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Bioleaching of cadmium(Cd) and zinc(Zn) from the contaminated soil using bacteria from wastewater sludge

D.A. Andrzejewska-Górecka*, B. Macherzyński, M. Wszelaka-Rylik

Faculty of Biology and Environmental Science, Department of Environmental Engineering, Cardinal Stefan Wyszynski University in Warsaw, Wóycickiego 1/3, 01-938 Warsaw, Poland, emails: d.andrzejewska@uksw.edu.pl (D.A. Andrzejewska-Górecka), b.macherzynski@uksw.edu.pl (B. Macherzyński), m.wszelaka-rylik@uksw.edu.pl (M. Wszelaka-Rylik)

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ABSTRACT

The aim of this work was to determine the influence of various variants of bioleaching on the effectivity of releasing metal cadmium and zinc from contaminated soil using bacteria from activated sludge. The initial zinc and cadmium concentration in the soil was adequate – 957 and 252 mg kg^{-1} dry-weight. The research was conducted in variants that considered different process factors such as mixing and aeration. The research was conducted in 3 variants of bioleaching – mixing and aerating, only mixing, only aerating and in 2 control variants – aerating and mixing. After the 7th and 14th day of the process the markings were made: the index of sulfur-oxidizing bacteria, metals content, sulfate concentration, pH. Determination of the elemental composition was performed by inductively coupled plasma – optical emission spectrometry. The best result of bioleaching for both metals was achieved in variants with mixing and aerating. For zinc, the efficiency of the bioleaching was 66% after 14 d of leading the process and cadmium was 99% after 7 d of leading the process. In the case of cadmium leading the process longer had a negative impact on bioleaching. The bacteria activity, sulfate concentration and pH measurement correspond to effectivity metals removal in bioleaching all variants.

Keywords: Bioleaching; Chemical leaching; Contaminated soil; Heavy metals

1. Introduction

Industries, mining, agriculture as well as progressive urbanization have a negative impact on various ecosystems. The soil environment is particularly exposed to the toxic effects of xenobiotics and heavy metal emissions. Heavy metals are of common concern in the environment due to their high toxicity and lack of biodegradability [1–3]. The risk of heavy metals comes directly from their ability to accumulate and move in a trophic chain [4]. Heavy metals may have a stimulating or toxic impact on organisms. It depends on metal types, forms in which they occur and concentration in the environment. Some heavy metals are necessary elements for the proper course of metabolic processes but can be harmful to organisms. Zinc is an example of that group of metals. It provides very important functions in the human body, such as the creation of enzymes regulating the metabolism of proteins and carbohydrates, regulating the work of the circulatory, reproductive and skeletal systems. On the other hand, the high concentration of zinc deposits in the kidneys and liver causes anemia [2,4]. There is a group of metals, for example AS, Hg, Pb, Cd, that even in low concentration is highly harmful to humans, animals and has a negative impact on plant growth and development [5,6]. An example of these metals is cadmium.

* Corresponding author.

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According to the Agency for Toxic Substances and Disease Registry, which publishes the list of the most dangerous substances with high priority of disposal, cadmium is in 7th place [7]. Cadmium is harmful due to its rapid absorption by living organisms and its easy accumulation in plant and animal tissue. The most common effects of accumulation of this element in humans and animals include liver, kidney and lung damage, osteoporosis, anemia and various tumors. The presence of cadmium in plants contributes to changes in the functionality of cell membranes, inhibiting cell division and reducing the efficiency of photosynthesis and oxygen evolution [8]. The final effect of heavy metal's influence on the environment depends on numerous factors: type of metal, it is potential and real bioavailability, pH, concentration, water solubility and chemical reactivity [9,10]. The permissible concentration of heavy metals in the soil is specified in the Polish regulation of the Minister of the Environment of September 1, 2016, on methods of earth diagnostics [11]. Due to the high impact of heavy metals on all elements of the trophic chain, implementing the appropriate method of soil remediation is a key issue.

There is a number of research in which the ability of microorganisms like bacteria, fungi or plants was tested as a soil remediation method [12–15]. An example of this method is bioleaching, which involves the natural ability of microorganisms to produce organic acids, complexing compounds and also bacteria oxidizing the sulfur compounds and creating sulfuric acid [16–18]. Bioleaching can be used as an alternative to traditional methods based on physical separation or chemical extraction [19]. Excavation and solidification or stabilization generate some disadvantages as cost-effectiveness limitations, generation of hazardous by-products or inefficiency. Biological methods could potentially solve these issues. Additionally, this method is easy to operate and environmentally friendly due to less secondary pollutions [20,21].

The important factors influencing the effectiveness of bioleaching such as temperature, oxygen availability, carbon and energy source. The significant impact depends also on the type, form and concentration of metal and microorganism ability [22–26]. The aim of this work was to determine the influence of various variants of bioleaching on the effectivity of releasing metal cadmium and zinc from contaminated soil using bacteria from activated sludge, enriched by 1% of dusty sulfur. The experiment was conducted in variants including different ways of aeration. The bioleaching media was prepared based on activated sludge coming from a municipal waste disposal facility, which was a source of microorganism, in detail – active autotrophic and heterotrophic bacteria.

2. Materials and methods

2.1. Waste characteristics

Contaminated soil was used in the research. The sample was taken from the embankment based in Poland, from deep-sea between 0.3–0.5 m. The contamination of zinc and cadmium was detected. The metals concentration in the soil were:

- Zn – 957 mg kg^{-1}
- Cd – 252 mg kg^{-1}

According to the Regulation of the Minister of the Environment of September 1, 2016, on methods of earth diagnostics, the content of zinc and cadmium was exceeded in the sample. Permissible content of the risk increasing package for the lowering of the IV level of land for soil and ground, for deep-sea 0.25 m below ground and water permeability of 1×10^{-7} ms^{-1} or greater amounts accordingly:

- Zn – 300 mg kg^{-1}
- Cd – 6 mg kg^{-1}

2.2. Bioleaching media

The bioleaching culture was prepared based on activated sludge coming from a municipal waste disposal facility, which was a source of microorganism, in detail – active autotrophic and heterotrophic bacteria. The culture consisted of activated sludge and distilled water in a 1:2 ratio and 1% of dusty sulfur. The process of leaching culture's adaptation was conducted in conditions of shaking (120 rpm) and temperature of 24°C in order to achieve a pH level below 2 and sulfate concentration on a level of 1%.

2.3. Experimental procedure

Metal leaching from the waste was conducted in 300 mL Erlenmeyer flasks containing 200 mL of leaching media or 1% sulfuric acid and 10 g of waste. The research was conducted in 5 variants with a temperature of 24°C. Variants 4 and 5 were control variants consisting of waste samples put in distilled water. The leaching process variants are shown in Table 1. Additionally, two control variants were conducted. The control variants were conducting in distillation water with 120 rpm speed of mixing and aeration with compressed air.

2.4. Control analyses

The process lasted for 14 d. After the 7th and 14th day of the process the markings were made:

- Metals concentration – measurements were conducted using a Shimadzu ICPE-9820 plasma spectrometer;
- Sulfates concentration – measurements were conducted using a Shimadzu ICPE-9820 plasma spectrometer;
- General number of bacteria – in accordance with PN-EN ISO 6222 norm;
- Index of sulfur-oxidizing bacteria – done using the breeding method on a WR (water on rock) subgrade. The WR medium composition was: $(NH_4)_2SO_4$ (1.0 g), K_2HPO_4

Table 1
The variants of bioleaching process

Variant	Speed of mixing	Aeration with compressed air	Type of leaching media
1	120 rpm	Yes	Bioleaching culture
2	No	Yes	Bioleaching culture
3	120 rpm	No	Bioleaching culture

(0.5 g), sulfur (10.0 g), 1% bromocresol purple solution (0.5 mL), 10% solution of yeast extract (few drops);
- pH – in accordance with PN-90/C-04540/01;

2.5. Effectivity calculation

The effectivity of the process (1) was calculated according to the formula:

$$X = \frac{100 \times C_r \times V_s}{m_s \times M_s} \quad (1)$$

where X – bioleaching effectivity (%); C_r – concentration of metal release in a leaching culture (mg L^{-1}); V_s – volume of leaching culture (L); m_s – mass of waste sample taking into the experiment (kg); M_s – metal content in waste (mg kg^{-1}).

2.6. Metal concentration in soil calculation

The metal concentration in the soil after bioleaching (2) was calculated according to the formula:

$$M_k = M_s - \left(C_r \times V_s \div m_s\right) \quad (2)$$

where M_k – metal content in waste after bioleaching (mg kg^{-1}); C_r – concentration of metal release in a leaching culture (mg L^{-1}); V_s – volume of leaching culture (L); m_s – mass of waste sample taking into the experiment (kg); M_s – metal content in waste (mg kg^{-1}).

3. Results and discussion

The results of metal biological and chemical leaching from printed board samples are presented in Figs. 1 and 2.

The results of zinc bioleaching depended on the type of aeration and process duration. In the variant with only mixing the effectively was 20% on 7th day and 42% on 14th day. In the variant with the only aeration the effectively was 16% on 7th day and 56% on 14th day. In the variant with simultaneous mixing and aeration the effectively was 38% on 7th day and 66% on 14th day.

On the 7th day of conducting the experiment, the release effectivity of zinc was the highest in variant with

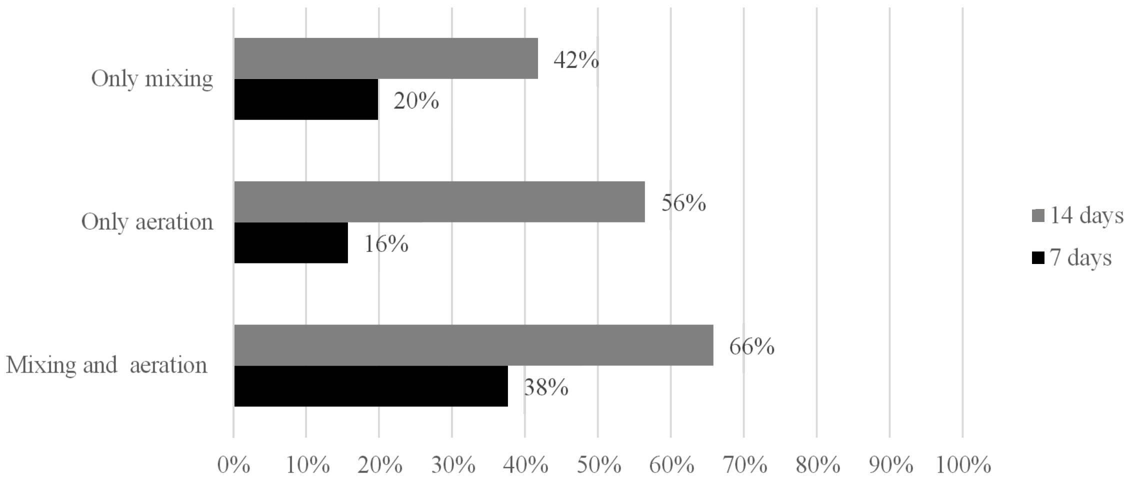

Fig. 1. Effectivity of zinc release in bioleaching variants on 7th and 14th day conducting the process.

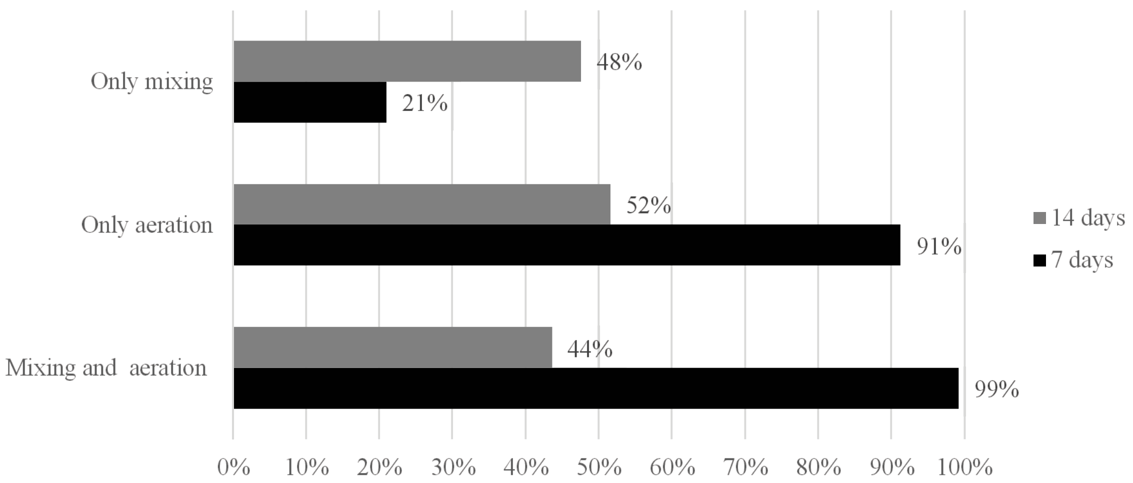

Fig. 2. Effectivity of cadmium release in bioleaching variants on 7th and 14th day conducting the process.

simultaneous mixing and aeration conditions. The effectivity amounted to 38%. The effectivity in variants with only aeration and only mixing were accordingly 16% and 20%.

On the 14th day of conducting the experiment, the release effectivity of zinc was the highest in variant with simultaneous mixing and aeration conditions. The effectivity amounted to 66%. The effectivity in variants with only aeration and only mixing were accordingly: 56% and 42%.

In control variants, the release efficiently of zinc in both only aerating and only mixing variants was below 5%.

Summing up, the best result was achieved on the 14th day in variant with simultaneous mixing and aeration conditions. The results achieved in variant with the only aeration and only mixing conducted for 14 d were accordingly 10% lower and 24% lower. The extension of the bioleaching process had a positive effect on efficiency.

The results of cadmium bioleaching depended on the type of aeration and process duration. In the variant with only mixing the effectivity was 21% on 7th day and 48% on 14th day. In the variant with the only aeration the effectivity was 91% on 7th day and 52% on 14th day. In the variant with simultaneous mixing and aeration the effectivity was 99% on 7th day and 44% on 14th day.

On the 7th day of conducting the experiment, the release effectivity of cadmium was the highest in variant with simultaneous mixing and aeration conditions. The effectivity amounted to 99%. The compared result was achieved in variant with the only aeration. The effectivity, in this case, amounted to 91%. The effectivity in variant with only mixing was 21%.

On the 14th day of conducting the experiment, the release efficiency of cadmium decreased in variants with simultaneous mixing and aeration conditions and only with aeration decreased accordingly to 44% and 45%. In variant with only mixing the efficiency increased more than twice to 48%. It was the highest result.

A decrease of effectivity on the 14th day in a can of cadmium could be caused by secondary sorption that took place in the biomass of microorganisms or in the soil itself. The analysis of this phenomenon requires further research [27].

In control variants, the release efficiency of cadmium in both only aerating and only mixing variants were below 1%.

Summing up, the best results were achieved on the 7th day in variant with simultaneous mixing and aeration conditions. A similar result, lower by only 8% was achieved in variants with the only aeration. The extension of the bioleaching process had a negative effect on efficiency in variants with simultaneous mixing and aeration conditions and only aeration.

During the conducting of the bioleaching process, the sulfate concentration and pH were also marked. The results have been shown in Fig. 3.

The sulfate concentration with simultaneous mixing and aeration variant was 4,097 mg L^{-1} on the 7th day and 5,530 mg L^{-1} on the 14th day. In case of only aeration variant, the sulfate concentration was 3,443 mg L^{-1} on the 7th day and 4,097 mg L^{-1} on the 14th day. In variant with only mixing on 7th day sulfate concentration was 1,515 mg L^{-1} and on the 14th day was 3,443 mg L^{-1}.

On the 7th day of conducting the bioleaching process, the sulfates concentration was the highest in the variant with simultaneous mixing and aeration variant. In this variant, the sulfate concentration was on a level of 4,097 mg L^{-1}. The sulfates concertation in the only aeration and only mixing variant were accordingly 3,444 and 1,515 mg L^{-1}.

On the 14th day of conducting the bioleaching process, the sulfates concentration increased in all variants. The highest level of sulfates concentration 5,530 mg L^{-1} was observed in variant with simultaneous mixing and aeration conditions. The sulfates concentration in the only aeration and only mixing variants were accordingly 4,097 and 3,443 mg L^{-1}.

Summing up, the extension of the bioleaching process had a positive effect on increasing the sulfur concentration. The sulfates concentration increased in all variants accordingly: simulations mixing and aeration conditions – 35%, only aeration – 18% and only mixing – more than 100%.

The changes of pH in all variants have been shown in Fig. 4.

The initial pH level was below 2. On the 7th and 14th day the lowest pH was in variant with simultaneous mixing

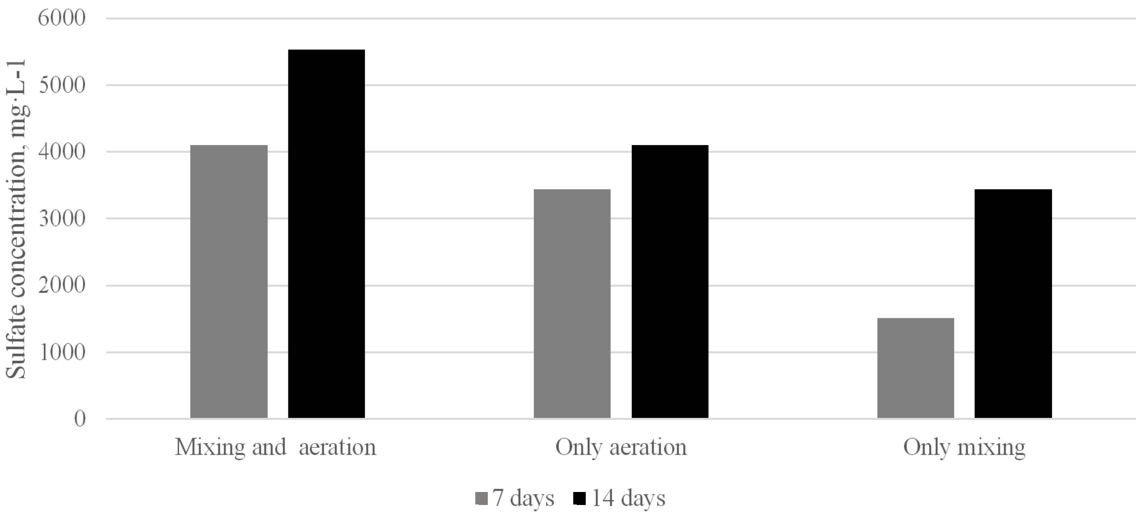

Fig. 3. Sulfate concentration in various variants of bioleaching media on the 7th and 14th day of conducting the process (mg L^{-1}).

and aerating conditions. The pH was accordingly – 3.8 on the 7th day and 3.2 on the 14th day. The pH in variant with only aeration was accordingly – 4.1 on the 7th day and 3.3 on the 14th day and with only mixing was accordingly – 4.5 on the 7th day and 3.4. Extension of the bioleaching process had a positive impact to decrease pH. The pH measure in all variants corresponded with sulfate concentration. The low pH at the end of the process led to soil acidification. This issue should be the subject of further research.

Bioleaching requires the presence of microorganisms and bacteria that oxidize sulfur or its volatile compounds. The source of active microflora may be activated sludge from the municipal sewage treatment plant [22,27].

The general number of bacteria and the index of sulfur-oxidizing bacteria are shown in Tables 2 and 3.

A general number of bacteria on the 7th day with simultaneous mixing and aeration variant was 2.4×10^7 CFU mL^{-1} and the index of sulfur-oxidizing bacteria was 10^{-7}. In the case of only aeration general number of bacteria was 1.8×10^6 CFU mL^{-1} and the index of sulfur-oxidizing bacteria was 10^{-7}. In variant with only mixing general number of bacteria was 1.5×10^{-6} CFU mL^{-1} and the index of sulfur-oxidizing bacteria was 10^{-5}.

A general number of bacteria and index of sulfur-oxidizing bacteria on the 7th day of conducting the bioleaching process were the highest in variant with mixing and aerating conditions. The general number of bacteria was on a level 2.4×10^7 CFU mL^{-1} and the index of sulfur-oxidizing bacteria was 10^{-7}. The general number of bacteria and index of sulfur-oxidizing bacteria in other variants were: 1.8×10^6 CFU mL^{-1} and 10^{-5} in only aerating and 1.8×10^6 CFU mL^{-1} and 10^{-5} in only mixing.

A general number of bacteria on 14th day with simultaneous mixing and aeration variant was 2.6×10^7 CFU mL^{-1} and index of sulfur-oxidizing bacteria was 10^{-7}. In case of only aeration general number of bacteria was 2.1×10^6 CFU mL^{-1} and index of sulfur-oxidizing bacteria was 10^{-6}. In variant with only mixing general number of bacteria was 1.9×10^{-6} CFU mL^{-1} and index of sulfur-oxidizing bacteria was 10^{-5}.

A general number of bacteria and index of sulfur-oxidizing bacteria on the 14th day of conducting the bioleaching process were the highest in variant with mixing and aerating conditions. The general number of bacteria was on a level 2.6×10^7 CFU mL^{-1} and the index of sulfur-oxidizing bacteria was 10^{-7}. The general number of bacteria and index of sulfur-oxidizing bacteria in other variants were: 2.1×10^6 CFU mL^{-1} and 10^{-6} in only aerating and 1.9×10^6 CFU mL^{-1} and 10^{-6} in only mixing.

Summing up, the effectivity of the bioleaching process corresponds with the activity of microorganisms in media marked as a general number of bacteria and index of sulfur-oxidizing bacteria.

The conducted variants were verified whether could be used to remediate metals from soil in order to achieve the requirements of the regulation of the Minister of the

Table 2
General number of bacteria and the index of sulfur-oxidizing bacteria in bioleaching culture on the 7th day

Variants	General number of bacteria	Index of sulfur-oxidizing bacteria
Mixing and aeration	2.4×10^7 CFU mL^{-1}	10^{-7}
Only aeration	1.8×10^6 CFU mL^{-1}	10^{-5}
Only mixing	1.5×10^6 CFU mL^{-1}	10^{-5}

Table 3
General number of bacteria and the index of sulfur-oxidizing bacteria in bioleaching culture on the 14th day

Variants	General number of bacteria	Index of sulfur-oxidizing bacteria
Mixing and aeration	2.6×10^7 CFU mL^{-1}	10^{-7}
Only aeration	2.1×10^6 CFU mL^{-1}	10^{-6}
Only mixing	1.9×10^6 CFU mL^{-1}	10^{-6}

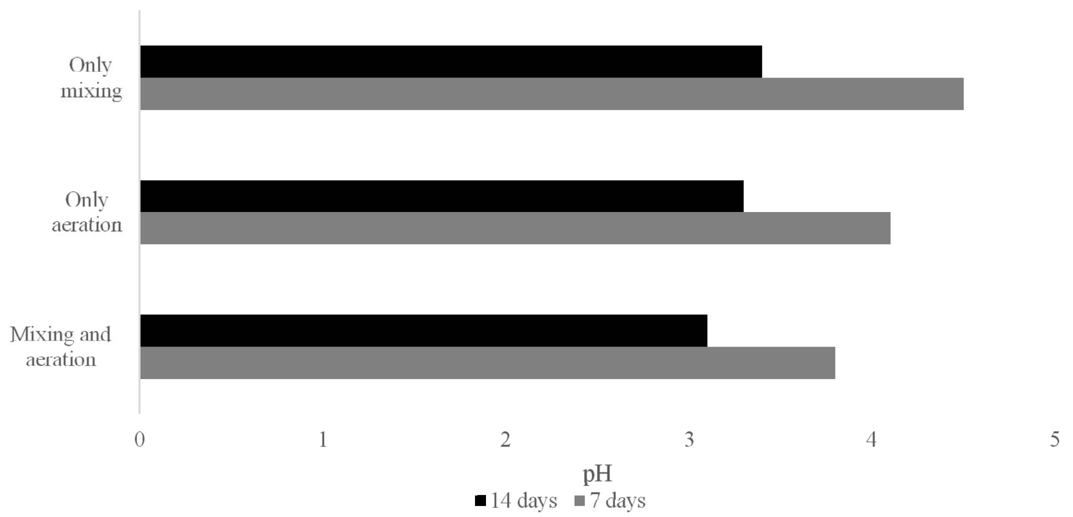

Fig. 4. Changes of pH in all variants.

Environment of September 1, 2016, on methods of earth diagnostics. To verify this issue the concentration of metals in the soil after bioleaching were calculated.

The metals concentrations in soil in case of zinc and in cadmium after bioleaching are presented in Figs. 5 and 6.

In case of zinc, the best result was achieved in variant with simultaneous mixing and aeration condition. The concentration of the zinc on 14th day of conducting the process decreased more than two times. Finally achieved result was above the maximum content of metal in the soil according to the regulation of the Minister of the Environment of September 1, 2016, on methods of earth diagnostics. The extending the process to 14 d had a positive impact. The recommendation, in this case, is to conduct a process for more than 14 d.

In the case of cadmium, the best result was achieved in variant with simultaneous mixing and aeration condition. The concentration of the cadmium on the 7th day of

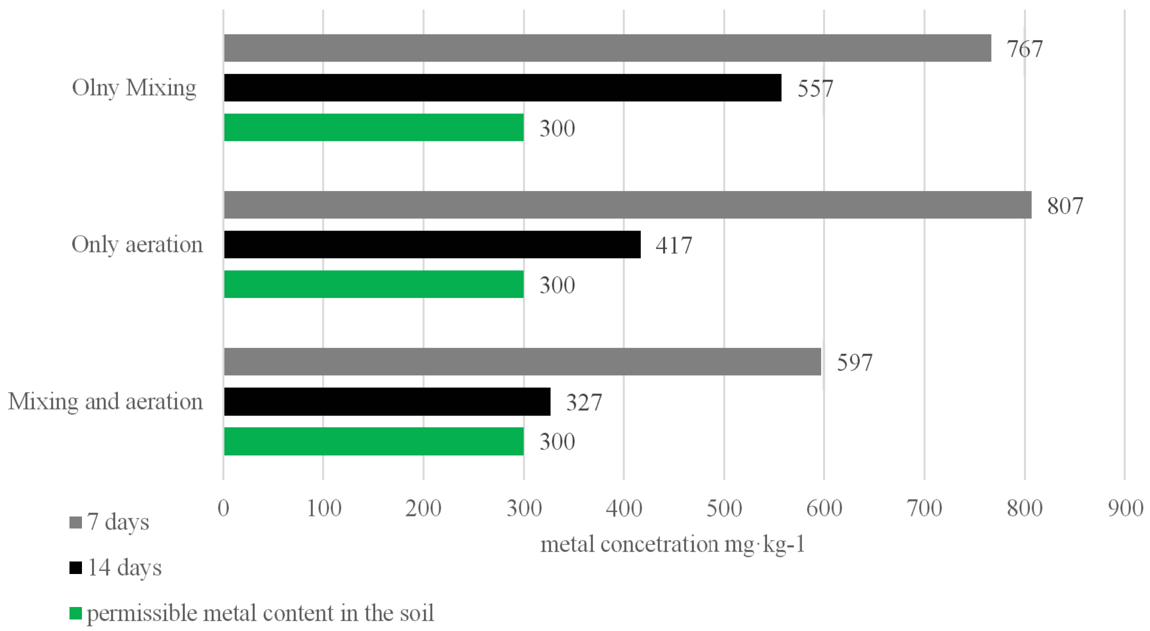

Fig. 5. Zinc concentration in the soil after bioleaching process.

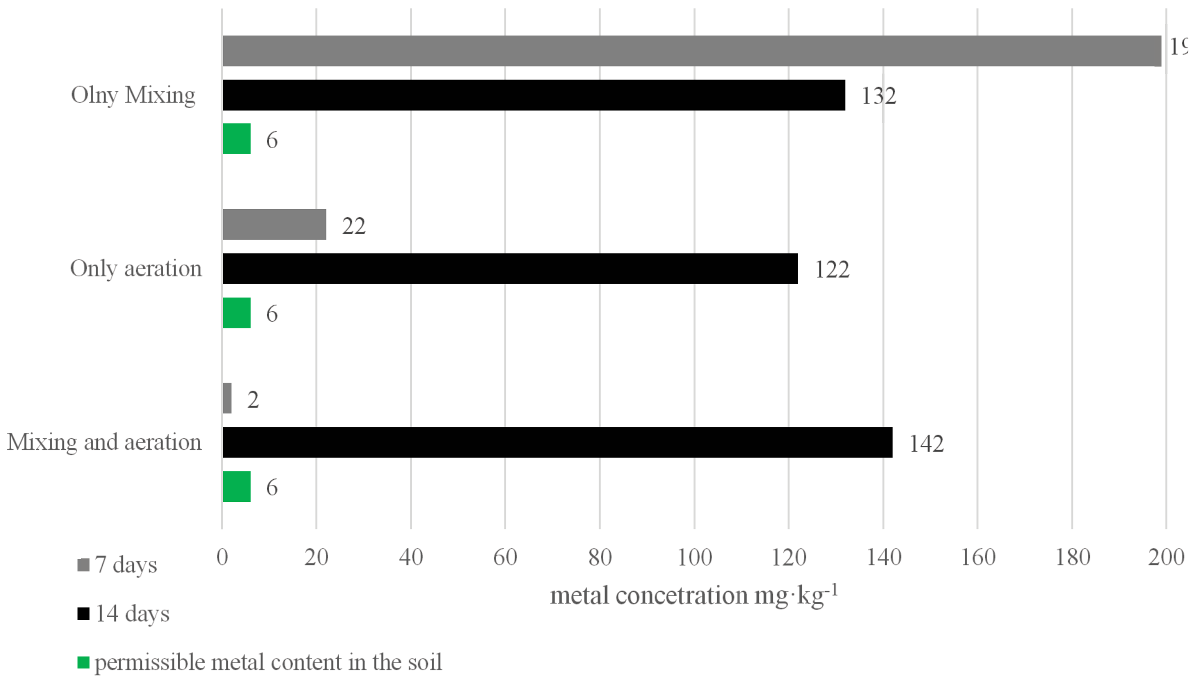

Fig. 6. Cadmium concentration in the soil after bioleaching process.

conducting the process achieved requirements of metal content in the soil according to the regulation of the Minister of the Environment of September 1, 2016, on methods of earth diagnostics. Extension of the process duration had a negative impact on the process. The effectivity of the metal release process decreased most probably because of secondary sorption that took place in the biomass of microorganisms or in the soil itself [27].

The mechanism of removing metals from the soil is based on physicochemical and biological processes. Metals are being washed out by bioleaching various medium from soil using mixing or aeration. Leaching metals could be conducted in chemical or biological mediums. Metals release using biological medium brings measurable benefits [22]. Biological medium is used the natural ability of bacteria to produce organic acids, complexing compounds and also bacteria oxidizing the sulfur compounds and creating sulfuric acid [16–18]. In this studies, activated sludge bacteria oxidizing the sulfur compounds and creating sulfuric acid were used. This type of microorganism require oxygen conditions, which affect efficient sulfuric acid production [17,18]. The high concentration of sulfuric acid impact on pH and have a brings measurable benefits in bioleaching process, what was observed during the research. Simulations mixing and aeration variants were the most effective. It was aligned with markers as a general number of bacteria, sulfate concentration and pH. There was also observed that too long a bioleaching process may be disadvantageous due to the process of metal secondary sorption on soil particles or biomass.

Based on literature data and results achieved in conducted research it can be established that bioleaching is an effective method of metals removal from contaminated soil. Nareshkumar and Nagendran [26] showed in their research that the effectivity of zinc and cadmium release can be achieved on the level of 97% and 93% accordingly by conducting the process for 4 weeks. Deng et al. [21] reported that the result effectivity of cadmium release was 74.93% to 92.76%. The research conducted by Diaz et al. [19] showed that bioleaching in cyclic treatment combining with the addition of biosurfactants can remove Zn from the soil with an effectivity of 70%. The key issue is to achieve the internal and external regulations' requirements regarding maximum concentrations of metals in contaminated soil.

4. Conclusion

The effectivity of the bioleaching process depends on the type of the metal. Cadmium characterized better susceptibility to biological leaching than zinc. In the case of zinc, the best result was achieved in variant with simultaneous mixing and aeration condition, after conducting a process for 14 d. The effectivity of the process was 66%. The final result was above the maximum content of metal in the soil according to the Regulation of the Minister of the Environment of September 1, 2016, on methods of earth diagnostics. In the case of cadmium, the best result was achieved in variant with simultaneous mixing and aeration conditions, after conducting a process for 7 d. The effectivity of the process was 99%. The final result fulfilled the requirements of regulation of the Minister of the Environment of September 1, 2016, on methods of earth diagnostics. The duration of the process should be adjusted to the type of metal. In the case of cadmium better result was achieved after 7 d of conducting the bioleaching process but in the case of zinc better result was achieved after 14 d. Too long a bioleaching process may be disadvantageous due to the process of metal secondary sorption on soil particles or biomass. The appropriate aeration has a significant impact on the bioleaching process. The best result was achieved in variants with simultaneous mixing and aeration in the case of both metals. The aeration with compressed air gave a better results than aeration by mixing.

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