

BIOLOGICAL CHARACTERISTICS OF “WARTOWICE” POST-FLOTATION TAILINGS POND (LOWER SILESIA, POLAND)

JUSTYNA RYBAK*, BARBARA KOŁWZAN, KAZIMIERZ GRABAS,
GRZEGORZ PASTERNAK, MAŁGORZATA KRAWCZYŃSKA

Institute of Environmental Engineering, Technical University of Wrocław
Wybrzeże Wyspiańskiego 27, 50-370 Wrocław

*Corresponding authors e-mail: justyna.rybak@pwr.wroc.pl

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Abstract: “Wartowice” tailings pond was closed in 1989, resulting in 232,4 ha tailings pile requiring reclamation. The major problem is heavy metals presence and poor nutrient conditions and physicochemical structure of soil which disturbs the plants development. In order to assess the real condition of studied area the complete biological characteristic has been done. The physicochemical conditions were assessed altogether with phytosociological, microbiological and toxicological studies of deposits. We recorded only 27 species of vascular plants belonging to 15 families on the tailings pond of which 5 belong to *Rosaceae*, 4 to *Asteraceae* and 3 to *Poaceae* and *Saliceae*. Species inhabiting the tailings depended on their dispersal capacity, metal tolerance and rhizome strategy. Microbiological analyses revealed the low number of bacteria and fungi on the tailings pond, apart from the small uplift area where the plants were indentified. Bacteria identified on the tailings pond were classified to 8 genera. The low number of bacteria suggests the lack of nutrients which affects the development of soil microflora. Toxicity tests showed that post-flotation sludge is not toxic to microorganisms because of its high pH. Some plants, such as lucerne could even influence positively the microorganisms development what has been proved in our studies. The tailings toxicity was higher towards producers, where *Secale cereale* appeared to be the most sensitive species. Amendment with topsoil from adjacent areas can influence positively the phytotoxic properties of tailings and enrich them into native seeds.

INTRODUCTION

Waste after the flotation of copper ore, due to the considerable content of copper and other heavy metals, is unusually troublesome for the environment [21]. Keeping it in an artificial containers is usually connected with a possibility of dispersing pollutants into air, water and surrounding grounds [18]. Heavy metals present in the waste pose a threat to living organisms and can influence the biocenosis composition [7]. Copper alone, in low concentrations, can stimulate a plant growth, although inhibits it in higher concentrations [6, 29].

The waste stored in “Wartowice” tailings pond can be characterized by high pH ranged from 8.0 to 8.5 which derived from the large content of CaO. As a consequence heavy metals compounds (Cu, Pb) exist in insoluble form, thus they do not enter the

water solution and are not absorbed by plants. The alkalinity of waste is at the same time the limiting or entirely blocking factor when it comes to the availability of nitrogen and phosphorus for plants [8, 10, 11, 20, 27]. Metal presence, low levels of major plant nutrients and poor physical structure of waste are the reasons why it is hard to restore biological life in places when the waste is stored [8, 10, 11, 20, 27]. The natural succession of the biocenosis occurs very slowly and the reclamation of tanks does not always end with the success. What is more, cleaning the terrain of contamination requires also a lot of money [6]. Therefore, methods based on the waste stabilization both with the application of physical, chemical as well as biological methods are very promising [22].

The invention and introduction of new technologies of the reclamation of mine tailings requires the preliminary studies containing a complete biological characteristic of the studied area, including phytosociological, microbiological and toxicological analyses of deposits. Such characteristic can serve as a basis for the subsequent selection of plants which should be used for the tank reclamation. A close relationship between the stability of the plants community and quantitative and qualitative composition of microorganisms inhabiting the degraded area was also underlined in many papers [2, 4, 6]. Authors showed that the autochthonous microflora and symbiotic microorganisms can have a fundamental influence on the tolerance of high concentrations of metals by plants and can play an important role in the restoration process of contaminated areas. The complete biological characteristic will also provide the ecotoxicological evaluation with regard to both function of waste, as habitat and retaining function [9]. Contaminants washed out from the tailings pond can migrate to surface and underground water along with rain posing a threat to the surrounding biocenosis. To summarize, the aim of the study was to determine the composition of microorganisms in both ways: quantitative and qualitative, inhabiting the tailings pond and to evaluate deposits using bioassay techniques. The studied tank along with “Żelazny Most” is the biggest, degraded area of this type in Lower Silesia.

MATERIAL AND METHODS

Tailings pond was created by disposing tailings from a former mine “Z.G. Konrad” in Iwiny, which is situated near Bolesławiec in the western part of Poland. The object is the youngest and biggest in the area. “Wartowice” no. 3 (fig. 1), started to operate in 1971. It has 232 ha. The maximum height of post-flotation tank is 32 m and the target functional capacity has been assessed as 19.4 mln m³ at an average depth of 10 m and maximum of 30 m [8, 27].

Post-flotation sediments contain lots of calcium oxides, silicon, aluminum and magnesium (CaO, SiO₂, Al₂O₃, MgO). Copper and lead predominate among heavy metals. The content of copper (3032 mg/kg) exceeds permissible values of concentrations of this element in the soil (600 mg/kg according to Decree of the Ministry of Environment on Soil and Land Quality Standards, dated 9.09.2002 [Journal of Laws (Dz. U.) No. 161, Item 1359]). Particles size of deposits with the majority of fractions with 0.06 mm of diameter facilitates the dust dispersion into bordering areas and influences flora growth [10, 11, 20, 27]. Tailings samples were collected from the top 20 cm of tailings pond. Samples were air-dried and passed through a 1-mm sieve. Analyses of chemical properties of sludge (aqueous extracts) presented in Table 1 were done with ICP MS method. They were

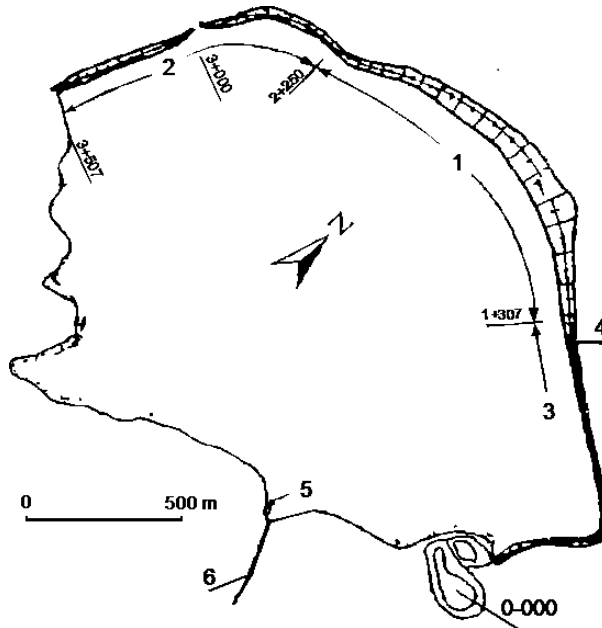


Fig. 1. Location and appearance of the "Wartowice" no. 3 post-flotation pond [14, 19, 26]

performed in the Hydrogeochemical Laboratory of University of Science and Technology in Cracow. As shows Table 1, none element exceeds the permissible value. The presented results are mean values, for example CuO content ranged from 0.184 to 0.469% [8].

Species composition and characteristics

Field studies were conducted from the beginning of May to the end of August 2009. Because the examined area was almost completely devoid of vegetation (about 90%) and the most of the plants grew only on the small uplift, all vascular plants growing on tailings were recorded. The whole examined area was divided into 3 smaller sites (Fig. 1 shows the map of the examined area). The method of Braun-Blanquet was applied in phytosociological studies [5]. The whole area (measuring 15×10 meters) was covered with phytosociological relevés, in order to recognise plant communities. Each species was named according to "Critical list of vascular plants in Poland" [19]. The vascular plants were also studied regarding their participation as the geographic-historical elements, life forms and phytociological classification [16, 28, 30].

Number of microorganisms

Samples derived from the tailings pond (T), uplift covered with plants (U) and from nearby farmland (F) and forest (FT). They were collected from the soil surface (depth of 30 cm). A suspension was prepared as follows: 10 grams of soil was placed in 90 cm³ of physiological solution and shaken thoroughly 10 minutes. We examined samples after the deposit sedimentation (5 minutes). A quantitative determination of microorganisms was

Table 1. Chemical composition of aqueous extract from tailings pond (average values) [8]

| Element | Content in aqueous extract [mg/dm ³] |
|-----------------|--|
| Sb | 0.00052 |
| Cr | 0.0018 |
| Zn | 0.015 |
| Al | 0.023 |
| Cd | 0.0015 |
| Mg | 133.1 |
| Mn | 0.033 |
| Cu | 0.052 |
| Ni | 0.0112 |
| Pb | 0.0010 |
| K | 54.72 |
| Hg | 0.00004 |
| SO ₄ | 876.0 |
| Na | 21.40 |
| Fe | 0.002 |
| Ca | 182.7 |

done by counting the colonies. Results of analyses were given appropriately in CFU/1 gram of soil. In the study we determined:

- the number of saprophytic bacteria on nutrient agar (Agar L Difco) cycloheximide, with the application of Koch's method after 72 hours of incubation in 20°C,
- the number of Actinomycetes on Pochon's medium (incubation: 5 days in 20°C),
- the number of fungi on Martin's and Saboraud's medium after 5 days of incubation in 26°C.

Identification of soil bacteria

The morphological, physiological and biochemical features of isolated strains were identified in order to determine the genera they belong to. The obtained results were confirmed with the application of Biomerieux company tests. Genotyping of bacteria strains was conducted with the cooperation of Laboratory of Molecular Biology IBB PAN in Warsaw.

Enzymatic studies of soil

The determination of soil's dehydrogenase activity was conducted according to methodology given by Show and Burns [24]. In the method a principle was used that chloride idonitrotetrazolium [INT], as the acceptor of hydrogen and electrons (with greater affinity than oxygen), is colorless in the water solution. After taking hydrogen and electrons from dehydrogenases, INT is reduced to colorful (red) formazone (INTF), the colour intensity is an indicator of the dehydrogenase activity of studied material. The

endogenic activity of soil microorganisms and the activity with the addition of easily resolvable substrate (peptone) were calculated.

Toxicity tests

The following species of plants were used for testing: *Brassica napus* L., *Phacelia tanacetifolia* Benth., *Triticum aestivum* L. and *Secale cereale* L. Before use their germination potential was examined at $20\pm 1^\circ$ in the dark. Germination rates over 90% guaranteed the viability of the seeds.

Early seedling growth tests

Sediments from the area of studies and sediments sown with the lucerne *Medicago* sp. and the clover *Trifolium* sp. named in this paper as "lucerne" and "clover" were used for the seedling growth tests. Examinations were performed with the vase method in three replicates according to the ISO/DIS norm 11269-2. Different proportions of tailings were thoroughly mixed with the appropriate weight of farm soil from an adjacent area. Tests were carried out in a greenhouse, where the following conditions were maintained: 16 h light/ 8 h dark cycle, light intensity 16,000 lux, temperature $20/16\pm 1^\circ$ (day/night), relative humidity 40–55%. Plants were placed in pots containing different proportions of tailings and control soil. 20 seeds were sown in each pot (diameter 14 cm). The constant level of soil moisture (80% of WHC, the whole water capacity) was kept during the experiment. Plants were harvested after 14 days. Plants were then removed and the measurements were performed (after washing with deionized water). Plant tissues were dried to constant weight at 70° and the total dry weights were recorded.

***Lemna minor* bioassay**

Aqueous extracts of deposit were made with the accordance of the norm of DIN 38414 [12]. The proportion of the sludge to distilled water was 1:2. The samples were shaken for 24 h and then filtered applying the membrane filter (0.22 μm) after spinning.

Growth test on *Lemna minor* L. was used for the evaluation of the toxicity of aqueous extracts of soil (chlorophyll a+b content) according to methodology based on EPA recommendations for the evaluation of the toxicity of substance in water solutions [23] and on methodology given by Lazorchak et al. [13] and Sikorski et al. [26]. The clean culture of this species was used for the assessment of the impact of the water extract on aquatic organisms (producers). Due to the slow growth of plants on control medium, a mineral nutrient was introduced instead of distilled water when preparing aqueous extract. Different concentrations were vaccinated with one-frond *Lemna minor* specimens. The experiment was conducted under 16 h light/8 h dark, 300 lux light intensity, at temperature $20/16\pm 1^\circ$ (day/night) and relative humidity 40–55%. The number of fronds, biomass, overall condition and chlorophyll a+b content of each specimen were assessed after 14 days. The experiment was prepared in three replicates.

***Microtox*® bioassay**

Bacterial luminescence inhibition bioassay was carried out using the protocol *Microtox*® system operating manual, by the measurement of the luminescence decrease of the bacterium *Vibrio fischeri* exposed to different samples. No color was observed among all of the samples, pH value was adjusted with HCl 1M and NaOH 1M, to reach value of

7.0 when it was needed. Soil samples were moistened (10% of capacity). Toxicity tests were carried out for 3 samples containing post-flotation sediments and control sample. A soil taken from surroundings (F) constituted the control sample. Other samples were as follows: sediment obtained from the area of studies and sediments sown with the lucerne *Medicago* sp. and the clover *Trifolium* sp. In order to assess the availability and the influence of heavy metals on microorganisms an aqueous extracts were prepared. The samples were shaken (speed 140 rpm in the temperature 20°C, 10 g per 30 ml of distilled water) for 24 hours. Then the supernatant was filtered with the blotting paper filter and whirled (speed 6000 rpm) for 5 min. All samples were performed in two replicates.

Statistic analyses

Experimental results were presented and statistically analyzed [3]. Microbiological tests were conducted with R Gui. A test used for checking normal distribution of results was Shapiro-Wilk test with 0.95 confidence level. When p value was lower than 0.05 the data were logarithmically transformed or transformation of square root was done. Range of confidence values and mean for transformed data was calculated with t-test and was retransformed to the initial form. EC_{50} and EC_{20} values were calculated by logit method.

RESULTS

Species composition and characteristics

Relevés were made on the small uplift (U) where plants were present. We found 27 species of vascular plants belonging to 15 families. The area of post-flotation tank was covered in 55.6% with grass and herbs (C). Trees constitute 29.6% (A) and bushes 14.8% (B). The considerable part of specimens grows separately and the rest of plants form clumps or groups. Most numerous were hemicryptophytes and phanerophytes then geophytes (Table 2).

The syntaxon *Epilobietea angustifolii* which includes two communities *Epilobio-Salicetum capreae* and *Calamgrostietum epigeji* (Table 3) was identified. *Epilobietea angustifolii*, a class of terophytes initiating a secondary succession is still poorly known. However, herbs and grasses (such as *Calamagrostis epigejos* (L.) Roth., *Chamaenerion angustifolium* L., *Fragaria vesca* L.) are characteristic for this class which can grow even on barren soil [16].

For the first identified community *Epilobio-Salicetum capreae* such species as *Salix caprea* and *Sambucus nigra* are the most characteristic. They form a relatively, long-lasting community, participating in the regeneration of forest, not only on barren soil, but also on a drier and mesotrophic coarse-grained or rocky soil. A role of accompanying species such as *Betula* sp. or *Populus tremula* L. increases in late stages, where they finally replace *Salix caprea* [16]. The second identified community *Calamagrostietum epigeji* represents grassy flora, common in Poland, in lowland. It can be a pioneer community covering sandy areas on poor soil devoid of mineral nitrogen. It could have a dynamic character and be quite long-lasting which may significantly inhibit the regeneration of the forest [16].

The participation of plants from the *Fabaceae* family is low (4%) what influences negatively the soil quality of the degraded area.

Table 2. Alphabetical list of identified species with characteristics*

| No. | Species | Family | G | F | H | Tr | A |
|-----|---|-----------------|----|-----|-----|-----|-----|
| 1. | <i>Agrostis stolonifera</i> L. | Poaceae | n | H | 4 | 3-4 | 3-5 |
| 2. | <i>Anthemis cotula</i> L. | Asteraceae | Ar | T | 3 | 4-5 | 4 |
| 3. | <i>Betula pendula</i> Roth | Betulaceae | Ap | M | 3 | 2-3 | 3-4 |
| 4. | <i>Calamagrostis epigeios</i> (L.) Roth | Poaceae | Ap | G,H | 3 | 3 | 3 |
| 5. | <i>Cerastium brachypetalum</i> Pers. | Caryophyllaceae | n | T | 2 | 2 | 5 |
| 6. | <i>Cerasus vulgaris</i> Mill. | Rosaceae | n | M | - | - | - |
| 7. | <i>Chamomilla suaveolens</i> (Pursh) Rydb. | Asteraceae | Kn | H | - | - | - |
| 8. | <i>Chamaenerion angustifolium</i> L. | Onagraceae | n | H | 3 | 3-5 | 3-4 |
| 9. | <i>Daucus carota</i> L. | Apiaceae | Ap | H | 3 | 4 | 4-5 |
| 10. | <i>Elymus repens</i> L. | Poaceae | Kn | G | 3 | 3-4 | 3-5 |
| 11. | <i>Equisetum arvense</i> L. | Equisetaceae | Ap | G | 3-4 | 3-4 | 3-4 |
| 12. | <i>Fragaria vesca</i> L. | Rosaceae | n | H | 3 | 3 | 3-4 |
| 13. | <i>Malus sylvestris</i> (L.) Mill. | Rosaceae | n | M | 3 | 4 | 4 |
| 14. | <i>Picea abies</i> (L.) H.Karst | Pinaceae | n | M | 3-4 | 2-3 | 1-3 |
| 15. | <i>Pinus sylvestris</i> L. | Pinaceae | Ap | M | 2-4 | 1-3 | 1-5 |
| 16. | <i>Populus alba</i> L. | Salicaceae | n | M | 3-4 | 4 | 5 |
| 17. | <i>Populus tremula</i> L. | Salicaceae | Ap | M | 3 | 3 | 3 |
| 18. | <i>Quercus robur</i> L. | Fagaceae | Ap | M | 3-4 | 3-4 | 3-4 |
| 19. | <i>Rosa canina</i> L. | Rosaceae | n | N | 3-4 | 3-5 | 3-4 |
| 20. | <i>Rubus fabrimontanus</i> Spreng. | Rosaceae | n | C | 3 | 3 | 2-4 |
| 21. | <i>Rumex acetosa</i> L. | Polygonaceae | Ap | H | 3-4 | 4 | 4 |
| 22. | <i>Salix caprea</i> L. | Salicaceae | Ap | N | 3-4 | 3-4 | 3-4 |
| 23. | <i>Taraxacum officinale</i> F. H. Wigg | Asteraceae | Ap | H | 3 | 4 | 4-5 |
| 24. | <i>Trifolium pratense</i> L. | Fabaceae | Ap | H | 3 | 4 | 4 |
| 25. | <i>Tussilago farfara</i> L. | Asteraceae | Ap | G | 3-4 | 3-4 | 4 |
| 26. | <i>Urtica dioica</i> L. | Urticaceae | Ap | H | 3-4 | 4-5 | 4 |
| 27. | <i>Vaccinium myrtillus</i> L. | Ericaceae | n | Ch | 3-4 | 2-3 | 2-4 |

* This analysis was done within the confines of master's thesis of Beata Brzezicka conducted on Wrocław University of Technology under dr J. Rybak supervision

Legend:

G – geographic-historical groups: Ap – apophytes, Ar – archeophytes, Kn – kenophytes; n – not classified

F – life forms according to Raunkia: C – herbaceous chamaephyte, Ch – arboreal chamaephyte,

G – geophyte, H – hemicryptophyte, M – megaphanerophyte, N – nanophanerophyte, T – terophyte,

H – humidity rate: 1 – very dry site 2 – dry, 3 – fresh, 4 – humid, 5 – wet,

Tr – trophic rate: 1 – very poor soil, 2 – poor soil, 3 – moderate soil, 4 – rich soil, 5 – very rich soil,

A – acidity rate: 1 – very acid $\text{pH} < 4$; 2 – acid $4 \leq \text{pH} < 5$; 3 – slightly acid $5 \leq \text{pH} < 6$; 4 – neutral $6 \leq \text{pH} < 7$; 5 – alkaline $\text{pH} > 7$

Table 3. Community identification

| Relevé no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Constancy |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----------|
| Class, order: | | | | | | | | | |
| Epilobietea angustifolii | | | | | | | | | |
| ChCl, ChO: | | | | | | | | | |
| <i>Calamagrostis epigeios</i> | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | - | 1.2 | 2.2 | V |
| <i>Chamaenerion angustifolium</i> | 2.2 | - | 3.2 | 2.2 | 4.2 | - | - | - | III |
| <i>Fragaria vesca</i> | - | - | 1.1 | - | - | - | - | 1.2 | II |
| Association: | | | | | | | | | |
| Sambuco-Salicion | | | | | | | | | |
| ChAll: | | | | | | | | | |
| <i>Salix caprea</i> | +1 | +1 | +1 | r.1 | +1 | - | 4.1 | 4.1 | V |
| DAll: | | | | | | | | | |
| <i>Betula pendula</i> | 5.2 | 5.2 | 5.2 | 4.2 | 3.2 | 5.2 | 2.1 | 4.2 | V |
| <i>Populus tremula</i> | 3.2 | 4.2 | 4.1 | 4.1 | 2.2 | 4.1 | - | - | IV |
| Community: | | | | | | | | | |
| Epilobio-Salicetum capreae | | | | | | | | | |
| ChAss: | | | | | | | | | |
| <i>Salix caprea</i> | +1 | +1 | +1 | r.1 | +1 | - | 4.1 | 4.1 | V |
| DAss: | | | | | | | | | |
| <i>Betula pendula</i> | 5.2 | 5.2 | 5.2 | 4.2 | 3.2 | 5.2 | 2.1 | 4.2 | V |
| <i>Populus tremula</i> | 3.2 | 4.2 | 4.1 | 4.1 | 2.2 | 4.1 | - | - | IV |
| Calamgrostietum epigeji | | | | | | | | | |
| ChAss: | | | | | | | | | |
| <i>Calamagrostis epigeios</i> | 3.2 | 3.2 | 3.2 | 3.2 | 3.2 | - | 1.2 | 2.2 | V |
| Accompanying species | | | | | | | | | |
| <i>Elymus repens</i> | 2.1 | 2.3 | 3.2 | +2 | 2.1 | 2.2 | 1.2 | 3.2 | V |
| <i>Quercus robur</i> | 2.1 | 2.2 | +1 | 1.1 | 3.2 | r.1 | r.1 | - | V |
| <i>Tussilago farfara</i> | +1 | 1.1 | 1.1 | 2.2 | 1.2 | - | 1.1 | 1.1 | V |
| <i>Pinus sylvestris</i> | - | +1 | +1 | 1.1 | 1.1 | +1 | - | +2 | III |
| <i>Anthemis cotula</i> | - | - | 1.2 | - | 2.2 | 1.2 | - | +2 | III |
| <i>Agrostis stolonifera</i> | - | - | +2 | - | - | - | - | 1.2 | II |
| <i>Cerastium brachypetalum</i> | - | +1 | 1.1 | 1.1 | - | - | - | - | II |
| <i>Daucus carota</i> | - | - | +1 | - | - | - | - | +1 | II |
| <i>Equistum arvense</i> | - | - | 3.2 | - | - | +2 | - | 1.2 | II |
| <i>Vaccinium myrtillus</i> | - | - | 3.3 | 2.3 | - | - | - | - | II |
| <i>Rumex acetosella</i> | - | 1.2 | - | - | - | 2.2 | - | - | II |
| <i>Rosa canina</i> | - | r.1 | - | - | +2 | - | - | - | II |
| <i>Rubus fabricomontanus</i> | - | - | - | - | r.1 | - | - | 1.1 | II |
| <i>Trifolium pratense</i> | - | - | 1.1 | 1.1 | - | - | - | - | II |
| <i>Picea abies</i> | - | - | +1 | - | +1 | - | - | - | II |
| Accessory species | | | | | | | | | |
| <i>Populus alba</i> | 2.2 | - | - | - | - | - | - | - | I |
| <i>Chamomilla suaveolens</i> | +3 | - | - | - | - | - | - | r.3 | I |
| <i>Urtica dioica</i> | - | +2 | - | - | - | - | - | - | I |
| <i>Malus sylvestris</i> | - | - | - | - | - | - | - | r.1 | I |
| <i>Cerasus vulgaris</i> | - | - | - | - | - | - | - | r.1 | I |
| <i>Taraxacum officinale</i> | - | - | - | - | 1.1 | - | - | - | I |

Microbiological analyses

In the post-flotation sediments and in the soil surrounding the tailings pond a presence of microorganisms of both the bacteria and fungi was recorded. The number of bacteria was higher than that of fungi. Although, the number of Actinomycetes, typical soil bacteria, was low. Probably the majority of microorganisms occurred in spore forms or in anabiosis state what proves the low enzymatic activity of the studied samples. The addition of the organic source of carbon such as peptone and glucose does not trigger the enzymatic processes in most cases. The analysis of the taxonomical status of strains inhabiting the tailings pond revealed 8 genera: *Bacillus*, *Stenotrophomonas*, *Sphingopyxis*, *Brevundimonas*, *Xanthomonas*, *Pseudomonas*, *Nexibacter* and *Methylobacterium*. These bacteria are resistant to heavy metals occurring in the degraded areas. The results of the analysis of 40 soil samples are presented in Table 4. The lowest number of microorganisms was detected in samples collected in tailings pond (T) (Fig. 2). On the other hand, the most numerous microorganisms were found in soil samples collected from farmland (F) and the small uplift (U) where plants were present. However, in the soil samples collected from the forest (FT), the number of bacteria was relatively low, although about 4 times higher than in the pond (T) alone. The number of fungi was about 300 times higher than in the tank area (T). The low number of microorganisms and low dehydrogenase activity obtained for the forest soil (FT) could be connected with the low pH of forest soil (FT) where coniferous trees are present. The high number of microorganisms on the small uplift (U) is surprising in spite of dust presence and lack of nutrients. The dehydrogenase activity of all samples was low, which suggests the low metabolic activity of soil microorganisms and bad physiological condition. The farmland (F) contained more active metabolic microorganisms than the rest of the area (FT, T, U). The introduction of small amounts of the organic carbon source results in the increase of the enzymatic activity, what was well demonstrated in the samples collected from the small uplift (U). The lack, low or even negative correlations among the number of microorganisms and dehydrogenases activity were revealed which resulted from the very low number of bacteria, thus slight fluctuations of their number (on the level of 10^2 – 10^3) were too low in order to influence dehydrogenases activity. Additionally, bad physiological factors also had a great impact on the obtained results of calculations. Our examinations showed that lack of biogenic substances affect the development of soil microorganisms. A surface layer of soil was inhabited relatively well by microorganisms up to the depth of 30 cm where a distinct decrease of number of microorganisms and low enzymatic activity was observed. The small size of soil particles restricts the oxidation of its deeper layers and reduces the development of both plants and soil microorganisms.

Toxicity tests

The studies showed the low toxicity of deposits towards applied tests. It resulted mainly from the alkalinity of studied samples, thus the low water solubility of heavy metals occurring in the deposit.

The inhibition of lighting of luminous bacteria on the toxic level was not observed. However, a fluctuation in the intensity of luminescence was recorded when compared to the control. Figs 3 and 4 present the results concerning the intensity of luminescence of undiluted samples. A low inhibition of luminescence of deposit was observed (about 6% compared to the control). The samples collected from the area, where previously clover

Table 4. Microbiological analysis of soil samples

| | | Total number of bacteria | Total number of fungi | Total number of Actinomycetes | Dehydr. activity | Dehydr. activity, sample with peptone | Sample type |
|-------------------------|-------|--------------------------|-----------------------|-------------------------------|----------------------------|---------------------------------------|--------------------------------|
| | | CFU/1g d.w. | CFU/1g d.w. | CFU/1g d.w. | µg INTF/g d.w. soil/24 hrs | µg INTF/g d.w. soil/24hrs | |
| Mean | | 2.62E+04 | 5.E+01 | 2.E+01 | 19 | 30 | tailings pond (T) |
| 95% confidence interval | Lower | 9.22E+03 | 1.E+01 | 2.E+00 | 8 | 17 | |
| | Upper | 4.31E+04 | 8.E+01 | 4.E+01 | 30 | 52 | |
| Mean | | 3.88E+05 | 4.97E+03 | 7.59E+02 | 28 | 185 | uplift covered with plants (U) |
| 95% confidence interval | Lower | 1.52E+05 | 1.67E+03 | 1.13E+02 | 10 | 82 | |
| | Upper | 6.24E+05 | 1.47E+04 | 5.12E+03 | 80 | 419 | |
| Mean | | 3.31E+05 | 1.09E+04 | 1.06E+04 | 96 | 353 | farmland (F) |
| 95% confidence interval | Lower | 1.41E+05 | 5.10E+03 | 6.00E+02 | 48 | 123 | |
| | Upper | 7.78E+05 | 1.67E+04 | 3.28E+04 | 193 | 583 | |
| Mean | | 4.28E+04 | 3.66E+02 | 7.90E+02 | 24 | 71 | forest (FT) |
| 95% confidence interval | Lower | 1.62E+04 | 8.E+01 | 1.91E+02 | 12 | 42 | |
| | upper | 1.13E+05 | 1.70E+03 | 3.26E+03 | 51 | 121 | |

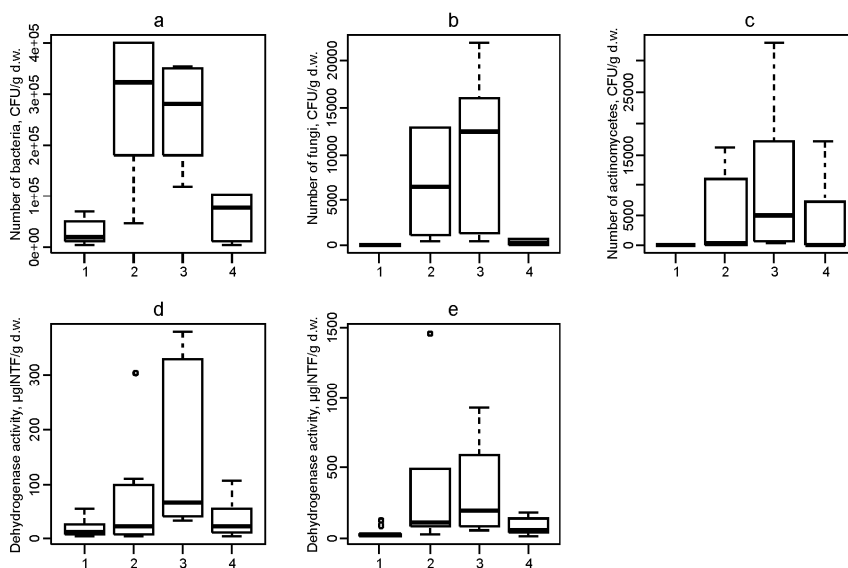


Fig. 2. Box charts for all studied sites 1 – tailings pond (T), 2 – uplift covered with plants (H), 3 – farmland (F), 4 – forest (FT). Charts a, b, c illustrate the number of microorganisms for the readability distant values were not marked. Chart d illustrates dehydrogenase activity, d – dehydrogenase activity with addition of peptone

Trifolium sp. was planted, caused the inhibition of luminescence up to 32.5%. However, no inhibition was observed in the samples with lucerne *Medicago* sp. The increase of luminescence has even been observed in more diluted samples which in consequence led to the decline of the effect of the inhibition below zero. The rise of luminescence was highest for the 40% concentration and form a distinct trend showed in the function of extract concentration (Fig. 4).

The toxicity of post-flotation deposit towards producers was higher when compared to the bacteria. It probably resulted from the possibility of absorbing by plants of water-insoluble substances, thanks to the secretion of peculiar metabolites increasing the solubility and facilitating the absorption. In most cases it was possible to establish EC_{50} value. The influence of deposit on the length of both: shoot and root was observed (Fig. 5, 6). The most sensitive were *B. napus* and *S. cereale* (Table 5). However, 50% inhibition of

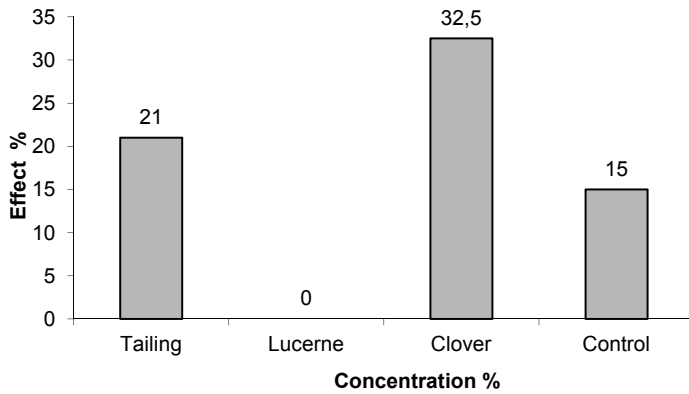


Fig. 3. Microtox bioassay. The low effect value obtained for the highest tested concentration of tailings (81%)

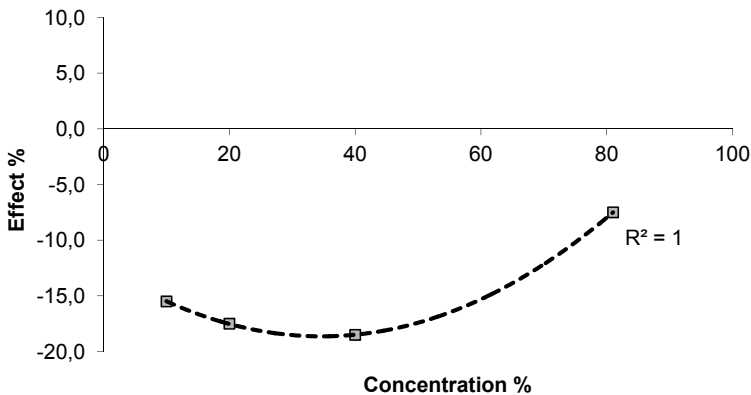


Fig. 4. The relationship between the concentration of aqueous extract "Lucerne" and luminescence intensity obtained in Microtox test

root development of *S. cereale* was observed in the 26.34% of deposit concentration. Due to its sensitivity only *S. cereale* was applied for the toxicological evaluation of the tailings collected after the plants cultivation. It was showed that the phytotoxicity of deposits after the plants growth had been much lower when concerning the root length (Table 5). However, the level of inhibition of shoot height depended on the planted species and was more effective in the case of lucerne.

The inhibition of chlorophyll production in *L. minor* has been observed. However, no phytotoxic effect was recorded before and after the cultivation of plants (Table 5).

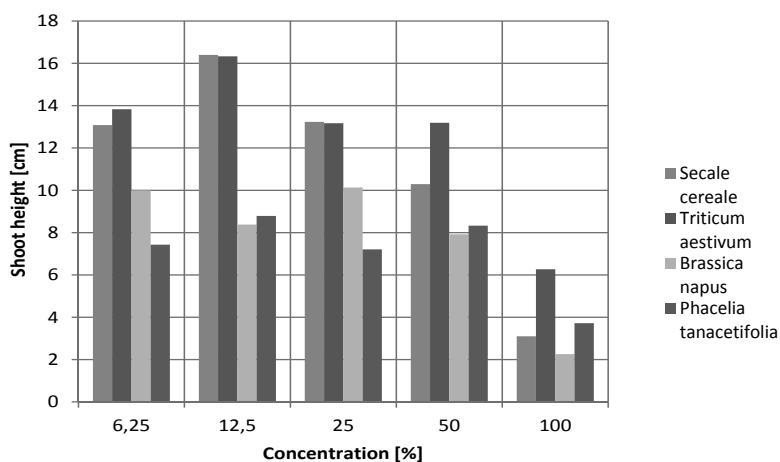


Fig. 5. Shoot height of four studied species in different extract of soils and tailings

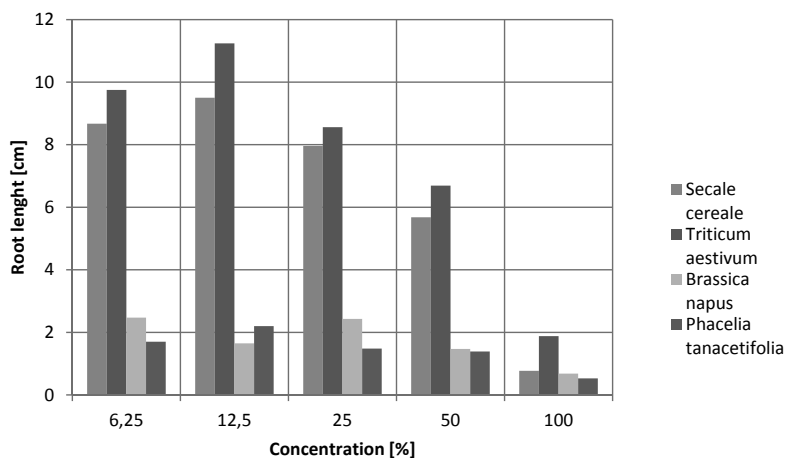


Fig. 6. Root elongation of four studied species in different extract of soils and tailings

Table 5. Bioassay results

| | Test species | Parameter | EC ₅₀ [%] | EC ₂₀ [%] |
|---------------------------------------|-------------------------------|--------------|----------------------|----------------------|
| Tailing | <i>Secale cereale</i> | Shoot height | 26.34 | 5.47 |
| | <i>Triticum aestivum</i> | | 51.47 | 11.81 |
| | <i>Brassica napus</i> | | 43.41 | 4.44 |
| | <i>Phacelia tanacetifolia</i> | | 51.72 | 4.01 |
| | <i>Secale cereale</i> | Root length | 81.76 | 13.70 |
| | <i>Triticum aestivum</i> | | >100 | 48.17 |
| | <i>Brassica napus</i> | | 66.07 | 6.68 |
| | <i>Phacelia tanacetifolia</i> | | >100 | 10.32 |
| Tailing after lucerne harvest | <i>Secale cereale</i> | Shoot height | 68.99 | 28.82 |
| | <i>Secale cereale</i> | Root length | 64.26 | 8.59 |
| Tailing after clover harvest | <i>Secale cereale</i> | Shoot height | 93.04 | 12.44 |
| | <i>Secale cereale</i> | Root length | 38.94 | 5.52 |
| Aqueous extract of tailing | <i>Lemna minor</i> | Chlorophyll | 39.57 | 5.29 |
| Aqueous extract after lucerne harvest | <i>Lemna minor</i> | | 40.16 | 5.92 |
| Aqueous extract after clover harvest | <i>Lemna minor</i> | | 44.28 | 5.37 |

DISCUSSION

Mine tailings impose various adverse effects on plant growth, particularly high levels of heavy metals and other elements in toxic concentrations but also low amounts of plant nutrients, acidity, salinity, alkalinity and poor physical structure [1, 10, 25]. Normal growth of plant is achieved when pH ranges from 4 to 9, therefore pH value of the studied tailing site is within the normal ranges for plant growth. Other nutrients like N, P and organic matter are the main factor influencing plant growth [1, 25]. Organic matter releases nutrients in soluble form and contributes to soil physical properties [1, 25].

The present study suggests that the natural colonization of plants was limited to small uplift (U) the only place inside the pond where plants were present. Probably this is the case when vegetation succession is dependent on physicochemical properties of the mine waste [15]. Shu et al. [25] revealed that wind-borne seeds of great immigration capacity (small, light or with pappus) have the major impact to colonize newly formed wastelands. Among 27 species colonizing the tailings pond, 5 belong to *Rosaceae*, 4 to *Asteraceae* and 3 to *Poaceae* and *Salicaceae*, which indicates their great immigration capacity. *Rosaceae* is a large family with seeds which are easily dispersed by animals. Most *Asteraceae* species have small and wind-borne seeds. These families altogether with *Caryophyllaceae* (recorded also in the studied area) and *Poaceae* are among metal tolerant taxa [25]. Only 4 rhizomatous species (*C. epigeios*, *Daucus carota* L., *Elymus*

repens L. and *Tussilago farfara* L.) were found among 27 growing in the deposit. However, they occurred constantly and they were abundant which suggest that they could play an important role during colonization of tailings pond. It is known that rhizomatous species can easily adapt to mine tailings, such phenomenon is called rhizome strategy [25]. Therefore, an artificial introduction of seeds (by applying topsoil from adjacent areas) should accelerate the colonization of plants on these wastes.

The stability of the community of plants depends on quantitative and qualitative composition of microorganisms inhabiting the studied area [2, 4, 6]. Unfortunately, microorganisms appeared in small numbers in the studied area. Again poor physical structure of deposit and lack of biogenic substances, mainly organic source of carbon inhibits the bacteria development. The analysis of enzymatic activity additionally confirms this thesis [17].

Toxic tests showed that post-flotation deposit probably because of its alkalinity is not toxic to microorganisms. Some plants can play the stimulating role for the microorganisms' development through the secretion of extracellular substances, what suggest the results obtained from Microtox® test (samples with lucerne). Unfortunately, it is possible that substances secreted by plant roots can also launch metals and contribute to enormous uptake of them by plants or to their migration with rain water.

Our studies showed that *S. cereale* was the most sensitive species to post-flotation deposit. When the tailings were mixed with farm soil (from the adjacent areas) the greater proportion of farm soil in the mixture, the greater were the results achieved (concerning shoot height and root length). This could be due to a reduction in the toxicity or improvement in nutrient availability or in soil physical properties.

CONCLUSION

- Plant development in the studied area is inhibited by the lack of nutrients, mainly nitrogen, phosphorus and organic carbon and physical structure of deposit alone.
- Only 27 species of vascular plants were recorded within uplift and the very close surroundings of dam crown area. Because of slow natural colonization artificial acceleration would be recommended. Amendment with topsoil from the adjacent areas can ameliorate the phytotoxic properties of tailings and enrich them into native seeds.
- Microbiological analyses showed the low number of bacteria and fungi which is connected with nutrients deficiency. The only exception was uplift area (where the plants described above were identified).
- Toxicity tests showed that sludge, because of its high pH, is not toxic to microorganisms. Toxicity was higher towards producers, where *Secale cereale* appeared to be the most sensitive species.
- The application of biopreparation on the basis of the autochthonous microflora in order to make the pond rich into microorganisms is necessary. Additionally, the introduction of some native, rhizomatous grasses such as *Calamagrostis epigeios* could contribute significantly in the reclamation of this area.
- However, both nutrient addition and colonization by plants can potentially contribute to the launching of heavy metals. Therefore, field and lysimetric study should be conducted before the beginning of the reclamation of this area.

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CHARAKTERYSTYKA BIOLOGICZNA ZBIORNIKA OSADÓW POFLOTACYJNYCH „WARTOWICE”

Osadnik po flotacji miedzi „Wartowice” został zamknięty w 1989 roku, co skutkowało pozostawieniem 232,4 hektarów osadów, które wciąż wymagają rekultywacji. Podstawowy problem stanowią: niedobór substancji odżywczych, obecność metali ciężkich oraz fizyczno-chemiczna struktura gleby, który zaburza rozwój roślin. Aby dokonać oceny czynników wpływających na rozwój organizmów żywych przeprowadzono kompleksową biologiczną charakterystykę terenu badań. Badania objęły fizyczno-chemiczne analizy, badania fitosocjologiczne, mikrobiologiczne i toksykologiczne. Na obszarze badań zarejestrowano tylko 27 gatunków roślin naczyniowych (obszar niewielkiego wzniesienia) należących do 15 rodzin, 5 z nich należało do rodziny *Rosaceae*, 4 do *Asteraceae* i po 3 do *Poaceae* i *Saliceae*. Obecność gatunków zależała od ich potencjału kolonizacji, tolerancji na metale oraz morfologii (strategia kłęczca). Analizy mikrobiologiczne wykazały niską liczbę bakterii i grzybów na obszarze osadnika za wyjątkiem małej wysepki (wzniesienie) gdzie wcześniej stwierdzono obecność roślin. Bakterie obecne na obszarze badań zostały zaklasyfikowane do 8 rodzajów. Niska liczba bakterii sugeruje brak substancji odżywczych, który z kolei upośledza rozwój mikroflory glebowej. Badania toksykologiczne wykazały niską toksyczność osadu w stosunku do mikroorganizmów, co wynika z jego zasadowego pH. Pewne gatunki, jak na przykład lucerna, mogą wpływać korzystnie na rozwój mikroorganizmów glebowych, co zademonstrowano w prezentowanych badaniach. Toksyczność odpadów po flotacji miedzi była wyższa w stosunku do producentów. Żyto zwyczajne (*Secale cereale*) okazało się być najwrażliwszym gatunkiem. Zastosowanie wierzchniej warstwy gleby z obszarów przyległych mogłoby znacznie obniżyć fitotoksyczne własności odpadów i jednocześnie wzbogacić je w rodzime nasiona, które dałyby początek roślinom odpornym na niekorzystne warunki siedliskowe.