with medium, and after three days watered to 60% WHC (water holding capacity) followed by the inoculation of the seeds, which had earlier been subjected for the treatment. To stimulate growth, one used three types of bio-preparations for seed treatment before inoculation on a surface containing sediment after flotation of copper ore. It was a suspension A of nitrogen-fixing bacteria of *Azotobacter* genus, biosurfactant B and a mixture AB composed of nitrogen-fixing bacteria and biosurfactant AB. The seed treatment solution constituted 4% of the seed weight. The seeds were kept in the solution for 1 h and then plated into the pots. The control seeds were not subjected to the seed treatment. The pot experiment was conducted in four replicates for 40 days with daily supplementation of water loss. After 40 days, the plants were cut, then dried and the dry matter yield was measured. The content of elements in the dry matter was determined by burning the plant material in a muffle furnace at 475 °C for 6 h, then the ash was diluted with concentrated nitric acid followed by the evaporation to dryness. The residue was quantitatively transferred with 1 mole of HNO_3 to 50 cm^3 volumetric flasks. Measurements were conducted by the flame emission for Ca and K, and the absorption emission for Cu, Zn, Mn, Fe, and Mg, phosphorus was determined colorimetrically according to the method by Murphy–Riley [18].

3. RESULTS OF THE STUDY

Collected flotation tailings are characterized by unfavorable properties of colonization by living organisms. In the pond, one detected the presence of only bacteria and fungi. The number of bacteria in the layer to the depth of 10 cm was 1000-fold (29 000 cfu/g) higher in comparison to fungi (68 cfu/g), while in the deeper layer to the depth of 20–30 cm, the number of fungi was an order of magnitude higher than that for bacteria (23 500 cfu/g) and accounted for 310 000 cfu/g [19]. The result of the prevailing conditions is the lack of plants capable of existence in unfavorable conditions.

Table 1

Chemical composition of post-flotation sediment
from "Konrad 3" tailings [20]

Element	Content [wt. %]	Element	Content [wt. %]
Na_2O	0.107	As_2O_5	0.008158
Li_2O	0.0118	SeO_2	0.000052
K_2O	3.25	MoO_3	0.001103
CaO	22.75	CrO_3	0.0132
MgO	3.96	WO_3	0.000011
SO_3	0.462	CdO	0.0000406
BO_2	0.1004	PbO	0.00396
ZnO	0.0082	CoO	0.002866
P_2O_5	0.1749	NiO	0.00287
Al_2O_3	11.74	BeO	0.001836
Fe_2O_3	2.58	V_2O_5	0.01835
SiO_2	53.90	CuO	0.22199
Mn_2O_5	0.268	Ag_2O	0.00218
SrO	0.0542	TiO_2	0.124
BaO	0.0212	ZrO_2	0.00217
SnO_2	0.000684	HgO	0.00019
TlO_2	0.0001843	UO_3	0.000898

The results of the studies listed in Table 1 [20] showed, that the tailings collected in the "Wartowice" pond contain significant amounts of copper and other elements, but they do not pass into the aqueous extract (Table 2). pH of tailings above 8.0 promotes immobilization of metals, including Cu and prevents their transfer to the plants, however a side effect of high pH is the reduction of availability of essential nutrients such as phosphorus, zinc and iron. Toxicological studies with the use of luminescent bacteria showed that impurities contained in the extract from the tailing, are non-toxic to living organisms.

Taking into account the results of our studies and studies conducted in the area of "Wartowice" pond, it was approved that the factor reducing the plant growth is the lack of biogenic components, low aeration of tailings, and the lack of soil air in wet periods. In addition, water uptake by plants is hampered by high retention of rainwater in the form of hygroscopic and pellicular water, and diverse brevity and permeability of each layer of tailings. It is necessary to change the structure of post-flotation tailings leading to their better aeration and changes in p*F* curve, that is the increase of share of water available for plants and the enrichment of tailing in biogenic components.

Table 2

Results of the analysis of aqueous extracts from post-flotation tailings

Parameter	Solid waste "Wartowice"	Solid waste "Wartowice" + pulps
pH	7.83	7.88
Conductivity, mS/cm	2.02	1.59
Total alkalinity, val/m^J	0.9	5.0
Dissolved organic carbon, g C/m^3	9.2	12.0
Total dissolved solids, g/m^3	2147	8377
Pb, g/m^3	0.025	0.194
Mn, g/m^3	0.07	0.29
Cu, g/m^3	0.020	10.785
Fe, g/m^3	0.02	0.0
Ni, g/m^3	0.002	0.008
Cd, g/m^3	0.0	0.0
Zn, g/m^3	0.011	0.037
Toxicity (EC_{50}), %	non-toxic	60

One tried to establish, what effect on the migration of metals, organic fertilization provides. It was considered that the improvement of the quality of the surface can be obtained by its enrichment with cheap waste from the food industry rich in organic matter. In the conducted experiment, pulps from the sugar production process were used. The introduction of pulps clearly changed the properties of the extracts and increased the content of metal ions. Copper ion content was at least three orders of magnitude higher. Increase in the content of organic carbon as well as the increase in the toxicity of studied sediment were observed. It resulted probably from the increasing degree of leaching from wastes of trace element characterized by a toxic effect on living organisms. The reason for this phenomenon may be intensive microbiological processes leading to the formation of metabolites that facilitate the passage of metal ions to a solution [12].

It was confirmed that the supply of waste in biogenic components can serve only by using such mineral fertilization necessary for the proper growth of plants, which will not lower pH of the ground [20]. It was established that only oligotrophic microorganism growth is possible here, for which the demand for organic carbon is low, as well as autotrophic microorganisms, for which energy is derived from the oxidation of inorganic compounds. Such microorganisms should also be characterized by the resistance to trace metal ions and the ability to grow in such specific environmental conditions [21, 22]. It was considered that the stimulation of the development of the microflora can be achieved by preparing special biopreparations containing appropriate microorganisms and biosurfactants. The results of pot experiment indicate significant differences in the intensity of nutrient uptake by the test plants growing on the post-flotation tailing in control vases. It appears that in this process a crucial role is played by species variability (Figs. 1, 2).

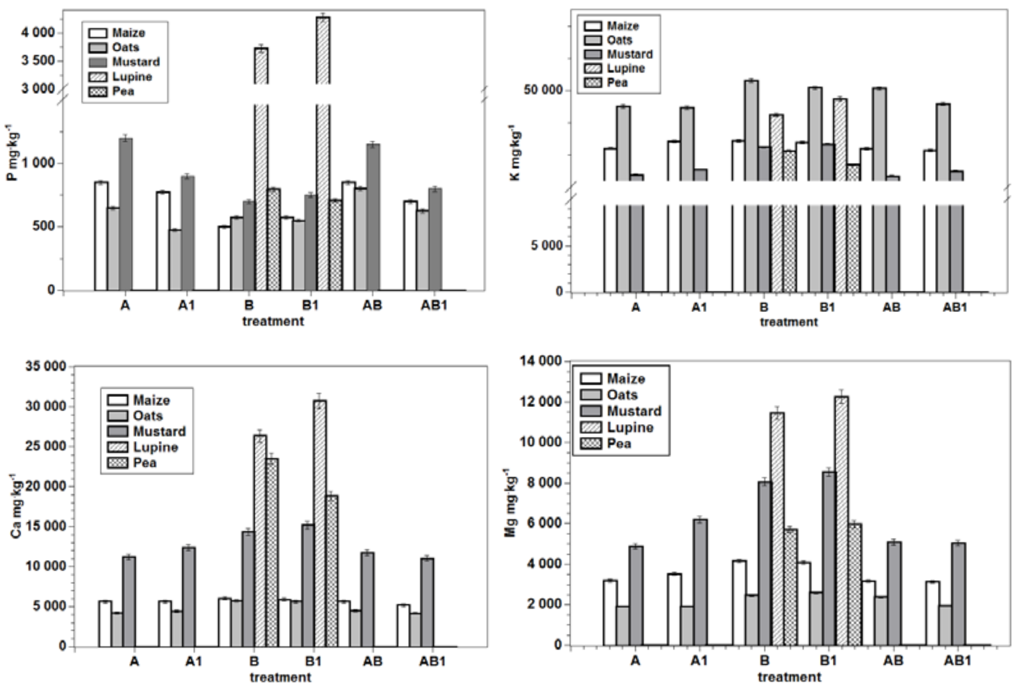

Fig. 1. The effect of ennoblement of seeds on the content of macroelements in the dry matter of plants:
A – seeds ennobled with *Azotobacter* strain, A1 – control sample in A variant,
B – seeds ennobled with biosurfactant, B1 – control sample in B variant,
AB – seeds ennobled with *Azotobacter* strain and biosurfactant, AB1 – control sample in AB variant

Analyzed legume (pea, lupine) species as best adapted to uptake of elements in difficult bioavailable forms especially phosphorus [23, 24], calcium, magnesium, zinc, manganese and iron, accumulated significantly more of analyzed components in comparison to the others. Among monocotyledonous (maize, oat), one should pay attention to the oats, because in its biomass, the most potassium was detected. Oat roots have a very high capability to uptake the nutrients contained in the ground in a hard to reach form available for the plants.

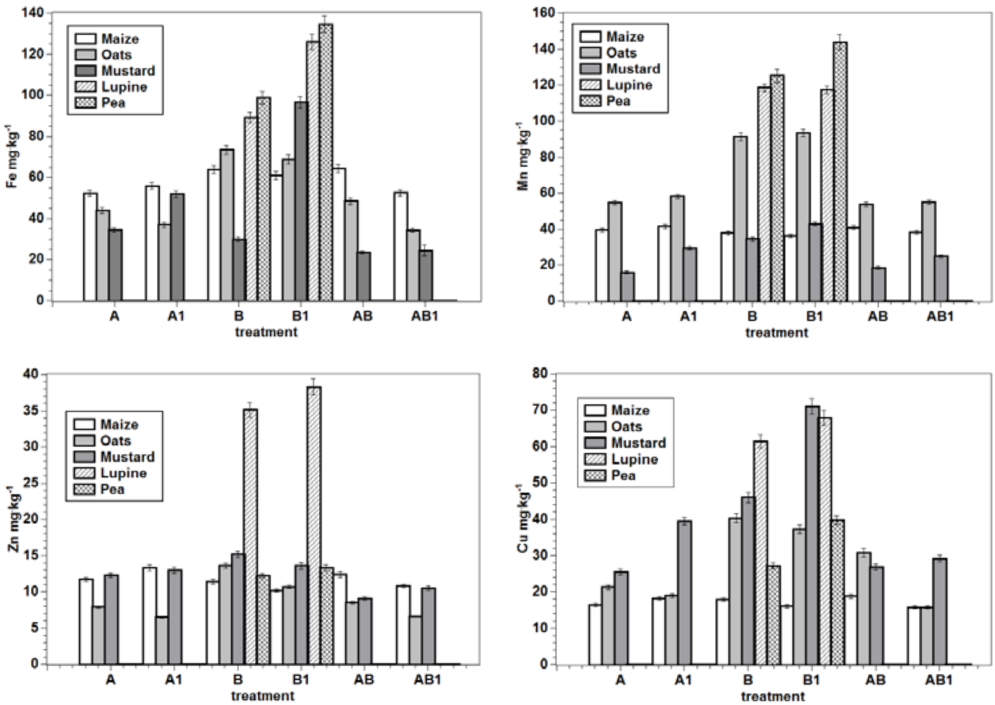

Fig. 2. The effect of ennoblement of seeds on the content of microelements in the dry matter of plants:
A – seeds ennobled with *Azotobacter* strain, A1 – control sample in A variant,
B – seeds ennobled with biosurfactant, B1 – control sample in B variant,
AB – seeds ennobled with *Azotobacter* strain and biosurfactant, AB1 – control sample in AB variant

It has been shown that bio-preparations exerted an effect on the uptake of basic nutrients by the analyzed plants. Preparation of seeds contributed to the significant increase in the content of the analyzed elements in the biomass of plants. It related mainly to the stimulation of phosphorus uptake, while the effect of bio-preparations on the uptake of the remaining elements was dependent on both, the type of plant as well as the type of bio-preparation.

It seems that monocotyledonous were more sensitive to the action of bio-preparations. Ennoblement of seeds with biosurfactant and its mixture of nitrogen-fixing bacteria suspension of *Azotobacter* genus had an effect on the uptake of analyzed elements by maize. A higher content of K, Ca, Mg, P, Zn, Mn, Fe and Cu in the aerial parts of maize was observed. In the aerial parts of oats, investigated bio-preparations caused an increase in the content of all elements with the exception of manganese. The magnesium content was higher only in plants, which seeds were ennobled by bacteria A and biosurfactant B, while the ennoblement with bacteria A did not cause the increase of calcium content in the aerial parts of oats.

Mustard is a plant for which a relatively low importance was attributed taking into account the ennoblement of seeds by tested bio-preparations. Most bio-preparations had an influence on the decrease in the content of the analyzed elements in its biomass. Particular attention should be paid to more than two-fold lower iron content in plants ennobled with the biosurfactant. Also, the content of manganese and copper was significantly lower in plants grown from ennobled seeds. Biosurfactant had usually no effect on better assimilation of biogenic elements by peas and lupins. In the biomass of peas ennobled with the biosurfactant, more phosphorus was detected, while in the biomass of lupine more calcium and manganese were detected. The effect of ennoblement, regardless of the method, significantly reduced the accumulation of copper in all tested species, which in the case of a ground with a high content of Cu is particularly significant.

4. CONCLUSIONS

The study showed that the phytostabilization post-flotation sediment may not be supported by organic fertilization. The introduction of the pulp, waste from sugar production, resulted in an increase leaching of trace metals, particularly copper, as well as an increase in the toxicity of extracts. The content of copper ions in the aqueous extract was 10.785 g/m^3 and was more than 500 times higher than in controls. Preferred from the viewpoint of phytoremediation process, we found the use of biopreparations for seed treatment. Plants grown from treated seeds characterized by an improved bioavailability of biogenic elements compared to the control. Biopreparations clearly stimulated accumulation of phosphorus by all the test plants. Particularly susceptible to the effects of biopreparations are monocotyledonous (maize, oats) which also accumulate higher amounts of calcium and magnesium in aerial parts. Seed treatment with biosurfactant (biopreparations: B, AB) increased the accumulation trace elements (Zn, Mn, Fe, Cu) by plants. Such an effect was not observed in the case of using a strain of *Azotobacter* for seed treatment. Biological stimulation did not cause accumulation of trace elements by dicotyledons (mustard, lupine, pea). Their content in the aerial parts of the plants grown from the treated seeds was lower than in the control.

The obtained results are promising and will be particularly useful to develop a strategy of reclamation of post-flotation ponds. A very important drawback in achievement of this objective is a large scale of conducted experiments and the complexity of the problem. An increase in the amount of trace elements absorbed by the plants, which can inhibit plant growth and can be subjected to concentration in food chain, may also be a problem.

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