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Use of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* in the Recovery of Heavy Metals from Landfill Leachates

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Abstract: Among the methods used to remove metals and their compounds from landfill leachates with low application costs and high efficiency are bioleaching and biosorption. The most effective bacteria used in the metal removal process are *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. The aim of the study was to determine the usefulness of the *A. ferrooxidans* and *A. thiooxidans* population in removing heavy metals from landfill leachate. In addition, development opportunities for bacterial population using landfill leachate as growth medium were identified. The substrate for the research was the raw leachate before the reverse osmosis process. In order to increase the efficiency of trace elements removal and recovery from leachate, variable combinations have been used which differ by the addition of sulfuric acid, *A. ferrooxidans* culture, *A. thiooxidans* culture, mixed culture containing populations of both bacteria, and elemental sulfur. Based on the research, it was found that the removal of heavy metals from leachate was a selective process. High bioleaching efficiency, from 80% to 90%, was obtained for all metals for which the sample acidification or sulfur addition was used. The simultaneous combination of both these additives turned out to be the most advantageous. The *A. thiooxidans* culture was the most effective in bioleaching reverse osmosis effluents. For the *A. ferrooxidans* culture used, much lower efficiencies were obtained, while by contrast, the use of mixed culture of two bacterium species had no significant effect.

Keywords: metals recovery; landfill leachate; *Acidithiobacillus thiooxidans*; *Acidithiobacillus ferrooxidans*



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1. Introduction

The leachates from municipal landfills are generated in chemical, physical and biological transformations taking place during waste landfilling and also for many years after the disposal site closure [1]. The leachates are highly contaminated and concentrated liquids containing biodegradable and non-biodegradable organic matter, inorganic pollutants [2], and hazardous substances, e.g., heavy metals or xenobiotics [3]. The formed leachate, depending on the characteristics of the deposited waste and the age of the landfill, is a habitat for functionally very different types of bacteria [4]. The leachate is also a reservoir of microplastics (concentrations of 0–291 particles per L), [5] especially polyethylene and polypropylene [6].

The physical and chemical properties of leachate from active and inactive landfills differ significantly. The main difference stems from the method of segregation, treatment, and disposal of waste during long-term operation of the landfill [7]. New landfill sites are often exposed to high concentrations of heavy metals due to the acidic phase of anaerobic digestion of landfilled waste. With the landfill ageing, the pH value increases, thus limiting the solubility and leaching of metals [8]. Therefore, it is necessary to constantly monitor many indicators, including heavy metals, mainly Cd, Cu, Cr, Hg, Pb, Ni, and Zn, for example, due to the effectiveness of the leachate treatment technology [9].

A number of physical, chemical, biological, and combined methods are used in the treatment of leachate. Biological methods are mainly applied to leachate water from young landfills characterized by a high ratio of BOD₅/COD [9–13]. For biological leachate treatment, it is possible to apply aerobic and anaerobic processes and reactors such as sequencing batch reactors (SBR) or upflow anaerobic sludge blanket reactor (UASB) [13,14]. Physicochemical methods including coagulation, chemical oxidation, advanced oxidation processes [15,16] adsorption, and ion exchange [17] are widely used in the treatment of leachate. High metal removal from leachate can also be achieved by using biological technologies such as phytoremediation or artificial wetlands [18].

However, physicochemical techniques also have disadvantages such as operational problems, high energy requirements and costs, in some cases, unsatisfactory efficiency [19]. In the case of biological technologies, there is a risk of generating toxicity in microorganisms by pollutants contained in the leachate [18].

One of the methods of metal extraction is biological leaching so far used in biohydrometallurgy. In the bioleaching process, metals (heavy metals) are solubilized using microorganisms that ensure the oxidation of sulfur and/or iron from insoluble metal sulfides to soluble sulfates. The most effective bacteria in bioleaching are considered *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. Generally, the effectiveness of bioleaching depends on the conditions of the process and especially on the requirements of the microorganisms which determine their activity [20,21]. Bioleaching has been tested in the treatment of sewage sludge with promising results. This method offered many advantages, including low cost, ease of use, low energy requirements, high metal solubility, and harmless by-products, making it compliant with the assumptions of a green deal [22–25].

There are many applications that use *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* focused on bioleaching metals from various matrices, e.g., sewage sludge [22,23], soil [26], municipal waste incineration fly ash [27], or plating sludge [28]. The novelty of the presented work is the use of landfill leachate as a starting matrix for bioleaching bacteria. The application of a new substrate to the well-known process of bioleaching requires research on optimization of process conditions in order to achieve high efficiency. Research in this field is necessary to consider bioleaching as method for the pretreatment of leachates before their final purification processes.

The leachates for testing were taken from a landfill where reverse osmosis technology was used for their purification. In the research, an attempt was made to recover metals using the biological method, which in this case is not used in practice for leachate treatment. The aim of the research was to determine the possibility of bioleaching metals from landfill leachate with the use of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. The efficiency of metal recovery was assessed on the basis of changes in heavy metal concentration monitored during the process.

2. Materials and Methods

2.1. Substrate

The leachate from the municipal waste landfill located in the Silesian Voivodeship (near Czestochowa city, Poland) was tested. The leachates from this landfill were collected in a buffer tank (sedimentation tank made of concrete). The buffer tank is supplied with 7 types of leachate/water: (1) leachate from mechanical-biological process—composting of organic fraction of municipal waste; (2) leachate from closed two quarters of landfill; (3) leachate from an active quarter (from 2 years); (4) effluent from deodorization installations; (5) effluent from biogas treatment and combustion installations; (6) effluent from landfill activities (cleaning of machinery, transport, etc.); (7) rainwater and meltwater. The amount of leachate generated from individual reservoirs is not monitored in the landfill. The leachate from 7 plots are collected in one buffer tank, from which they are then treated with a reverse osmosis installation. The test substrate was the leachate collected from the buffer tank, so it was a mixture of all leachates generated in the landfill.

The research was carried out in two stages, denoted as Stage I and Stage II. During Stage I, leachate was collected in March (early spring), whereas during Stage II, it was collected in May (late spring). Table 1 presents a general characterization of the substrate used in the study. The metal content (Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn) in the substrates is presented in Table 2. Due to the low concentration of Cd, Ni, Pb, the bioleaching efficiency was not analyzed for these metals.

Table 1. Physicochemical parameters and elemental composition of landfill leachate, collection I (Stage I) and II (Stage II).

Index	Units	Stage I	Stage II
pH	-	7.87	8.2
Dry solids (DS)	(g/L)	6.2	5.7
Volatile solids (VS)	(g/L)	1.3	1.1
% vs. in DS	(% DS)	20.9	20.0
Alkalinity	(mg CaCO ₃ /L)	-	3325.0
Dissolved organic carbon	(mg C/L)	530.7	505.6
Dissolved inorganic carbon	(mg C/L)	3.63	4.8
Total Kjeldahl nitrogen	(mg N/L)	1411.3	481.6
Ammonium nitrogen	(mg N-NH ₄ ⁺ /L)	627.2	425.6
Carbon content	(% DS)	9.24	8.25
Hydrogen content	(% DS)	1.1	1.28
Nitrogen content	(% DS)	0.46	0.50
Sulfur content	(% DS)	0.56	2.32
Phosphorus content	(% DS)	0.1433	0.1828

Table 2. Metal content in the tested landfill leachate.

Element (ppb)	Stage I	Stage II
Al	136.7 ± 11.3	630.5 ± 31.2
Ca	216,005 ± 1480	230,620 ± 2520
Cd	under detection limit (6.26)	under detection limit (6.26)
Cr	302.6 ± 12.6	169.5 ± 7.3
Cu	208 ± 7.4	94.6 ± 8.1
Fe	2709 ± 69.3	3008.8 ± 84.3
Mg	240,609 ± 2842.3	176,958 ± 1966.1
Mn	933.0 ± 36.3	716.6 ± 26.4
Ni	43.4 ± 7.2	under detection limit (8.67)
Pb	under detection limit (34.88)	under detection limit (34.88)
Zn	449 ± 16.3	408 ± 10.4

2.2. Inoculum Preparation

Culture of bioleaching bacteria was isolated from the activated sludge (AS) from wastewater treatment plant. For the cultivation, 9K medium (autoclaved at 112 °C for 30 min) with the addition of 44.2 g/L of FeSO₄·7H₂O as the energy source was used (for *A. ferrooxidans*). For *A. thiooxidans* cultivation 10 g/L of elemental sulfur to 9K medium was added. Both S and FeSO₄ were added for mixed cultivation. The cultivation of the microorganisms was performed in 150 mL flasks using 100 mL of medium and about 2 g of AS. The pH was adjusted to 2 with 5N sulfuric acid. The flasks were shaken in an orbital shaker at 180 rpm at the growth temperature of 30 °C for 7 days. The obtained microorganisms were transferred to fresh medium. Further cultivation was carried out under the same conditions. This procedure was repeated 4 times, the last repetition being the inoculum for the experiments. The basic parameters of the inoculum are presented in Table 3.

Table 3. Parameters of the inoculum used in the experiments.

Inoculum	pH	ORP, mV	DS, g/L	VS, g/L
<i>A. thiooxidans</i>	1.81 ± 0.1	536 ± 0.1	38.8 ± 0.1	34.9 ± 0.1
<i>A. ferrooxidans</i> + <i>A. thiooxidans</i>	1.90 ± 0.1	506 ± 0.1	36.6 ± 0.1	32.3 ± 0.1
<i>A. ferrooxidans</i>	1.91 ± 0.1	498 ± 0.1	35.8 ± 0.1	31.7 ± 0.1

Bacterial species identification was performed to confirm the presence of *A. ferrooxidans* and *A. thiooxidans* as described previously [24]. Polymerase chain reaction (PCR) analysis with species-specific primers was used. Genomic DNA from the putative *A. ferrooxidans* and *A. thiooxidans* isolate was extracted using GeneMATRIX Gram Plus & Yeast Genomic DNA Purification Kit (Eurx, Poland). Results confirmed the species belonging of the isolated bacteria strain to *A. ferrooxidans* and *A. thiooxidans*.

2.3. Bioleaching Experiments

The experiments were carried out in 300 mL flask with a working volume of 0.15 L. The volume of the leachates was 90% (*v/v*). The active inoculum was 10% (*v/v*). The flasks were incubated in a thermostatic shaker at 180 rpm and temperature 30 °C for 12 days. The studies were divided into 9 major combinations (A–I). The variable parameters in the combinations were addition of *A. thiooxidans* and *A. ferrooxidans* culture, the addition of sulfuric acid (to adjust pH to 2.0), and the addition of elementary sulfur—1.5 (*w/v*) (Table 4). Course of the process was assessed based on the effectiveness of bioleaching of heavy metals (Al, Ca, Cr, Cu, Fe, Mg, Mn, Zn) during the process, pH, and redox potential. The analytical test was performed on the 3rd, 6th, 9th, and 12th day of the process.

Table 4. Combinations analyzed in Stage I and Stage II based on the use of landfill leachate.

Treatment	Substrates
A	270 mL leachate + 30 mL deionized water
B	270 mL leachate + 30 mL deionized water with acid *
C	270 mL leachate + 30 mL <i>A. ferrooxidans</i>
D	270 mL leachate + 30 mL <i>A. ferrooxidans</i> / <i>A. thiooxidans</i>
E	270 mL leachate + 30 mL <i>A. ferrooxidans</i> / <i>A. thiooxidans</i> + acid *
F	270 mL leachate + 30 mL <i>A. thiooxidans</i>
G	270 mL leachate + 30 mL <i>A. thiooxidans</i> + acid *
H	270 mL leachate + 30 mL <i>A. thiooxidans</i> + 3 g S
I	270 mL leachate + 30 mL <i>A. thiooxidans</i> + acid * + 3 g S

* pH adjusted to 2.0.

2.4. Physical and Chemical Analyses

The dry solids (DS) and volatile solids (VS) were determined using the weighing method according to PN-EN 12880:2004 and PN-EN 12879:2004, respectively.

Total Kjeldahl nitrogen (TKN) was determined using the titration method according to PN-EN 16169:2012.

Ammonium nitrate (N–NH₄) was determined using the titration method according to PN-EN 14671:2007.

Redox potential (electrochemical method, M781 IO-1) and pH (potentiometric method, PN-EN ISO 10523) were measured by means of calibrated electrodes and Elmetron measuring device.

Heavy metals were determined in accordance with PN-EN ISO 11885:2009 using inductively coupled plasma optical emission spectrometry (ICP-OES; Multi View Arcos, Ametek, Germany).

Determination of the elementary content of carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) was performed using LECO TruSpec multi analyzer.

Determinations of the concentration of dissolved inorganic carbon and dissolved organic carbon were performed by means of Analytik Jena multi N/C 3100 apparatus using the NPOC method (non-purgeable organic carbon).

The alkalinity of raw leachate liquid (Z) was determined using the potentiometric method according to PN-EN ISO 9963-1.

The determinations were made in samples obtained after centrifugation and filtration of leachate water (12,100 rcf centrifugation, 15 min, filtration 0.45 microns membrane filters).

Samples for the analyses were collected after 3, 6, 9, and 12 days of the bioleaching process. Each analysis was performed in triplicate.

The results of the analyses are presented as the arithmetic mean with standard deviation. The efficiency of removed metals was calculated as the quotient of the metals content in the dissolved phase to the total metals content in the leachates (Table 2 shows the total metal content).

2.5. Statistical Analysis

The aim of the analysis was to determine differences in the efficiency of bioleaching depending on the combination of research (A–I). STATISTICA software was used (STATISTICA 13.3, TIBCO Software Inc., Palo Alto, CA, USA). To verify the homogeneity of variance, Levene's test was used. The results of Levene's test were statistically significant, therefore, the hypothesis of homogeneity of variance was rejected. Tukey's post hoc test was performed subsequently. The adopted alpha significance level was 0.05. The results marked with different letters (in the graphs, in the bar for combinations) indicate which combinations are significantly different.

3. Results and Discussion

3.1. Changes in pH and Oxidation-Reduction Potential during Study

The oxidation-reduction potential (ORP) and pH are the basic indicators in the monitoring of bioleaching. Low pH and a high ORP are considered effective conditions for the bioleaching process. The accurate pH for the development of the biomass of bioleaching bacteria was found in samples of combinations B, E, G, I (Figures 1 and 2). In step II of the study, the pH of the samples was below 2.0. These were combinations in which sample pre-acidification was used. Fluctuations in pH were noted in combinations of F and H. Especially in step I, a decrease in pH was noted as a function of the process time. In combinations A, C, D, the pH of the samples was within the range of 6.5–8.5 (Stage I), and 5.5–8 (Stage II).

Sulfur added to the leaching mixture is a substrate for microorganisms that oxidize it to sulfuric acid, and as a result, the pH is lowered, thus increasing the solubility of metals. This effect was observed by Zheng et al. [29] during research on the leaching of heavy metals from soil contaminated by acid rain. Lowering the pH during bioleaching is also quite characteristic of this process with the addition of culture, as confirmed by studies by Zhang et al. [30].

According to literature, acidifying the sample at the beginning of the study may increase the activity of microorganisms in bioleaching and improve the mobility of metals in the liquid phase [31–33]. *A. thiooxidans* and *A. ferrooxidans* can oxidize elemental sulfur, which is accompanied by a decrease in pH and leads to dissolution of metals from the metal sulfide fraction (transition from solid to liquid phase) [34].

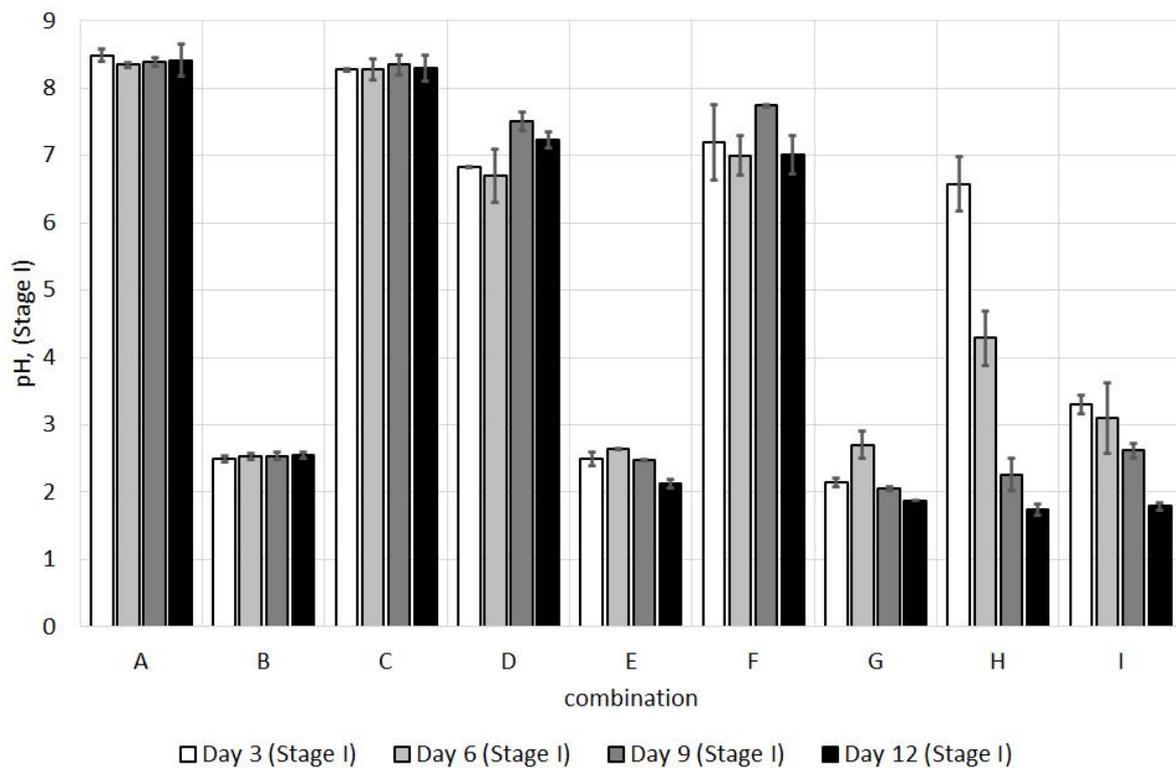


Figure 1. Changes in pH in the combinations tested in Stage I.

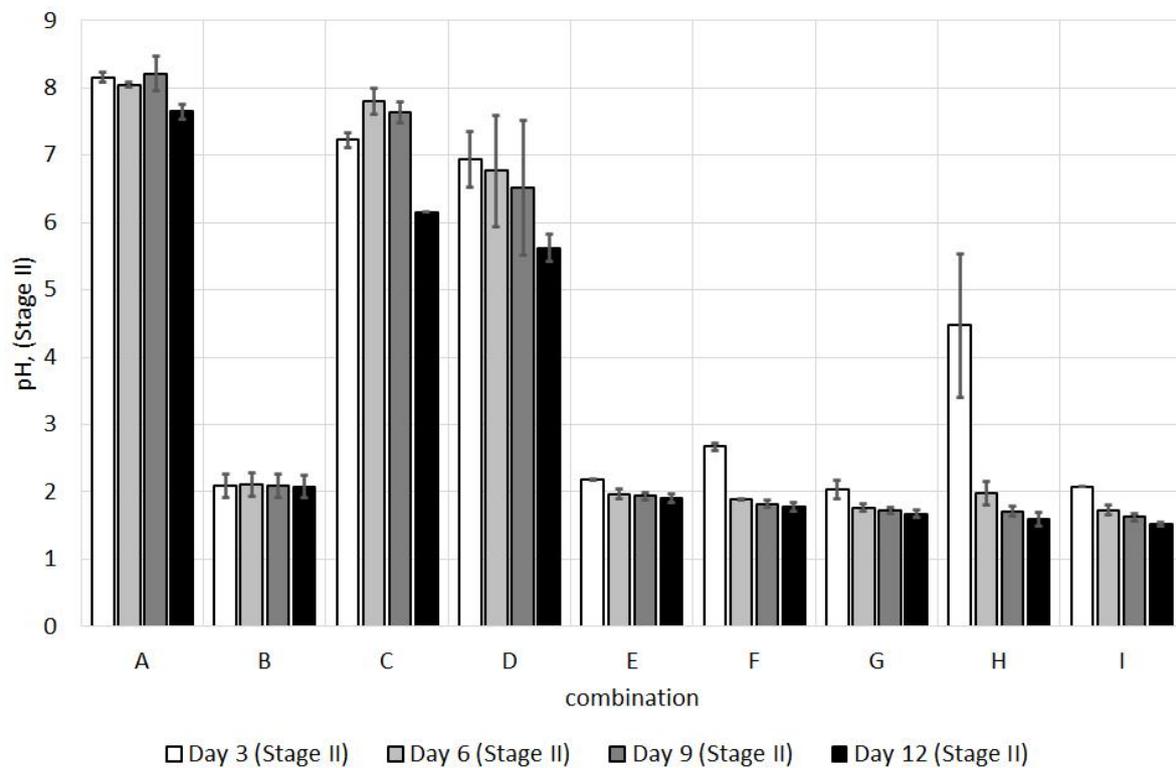


Figure 2. Changes in pH in the combinations tested in Stage II.

The ORP changed dynamically, depending on the process time and the used combination (Figures 3 and 4). Overall, the increasing ORP trend as a function of time was observed. For the E–I combination, the highest ORP values of 450 mV were achieved on day 12 of the

process (Stage I, Stage II). The maximum value of the oxidation and reduction potential for samples A–D did not exceed 300 mV. The ORP of the sludge should be greater than 250 mV to initiate proper solubilization of metals [35]. The dynamics of ORP changes during bioleaching are closely related to the enrichment of the mixture with elemental sulfur and the activity of microorganisms such as *A. ferrooxidans* or *A. thiooxidans* [22,30,36]. According to the literature data, the mobility of metals increases with a decrease in pH and an increase in ORP; often, these conditions are described as critical [37–39]. The solubility of metals increases with a decrease in pH and an increase in ORP and high ORP can cause the dissociation of the complicated sludge solid to release the heavy metals [40]. ORP increases with the bacterial activity which benefits bioleaching of the metal. However, the redox potential during bioleaching is not significantly affected by the amount of S provided with the increase in bacterial activity, an increase in ORP and the effectiveness of metal bioleaching are observed. The amount of elemental sulfur supplied does not affect the increase in the redox potential [31,33].

3.2. The Removal of Heavy Metals during Bioleaching

The efficiency of bioleaching of metals—Al, Ca, Cr, Cu, Fe, Mg, Mn, Zn—for the tested combinations of the 1st and 2nd stage is shown in Figures 5–20. The efficiency of bioleaching on particular days of the experiment—3, 6, 9, 12—was calculated each time in relation to the initial metal concentration.

The intensity of Al bioleaching was dependent on the time of the process and the tested combination. It was observed that the course of the bioleaching process in Stage I was comparable to Stage II. The greatest increase in dissolved metal occurred until the 9th processing day. On the 12th day of the process, a smaller increase in leaching efficiency was noted. Combinations G, H, and I were the most effective combinations in bioleaching, and these were combinations in which the substrate was acidified, the efficiency obtained was over 90% (Stage II). Statistical analysis showed that the obtained bioleaching efficiency did not generally differ between the combinations B, F, G, I. Overall, the post-hoc tests also showed similar efficiency of bioleaching in combinations C, D, F (the maximum efficiency ranged in the range of 65–81%). Only combination A differed from all other combinations and was also the least effective (Figures 5 and 6).

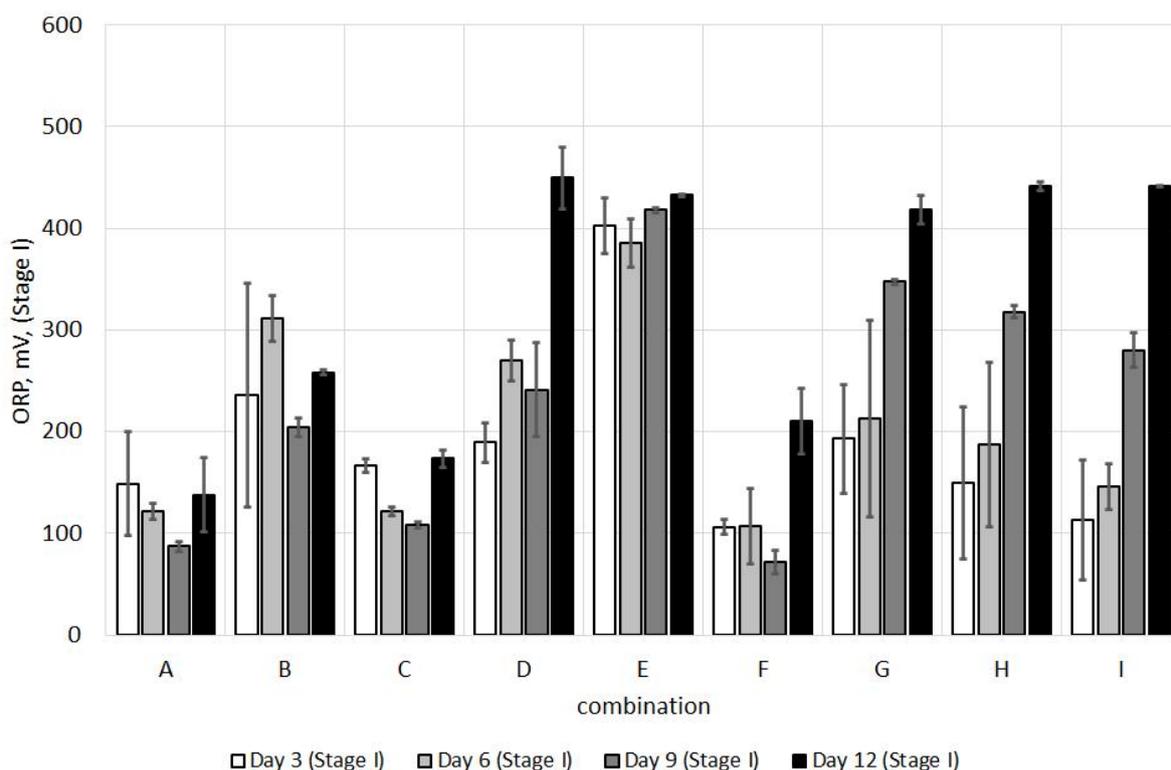


Figure 3. The oxidation and reduction potential of the samples analyzed in Stage I.

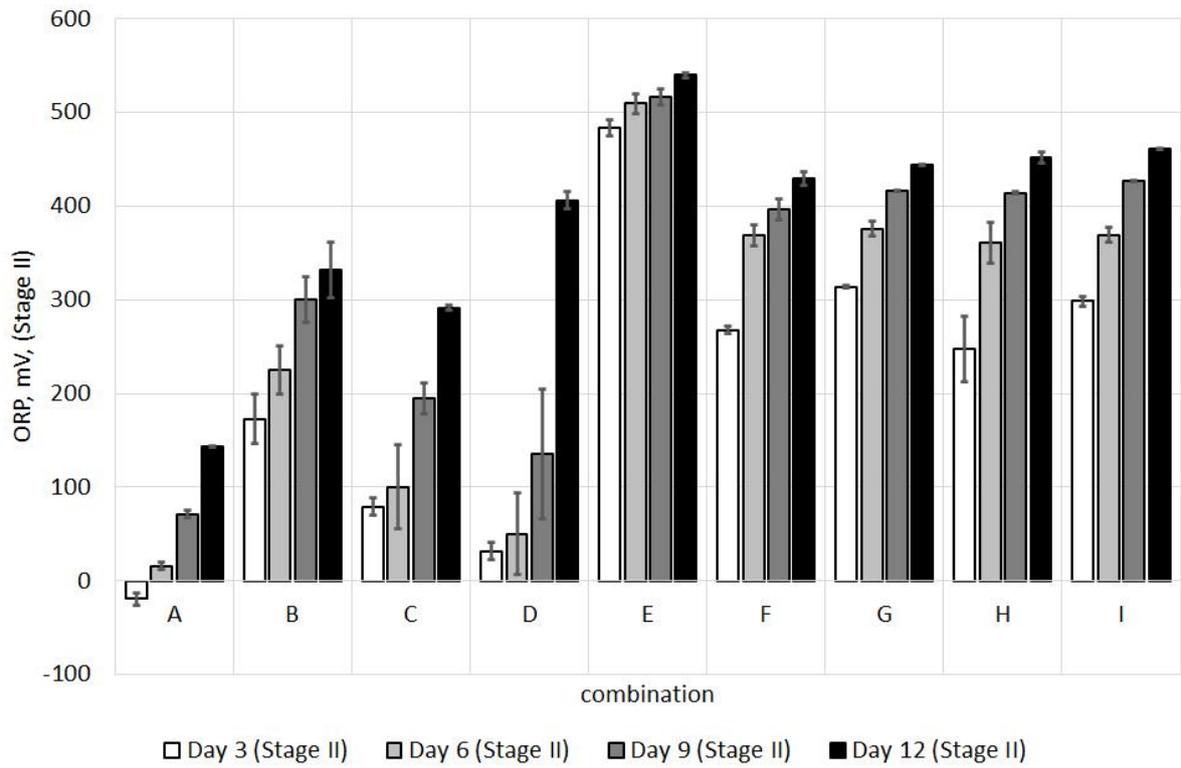


Figure 4. The oxidation and reduction potential of the samples analyzed in Stage II.

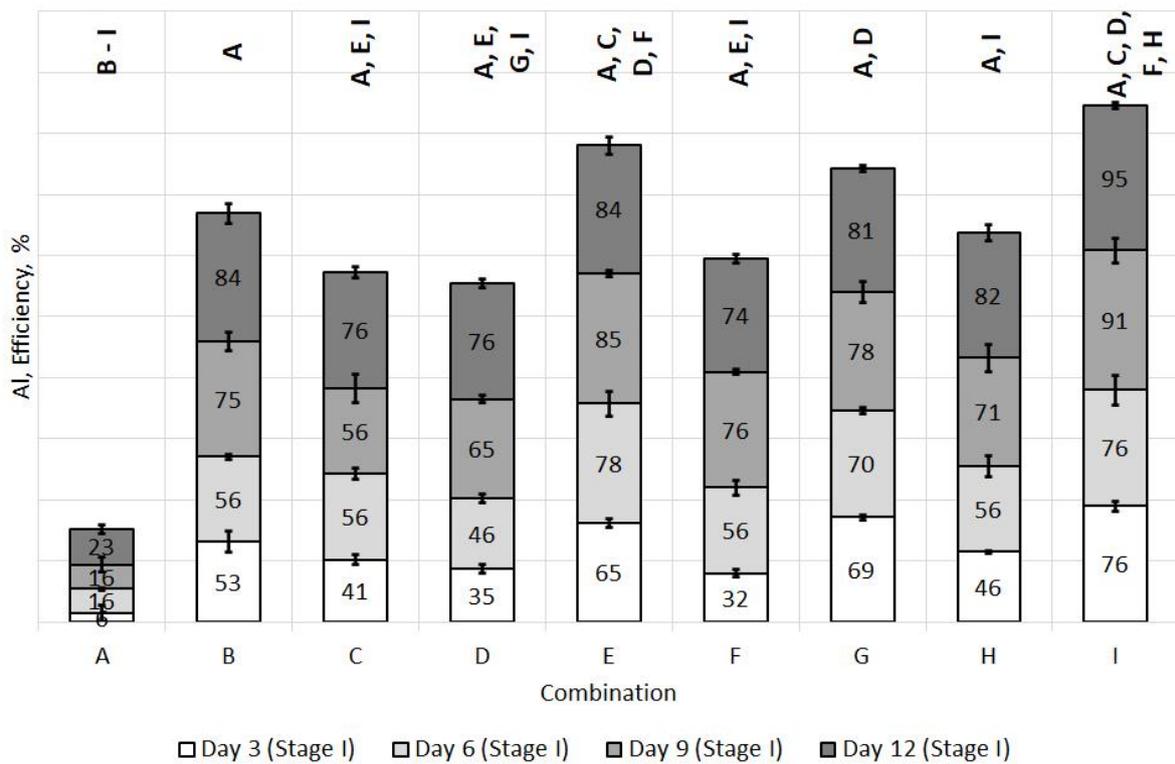


Figure 5. Effectiveness of Al release to the dissolved fraction of leachate (Stage I).

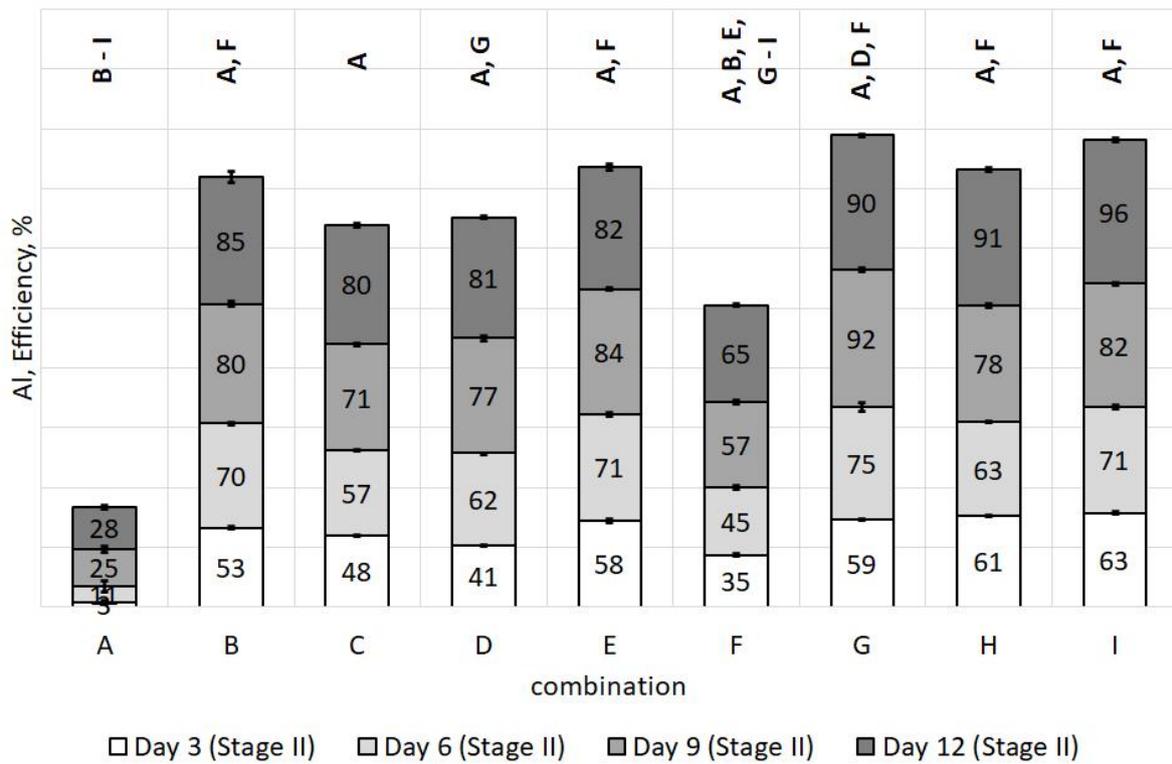


Figure 6. Effectiveness of Al release to the dissolved fraction of leachate (Stage II).

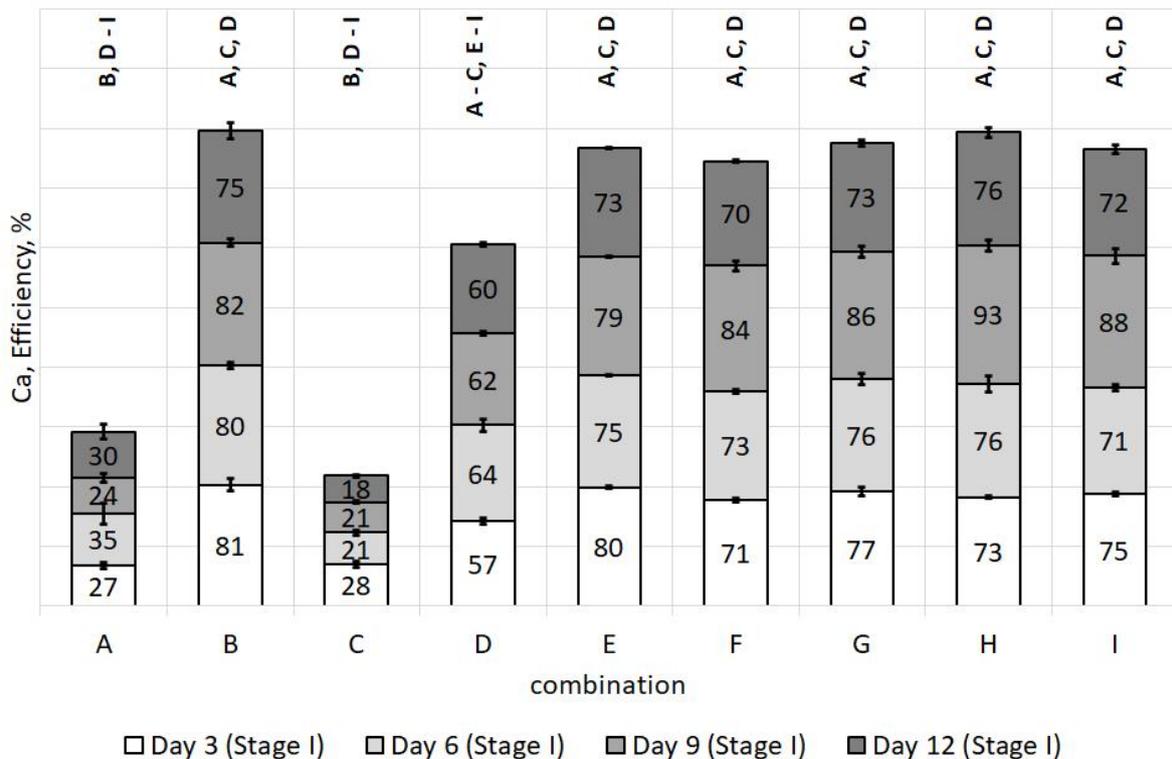


Figure 7. Effectiveness of Ca release to the dissolved fraction of leachate (Stage I).

The results of the Ca bioleaching efficiency for Stages I and II are presented in Figures 7 and 8. Combinations B and D-I proved to be highly effective in Ca bioleaching. Already on the 3rd day of the process, the efficiency was about 80% (Stage I and Stage II). In the following days, the efficiency of bioleaching was still around 80%. Moreover, the statistical

analysis showed that the bioleaching efficiencies of the combinations B, E–I were equal to each other. Statistically different effects were obtained for combinations A, C, D. The bioleaching efficiency in combination D was about 60–70%. Statistically, combination D showed differences from all other combinations. Combinations A and C caused the release of calcium from leachates in a percentage not exceeding 50%, with the highest efficiencies noted on the 3rd and 6th day of the process.

High efficiency of chromium bioleaching was obtained for the combination E–I. The maximum efficiency was achieved on the 12th day of the process, in the first stage it was approximately 80%, and in the second stage approximately 70%. The process time was important in the chromium bioleaching. In combinations B–I, an increase in the amount of leached metals took place after every 3 days of the process. The statistical analysis of the efficiency of Cr leaching was complex. There were many significant differences between the prepared combinations. Statistical analysis showed that in Stages I and II, the combinations E–I were equal to each other in terms of leaching efficiency. D–A combinations were characterized by decreasing bioleaching efficiency, but also by irregular statistical relationships (Figures 9 and 10).

In Cu bioleaching, an increase in efficiency was noted along with the extension of the process time (Figures 11 and 12). High efficiency was obtained in combinations E, G, I, in which sample pre-acidification was used. The maximum values of Cu bioleaching efficiency were characteristic for combination I and were 93% (Stage I) and 86% (Stage II). Statistical analysis generally showed no differences in the effects of bioleaching in the first stage of the study. In Stage II, combinations A, C, D were clearly outlined, and their efficiency on the 12th day of the process did not exceed 45%, 44%, and 64%, respectively.

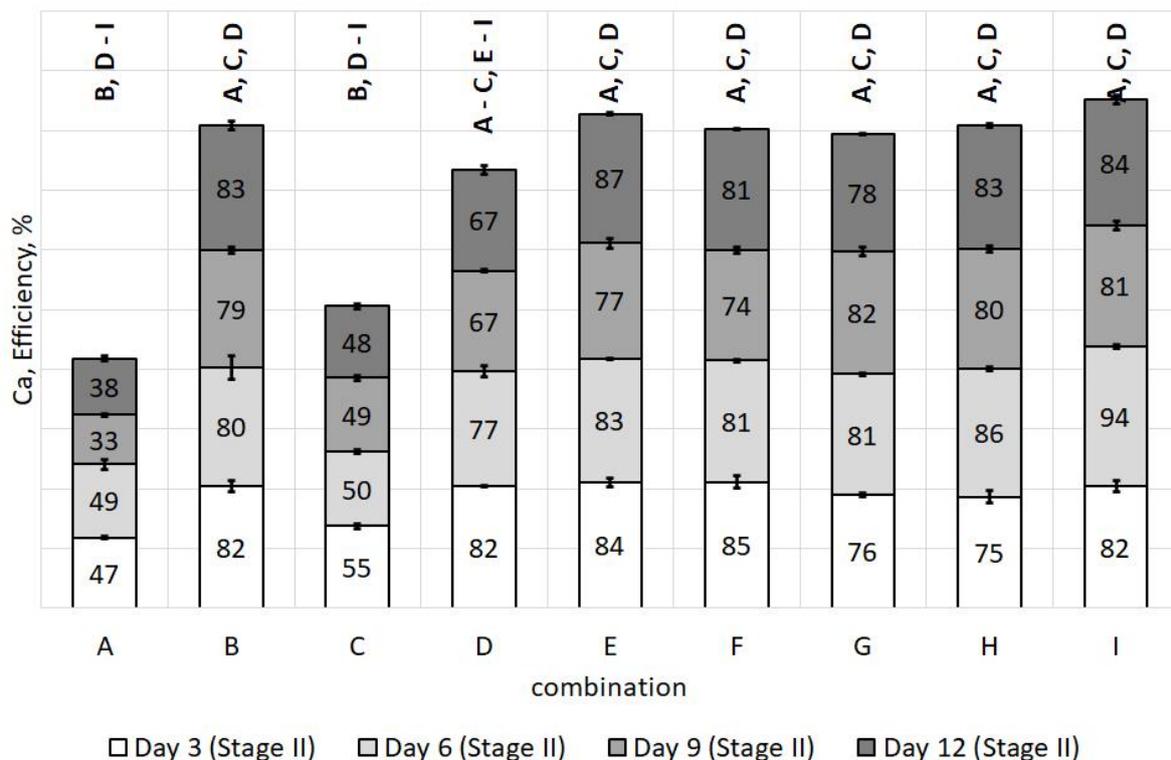


Figure 8. Effectiveness of Ca release to the dissolved fraction of leachate (Stage II).

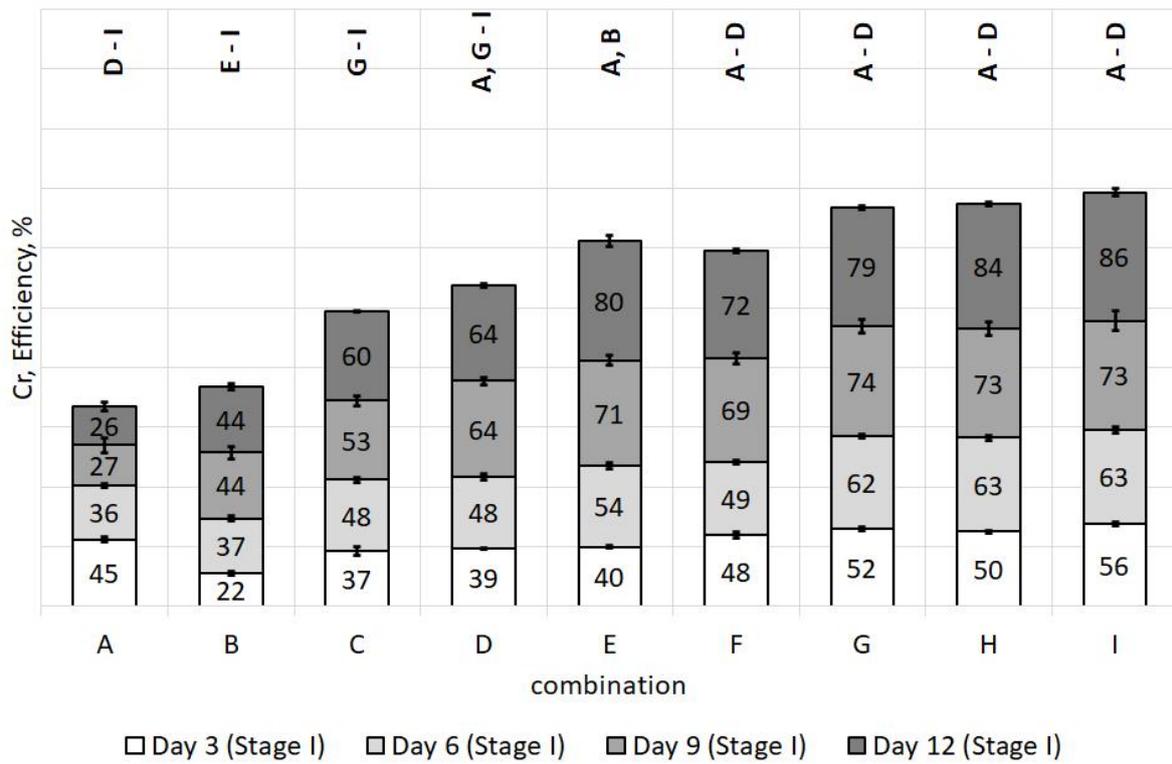


Figure 9. Effectiveness of Cr release to the dissolved fraction of leachate (Stage I).

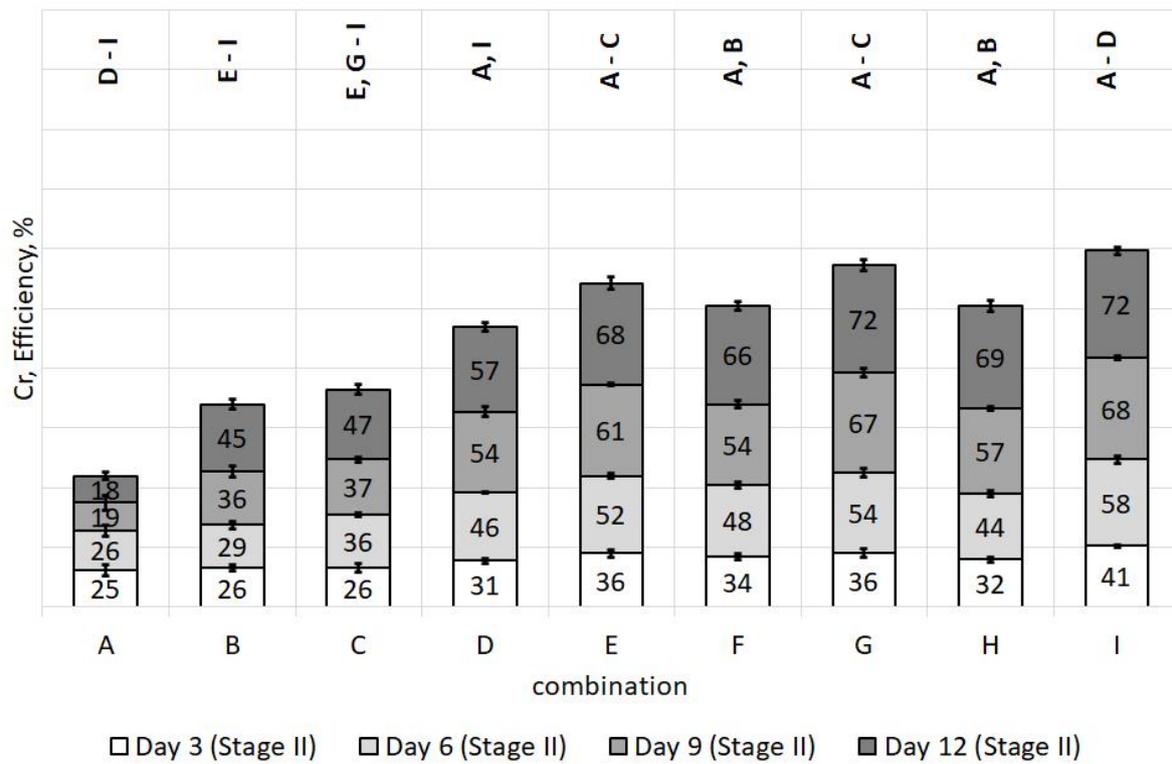


Figure 10. Effectiveness of Cr release to the dissolved fraction of leachate (Stage II).

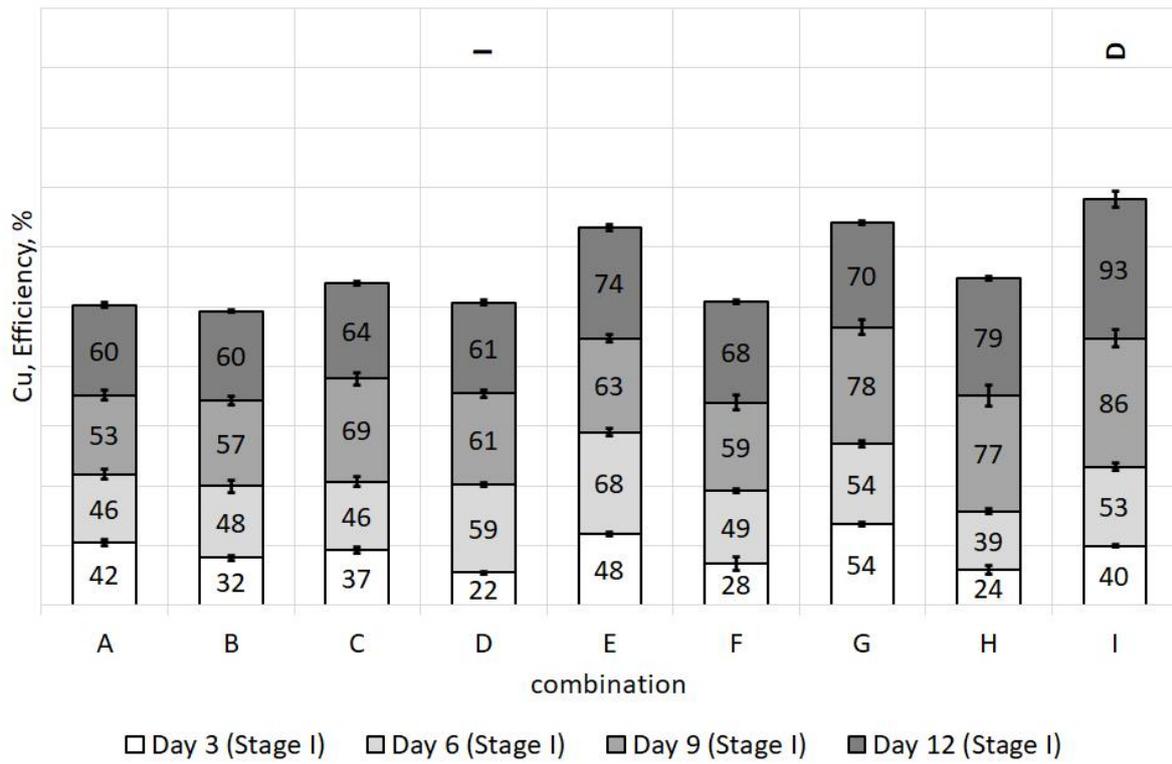


Figure 11. Effectiveness of Cu release to the dissolved fraction of leachate (Stage I).

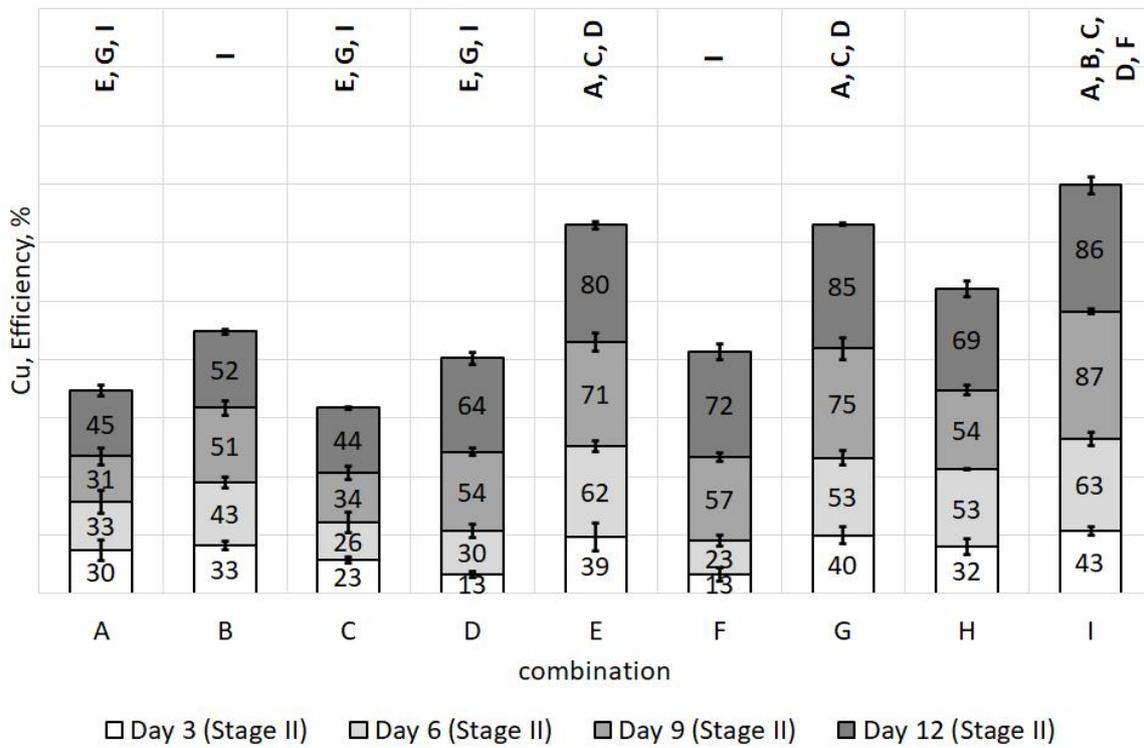


Figure 12. Effectiveness of Cu release to the dissolved fraction of leachate (Stage II).

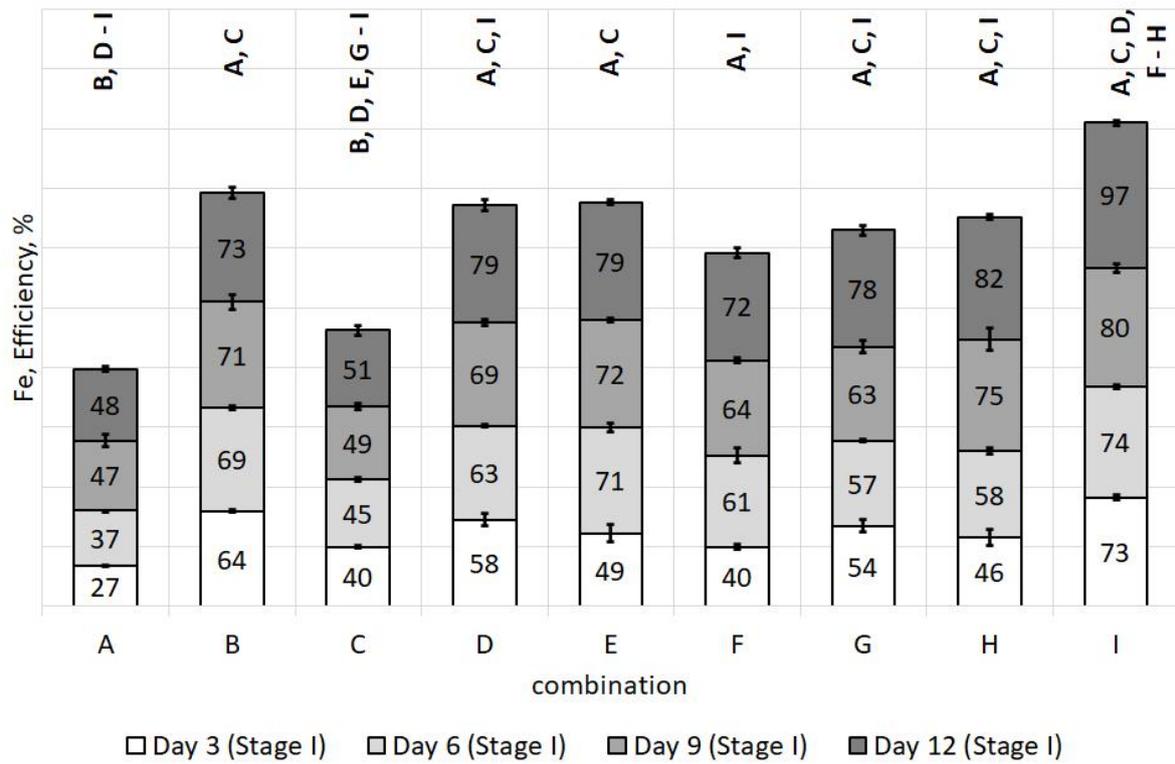


Figure 13. Effectiveness of Fe release to the dissolved fraction of leachate (Stage I).

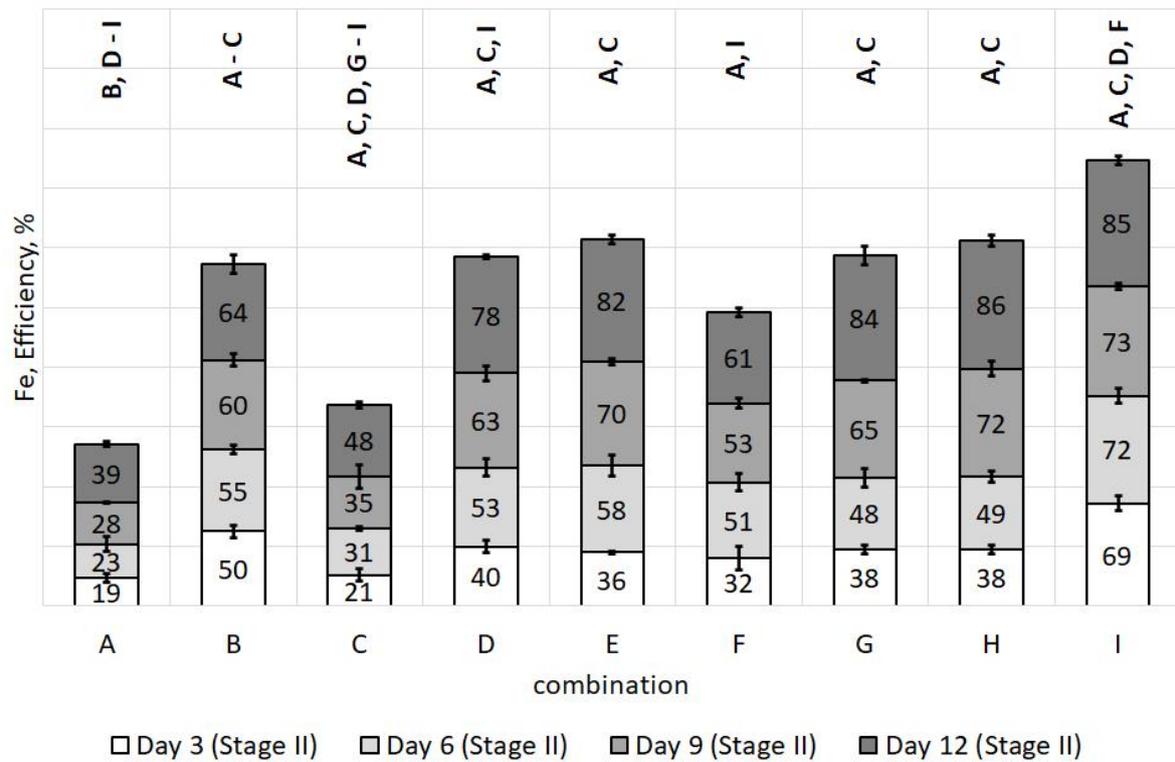


Figure 14. Effectiveness of Fe release to the dissolved fraction of leachate (Stage II).

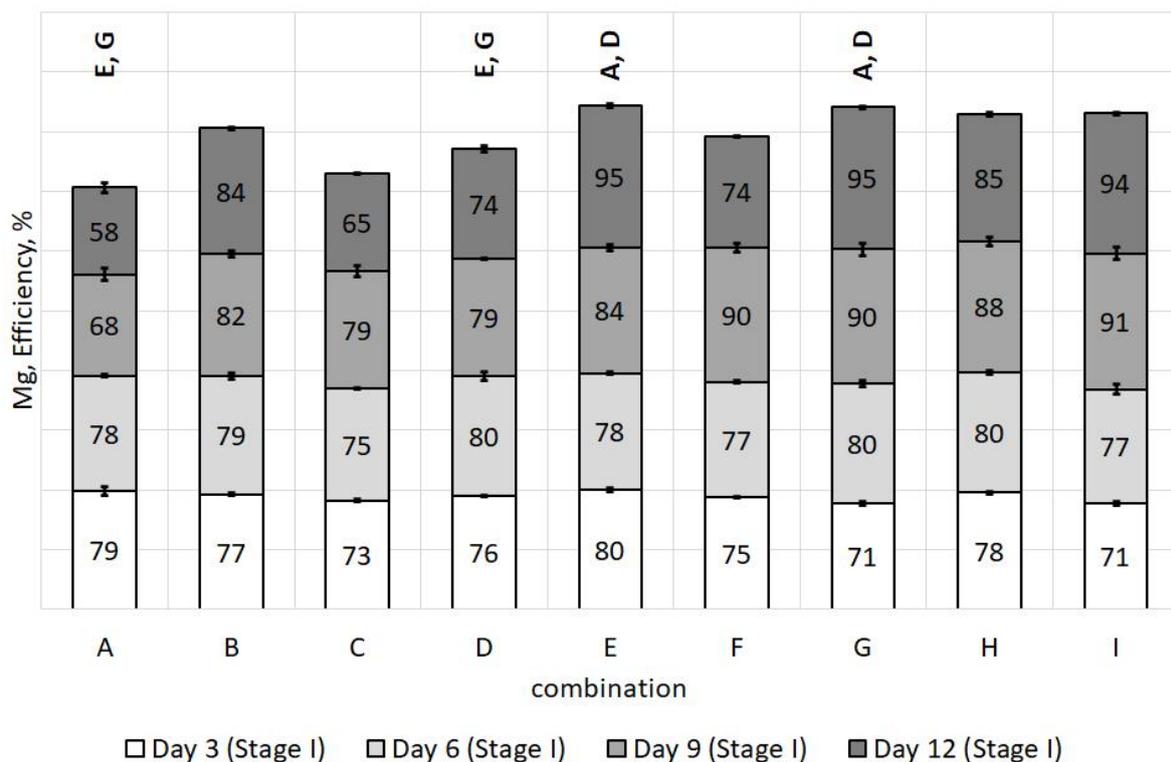


Figure 15. Effectiveness of Mg release to the dissolved fraction of leachate (Stage I).

In bioleaching of Fe, combination I was the most advantageous. High process efficiency was achieved for this combination already on the 3rd day of the process (Stage I—73%, Stage II—69%). High efficiency was also obtained in combinations of B, D, E, G, and H. Importantly, the condition for obtaining high solubility of metals was the use of a 12-day process (Figures 13 and 14).

The efficiency of magnesium bioleaching was high for most combinations. The use of various additives or the acidification of the sample did not affect the efficiency of magnesium bioleaching. Efficiency of about 90% was obtained for almost all combinations, especially E, G, H, and I. Less preferred combinations were A, C, D (Figures 15 and 16).

It was found that the Mn bioleaching is a strongly time-dependent process and significantly influenced the effectiveness of manganese leaching during each testing point of the study. The highest increase in efficiency was obtained by the 9th process day, and was lower on the 12th day. It seems that the substrate preparation method (combination) had a smaller impact on the differences in the bioleaching effects, which is particularly evident in the results of Stage II. This is confirmed by statistical analysis, which generally qualifies combinations B, F–I as equally effective in bioleaching (Figures 17 and 18).

The effects of Zn bioleaching were mainly dependent on the tested combination. Approximately 90% of the dissolution efficiency was obtained using the combinations E, G, H, I. Moreover, higher efficiency of bioleaching was noted in Stage II. It is also confirmed that among the tested combinations, the combinations A, C, D are considered the least successful.

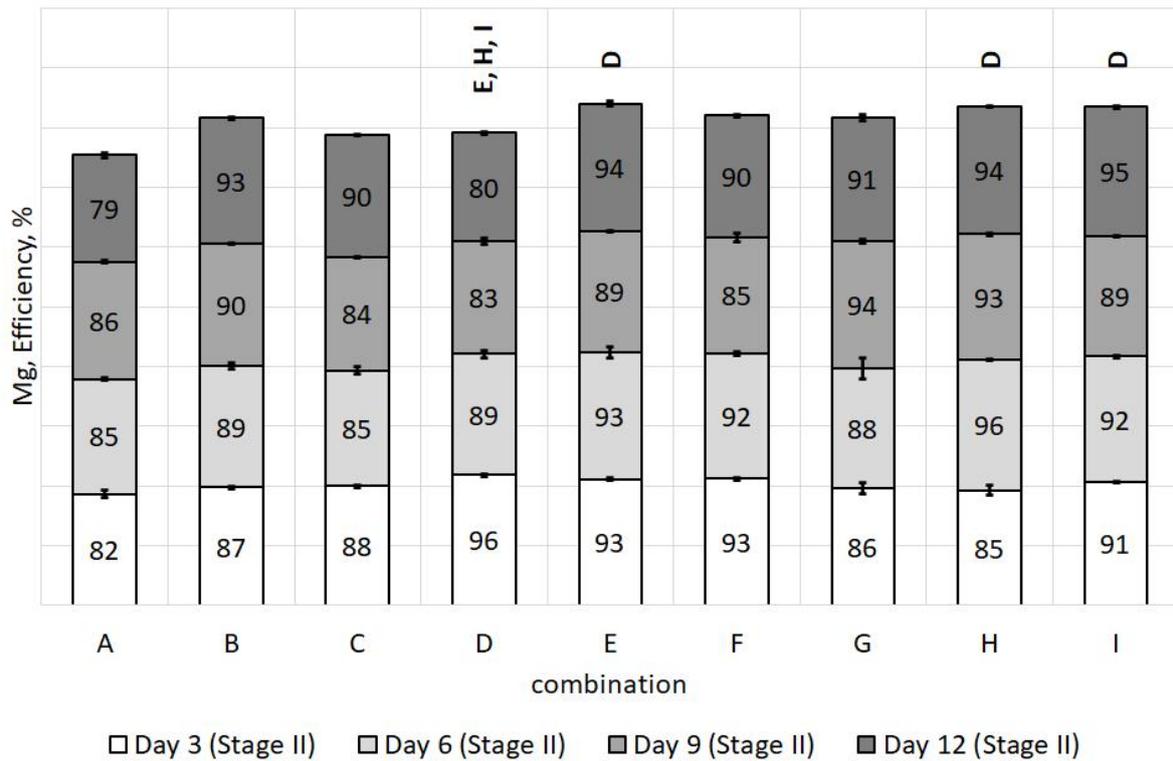


Figure 16. Effectiveness of Mg release to the dissolved fraction of leachate (Stage II).

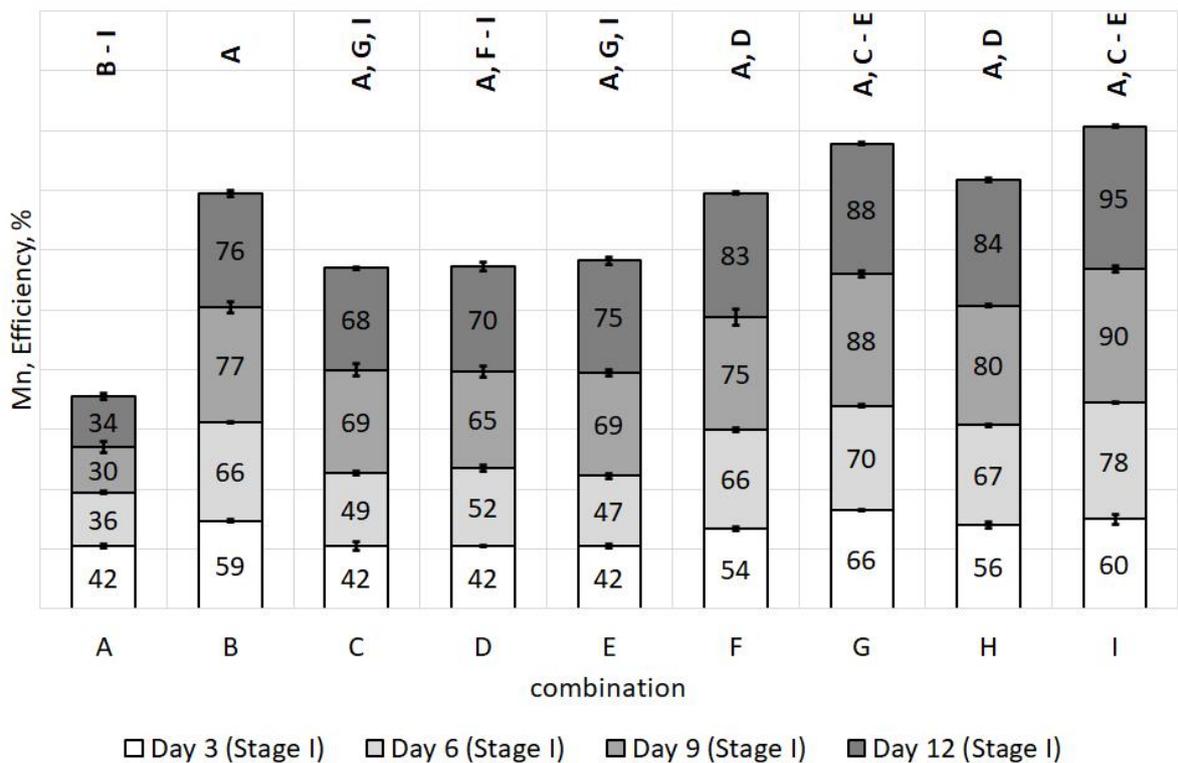


Figure 17. Effectiveness of Mn release to the dissolved fraction of leachate (Stage I).

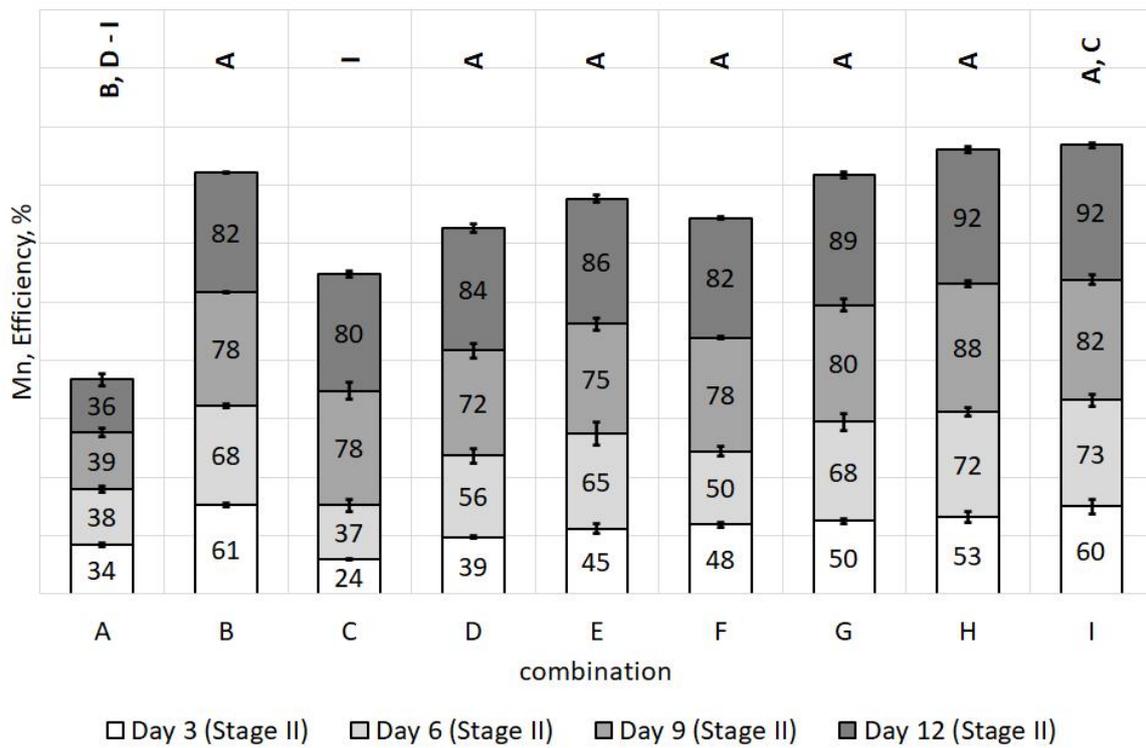


Figure 18. Effectiveness of Mn release to the dissolved fraction of leachate (Stage II).

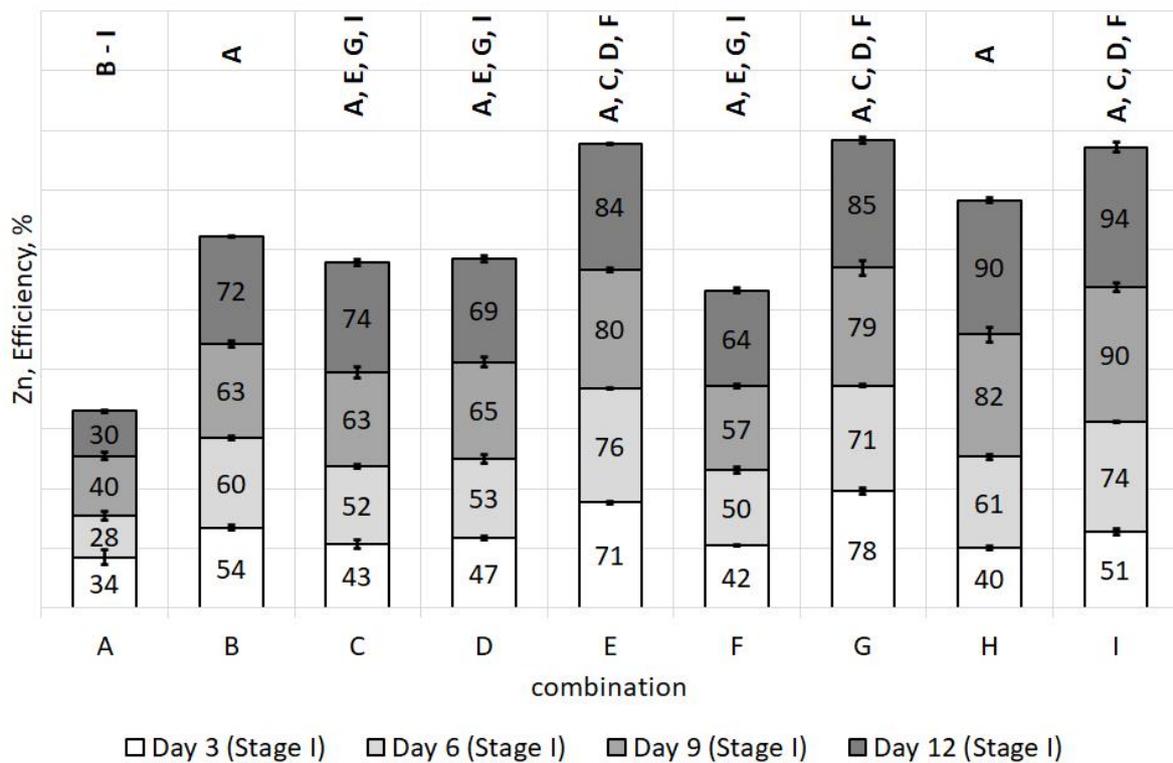


Figure 19. Effectiveness of Zn release to the dissolved fraction of leachate (Stage I).

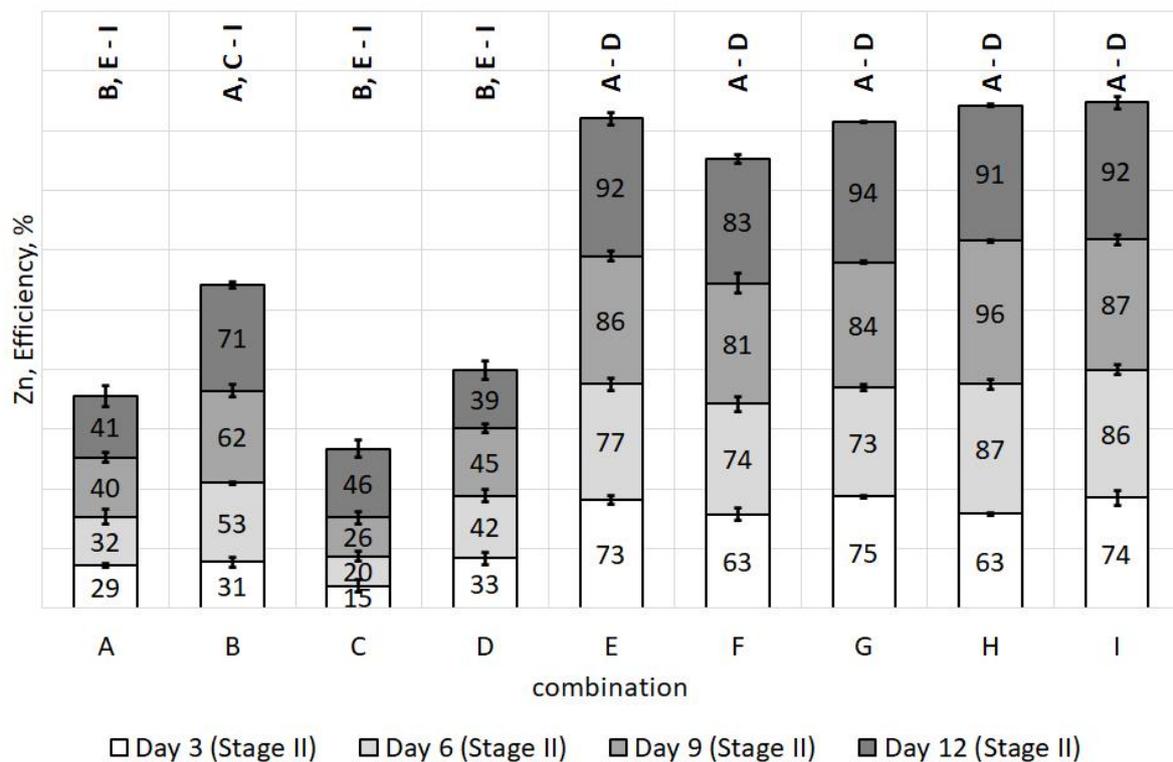


Figure 20. Effectiveness of Zn release to the dissolved fraction of leachate (Stage II).

Table 5 lists the combinations with the highest efficiencies (above 80% and 90%) of bioleaching for the tested metals in the 1st and 2nd stage. Based on the results, it was found that high process efficiency was obtained for the combination with the addition of sulfur and the acidification of the sample (combinations G, H). The most effective combination for leaching the tested metals turned out to be combination I, for which sulfur addition, sample acidification and the *A. thiooxidans* strain were used. Much lower yields were obtained for bioleaching with the *A. ferrooxidans* strain; when a mixed culture was used, no significant effect was observed.

Table 5. Combinations with the highest leaching efficiencies and the most favorable process time.

Element	Stage I		Stage II	
	Treatment (Efficiency, %)	Process Day, d	Treatment (Efficiency, %)	Process Day, d
Al	I (>90)	9	G, H, I (>90)	12
Ca	B, F, G, H, I (>80)	9	B, E, F, H, I (>80)	12
Cr	H, I (>80)	12	G, I (>70)	12
Cu	I (>90)	12	E, G, I (>80)	12
Fe	H, I (>80)	12	E, G, H, I (>80)	12
Mg	E, G, I (>90)	12	B, G, H, I (>90)	12
Mn	F, G, H, I (>80)	12	B, D-I (>80)	12
Zn	H, I (>90)	12	E, G, H, I (>90)	12

The selectivity of the bioleaching of metals from landfill leachate was found on the basis of the conducted tests. Higher content of selected metals (Ni, Cr) in leachate waters was obtained for the combination of mixed culture used. The results presented by Pacholewska et al. [41] and Ojumu et al. [42] confirm the effectiveness of the use of *A. ferrooxidans* and *A. thiooxidans* for the effective bioleaching of Zn, Ca, Mg, Pb and even Cd from metal ores. Similarly, the results of Li et al. [24] indicate an increase in the effectiveness of the bioleaching process using mixed cultures rather than individual *A. ferrooxidans* or *A. thiooxidans* cultures. The high efficiency of the combined culture in bioleaching of Cr, Cu, Ni, and Zn was also noted. The application of single cultures of *A. thiooxidans* or *A. ferrooxidans* was definitely less effective in recovery of Cu, Ni, Pb, and Zn from waste printed circuit boards than the mixed culture. To make the process of bioleaching metals from various substrates practical and effective, it is recommended to use mixed cultures of bioleaching bacteria [24,36]. Numerous studies confirm the higher efficiency of bioleaching with the use of *A. thiooxidans* culture with the addition of elemental sulfur. Sulfur added to the medium has a significant effect on cell growth [43]. The efficiency of bioleaching depends to a large extent on the substrate, the bioleaching of Cr, Zn, Cu, Pb, and Cd from contaminated soil ranged from 11 to 99% with *A. thiooxidans* bacteria [26]. Use of *A. ferrooxidans* culture to tackle anaerobically digested sludge obtained the removal of Cr, Cu, Zn, Ni, and Pb which was between 16.2 and 91.5% [44] from coal fly ash using *A. thiooxidans* recovery yields of 25% Al and 22% Fe [45]. Studies of Rastegar et al. [46] also showed an increase in the recovery of Ni and Cu by 16 and 8%, respectively, in samples acidified with sulfuric acid. The results presented in the current study show the same relationship-increased efficiency of the bioleaching process as was observed in the tests with sulfur and acid addition.

It was also correlated with an increase in dry solids and volatile solids (Figure 21). In combinations H and I where the sulfur addition was 1.5 (*w/v*), DS and vs. increased to over 30 mg/L and 25 g/L, respectively (measurement on the 12th day of the process). In general, in all combinations, there was an increase in biomass in relation to the collected leachate 0 (S I—Stage I) and 0 (S II—Stage II). However, this increase was not as significant as in the case of combinations of H and I. An exception was combinations B, E, G (especially in Stage II), in which initial acidification was applied, which resulted in an increase in the value of DS and VS.

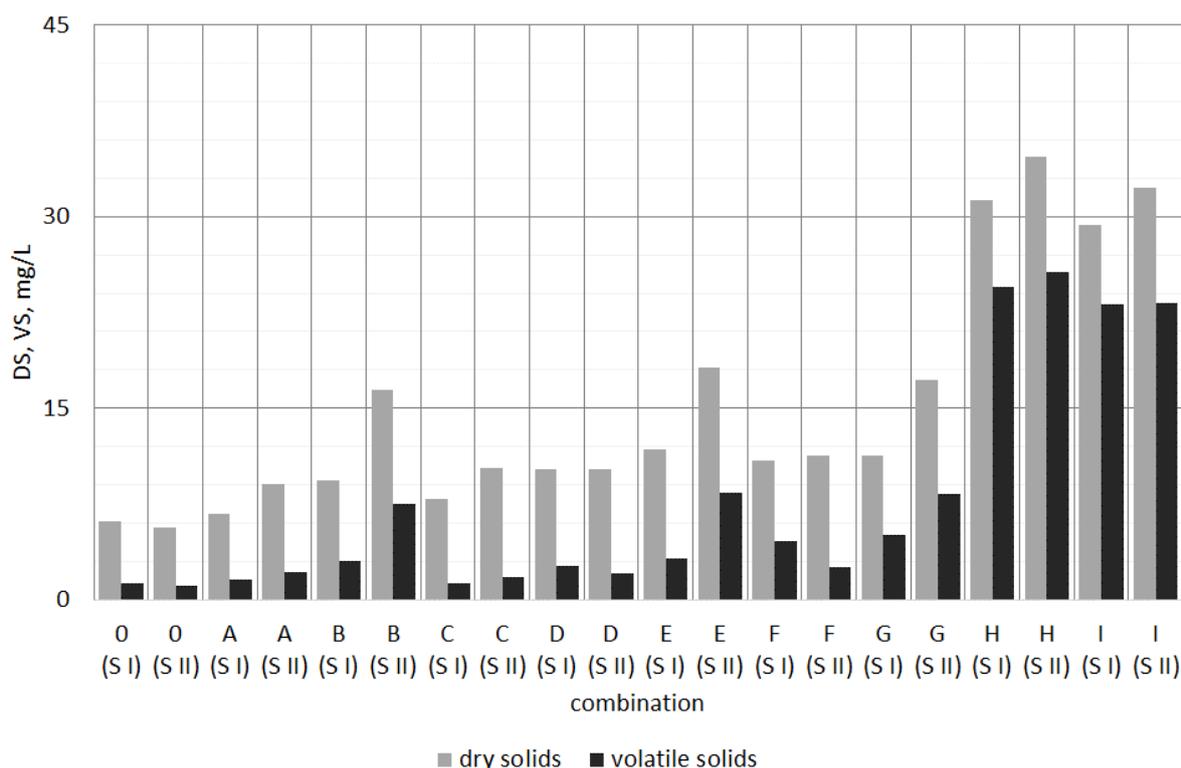


Figure 21. Dry solids and volatile solids in samples after 12 days of the process.

Bioleaching is considered a promising environmental technology for the recovery of metals from various substrates, as well as a technology with low energy and chemical consumption. Nevertheless, the practical usefulness and durability of this technology has not been demonstrated on a larger scale. Laboratory scale research focuses on identifying the best working conditions (e.g., substrate concentration, substrate treatment, microbiology consortium, temperature). Industrial bioleaching is still undergoing research and is being tested mostly in pilot installations. As of today, the process is used to extract metals from low-quality ores and is a long-term process. Still, an independent bioleaching process is not effective enough and is therefore not widely used in industry unless it is combined with other methods. Bioleaching of leachate waters of different origin is not used, while usually chemical methods are more popular [30].

In this study, bioleaching was used as a method to eliminate metals from landfill leachate. The obtained results confirm the high efficiency of the proposed method. In addition, the produced biomass, after separation from the remaining post-process liquid, can be used as a biopreparation to intensify the decomposition of deposited waste. Currently, the high cost of periodic replacement and cleaning membranes in reverse osmosis installations contribute to the search for methods supporting the treatment of leachate.

Literature data indicate that the integration of biological and physicochemical processes results in higher sewage, waste, fly ash treatment efficiency, while no data can be found on leachates [10,47]. Systems using biological methods followed by reverse osmosis make it possible to discharge the purified leachate to surface waters, while maintaining that treated leachates are directed to the municipal sewage treatment plants.

The proposed solution would comprehensively improve the functioning of the landfill, on the one hand, cleaning and recovery of metals from leachate, as a consequence of relieving the reverse osmosis installation, and on the other hand, obtaining microorganisms biomass that would improve the biological degradation processes of the deposited waste. The diagram (Figure 22) shows the possible, integrated modifications and solutions for the treatment of landfill leachate.

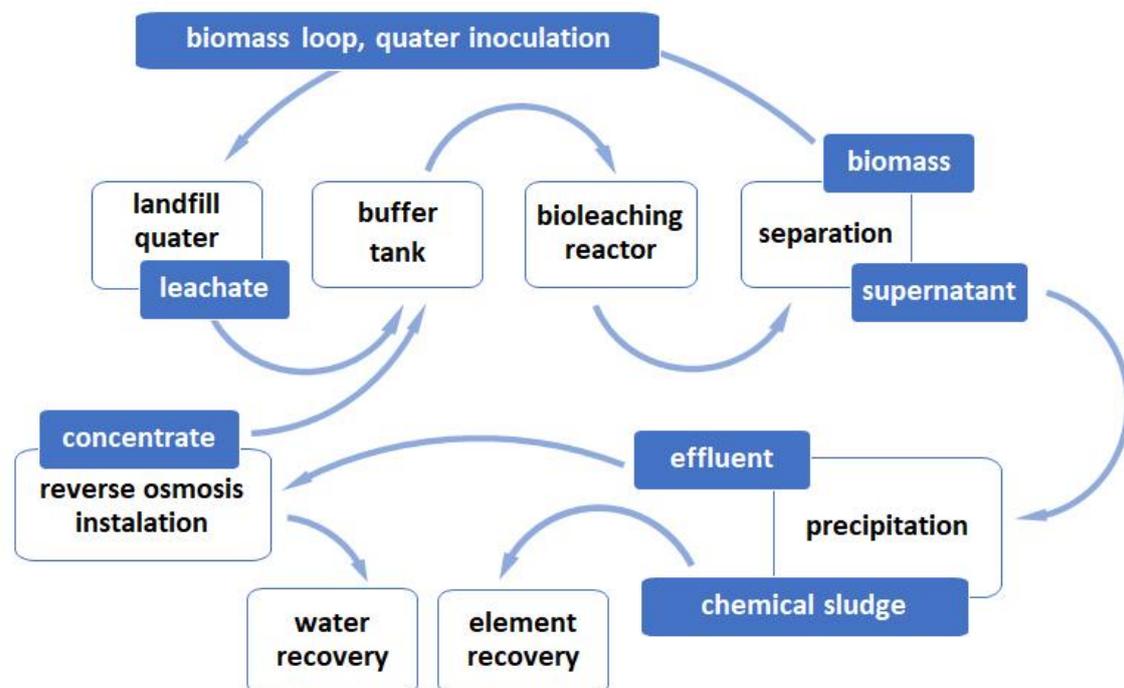


Figure 22. Scheme of an integrated solution for the treatment of landfill leachate.

4. Conclusions

The content of metals in the leachate hinders their treatment and increases the level of nuisance to the environment. Metals and inorganic compounds presented in leachate are difficult to separate, thus, various methods are used, which have some disadvantages. This mainly applies to process costs, efficiency, and environmental impact. Another aspect of metals removal from leachate is the opportunity to recover rare and precious metals. This procedure not only reduces the environmental impact, but also enables the reuse of recovered metals in industrial processes. These current conducted preliminary tests confirm the possibility of using bioleaching for landfill leachates treatment, in order to pre-clean them from heavy metals. This is a biological process, thus, very important is the selection of optimal conditions for bioleaching making the process more efficient.

Based on the literature review and own research, it was found that bioleaching requires the selection of a number of process parameters, including used strain or mixed culture, pH correction, type of additives and their amount, process temperature. All these factors must be adapted to the type of substrate in each case, due to a high leachate inhomogeneity.

Based on the experiment, selectivity in metal removal was found, especially acidification of the samples, as well as the addition of sulfur were factors increasing the efficiency of bioleaching.

High bioleaching efficiency, from 80% to 90%, was obtained for all metals for which the sample acidification and sulfur addition were used (combinations G, H). The simultaneous combination of both mentioned additives turned out to be the most advantageous (combination I). The *A. thiooxidans* culture was the most effective in bioleaching. For the *A. ferrooxidans* culture used, much lower efficiencies were obtained; by contrast, the use of mixed culture had no significant effect.

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