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Fractionation study in bioleached metallurgy wastes using six-step sequential extraction

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ABSTRACT

The stored metallurgy wastes contain residues from ore processing operations that are characterized by relatively high concentrations of heavy metals. The bioleaching process makes use of bacteria to recover elements from industrial wastes and to decrease potential risk of environmental contamination. Wastes were treated by solutions containing bacteria. In this work, the optimized six-stage sequential extraction procedure was applied for the fractionation of Ni, Cr, Fe, Mn, Cu and Zn in iron–nickel metallurgy wastes deposited in Southern Poland (Szklary). Fractionation and total concentrations of elements in wastes before and after various bioleaching treatments were studied. Analyses of the extracts were performed by ICP-MS and FAAS. To achieve the most effective bioleaching of Zn, Cr, Ni, Cu, Mn, Fe the usage of both autotrophic and heterotrophic bacteria in sequence, combined with flushing of the residue after bioleaching is required. 80–100% of total metal concentrations were mobilized after the proposed treatment. Wastes treated according to this procedure could be deposited without any risk of environmental contamination and additionally the metals could be recovered for industrial purposes.

1. Introduction

Environmental pollution by heavy metals originated from abandoned mines and/or dump metallurgy waste are very important sources of soil and water contamination. In the Lower Silesia region of Poland there are a lot of dumps where various industrial wastes are deposited. One of such heaps is located in Szklary. An iron–nickel alloy was produced there until the end of 1970s. The mining and metallurgy wastes are characterized by relatively high concentrations of most of the elements, some of them are particularly toxic. There are many efforts to recover valuable elements from industrial wastes and to decrease potential risk of environmental contamination. One of the recently applied methods is bioleaching (the solubilization of metals from solid substrates either directly by the metabolism of leaching bacteria or indirectly by the products of their metabolism [4]) Bioleaching is a simple, economical and effective process for metal solubilization from industrial wastes or biosolids [1–3]. Metal solubilization from solid wastes or other solids is achieved through the activity of some chemolithotrophic bacteria for example autotrophic or heterotrophic bacteria. Autotrophic bacteria e.g. *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Thiobacillus thioparus* which can catalyze the oxidation of sulfur compounds to sulfuric acid causing pH lowering. Activity of heterotrophic bacteria e.g. *Pseudomonas fluorescens*, *Bacillus cereus* and *Bacillus thuringiensis* causes decomposition of organometallic compounds. Organic acids and phenols are the main products of bacteria metabolism [5]. These compounds may take part in decomposition of minerals available in industrial wastes.

The mobility and bioavailability of elements in the environment depends strongly on their chemical forms. Elements in soils, sediments and wastes occur in several different physico-chemical forms, i.e. as simple or complex ions, as easily exchangeable ions, as organically bound, as occluded by or coprecipitated with metal oxides, carbonates, phosphates and secondary minerals or as ions in crystal lattices of primary minerals [6]. The solid–liquid extraction is a useful tool to evaluate the elements binding. Many different sequential extraction procedures were applied to evaluate the contamination risk for soil [7–10] and sediment [11–13]. However, the number of proposed schemes for mining and metallurgy wastes was limited [14].

The aim of the study was to apply the optimized extraction procedure and to compare mobility of selected elements (Cr, Cu, Fe, Mn, Ni, and Zn) and their distribution between operationally defined phases in wastes before and after bioleaching processes. The sequential extraction was used to estimate the efficiency of mobilization of studied elements after application of bioleaching procedure. During preliminary studies the following fractions were defined: water-soluble, carbonate, Mn oxides, Fe oxides, organic and sulfide and residual. The concentration of selected extractants, temperature and duration of extraction procedure were optimized.

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Based on the obtained results the mobility of metals in residues and usefulness of bio-extracts for recovery of some valuable elements were assessed.

2. Experimental

2.1. Reagents

The following reagents were used: hydroxylamine hydrochloride and oxalic acid (puriss p.a.) (Fluka, UK); ammonium oxalate, hydrogen peroxide (puriss p.a.) (Sigma–Aldrich, Germany) and nitric acid, acetic acid, perchloric acid, hydrofluoric acid (supra pure) (Merck, Germany).

Standard solutions were prepared by dilution of Spectroscan solutions (1000 mg L^{-1}) of the appropriate element. Ultrapure water obtained form a Milli-Q-Water System (Millipore, USA) was used throughout the work.

2.2. Instrumentation

The total concentrations of Ca, Fe, Mg, Mn, Zn in samples and in extracts were measured using flame atomic absorption spectrometer 3110 (Perkin Elmer, USA). Total contents of Cr, Cu, Ni were measured using inductively coupled plasma mass spectrometer Elan 6100 DRC (Perkin Elmer SCIEX, Canada) with Meihard-type nebulizer and Scott-type spray chamber. Microwave Digestion System Ethos 1 (Milestone, Italy) was used for sample digestion. An Elpan 357 water bath shaker (Elpan, Poland) was used for sample extractions. Total concentrations of macroelements were measured using Scanning Electron Microscope equipped with Energy Dispersive Spectroscopy analyzer (Zeiss, LEO 435 VP) Röntec M1, Germany).

2.3. Sampling and sample preparation

Waste samples were collected in 2006 in Szklary in the Lower Silesia region of Poland. Waste samples were collected – using Zig-Zag method – from dump where metallurgy wastes were deposited. A total amount of samples of 1.5 kg were collected from 0 to 10 cm depth layer. Samples were air-dried, milled in agate ball mill and stored in polypropylene containers in room temperature.

2.4. Bioleaching procedures

Bioleaching process was performed using two different procedures. Each process was performed in Erlenmeyer flasks. The first bioleaching process with autotrophic bacteria was carried out for 65 days, while the second one consists of 35 days heterotrophic pretreatment and then 35 days autotrophic bioleaching (in sequence). In the first process 250 mL of leaching medium, containing some inorganic ions such as: Fe^{2+}, Ca^{2+}, K^{+}, Mg^{2+}, NH_4^{+}, NO_3^{-}, SO_4^{2-}, HPO_4^{-}, Cl^{-}, was added to each of three flasks containing 50 g of pretreated solid waste. The leaching medium was inoculated with a mixture of autochthonic bacteria strains *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* [former name *T. ferrooxidans* and *T. thiooxidans*] before addition to the flasks. The process was carried out for 65 days at 25 °C, pH 2. Both bacterial systems as well as control one were aerated and stirred with magnetic stirrers. pH was adjusted daily to the value 2 using 5 mol L^{-1} H_2SO_4.

In the second bioleaching process, with heterotrophic bacteria, 250 mL of mineral solution (pH 7) containing some inorganic ions such as: K^{+}, Mg^{2+}, NH_4^{+}, SO_4^{2-}, HPO_4^{-}, $H_2PO_4^{2-}$ was added to each of three flasks, containing 50 g of solid waste. After that, flasks were inoculated with a mixture of active, autochthonic bacteria strains (*P. fluorescens*, *B. cereus* and *B. thuringiensis*). The process was performed for 35 days at 25 °C. All solutions were stirred with magnetic stirrers. Solid phase after first step of bioleaching procedure (with heterotrophic bacteria) was flushed, dried and treated with autotrophic bacteria. The dried material was flushed with H_2SO_4 solution and pH was adjusted to value 2. After that operation, autotrophic bioleaching was started using leaching medium with autochthonic bacteria strains *Acidithiobacillus ferrooxidans* and *Acidithiobacillus* mixed in the ratio of 1:1. All solutions were stirred on magnetic stirrers. Bioleaching process was performed for 35 days at 25 °C, pH of the solutions was adjusted to value 2 and controlled throughout the experiment.

Control sample were prepared only in autotrophic and sequential bioleaching procedures. It contained only solid waste and leaching medium without bacteria. Thymol as a bacteriostatic substance was added to the both control flask. After all described bioleaching processes samples were rinsed with Milli-Q water, air-dried and homogenized by grinding in agate mill.

2.5. Total metal determination

Approximately 200 mg of dried material and a mixture of concentrated acids (2 mL of HNO_3 and 1 mL $HClO_4$) were placed in PTFE vessels and digested in a microwave digestion system. A three-stage program with a maximum temperature of 200 °C and maximum microwave power of 1000 W was used. In the second step 0.5 mL HF was added and the same three-stage program was applied (5 min: 20–90 °C; 10 min: 90–170 °C; 50 min: 170–200 °C). Digested samples were transferred into 50 mL volumetric flasks and diluted to the volume with Milli-Q water. Digestion of all samples was triplicate. Digested samples were diluted and the concentrations of studies elements were measured using FAAS and ICP-MS. ICP-MS was used for determination of Cr, Cu and Ni. ICP-MS measurements were performed under following conditions: sweep 5, replicates 5, dwell time 100 ms, ICP RF power 1100 W, lens voltage 8 V, plasma gas flow 15 L min^{-1}, auxiliary gas flow 1.2 L min^{-1}, nebulizer gas flow 0.9 L min^{-1}, measured isotopes: ^{52}Cr; ^{63}Cu; ^{58}Ni. FAAS was used to determine Ca, Mg, Fe, Mn and Zn. The air-acetylene flame was adjusted according to the manufacturer's recommendations. The following parameters of measurements were applied—HCL wavelength, current and slit width respectively: Ca – 422.7 nm, 7 mA, 0.5 nm; Mg – 202.6 nm, 7 mA, 0.7 nm; Fe – 248.3, 13 mA, 0.2 nm; Mn – 279.5, 10 mA, 0.2 nm; Zn – 213.9 nm, 10 mA, 0.7 nm. Quantitative determination of elements in both techniques was performed using calibration plot. Elementary analysis of main components of solid samples was performed using Scanning Electron Microscope equipped with EDS analyzer.

2.6. Extraction procedure

The six-step sequential extraction was applied to compare the mobility of Mn, Fe, Ni, Cr, Zn, and Cu in waste samples before and after bioleaching treatment. Extractions were carried out in triplicate. 1 g of dried solid waste sample was extracted with 50 mL of the extractant in 120 mL polyethylene container (steps 1–5). Extracts were centrifuged at 2000 rpm during 30 min and filtered through 0.45 μm cellulose acetate filter into a polyethylene container. Extracts after filtration were acidified with 50 μL of concentrated HNO_3 (to pH about 2) and stored at 4 °C before analysis.

Step 1. (Water-soluble fraction) Samples were shaken with Milli-Q water for 3 h in room temperature in horizontal position in a water bath shaker.

Step 2. (Carbonate fraction) 0.43 mol L^{-1} (24.6 mL of glacial acetic acid was diluted with water in 1 L volumetric flask) acetic

Table 1
Concentrations of macroelements elements in solid wastes before bioleaching process and after bioleaching process and in control sample measured using SEM [% m/m ± SD].

Element	Sample					
	Before bioleaching	After heterotrophic bioleaching	After autotrophic bioleaching	Control sample to autotrophic bioleaching	After sequential bioleaching	Control sample to sequential bioleaching
C	25.2 ± 2.0	13.3 ± 1.2	11 ± 1	11.3 ± 1.0	8.5 ± 0.8	11.3 ± 1.1
Ca	4.4 ± 0.2	8.2 ± 0.4	11.8 ± 0.7	12.4 ± 0.6	7.5 ± 0.4	8.3 ± 0.4
Cr	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Fe	5.5 ± 0.4	5.2 ± 0.5	6.5 ± 0.5	6.5 ± 0.5	5.8 ± 0.5	5.8 ± 0.5
K	0.7 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
Mg	7.7 ± 0.5	4.0 ± 0.3	2.5 ± 0.2	5.9 ± 0.4	4.0 ± 0.3	4.6 ± 0.3
Ni	0.7 ± 0.2	<0.2	<0.2	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.1
O	44.2 ± 3.6	44.8 ± 0.2	39.7 ± 3.4	43.8 ± 3.4	44.4 ± 3.7	45.6 ± 3.8
S	0.2 ± 0.04	3.6 ± 0.2	2.7 ± 0.2	2.3 ± 0.1	11.6 ± 0.6	12.2 ± 0.6
Si	18.3 ± 0.8	17.1 ± 0.9	17.2 ± 0.9	17.4 ± 0.9	13.2 ± 0.7	13.4 ± 0.8
Al	4.8 ± 0.2	2.3 ± 0.2	2.9 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.2 ± 0.2

acid was added to the residue from step 1. The extraction was carried out for 16 h in room temperature.

Step 3. (Easy-reducible fraction) Freshly prepared 0.04 mol L^{-1} hydroxylamine hydrochloride in 25% acetic acid (7.5 g $NH_2OH{\cdot}HCl$, 250 mL 100% CH_3COOH, diluted with water in 1 L volumetric flask) was added to the residue from step 2. The extraction was carried out for 5 h in room temperature.

Step 4. (Moderately reducible fraction) 0.2 mol L^{-1} oxalate buffer (24.82 g $NH_4C_2O_4$, 18.1 g $H_2C_2O_4$, diluted with water in 1 L volumetric flask) was added to the residue from step 3. The extraction was carried out for 7 h in room temperature.

Step 5. (Organic/sulfide fraction) 30% hydrogen peroxide and 50 μL of concentrated HNO_3 (pH 2) was added carefully to the residue from step 4. The extraction was carried out for 3 h at 90 °C in the water bath shaker.

Step 6. (Residual fraction) The solid residue and a mixture of concentrated acids (2 mL HNO_3 and 1 mL $HClO_4$) were placed in PTFE vessels and digested in microwave digestion system. Parameters of digestion are described in Section 2.5.

3. Results and discussion

3.1. Total metal determination

Total concentrations of main elements in samples before and after bioleaching processes were determined using SEM with EDS detector. Results are presented in Table 1. Concentration of carbon in samples before bioleaching process is relatively high. Leaching medium causes partial dissolution of carbonates included in samples. During bacteria activity even more carbonates are dissolved. Both leaching medium and bacteria activity, results in precipitation of calcium sulfate, therefore concentrations of calcium and sulfur increase in samples after all applied bioleaching processes. Aluminium concentrations in control samples and samples after bioleaching treatment decrease of 50%. In case of iron and nickel, the sensitivity of SEM measurements is not sufficient, since the changes in total concentrations of these elements in samples before and after treatment is negligible. Since the nutrient medium contain Fe, Ca and Mg the determinations of these elements in operationally defined fraction should be performed with a special attention. The total contents of Ca and Mg in waste samples were too low compared to their content in the nutrient medium, so in the further studies their determinations were not performed, contrary to Fe.

ICP-MS and FAAS were used to determine elements which concentrations were below 0.1%. The results are presented in Table 2. The total concentrations of Cr, Cu, Mn and Ni in samples after all applied bioleaching processes were lower than the total contents of these metals in samples before bioleaching. The bacteria transformed insoluble metal compounds into more soluble forms. In the samples after autotrophic bioleaching decrease: of 85% for Cu, 20% for Zn, 75% for Ni, 40% for Mn and 20% for Cr were observed. No changes were noticed in case of Fe. In the samples where heterotrophic bacteria were used decrease: of 80% for Cu, 10% for Fe, 25% for Zn, 75% for Ni, 40% for Mn and 15% for Cr were observed. In material after sequential bioleaching decrease of: 20% for Cu, 25% for Fe, 20% for Ni, 70% for Mn and 60% for Cr were observed. No changes were noticed in case of Zn.

3.2. Optimization of the extraction procedure

It should be emphasized that the results obtained during sequential extraction are strongly influenced by the nature of the sample [14,15], the concentration of reagents [16], the duration of the experiment [17], the solid to liquid ratio, as well as any pretreatment before analysis [8]. Most of the described reagents are related to the fractionation study of elements in soils [8,9] and sediments [12,13]. Only a few publications are connected with the estimation of mobility of elements in solid wastes [14], as in most of them only the water leaching test is described [18,19].

We particularly investigated the mobility changes of Cr, Cu, Fe, Mn, Ni and Zn in some mineral phases. Our choice of applied reagents was based on the literature data and our own experience [7,16–18,20]. Samples before bioleaching processes were used for optimization study (reagents, concentrations, time, temperature). For the estimation of especially mobile fraction called water-soluble fraction, the water extraction test was chosen according to the standard procedures. A 3 h duration was chosen according to the literature [18]. To isolate the carbonate fraction two solutions of

Table 2
Total concentration of elements in samples from Szklary before and after bioleaching processes and in control sample [mg/kg d.m.; $n = 6$].

Element/technique		Before bioleaching	Heterotrophic bioleaching	Autotrophic bioleaching	Control sample to autotrophic bioleaching	Sequential bioleaching	Control sample to sequential bioleaching
Cr	ICP-MS	3645 ± 80	3130 ± 35	2855 ± 25	2537 ± 57	1515 ± 26	2876 ± 25
Ni	ICP-MS	6175 ± 50	1715 ± 9	1667 ± 18	1854 ± 16	5185 ± 45	5432 ± 29
Cu	ICP-MS	163 ± 3	35.3 ± 0.4	21.6 ± 0.2	23.8 ± 0.2	130 ± 3	154 ± 4
Mn	AAS	1390 ± 20	848 ± 30	870 ± 40	980 ± 20	455 ± 20	820 ± 35
Fe	AAS	70000 ± 2000	65000 ± 2000	70000 ± 3000	70000 ± 2000	52000 ± 1000	70000 ± 2000
Zn	AAS	120 ± 8	89 ± 2	93 ± 3	94 ± 2	120 ± 10	120 ± 8

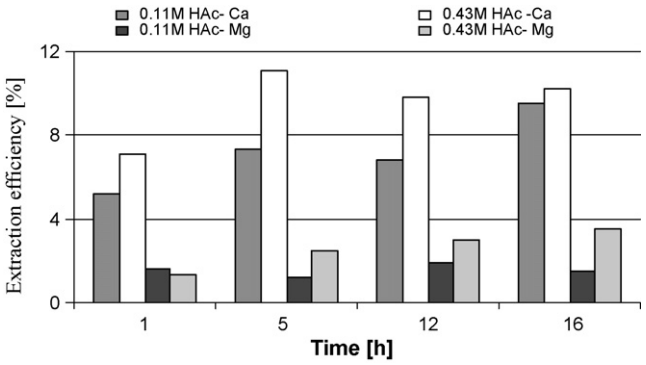

Fig. 1. Dependence of extraction efficiencies for Ca and Mg on HAc concentration and duration of extraction.

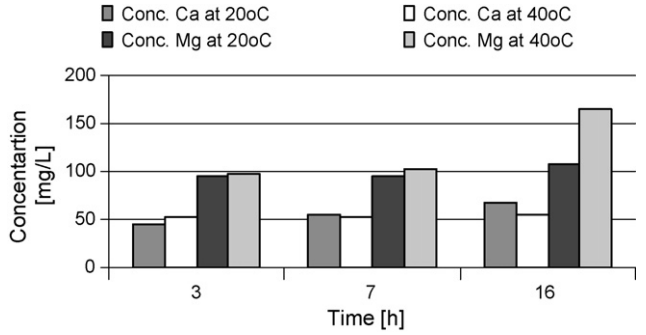

Fig. 2. Dependence of concentration for Ca and Mg on temperature and duration of extraction.

acetic acid were tested: 0.11 mol L^{-1} according to the latest BCR scheme [20] and 0.43 mol L^{-1} according to our experience with waste-sediments rich in carbonate minerals treatment [13]. The commonly used 0.43 mol L^{-1} acetic acid was found to be the most efficient extractant which leached the highest amounts of Ca and Mg after 5–6 h of extraction (Fig. 1). The further increase of the duration time fortunately did not cause the readsorption, therefore the 16 h extraction could be chosen according to BCR procedures, which allows to compare the obtained results with the literature data. The increase of temperature from 20 °C to 40 °C did not affect extraction efficiency for Ca and Mg (Fig. 2).

In our research the reducible fraction was split into 2 fractions: easily (MnOx) and moderately fraction (FeOx). According to the literature the solution of 0.04 mol L^{-1} $NH_2OH{\cdot}HCl$ in 25% HAc was chosen [16,21]. The time study showed that the extraction efficiency of Mn and Fe by 0.04 mol L^{-1} $NH_2OH{\cdot}HCl$ in 25% HAc hardly depended on the extraction time and reached the maximum just after 3–5 h (Fig. 3) (40% for Mn and 8% for Fe). The temperature did not influence the efficiency of the process. Therefore the extraction with $NH_2OH{\cdot}HCl$ at ambient temperature was chosen for further

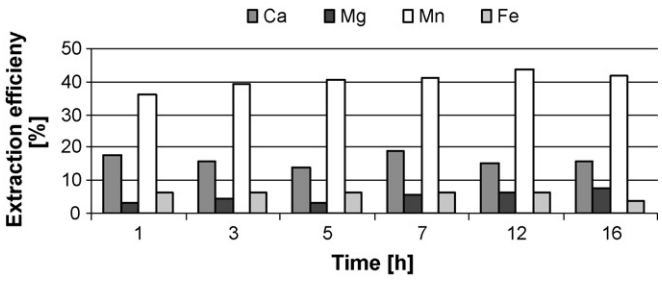

Fig. 3. Dependence of extraction efficiencies for Ca, Mg, Mn and Fe for 0.04 M $NH_2OH{\cdot}HCl$ in 25% HAc as an extractant on duration of extraction.

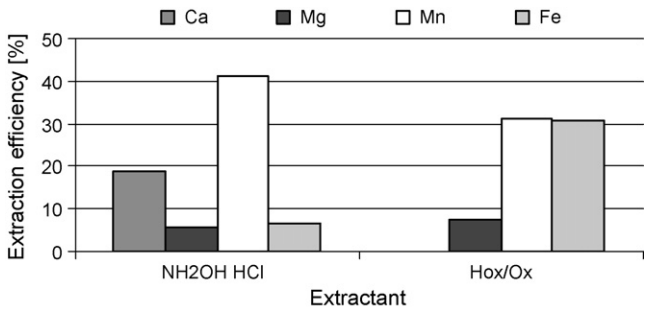

Fig. 4. Comparison of extraction efficiencies for Ca, Mg, Mn and Fe for 0.04 M $NH_2OH{\cdot}HCl$ in 25% HAc and oxalate buffer as an extractant.

studies. The use of 25% HAc as the medium for $NH_2OH{\cdot}HCl$ additionally enhanced the leaching of elements associated with carbonate residue. Hydroxylamine hydrochloride solution in 25% acetic acid leached the highest levels of Mn, comparing to other reagents which were used during the described experiments, and extraction efficiency obtained for Fe was relatively low (Fig. 4). This let to the conclusion that reducible fraction can be split into two separately defined fractions: easily and moderately reducible. So the next planned step of the experiment was to estimate moderately fraction, often called Fe-oxides fraction. The chosen oxalate buffer is a reagent which is able to reduce crystalline form of Fe oxide [8] and basing on the obtained results we could conclude that it enabled the highest extraction efficiency of Fe. Preliminary experiment indicated that ascorbic acid used as an extractant, also very efficient for Fe extraction caused some problems during determination by ICP-MS. The comparison of Mn, Fe, Ca and Mg extraction efficiencies, by different extractants is presented in Fig. 4. It is clearly illustrated that applying the chosen extractants in a sequence allowed to separate both reducible fractions—bound to MnOx and FeOx respectively. It should be noticed that Ca was not extracted while the oxalate buffer was used. It is due to the precipitation of calcium oxalate. To obtain the selective dissolution of FeOx phase the solution of 0.2 mol L^{-1} oxalate buffer according to Kersten and Foerstner [15] and Krasnodebka-Ostręga et al. [16] was chosen. The time study indicated that Mn extractability during 16 h was noticeably higher than during 7 h. In case of Fe a two time increase did not give expected results (Fig. 5). The amounts of Mn and Fe leached by oxalate buffer at 20 °C and 40 °C are comparable. Therefore for leaching of Fe-oxides fraction extraction in room temperature was chosen. To leach organic and sulfide fraction, solution of 30% H_2O_2 acidified to pH 2 with nitric acid, was used as an extractant. This reagent is widely accepted in fractionation studies. The duration of 3 h and the temperature 90 °C was chosen according to latest BCR scheme [20].

To estimate the amount of studied elements bound into residue the total digestion procedure using mixture of $HClO_4$, HNO_3 and HF

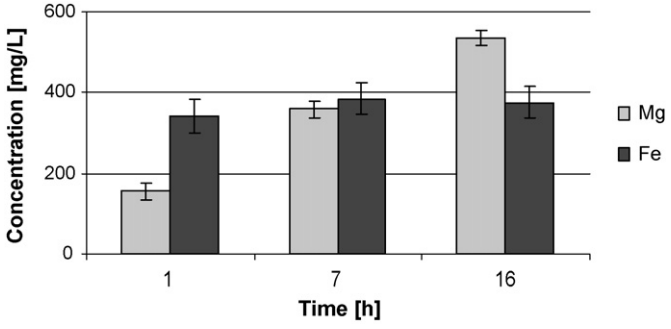

Fig. 5. Dependence of concentration for Mg and Fe on duration of extraction.

Table 3
Optimized scheme for six-step sequential extraction.

Step	Fraction	Reagent	Time	Temperature
1	Water-soluble	H_2O, pH 7	3 h	20 °C
2	Carbonate	0.43 mol/L HAc	16 h	20 °C
3	Mn oxide	0.04 mol/L NH_2·OH HCl in 25% HAc	5 h	20 °C
4	Fe oxide	0.2 mol/L oxalate buffer	7 h	20 °C
5	Organic and sulfide	30% H_2O_2, pH 2/HNO_3	3 h	90 °C
6	Residual	HNO_3, $HClO_4$, HF, concentration—microwave system	1.5 h	200 °C

was performed [7,11,22]. This step was conducted in the microwave digesting system, according to the program in Section 2.

3.3. Fractionation study

The six-step scheme (Table 3) was used to define the distribution of selected elements between phases in waste before and after bioleaching procedures and in control samples. Total concentrations of the investigated elements are presented in Table 2, as it was already mentioned. The sequential extraction procedure was performed three times and the results are always given as the mean with the standard deviation. The studied metals in extracts were determined using ICP-MS and FAAS. The results of the fractionation study for Cr, Cu, Fe, Mn, Ni and Zn are presented in Table 4.

3.3.1. Fractionation of the elements in control sample

pH of the leaching solutions used in the autotrophic bioleaching and in the second step of sequential bioleaching was 2. Sulfuric acid was the main component of these solutions. Therefore it was necessary to check whether the leaching solutions were able to leach the investigated elements by themselves. Basing on the obtained results (Table 2) it could be concluded that the use of leaching solution with and without bacteria (both procedures) caused some decrease of concentrations of studied elements in the residue. The comparison of fractionation in residue after sequential bioleaching and in control samples, presented as an example for Mn (Fig. 6). Concentrations of elements in residue after bioleaching procedure are significantly lower than concentrations in control sample (Table 2). The activity of bacteria in sequential process also caused some changes in the distribution of all studied elements comparing to the control samples. Activity of bacteria in the autotrophic bioleaching is noticeable. Distribution for most of studied elements was similar. It is related to solubility of minerals in leaching medium (H_2SO_4, pH 2).

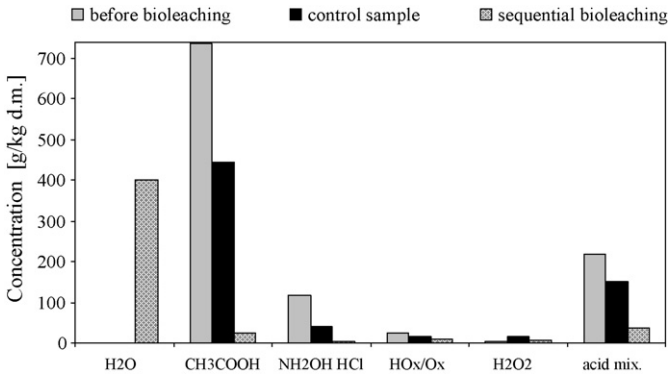

Fig. 6. Fractionation of Mn in sample before bioleaching treatment, after sequential bioleaching and in control sample (after sequential leaching without bacteria).

3.3.2. Fractionation of Cu, Cr, Fe, Mn, Ni and Zn in waste samples

During interpretation of these results it should be taken into consideration that total concentrations of metals in solid samples were lower after bioleaching process comparing to samples before bioleaching treatment. The changes are presented as a relative decrease (%) of concentration of studied elements in solid residue after bio-treatment comparing to sample before the described process.

3.3.2.1. Copper. The mobile fraction of Cu in sample before bioleaching was leached mainly from easy-reducible minerals, more than 35% of total concentration was leached with NH_2OH·HCl solution (Table 4). The oxalate buffer was able to leach only 20% of the total Cu. The steps 1, 2 and 5 were of little significance for the extractability of that metal. In the sample after autotrophic bioleaching decrease of 85% of total Cu concentration was observed (Table 2). The important changes in the distribution of Cu were noted in 3rd and 5th fractions and slightly increase for the moderately reducible fraction (Table 4). The irrelative especially mobile fraction (defined as the sum of 1 + 2) was changed and the carbonate fraction decreased (leaching medium pH 2). After bioleaching process with heterotrophic bacteria the total Cu concentration diminished of 80% (Table 2). The distribution of mobile copper after this process is similar to the distribution in sample after autotrophic process (Table 4). The irrelative especially mobile fraction (1 + 2) was not practically changed. Decrease in especially reducible and some increase in the oxidable fraction were observed. The carbonate fraction was not changed in residue after heterotrophic bioleaching treatment. After sequential bioleaching process decrease of 20% of total Cu concentration was observed. In this sample, important changes in the distribution of Cu were noted in the 1st and the 4th fractions. The irrelative especially mobile fraction (1 + 2) increased about 15 times, but the mobile fraction (1) increased 80 times itself. Carbonate minerals were dissolved under that condition. The sequential bioleaching process mobilized copper; more than 75% of residual copper was leached with water. The wastes after this bioleaching process should not be deposited without previous flushing.

3.3.2.2. Iron. The mobile fraction of Fe in the sample before bioleaching was bound mainly to carbonate minerals, about 40% of total concentration was leached with HAc (Table 4). Approximately 40% of Fe was found in residual fraction and about 13% of this element was leached in the 3rd step of the sequential extraction. The fractions obtained in the 4th and the 5th steps were negligible. It is important to note that although iron is present in leaching medium, the concentration of this element in the solution compared to concentration of extractable Fe content is very low and fractionation studies could be performed. The total Fe concentration in samples after treatment with autotrophic bacteria did not change. Similarly the iron distribution after that bioleaching treatment did not differ from the distribution of this element in sample before the process. On the contrary autotrophic treatment caused noticeable changes especially for HAc leaching (decrease), for leaching with the oxalate buffer (increase) and hydrogen peroxide (increase). The fraction mobilized in steps 1 to 5 accounts for 58% of total Fe concentration. It is important to note that the residue after the autotrophic bioleaching procedure contains iron oxide particles. In the sample after heterotrophic bioleaching decrease of 10% of total Fe concentration was observed. In this sample the leaching of Fe in the step 2 slightly decreased when in steps 4 and 5 slightly increased. The heterotrophic treatment did not change the total concentration of Fe in the residue and also did not influence the iron distribution. After sequential bioleaching process the decrease of 25% of total Fe concentration was observed. Some changes in the distribution of Fe were observed in this sample. The highest decrease

Table 4
Fractionation of investigated elements in samples before bioleaching process, after different bioleaching treatments and in control samples. Concentrations of elements were measured using ICP-MS and FAAS [C ± SD].

Element/sample	Extractant					
Cu [mg/kg]	H_2O	CH_3COOH	$NH_2OH{\cdot}HCl$	HOx/Ox	H_2O_2	HNO_3
Before bioleaching process	<1 ppb	5.4 ± 0.2	53.9 ± 0.4	31.0 ± 1.5	2.0 ± 0.1	60.6 ± 0.5
Autotrophic bioleaching control sample	<1 ppb	1.70 ± 0.05	8.30 ± 0.05	1.8 ± 0.1	15.0 ± 1.6	4.2 ± 0.4
Sequential bioleaching control sample	<1 ppb	5.3 ± 0.3	8.1 ± 0.1	15.3 ± 0.2	15.6 ± 0.1	66 ± 3
Heterotrophic bioleaching	<1 ppb	2.5 ± 0.1	10.7 ± 0.1	4.2 ± 0.1	13.1 ± 0.1	8.7 ± 0.1
Autotrophic bioleaching	<1 ppb	1.1 ± 0.1	7.9 ± 0.1	3.5 ± 0.1	11.3 ± 0.1	4.6 ± 0.1
Sequential bioleaching	83 ± 2	8.5 ± 0.1	7.2 ± 0.1	12.8 ± 0.2	4.7 ± 0.2	1.5 ± 0.1
Fe [g/kg]						
Before bioleaching process	<1 ppb	26.2 ± 1.5	9.1 ± 0.1	3.1 ± 0.2	0.17 ± 0.01	29 ± 0
Autotrophic bioleaching Control sample	<1 ppb	22.0 ± 0.2	5.1 ± 0.5	6.7 ± 0.3	2.4 ± 0.1	33 ± 1
Sequential bioleaching control sample	0.20 ± 0.01	19.3 ± 0.2	5.5 ± 0.1	3.2 ± 0.2	1.5 ± 0.1	40 ± 2
Heterotrophic bioleaching	<1 ppb	21.7 ± 0.2	6.0 ± 0.1	14.4 ± 0.2	0.8 ± 0.1	18.6 ± 0.1
Autotrophic bioleaching	<1 ppb	18.1 ± 0.1	8.1 ± 0.1	14.8 ± 0.1	2.8 ± 0.1	21.3 ± 0.2
Sequential bioleaching	8.3 ± 0.1	3.1 ± 0.1	8.9 ± 0.1	27.7 ± 0.2	1.0 ± 0.1	4.9 ± 0.1
Zn [mg/kg]						
Before bioleaching process	1.4 ± 0.1	42 ± 1	11.2 ± 0.3	6.8 ± 0.1	1.5 ± 0.1	38 ± 1
Autotrophic bioleaching control sample	0.15 ± 0.01	25.8 ± 0.5	5.1 ± 0.1	6.5 ± 0.2	3.3 ± 0.1	45 ± 2
Sequential bioleaching control sample	0.50 ± 0.01	38.2 ± 0.5	9.9 ± 0.2	6.7 ± 0.1	3.2 ± 0.1	57.5
Heterotrophic bioleaching	<1 ppb	32.2 ± 0.1	9.4 ± 0.1	10.9 ± 0.1	7.0 ± 0.1	26.6 ± 0.2
Autotrophic bioleaching	2.2 ± 0.1	26.5 ± 0.2	12.6 ± 0.1	9.6 ± 0.1	9.6 ± 0.1	26.6 ± 0.2
Sequential bioleaching	95 ± 2	8.3 ± 0.1	1.6 ± 0.1	2.1 ± 0.1	2.4 ± 0.1	10.4 ± 0.1
Ni [g/kg]						
Before bioleaching process	<1 ppb	1.4 ± 0.1	0.40 ± 0.01	0.20 ± 0.01	0.80 ± 0.05	3.0 ± 0.1
Autotrophic bioleaching control sample	0.0040 ± 0.0001	0.40 ± 0.01	0.070 ± 0.001	0.090 ± 0.001	0.53 ± 0.01	0.60 ± 0.01
Sequential bioleaching control sample	0.012 ± 0.001	0.85 ± 0.03	0.30 ± 0.01	0.40 ± 0.01	0.81 ± 0.01	3.0 ± 0.1
Heterotrophic bioleaching	0.010 ± 0.001	0.40 ± 0.01	0.070 ± 0.001	0.10 ± ± 0.01	0.50 ± 0.01	0.40 ± 0.01
Autotrophic bioleaching	0.010 ± 0.001	0.40 ± 0.01	0.080 ± 0.001	0.10 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Sequential bioleaching	3.8 ± 0.1	0.30 ± 0.01	0.20 ± 0.01	0.010 ± 0.001	0.030 ± 0.001	0.80 ± 0.01
Mn [mg/kg]						
Before bioleaching process	<1 ppb	738 ± 27	117 ± 15	25 ± 5	3.3 ± 0.1	218 ± 7
Autotrophic bioleaching control sample	<1 ppb	540 ± 18	71 ± 1	27.6 ± 1.2	23.1 ± 0.8	260 ± 1
Sequential bioleaching control sample	<1 ppb	445 ± 15	40 ± 2	15.0 ± 0.5	15.0 ± 0.3	250 ± 6
Heterotrophic bioleaching	2.8 ± 0.1	449 ± 25	61 ± 2	38 ± 2	4.5 ± 0.1	238 ± 15
Autotrophic bioleaching	3.0 ± 0.1	518 ± 26	69 ± 2	30 ± 2	20 ± 1	294 ± 13
Sequential bioleaching	375 ± 13	26 ± 2	3.7 ± 0.1	7.8 ± 0.1	5.4 ± 0.1	38
Cr [g/kg]						
Before bioleaching process	<1 ppb	0.97 ± 0.01	0.45 ± 0.01	0.15 ± 0.01	0.10 ± 0.01	1.5 ± 0.1
Autotrophic bioleaching control sample	<1 ppb	1.04 ± 0.04	0.24 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	1.32 ± 0.01
Sequential bioleaching control sample	<1 ppb	0.86 ± 0.02	0.18 ± 0.01	0.15 ± 0.01	0.05	1.42 ± 0.03
Heterotrophic bioleaching	<1 ppb	0.98 ± 0.05	0.36 ± 0.02	0.17 ± 0.01	0.15 ± 0.01	1.3 ± 0.1
Autotrophic bioleaching	<1 ppb	0.70 ± 0.01	0.29 ± 0.02	0.20 ± 0.01	0.12 ± 0.01	1.5 ± 0.1
Sequential bioleaching	0.35 ± 0.01	0.13 ± 0.01	0.25 ± 0.01	0.15 ± 0.01	0.070 ± 0.001	0.48 ± 0.02

of total concentration of Fe in residual fraction was observed. The bacteria treatment caused mobilization of reducible fractions in residue.

3.3.2.3. Zinc. The mobile fraction of Zn was bound mainly to carbonate minerals, less than 40% of total concentration is leached with HAc (Table 4) solution of $NH_2OH{\cdot}HCl$ was able to leach less than 10% of the total Zn. The fractions obtained in steps 1, 4 and 5 were not important in respect to Zn mobilization. More than 20% of total concentration of Zn was dissolved after acids treatment. The use of autotrophic bacteria in bioleaching process resulted in 20% decrease of total Zn concentration. The carbonate minerals as well as the reducible fractions bound the large mobile fraction of zinc. This process caused reduction of total concentration of Zn but the relative distribution was comparable to that observed in material before the bioleaching process. The use of heterotrophic bacteria led to decrease of 25% of total Zn amount in residue. The distribution of mobile zinc after the bioleaching process was similar to the sample after the autotrophic bioleaching process. The decrease of extractability in steps 2 and 3 was observed. The total Zn concentration did not change after sequential bioleaching process, however 80% of residual concentration of Zn was leached with water. Autotrophic bacteria as well as heterotrophic bacteria practically did not change the distribution of Zn in comparison to sample before bioleaching. After sequential bioleaching process the total Zn concentration did not change, but the activity of bacteria in this process effectively mobilized Zn. The flushing (step 1) of the residue after bioleaching treatment allowed to reduce concentration of Zn more than 80%. Application of sequential bioleaching treatment with addition of flushing step mobilized Zn more effective than the autotrophic or heterotrophic bioleaching process.

3.3.2.4. Nickel. The mobile fraction of Ni in the material before bioleaching treatment was mainly bound to carbonate minerals, about 25% of total concentration was extracted with HAc (Fig. 7). H_2O_2 solution was able to leach less than 13% of the total Ni. The fractions obtained in steps 1, 3 and 4 were negligible with respect to Ni mobilization. More than 50% of total concentration of Ni was dissolved after acids treatment. The use of autotrophic bacteria in bioleaching process resulted in 75% decrease of total Ni concentration in residue (Table 2). Significant changes in distribution of Ni in this sample were noted in 2 and 3 fractions (decrease) (Table 4). The irrelative especially mobile fraction (1 + 2) was not changed. The use of heterotrophic bacteria led to decrease of 75% of total Ni amount in residue. The distribution of mobile nickel after this process was similar to the distribution in sample after autotrophic

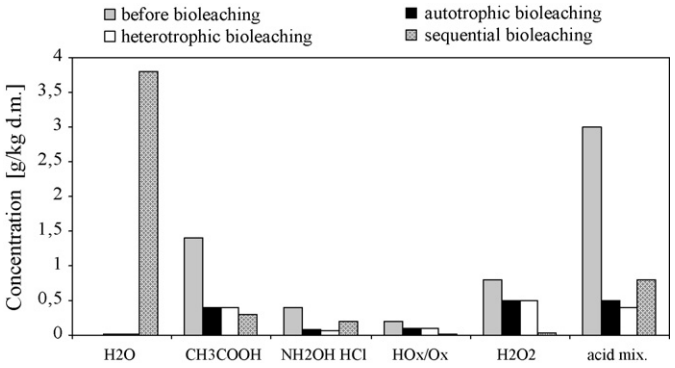

Fig. 7. Fractionation of Ni in sample before bioleaching treatment, in sample after: heterotrophic bioleaching, autotrophic bioleaching and sequential bioleaching.

bioleaching process. After sequential bioleaching treatment the Ni concentration led only to 20% decrease of Ni concentration in residue. However, this bioleaching process effectively mobilized nickel; more than 80% of residual concentration was found in the especially mobile fraction (1 + 2). This fraction had a special importance due to the high mobility of heavy metals from this dump metallurgy waste to the ground water.

3.3.2.5. Manganese. The mobile fraction of Mn in the material before bioleaching treatment was mainly bound to carbonate minerals, more than 50% of the total concentration was leached with HAc (Table 4). The residual fraction accounts for 15% of the total Mn concentration. The Mn-oxide fraction contained less than 10% of the total Mn concentration. The Fe-oxide fraction was also insignificant. The decrease of 40% of total Mn concentration in residue after bioleaching process with the use of autotrophic bacteria was observed (Table 2). Important changes in distribution of Mn in this sample were noted in 2 and 3 fractions (decrease), and in 5 and 6 fractions (increase) (Fig. 6). The irrelative especially mobile fraction (1 + 2) was not changed. After heterotrophic bioleaching process the total Mn concentration diminished of 40%. Manganese in this sample was leached in carbonate and residual fraction under the applied extraction conditions. The decrease of 70% of total Mn concentration in residue after sequential bioleaching process was observed (Table 2). Decreases in both reducible fractions (3 and 4) were noted. After sequential procedure the carbonate minerals contained manganese were totally dissolved (leaching medium pH 2). This process mobilized manganese, about 90% of residual manganese was leached with water, therefore wastes after this process should not be deposited without previous flushing.

3.3.2.6. Chromium. The most mobile fraction of Cr in the material before bioleaching treatment was bound to carbonate minerals, more than 30% of total Cr concentration was leached with HAc (Table 4). The residual fraction accounts for 50% but the Mn-oxide fraction accounts for 20% of total Cr concentration. The use of autotrophic bacteria in bioleaching process resulted in 20% decrease of total Cr concentration (Table 2). Decreases in 2, 3 and 4 steps were observed in that sample. The irrelative especially mobile fraction (1 + 2) decreased. The heterotrophic bioleaching process reduced the chromium concentration in residue of about 15% (Table 2). The most significant change in distribution of Cr after this process was noted in step 3. After sequential bioleaching process the total Cr concentration decreases of 60% in residue. About 25% of residual Cr was leached with water, but irrelative especially mobile fraction (1 + 2) was not changed comparing to sample before the procedure. The decreases of extractability in steps 2, 3 and 6 were observed. Wastes after this bioleaching process should not be deposited without previous flushing. After the second step of sequential bioleaching procedure carbonate minerals were dissolved (leaching medium pH 2).

4. Conclusion

The aim of the experiments was to define the especially mobile fraction (1 + 2) and to specify distribution of the studied elements in material before and after bioleaching processes. The phases were operationally defined under the six-step sequential extraction conditions. According to the obtained distributions for waste sample before the bacterial treatment, it could be concluded that Cu is bound to the reducible phase but other investigated metals are bound to the carbonate phase. Heterotrophic as well as autotrophic bioleaching reduced the total concentration of the investigated metals but the relative distributions slightly changed. The sequential bioleaching causes mobilization of all studied metals.

To reduce potential risk of environmental contamination caused by metallurgy it is necessary to reduce the amount of heavy metals in wastes. The remediation process could be conducted based on bacteria treatments. The bioleaching process could also be applied to recover valuable metals. We cannot choose one bioleaching procedure and one mechanism of recovery of the elements from dump metallurgy wastes. In case of Ni the single autotrophic bioleaching treatment as well as the heterotrophic bioleaching process allowed to recover about 75% of the total concentration. In case of Zn the heterotrophic bioleaching is the most effective process. The autotrophic bioleaching process should be proposed for Cu recovery. The most effective process for recovery of Mn, Fe and Cr is the sequential bioleaching. The sequential treatment mobilized all studied elements, most of the residual content is leached with water. It is necessary to stress for all these bioleaching procedures that the residue after bacteria treatment should not be deposited without previous flushing, because fraction obtained after leaching with H_2O and HAc (especially mobile fraction) is enriched with metals comparing to the material before bioleaching process. After our studies we can conclude that for recovery of metals from the metallurgy wastes, the most effective treatment is the sequential bioleaching process combined with flushing of the bioleaching treatment residue. In most cases the proposed procedure is able to mobilize 80–100% of total concentration of the investigated metals.

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