Bioaccumulation and Bioleaching of Cu, Pb, and Zn from Mining Waste

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Abstract

The subject of this study was to determine the effectiveness of Aspergillus niger (Pezinok) and Neosartorya fischeri strains in bioleaching and bioaccumulation of metals from mining waste from the Hodruša tailings pond. The waste used in the work contained 0.10 mm fractions weighing 1 and 2 g from different depths of the soil matrix, samples A: 10–20 cm, samples B: 20–30 cm. We focused on the elements copper, lead and zinc with the content of individual elements (mg/kg) copper – in samples A 487 mg/kg, in samples B 115 mg/kg. Lead – samples A 1208 mg/kg, samples B 582 mg/kg. Zinc – samples A 6970 mg/kg, samples B 6808 mg/kg. We confirmed the effectiveness of the Aspergillus niger strain in this process due to the production of organic acids (citric acid, oxalic acid, acetic acid). In the experimental part, we found that the most effective strain in determining the efficiency of extraction and concentration of metals in the solution after bioleaching was the Aspergillus niger strain. The Neosartorya fischeri strain appears to be most effective in accumulating metals in solution and accumulating metals in biomass. We can justify this phenomenon due to the different properties of the cell walls of individual fungal strains and it is necessary to keep these differences in mind when comparing biosorption properties.

Keywords: *Aspergillus niger, Neosartorya fischeri, bioleaching, bioaccumulation, copper, lead, zinc*

Introduction

The purpose of the study was experimental quantification of bioleaching of Cu, Zn and Pb using microorganisms from solid substrates of mining waste. Sub-objectives including evaluation of extraction efficiency of selected elements, quantification of their bioaccumulation and monitoring of these processes using an autochthonous microbiota enriched with Aspergillus niger and Neosartorya fischeri strains while increasing the efficiency of leaching processes. Monitoring and comparison of the influence of cultivation length and the amount of material used in the bioleaching process. The experiments are performed on native samples of flotation sludge taken on the model subpage Hodruša – Hámre with the studied sludge pond (304 m. a. s. l.), which are located in the part of Dolné Hámre (Masarovičová et al. 2000). It is a permanent repository of flotation sludge generated during mining activities.

Methods

The collection of the examined material took place in the locality of Hodruša – sludge pond, in the region of Banská Štiavnica. A line profile was chosen for sampling in the length range of 20 meters between the individual sampling points. The samples were marked HO–1 to HO–8 (HO – Hodruša), while HO – 1a, 2a, 3a, 4a, 5a, 6a, 7a, 8a represented the collected substrate in the depth range from the surface of 10 to 20 cm. Samples marked HO – 1b, 2b, 3b, 4b, 5b, 6b, 7b, 8b were taken in the depth range of 20 to 30 cm (in both cases they were mixed samples). The top contaminated layer formed by plastic clay was also taken from the samples. Sampling points are recorded on the created map (Google Earth map base, Fig. 1), which is supplemented by Table 1 indicating the individual coordinates of the sampling points.

The removed substrate from the sludge was allowed to dry at room temperature (20–22°C). The substrate was homogenized after drying by stirring and sieving through a sieve (0.10 mm fraction), and the obtained material was subsequently analyzed by F-AAS and HG-AAS methods. The solid samples (biomass and substrate along with filter paper) were decomposed with acids (H_2SO_4, HNO_3 and HCl) at high pressure under microwave irradiation to form a solution in which the elements were analyzed. The results of the analysis determined the composition of the substrate to the content of the elements monitored by us (mg/kg) recorded in Table 2.

Preparation of cultures of microscopis fungi

We prepared slant agar with Sabouraud medium (SAB) containing 20 g of glucose and 10 g of peptone pH 5.6 from Himedia (India) for culturing filamentous fungi and yeasts. We followed the standard procedure in the preparation of cultures. After solidification, the individual tubes were inoculated with A. niger and N. fischeri strains, which we cultured for 14 days in a dark environment at 4°C. We created two sets of flasks into which we weighed 1 g and 2 g of substrate from both samples for three replicates from each. We poured 50 ml of liquid nutrient medium into the samples. The first set was cultured for 20 days and the second for 40 days.

To calculate the extraction efficiency of individual elements (Pb, Cu, Zn) from samples of solid substrates, we used the formula according to the study of Yang et al. (2008) used in the calculation of leaching of As and Sb.

$$\text{metal extraction efficiency (\%)} = \frac{C_L \times V_L}{C_S \times M_S} \times 100$$

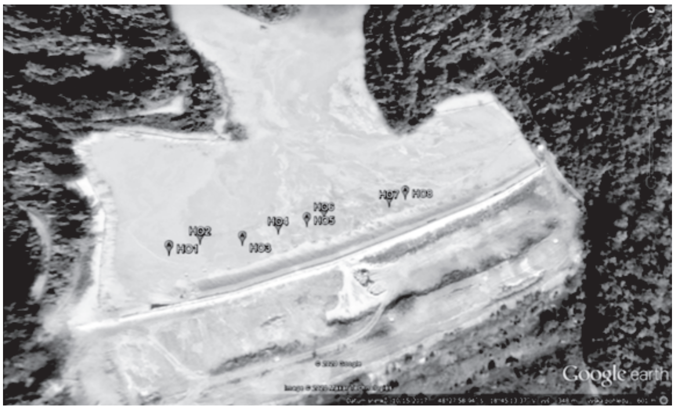

Fig. 1. Aerial view of the site where the sampling points are marked (Google Earth map background)
Rys. 1. Widok z lotu ptaka miejsca, w którym zaznaczono punkty poboru próbek (tło mapy Google Earth)

Tab. 1 Designation of the samples and the coordinates of the specific sampling point
Tab. 1. Oznaczenie próbek i współrzędne konkretnego punktu pobierania próbek

Sample marking	GPS coordination of the collection points
HO1	N48°27.959´ E018°45.149´
HO2	N48°27.964´ E018°45.161´
HO3	N48°27.966´ E018°45.179´
HO4	N48°27.970´ E018°45.195´
HO5	N48°27.973´ E018°45.208´
HO6	N48°27.976´ E018°45.216´
HO7	N48°27.980´ E018°45.247´
HO8	N48°27.983´ E018°45.255´

Tab. 2. Total content of monitored elements in individual samples
Tab. 2. Całkowita zawartość monitorowanych pierwiastków w poszczególnych próbkach

Element contents (mg/kg)	Sample A	Sample B	Measurement uncertainty
Cu	487	115	3%
Pb	1208	528	3%
Zn	6970	6808	2%

CL is the concentration of metals in the solution after leaching (mg/l), VL is the volume of the solution after leaching (l), CS is the concentration of metals in the substrate before leaching (mg/g) and MS is the amount of substrate added (g) (Yang, 2008). In the analysis of samples, we used the Student's test to determine the value of the statistical difference. The differences between the variability of the parameter of the control sample and the experiment were able to divide the results into 4 levels of significance. For this purpose, we created 24 sets, with three repetitions in each set. The results obtained represent the average of the three replicates we performed for each set. We changed the following parameters in the kits: weight of 1 or 2 g, substrate A or B, Aspergillus niger or Neosartorya fischeri strain used, cultivation time 20 or 40 days. We observed differences in the concentrations of individual metals after leaching into solution.

Used strains for bioleaching

Aspergillus niger Pezinok – isolated from stream sediment in the mining area in Pezinok (contamination with As, Sb and other elements, As 363 mg/kg; Sb 93 mg/kg; Fe 82.8 mg/kg), pH (H_2O) = 5.30.

Neosartorya fischeri Pezinok – strain isolated from the locality Pezinok – tailings pond (contamination with As, Sb and other elements) pH (H_2O) = 6.79.

Results and discussion

For samples focusing on zinc leaching, we evaluated A. niger for substrate B (zinc content in substrate: 6808 mg/kg) with a culture time of 20 days at a weight of 2 g (22.53 mg/l) as the most effective strain. Although the proportion of zinc in this sample was slightly lower, the concentration of zinc in the solution after leaching was higher (sample A contained: 6970 mg/kg zinc). We can substantiate this statement with the production of acids by the A. niger strain. We can substantiate this statement thanks to several works. Golab and Orlowska (1988) in their publication on the effectiveness of organic acids produced by the A. niger strain in these processes. This strain is one of the most efficient accumulators of heavy metals (Ren et al. 2009). The leaching of these metals is ensured by means of acids, especially citric, gluconic, oxalic or tartaric acid. In a set of samples in which we determined the concentration of metals in the solution after leaching (mg/l), according to a study by Hendrich et al. (2016), the duration of the bioleaching process should be one of the essential factors that limit the amount of leached elements from the substrate. According to their study, a longer duration of bioleaching should ensure higher values of the yield of elements from the substrate. However, we did not confirm their claim in our experiments. Some of our samples showed specific behavior, in which we observed that a shorter leaching time (20 days) was more effective: Copper – in the HO–A sample 1g for the A. niger strain as well as for the autochthonous microflora. For the HO–A sample 2g as well. For sample HO–B 2g for indigenous microflora. Lead – for sample HO–A 1g for autochthonous microflora, for sample HO–A 2g for strain A. niger, for sample HO–B 1g for strain A. niger as well as for autochthonous microflora and in sample HO–B

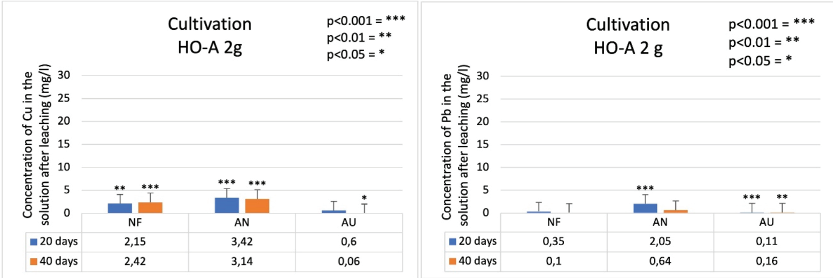

Fig. 2. Concentration of Cu and Pb in the solution after leaching (mg / l) in the sample HO–A 2g
Rys. 2. Stężenie Cu i Pb w roztworze po ługowaniu (mg/l) w próbce HO-A 2g

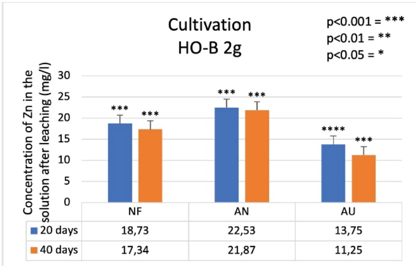

Fig. 3. concentration in the solution after leaching (mg / l) in the sample HO–B 2g
Rys. 3. stężenie w roztworze po ługowaniu (mg/l) w próbce HO-B 2g

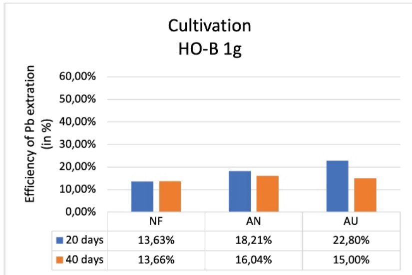

Fig. 4. Pb extraction efficiency (in%) in sample HO–B 1g
Rys. 4. Wydajność ekstrakcji Pb (w%) w próbce HO-B 1g

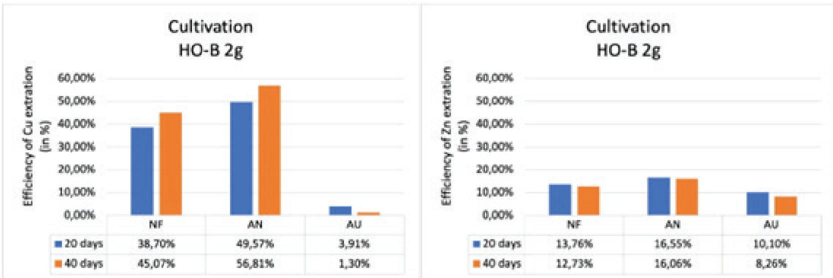

Fig. 5. Extraction efficiency of Cu and Zn (in%) in the sample HO–B 2g
Rys. 5. Wydajność ekstrakcji Cu i Zn (w%) w próbce HO-B 2g

2g for strain A niger. Zinc – for sample HO–A 2g for N. fischeri strain and autochthonous microflora and sample HO–B 2g for all strains. We can justify the behavior by a study by Boriová et al. (2014), which points to a process taking place over a longer period of bioleaching. Conversely, this process may reduce the concentration of leached elements due to immobilization in biomass or precipitation of metals. This immobilization at high concentrations of elements in biomass is one of the properties of microscopic fungi in the process of bioaccumulation and biosorption. Higher leached contents and accumulations, in case of higher batch weight, can lead to better bioavailability of fractions for fungi (if growth elements such as copper and zinc are needed). In the case of lead, on the other hand, this can lead to increased toxicity, which reduces the accumulation and leaching process.

When accumulating metals into the solution, we observed the same trend in almost all samples. The most effective strain was N. fischeri. Differences occurred in only two cases. For lead in the HO–A 1g sample, the A. niger strain was more effective in both culture time horizons, and for zinc in the HO–B 1g sample, after 40 days of cultivation, the A. niger strain. The studies of Dursun et al. (2003) demonstrate the effectiveness of the A. niger strain in the heavy metal biosorption process. We have proved the same conclusions in our work. Heavy metals such as copper cause inhibition of this strain, allowing a high yield of copper accumulation. The properties of the cell walls and their differences between the individual fungal strains, in our case A. niger and N. fischeri, naturally lead to different biosorbent behavior in our experiment. These differences should be borne in mind when comparing the biosorption properties of the two strains (Littera et al., 2011). At the end of the experiment, we can state that the A. niger strain was the most effective in the extraction efficiency and concentration of metals in solution. The highest value in the extraction efficiency for copper was recorded in the sample HO–B 2g with the value 56,81% after 40 days of cultivation. For zinc, it was a HO–B 2g sample with a value of 16.55% after 20 days of culture. Lead had the most effective autochthonous microflora in the HO–B sample of 1 g after 20 days of culture with a value of 22.80%. When determining the concentration of metals in the solution after leaching, we recorded the highest value for copper (3.42 mg/l) in the sample HO–A 2g after 20 days of cultivation. For lead, we measured the highest value (2.05 mg/l) in the HO–A sample of 2 g after 20 days of culture. For zinc, we recorded the highest value (22.53 mg/l) in the sample HO–B 2g after 20 days of cultivation.

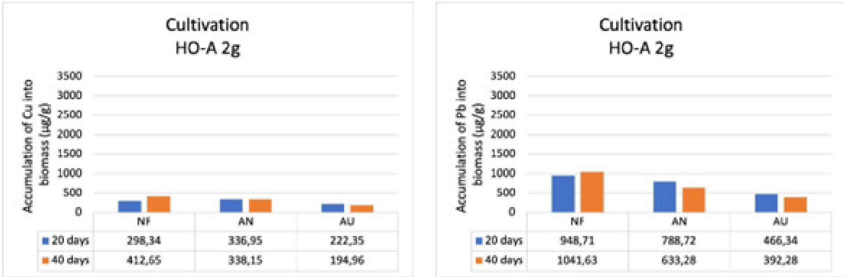

Fig. 6. Accumulation of Cu and Pb into biomass (μg / g) in the sample HO–A 2g
Rys. 6. Akumulacja Cu i Pb w biomasie (μg / g) w próbce HO-A 2g

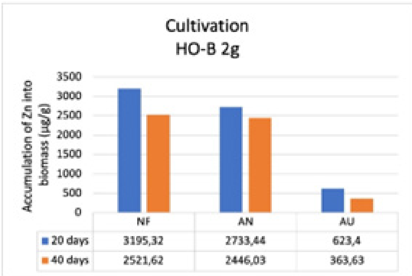

Fig. 7. Accumulation of Zn into biomass (μg / g) in the sample HO–B 2g
Rys. 7. Akumulacja Zn w biomasie (μg / g) w próbce HO-B 2g

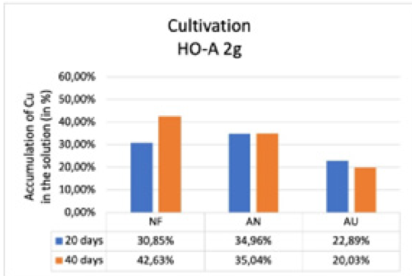

Fig. 8. Accumulation in Cu solution in% in sample HO–A 2g
Rys. 8. Akumulacja w roztworze Cu w% w próbce HO-A 2g

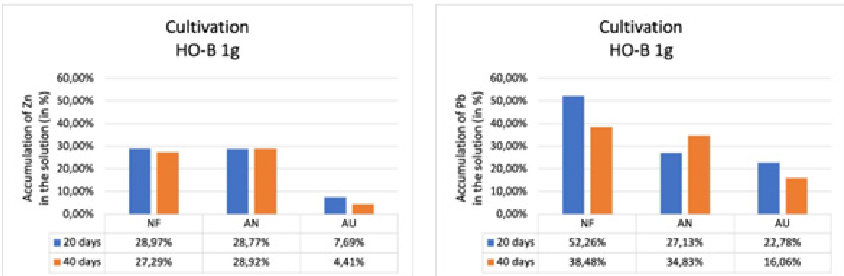

Fig. 9. Accumulation of a solution of Pb and Zn in% in a sample HO–B 1g
Rys. 9. Akumulacja roztworu Pb i Zn w% w próbce HO-B 1g

Conclusions

Based on our results, we can state that the highest concentration of metals found in the solution after leaching was achieved in a shorter time horizon of cultivation. On the contrary, a longer time reduced this value. When processing the results, we used the Student's test to determine statistical significance. In all three cases (Cu, Pb, Zn) it was a very high significance for the highest measured values.

We can justify the efficiency of the A. niger strain by its ability to produce citric, glucanic or tartaric acids. Tartaric acid is essential in the process of bioleaching zinc, because it is classified as a chelating agent. The formation of given acids significantly contributed to the efficiency of copper leaching, which we managed to prove experimentally in our work with the help of microorganisms in the process of conversion of contaminants, which led to partial or complete mineralization of the pollutant.

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Bioakumulacja i bioługowanie Cu, Pb i Zn z odpadów górniczych

Przedmiotem niniejszego badania było określenie skuteczności szczepów Aspergillus niger (Pezinok) i Neosartorya fischeri w bioługowaniu i bioakumulacji metali z odpadów górniczych ze stawu osadowego Hodruša. Odpady wykorzystane w pracy zawierały frakcje 0,10 mm o masie 1 i 2 g z różnych głębokości matrycy glebowej, próbki A: 10–20 cm, próbki B: 20–30 cm. Skupiliśmy się na pierwiastkach miedzi, ołowiu i cynku z zawartością poszczególnych pierwiastków (mg/kg) miedź – w próbkach A 487 mg/kg, w próbkach B 115 mg/kg. Ołów – próbki A 1208 mg/kg, próbki B 582 mg/kg. Cynk – próbki A 6970 mg/kg, próbki B 6808 mg/kg. Potwierdziliśmy skuteczność szczepu Aspergillus niger w tym procesie ze względu na produkcję kwasów organicznych (kwas cytrynowy, kwas szczawiowy, kwas octowy). W części eksperymentalnej stwierdziliśmy, że najskuteczniejszym szczepem w określaniu wydajności ekstrakcji i stężenia metali w roztworze po bioługowaniu był szczep Aspergillus niger. Szczep Neosartorya fischeri wydaje się być najbardziej efektywny w akumulacji metali w roztworze i akumulacji metali w biomasie. Zjawisko to można uzasadnić różnymi właściwościami ścian komórkowych poszczególnych szczepów grzybów i należy pamiętać o tych różnicach przy porównywaniu właściwości biosorpcyjnych.

Słowa kluczowe: *Aspergillus niger, Neosartorya fischeri, bioługowanie, bioakumulacja, miedź, ołów, cynk*