

**Mining and Metallurgy Institute Bor
West University Timisoara**

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Scientific Study 2
Remediation methods used to clean
waters contaminated with metals

Final Study



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Remediation methods used to clean waters contaminated with metals

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1. INTRODUCTION

Global mining activities (mineral and metal production processes) produce several billion tons of solid inorganic wastes or by-products, including liquid waste. According to the study made for DG Environment, European Commission, more than 4.7 billion tons of mining waste and 1.2 billion tons of tailings waste are stored all over European Union [1]. Open pit or underground mining operations generally have a serious negative impact on the surrounding environment, such as air pollution, the land use and biodiversity, and water availability. The common challenges derive from copper mining activities that inevitably will produce waste solid materials that will be constructed as hills. There are different types of materials in these dumps. Normally, different materials have to be disposed in separate dumps, but, unfortunately this is not the case in Bor dumps. In these dumps, there are waste rocks that contain some metals and tailings that are made up of very fine host rock and minerals that also contain other metals.

These waste materials have common characteristics; they contain a part of the sulfide and an increased part of the oxide mineralization of metals. This mineralization in contact with atmosphere is the main cause of the generation of acid mine water with pH values in the interval from 3 to 4. In the surrounding dumps at Bor area, there are around one billion of tons of waste containing around 1 million tons of copper. Acid mine drainage (AMD) degrades many rivers in Serbian mountains causing the loss of aquatic life, restricts use for recreation, public drinking water and industrial water supplies. It is not only an ecological concern to the states but a population health concern as well [2-4].

During the more than one hundred years of copper ore mining activities in southeast Serbia, the mine drainage water has been released to the downstream without any treatment through tributaries of Danube River and has provided the negative environmental impact on the river water of Danube River. Bor River, which is the most polluted river by mining activities in Europe starts near the Bor mine, collecting AMD, sewage water and metallurgical waste waters. After passing through the Slatina village, Bor River together with Krivelj River forms Bela River flowing into Timok River and further into Danube. During the last decades, flotation tailings were deposited as sediments in Bor River. The collection points for surface and AMD, sediments, flotation tailing deposited in the coastal Bor River area and the agriculture soil (out of the outflow impact samples) [5].

The Robule Lake which is formed by seepage and underground water from waste dump site is one of the characteristic acid mine drainage.



Figure 1. Robule Lake

Waste materials around Robule Lake consist of waste rocks, flotation tailings and low grade ores accumulated from the beginning of the mine operation in Bor area. Water of Robule Lake is characterized by low pH due to the high content of residual sulphuric acid and heavy metals, such as Cu, Cd, Zn, Pb, Ni, Co, Mn, etc [6]. Concentration of iron is more than 500 mg/L, copper concentration is more than 50 mg/L, concentration of manganese is exceptionally high, exceeding 100 mg/L. Multistep neutralization process will be applied with purpose to separate iron from the other valuable metals, especially copper. Environmental friendly and cheap easily available adsorbents will be used for adsorption of metals from raw or neutralized wastewater from Robule Lake.

2. MATERIAL AND METHODS

2.1. MATERIALS

Real AMD sample from Robule Lake (W1) was used for neutralization. Start pH of this water was 2.67. As a neutralizing agent are used $\text{Ca}(\text{OH})_2$, concentration 25 g/L, and CaCO_3 p.a. quality.

As a leaching reagents are used 0.5M-1.5M H_2SO_4 . For solvent extraction following extractants were used: 5 % LIX 984N and 5 % M5910. Re-extraction process was performed with 1.5 M H_2SO_4 . Lime is considered one of the most universal alteration compounds for stabilization of wastes, as it plays a critical role in accessibility of heavy metals, and the relevant environmental risks, as well as enhancing its agricultural benefits.

This procedure has been suggested for the advanced treatment of waste from neutralization process in the related EU working document on sludge application [7]. Lime addition is normally more gainful and easier than alternative options, and low-cost stabilization agent, in this area also very accessible. Plants for waste stabilization may be effortlessly added to the processes that have insufficient capacity to meet regulatory necessities. In this process, adequate lime is added to raw sludge to increase its pH (less than 13). Lime, added in form of $\text{Ca}(\text{OH})_2$, is an inexpensive chemical that is already used in AMD treatment process.

2.2. METHODS

2.2.1 Neutralization

Neutralization of selected AMD –W1 with $\text{Ca}(\text{OH})_2$ and CaCO_3 was done in several stages up to different pH values depending on chemical characterization of neutralized water, and depending on the legislation for surface water. Investigations were carried out with 1000 mL of real AMD samples. Lime milk, $\text{Ca}(\text{OH})_2$ and CaCO_3 was used as a neutralizer to reached pH 3; 3.5 and 4 in a first neutralization step. To AMD sample continually was added a certain amount of neutralization agent in a batch reactor. Stirring was performed with a magnetic stirrer, at a constant speed of 400 rpm, in order to avoid precipitation. After solution reached pH 3; 3.5 or 4, each solution was filtered by vacuum filtration to separate solid from liquid phase. Dewatered sludge was dried to constant mass at 40 °C. Liquid sample was collected and given on chemical analyses according to the chemical characterization of real sample. AMD sample neutralized in the first neutralization step up to pH 3; 3.5 or 4 were used as start sample for second neutralization step to reached pH 7; 7.5 and 8. As in the first neutralization step, liquid and solid phase were separated by vacuum filtration. Liquid phase was used for the chemical analyses and solid phase was dried at 40 °C and measured after reaching the constant mass value.



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2.2.2 Leaching

Acid sludge leaching was carried out with control of temperature and stirring during the experiments. Before and after experiments are analyzed metals concentrations in leaching reagents. For solvent extraction and re-extraction experiments, the glass separation funnels with regulated time and intensity of mixing the phases were used.

At the end of each extraction and re-extraction experiment, the separated aqueous phase was analyzed by the ICP method in order to determine the metal concentration in the aqueous phase.

2.2.3 Testing leachability of waste

The impact of generated waste during neutralization process on the environment was carried out by leaching test according to standard methods EN 12457-2 (Characterization of waste - Leaching - Compliance test for leaching of granular waste materials and sludges - Part 2: One stage batch test at a liquid-solid ratio of 10 l / kg for materials with a high content solids and particle size of less than 4 mm (with decreasing particle size, or without decreasing).

The results of waste testing are compared with limits given in Annex 10 of the Regulation on categories, testing and classification of waste [8].

2.2.4 Testing toxicity of waste

To determine the mobility of compounds, present in waste generated during neutralization process, Toxicity Characteristic Leaching Procedure (TCLP) is carried out by leaching test according to standard method EPA 1311. The results of waste testing is compared with limits given in Annex 10 of the Regulation on categories, testing and classification of waste [8].

Since direct disposal of contaminated waste to landfill is not allowed and it must be qualified the Landfill Disposal Criteria prior to disposal. TCLP is one of approved method used to assess the contamination concentration. If any TCLP test results of the sample exceed the disposal criteria, the contaminated soil must first be pre-treated or stabilized before disposal.

2.2.5 Stabilization of waste

The procedure of sludge stabilization performed aim to decrease mobility of waste component that have leachability properties.

Stabilization of sludge includes the following steps:

- determine the homogeneity of the waste,
- determine the moisture of the waste,
- determination optimal ration lime and waste,
- determination of optimal contact time needed for stabilization process
- confirmation defined parameters of stabilization by minimum five times repeated tests of leachability and toxicity of waste (procedure under 1.1 and 1.2)

3. RESULTS AND DISCUSSION

3.1. NEUTRALIZATION

Real AMD sample from Robule Lake was used for neutralization. Start pH of this water was 2.67. As a neutralizing agent are used $\text{Ca}(\text{OH})_2$, concentration 25 g/L, and CaCO_3 p.a. quality.

Neutralization of selected AMD with $\text{Ca}(\text{OH})_2$ and CaCO_3 was done in several stages up to different pH values depending on chemical characterization of neutralized water, and depending on the legislation for surface water. Lime milk and CaCO_3 was used as a neutralizer to reached pH 3, 3.5 or 4



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in a first neutralization step. To AMD sample continually was added a certain amount of neutralization agent in a batch reactor. Stirring was performed with a magnetic stirrer, at a constant speed of 400 rpm, in order to avoid precipitation. After solution reached pH 3, 3.5 or 4, each solution sample was filtered by vacuum filtration to separate solid from liquid phase. Dewatered sludge was dried to constant mass at 40 °C. Liquid sample was collected and given on chemical analyses according to the chemical characterization of the real sample. AMD samples neutralized in the first neutralization step up to pH 3, 3.5 or 4 were used as start samples for second neutralization step to reached pH 7; 7.5 or 8. As in the first neutralization step, liquid and solid phase were separated by vacuum filtration. Liquid phase was used for the chemical analyses and solid phase was dried at 40 °C and measured after reaching the constant mass value. In Table 1 are given the results of chemical analyses of AMD from the site.

Table 1. Chemical analysis of real AMD samples

Neutralization agents	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L	Cr, mg/L	S, mg/L	Se, mg/L
Ca(OH) ₂	322.6	90.8	34.7	12.8	0.0042	0.413	0.007	0.04	0.003	2376	0.008
CaCO ₃	506.2	86.1	51.0	19.5	<0.020	0.56	<0.020	0.11	0.014	3973	<0.033

3.1.1. Neutralization with Ca(OH)₂

Neutralization process is carried out with 2000 ml of real AMD to reach pH 3 or 4. As a neutralizing agent is used Ca(OH)₂, concentration 25 g/L. After reached the pH 3 or 4, solution is filtered by vacuum filtration with aim to separate solid from liquid phase. Dewatered sludge was dried to constant mass. Results of chemical analyses of solution are shown in the following Table 2.

Table 2. Chemical analysis of solutions after neutralization

Elements	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L	Cr, mg/L	S, mg/L	Se, mg/L
W1 start to pH 3	274.1	62.1	31.6	12.6	0.004	0.4	0.017	0.04	0.004	2245	0.01
W1 start to pH 3.5	12.32	60.9	31.2	12.1	0.001	0.4	0.006	0.04	0.006	2252	0.01
W1 start to pH 4	0.98	62.7	31.4	12.4	0.001	0.4	0.003	0.04	0.005	2022	0.01

From the Table 2, it can be seen that at pH 4 there is a significant drop in the concentration of iron ions while the concentrations of the other metal ions remained unchanged. It can be concluded that the iron ions precipitated in the sludge while the other metal ions remained in solution.

AMD neutralized up to pH 3, 3.5 or pH 4 was used as start samples for neutralization up to 7, 7.5 and 8. As in the first neutralization stage, liquid and solid phase are separated by vacuum filtration. Liquid phase is used for the chemical analyses and solid phase is dried and measured after reaching the constant mass value. Chemical analyses of solution are shown in the following Table 3 and 4.

Table 3. Chemical analysis of solutions after neutralization from pH 3 to pH7, 7.5 or 8

Elements	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L	Cr, mg/L	S, mg/L	Se, mg/L
W1 pH3 to pH7	0.14	38.2	0.06	0.44	0.0004	0.15	0.0007	0.01	0.005	1950	0.003
W1 pH3 to pH7.5	0.03	28.3	0.04	0.13	0.0004	0.08	0.0007	0.006	0.005	1976	0.003
W1 pH3 to pH8	0.09	20	0.03	0.03	0.0004	0.03	0.0007	0.002	0.006	1994	0.003

Table 4. Chemical analysis of solutions after neutralization from pH 3.5 to pH 7, 7.5 or 8

Elements	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L	Cr, mg/L	S, mg/L	Se, mg/L
W1pH3.5 to pH7	0.02	42.9	0.06	1.04	0.0012	0.24	0.0007	0.022	0.006	1919	0.003
W1pH3.5 to pH7.5	0.04	37.2	0.03	0.27	0.0005	0.12	0.0011	0.013	0.005	1849	0.003
W1pH3.5 to pH8	0.02	31.8	0.03	0.07	0.0007	0.07	0.0008	0.0072	0.006	1932	0.003

Table 5. Chemical analysis of solutions after neutralization from pH 4 to pH 7, 7.5 or 8

Elements	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L	Cr, mg/L	S, mg/L	Se, mg/L
W1pH4 to pH7	0.01	42.2	0.04	0.65	0.0009	0.21	0.001	0.02	0.0057	1962	0.004
W1pH4 to pH7.5	0.01	39.8	0.05	0.22	0.0008	0.14	0.0002	0.015	0.0057	1949	0.003
W1 pH4 to pH8	0.01	36.8	0.03	0.11	0.0008	0.1	0.0004	0.009	0.0056	1936	0.004

From the Tables 3, 4 and 5, it can be seen that all the metals ions from the solution precipitated into a sludge. Only the concentration of manganese ions remained unchanged due to the fact that manganese precipitates at higher pH values, which is around 10.

Table 6 shows the lime consumption during neutralization as well as the mass of the obtained sludge.

Table 6. Consumption of Ca(OH)₂ and sludge mass

Sample	Consumption of 25 g/L Ca(OH) ₂ , ml/L	Sludge mass, g
start to pH 3	3.5	0.7
from pH 3 to pH 7	65.4	2.9
from pH 3 to pH 7.5	67.2	3.3
from pH 3 to pH 8	70	3.7
start to pH 3.5	20.75	3.13
from pH 3.5 to pH 7	36	2.13
from pH 3.5 to pH 7.5	40	2.73

from pH 3.5 to pH 8	36	2.13
start to pH 4	25	3.93
from pH 4 to pH 7	31.8	1.1
from pH 4 to pH 7.5	33.8	1.3
from pH 4 to pH 8	40.3	2.1

3.1.2. Neutralization with CaCO₃

Neutralization process is carried out with 2000 ml of real AMD to reach pH 3 or pH 4. CaCO₃ is used as a neutralizing agent. After reached the pH 3 or 4, solution is filtered by vacuum filtration with aim to separate solid from liquid phase. Dewatered sludge was dried to constant mass. Chemical analyses of solution are shown in the following Table 7.

Table 7. Chemical analysis of solutions after neutralization to pH 3 or 4

Elements	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L	Cr, mg/L	S, mg/L	Se, mg/L
W1 start to pH 3	39.9	79.4	47.3	18	<0.020	0.53	<0.020	0.06	<0.005	3384	<0.033
W1 start to pH 4	196	85.9	47.3	18.6	<0.020	0.58	<0.020	0.08	<0.005	3163	<0.033

AMD neutralized to pH 3 or 4 was used as start samples for neutralization to pH 7, 7.5 or 8. As in the first neutralization stage, liquid and solid phase are separated by vacuum filtration. Liquid phase is used for the chemical analyses and solid phase is dried and measured after reaching the constant mass value. Chemical analyses of solution are shown in the following Table 8 and 9.

Table 8. Chemical analysis of solutions after neutralization from pH 3 to pH 7, 7.5 or 8

Elements	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L	Cr, mg/L	S, mg/L	Se, mg/L
W1 pH3 to pH7	<0.007	46.1	0.11	1.8	<0.02	0.24	<0.02	0.014	<0.005	1772	<0.033
W1 pH3 to pH7.5	<0.007	11.6	0.04	0.18	<0.02	0.16	<0.02	0.007	<0.005	2622	<0.033
W1 pH3 to pH8	<0.007	0.23	0.03	0.03	<0.02	<0.007	<0.02	0.012	<0.005	2240	<0.033

Table 9. Chemical analysis of solutions after neutralization from pH 4 to pH 7, 7.5 or 8

Elements	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L	Cr, mg/L	S, mg/L	Se, mg/L
W1pH4 to pH7	<0.007	32.1	0.05	1.1	<0.020	0.37	<0.020	<0.008	<0.005	1962	<0.03
W1pH4 to pH7.5	<0.007	8.8	0.03	0.2	<0.020	0.16	<0.020	<0.008	<0.005	1949	<0.03
W1 pH4 to pH8	<0.007	8.4	0.03	0.21	<0.020	0.1	<0.020	<0.008	<0.005	1936	<0.03

From the Tables 8 and 9, it can be seen that all the metals ions from the solution precipitated into a sludge. Manganese concentration also decreased, which was not the case when $\text{Ca}(\text{OH})_2$ is used as a neutralizing agent. The assumption is that a large amount of CaCO_3 consumption led to decreased manganese.

Table 10 shows the lime consumption during the neutralization process as well as the mass of the obtained sludge.

Table 10. Consumption of CaCO_3 and sludge mass

Sample	Consumption of CaCO_3 , g	Sludge mass, g
start to pH 3	2	2.15
from pH 3 to pH 7	125	128.9
from pH 3 to pH 7.5	200	202.3
from pH 3 to pH 8	275	279.6
start to pH 4	4	4.15
from pH 4 to pH 7	101	103.7
from pH 4 to pH 7.5	175	175.3
from pH 4 to pH 8	250	254.6

From the Tables 5 and 9, it can be seen that the consumption of calcium carbonate is much higher than the consumption of calcium hydroxide. As chemical analyzes have shown almost the same results of changes in metal ions concentration, it can be concluded that it is better to use calcium hydroxide to neutralize acidic mine water.

3.1.3. Two-Step Neutralization Treatment on Semi Industrial Plant – First selected water

Based on results of tests carried out in the laboratory condition conclusion was reached that the pH control process is effective for separate metal removal. All data obtained during the investigation of artificial and real wastewater from defined location in the area of copper mining activities in the southeast of Serbia were used for the design of a new semi industrial plant for continuous neutralization process. The new semi industrial plant was designed and manufactured in Japan, by Mitsui Mineral Development Engineering Co., during the activities of SATREPS project: “Research on the integration system of spatial environment analyses and advanced metal recovery to ensure sustainable resource development” from 2015 to 2019, which has been conducted by the Akita University, Japan Space System, Mitsui Mineral Development Engineering Co., Ltd., and the Mining and Metallurgy Institute Bor. The new plant was installed in the Mining and Metallurgy Institute Bor. Designed process capacity of the equipment is 2–7 L min⁻¹ and it was capable to use in the field continuous operation.

General description of semi industrial plant for two-step neutralization treatment

Specification of semi industrial plant (Figure 2.), which is consists of five Units, Compressor and Control Panel and that continuously perform pH control and flocculation-precipitation of the AMD in two steps is given in Table 11.

Table 11. General description of semi industrial plant for two-step neutralization treatment

Items	Description
Unit A: Wastewater supply	Receiving pump, flow rate: 20 L min ⁻¹ , used for transport the feed wastewater in the wastewater tank. Wastewater tank, for storage of feed wastewater. Wastewater pump, flow rate: 2 - 7 L min ⁻¹ , used for feed wastewater transport in unit B.
Unit B: pH control and flocculation-precipitation, configured from: Unit B-1, Unit B-2 and Unit B-3	Unit B-1: Thickener A, volume 410 L, used for solid-liquid separation of the feed water that has been done primary pH control (according to the first set pH value) and flocculation. Unit B-2: pH Control tank A/B (50 L each tank), Flocculation tank A/B, (50 L each tank). This unit is used for primary pH control (according to the first set pH value), secondary pH control (according to the second set pH value) and flocculation of the feed waste water. pH control agitator A/B is used for solution mixing in pH Control tank A/B. Flocculation agitator A/B is used for solution mixing in Flocculation tanks. Unit B-3: Thickener B, volume 410 L, used for solid-liquid separation of the feed water that has been done secondary pH control (according to the second set pH value) and flocculation.
Unit E: Filtration	Thickener A and Thickener B of Unit B should be visually checked regularly for slurry buildup, and as it has grown to a substantial level, manually operated valves under the respective thickeners should be opened for discharge of the slurry by slurry pump, flow rate 20 L min ⁻¹ , to Filter press, capacity 3 L. The filtrate is to be either discharged by a portable filtrate pump or returned to wastewater tank depending on the level of its water quality. Filter press will be of a fully automatic type with a pneumatic press function built in.
Unit C: Slaked lime feed	Slaked lime and water are to be charged into Lime slurry tank, volume 250 L, in advance for controlling its concentration to 2.5 mass %. The conditioned lime sludge will be sent automatically, by lime sludge pump A/B, (0.5 L min ⁻¹) to pH Control tank A/B according to their respective pH values.
Unit D: Flocculant feed	Polymer flocculant and water are charged into Flocculant tank, (volume 100 L), in advance for controlling its concentration up to 0.5 g L ⁻¹ . The conditioned flocculant will be sent to Flocculation tank A/B at a constant feed rate irrespectively of the feed water flow rate by flocculant pump A/B, flow rate 0.05 L min ⁻¹ .
Compressor	Compressor (370 L min ⁻¹) will be used in order to supply the compressed air to filter press and solenoid valves.
Control panel	Control panel will be used for control, operation and power supply of equipment

Storage tank for wastewater (volume 1000 L) is used for storage the sampled AMD from proposed location. Ambient conditions: 5-40 °C; Power Supply: 220 VAC x 3-phase x 50Hz, 13 kVA. Equipment is made from acid resistance material due to the fact that acidity of AMD is mostly between 2.5 and 4, that is some as for pH of AMD from Robule Lake.



Figure 2. Semi industrial plant for two-step neutralization treatment

Technological procedure for neutralization treatment on semi industrial plant

Real AMD sample (about 2000 L) from area of copper mining activities in southeast Serbia, was used for test operation. AMD was feed to the equipment and pH was set: for a first step on pH 4 and for a second step on pH 8. The wastewater from storage tank for wastewater was transported by receiving pump with constant flow rate of 20 L min^{-1} to Wastewater tank. Wastewater tank was with level control that switches off/on the receiving pump. Wastewater pump with constant flow rate of 5 L min^{-1} was used for wastewater transport to pH Control tank A. In the same time, to pH Control tank A was continually added 2.5 mass % lime milk from Lime slurry tank by lime sludge pump. In pH Control tank A is installed pH meter aim to control first set pH value. Due to the fact that primary pH value was set on value of 4, according to the pH value during the test, dosing of lime milk was corrected. Suspension from pH Control tank A overflowed in Flocculation tank A. Flocculant (0.05 mass %) was transported by flocculant pump A with constant flow rate of 0.025 L min^{-1} to Flocculation tank A. Sludge was precipitated in the bottom of Thickener A incrementally during the test. Neutralized AMD that overflowed from Thickener A was taken for chemical analysis and by gravity flow to pH Control tank B for secondary pH control (according to the second set pH value of 8).

Flocculation was followed in Flocculation tank B thereafter, and then solid-liquid separation was occurrence in Thickener B. Neutralized AMD that overflowed from Thickener B was also taken for the chemical analysis and discharged continuously by gravity as treated water. Thickener A and Thickener B were discharged one by one aim to filtrate the sludge pulp on a filter press. The filtrate was discharged by a portable filtrate pump as treated water. Dewatered sludge was measured and dried at $40 \text{ }^{\circ}\text{C}$ to constant mass and taken for chemical analysis.

During the two-step neutralization treatment, the following parameters were measured and controlled: pH value in the Tanks for pH control, AMD flow rate, lime milk, and flocculant flow rates. Process duration was 6 h.

Water samples for chemical analysis were taken from the Thickener A on each hour during the test. Sampling from the Thickener B started 3 h after starting the outflow of AMD neutralized in the second step. The results for chemical composition of the neutralized AMD during the two-step neutralization treatment are presented in Table 12.

Table 12. Results of neutralization experiment

Elements	pH	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L
W1 start	2.9	537.6	100.8	50.9	21.8	0.016	1.005	0.004	0.06
W1 pH 4	4.0	0.97	96.2	47.8	16.5	0.007	1.034	0.004	0.06
W1 pH 8	8.0	0.007	46.1	0.008	0.024	0.002	0.005	0	0
Reduction rate at pH4 (%)		99.82	4.62	6.03	24.41	58.09	0	0	0
Reduction rate at pH8 (%)		0.18	49.68	93.96	75.48	31.19	100	100	100

The experiment results suggest that the two-step pH control neutralization and precipitation method is effective and practical to recover iron and copper separately in the sludge generated along the process and is reliable in processing the water quality to the required levels.

3.1.4. Two-Step Neutralization Treatment on Semi Industrial Plant – Second selected water

Because inability of supplying a larger amount of water from another location, it was decided that we now work with the same water only from the dry period. Due to the increased manganese content in the water and after the second degree of neutralization, the adsorption process was brought manganese to acceptable values so that the purified water could be discharged into the recipient. Experimental procedure was carried out with real sample of AMD from location Robule Lake, generated in area of copper mining activities in southeast Serbia.

Results for major elements (Table 13) revealed that this water had a typically characteristics of AMD and this location was chosen for planned investigations.

Table 13. Chemical characterization of Robule Lake

Elements	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L
W1 start	323.5	68.3	33.4	13.6	0.007	0.47	0.002	0.05

Real AMD sample (about 2000 L) from area of copper mining activities in southeast Serbia, was used for test operation. AMD was feed to the equipment and pH was set: for a first step on pH 4 and for a second step on pH 8. The wastewater from storage tank for wastewater was transported by receiving pump with constant flow rate of 20 L min⁻¹ to Wastewater tank. Wastewater tank was with level control that switches off/on the receiving pump. Wastewater pump with constant flow rate of 5 L min⁻¹ was used for wastewater transport to pH Control tank A. In the same time, to pH Control tank A was continually added 2.5 mass % lime milk from Lime slurry tank by lime sludge pump. In pH

Control tank A is installed pH meter aim to control first set pH value. Due to the fact that primary pH value was set on value of 4, according to the pH value during the test, dosing of lime milk was corrected. Suspension from pH Control tank A overflowed in Flocculation tank A. Flocculant (0.05 mass %) was transported by flocculant pump A with constant flow rate of 0.025 L min^{-1} to Flocculation tank A. Sludge was precipitated in the bottom of Thickener A incrementally during the test. Neutralized AMD that overflowed from Thickener A was taken for chemical analysis and by gravity flow to pH Control tank B for secondary pH control (according to the second set pH value of 8).

Adsorption method

Adsorption removal tests for Mn recovery using chitosan as an adsorbent were mainly performed at room temperature. Basic tests were firstly carried out to confirm Mn adsorption capacity. For that, 0.5 mM of Mn solutions with various pH were prepared from manganese sulfate pentahydrate ($\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$), which was purchased from Wako Pure Chemical Industries, Ltd., Japan. The solution pH was adjusted using NaOH aqueous solution. Desired amount of chitosan (0.5 -5 g) was introduced into 50 mL of Mn solution. The Mn solutions including chitosan were stirred with a magnet bar for 24 h. After adsorption test, the solution samples were filtrated through PTFE membrane filter with a $0.45 \mu\text{m}$ mesh for a quantitative analysis.

As for application of adsorption method to AMD treatment, the real wastewater treated by neutralization was used. To the real AMD water derived from Robule Lake, the two-step neutralization including treatment at pH 4 and 7 was applied. The treated AMD water had the value of pH 8 and included 34 mg L^{-1} of Mn. Chitosan (5 g) was introduced into 50 mL of the treated AMD water. It was stirred with a magnet bar for 24 h. After adsorption removal test, the treated AMD water was filtrated through PTFE membrane filter with a $0.45 \mu\text{m}$ mesh for a quantitative analysis.

Table 14. Results of neutralization experiment

Elements	pH	Fe, mg/L	Mn, mg/L	Cu, mg/L	Zn, mg/L	As, mg/L	Ni, mg/L	Pb, mg/L	Cd, mg/L
W1 start	3.0	323.5	68.3	33.4	13.6	0.007	0.47	0.002	0.05
W1 pH 4	4.0	0.83	65.7	31.2	9.7	0.002	0.4	0.002	0.05
W1 pH 8	8.0	0.006	34.1	0.006	0.018	0.001	0	0	0
Reduction rate at pH4 (%)		99.74	3.8	6.58	28.68	71.43	0	0	0
Reduction rate at pH8 (%)		99.99	53	99.98	99.86	85.71	100	100	100

The above-mentioned results of the two-step neutralization treatment revealed that most of Fe (ferric iron) and Cu were separated and removed as precipitates from the real AMD water derived from Robule Lake at pH 4 and 8, respectively. But, approx. 30 mg L^{-1} of Mn till remained in the treated AMD water. Generally, Mn removed from the water can be induced by neutralization treatment at pH 9 or above, which is often more than the pH value for the effluent standard. In this

case, it is necessary for discharge to river or surrounding areas to lower pH to below the effluent standard. Thus, adsorption method using chitosan, a kind of polysaccharide, was investigated as a new approach for Mn removal.

To confirm Mn removal capacity of chitosan, adsorption removal tests were firstly carried out using simulated Mn solutions (0.5 mM). Mn removal from the aqueous solution at higher pH (more than 6.5) was identified, while Mn removal capacity by chitosan was not confirmed below pH 5. Figure 3, shows change in Mn removal rate when various input amounts of chitosan (0.5, 1, 2, 4 g) were added to 50 mL of the Mn solutions with pH 6.7 and 7. In case of 0.5 g of chitosan input, approx. 30 mass % of Mn removal rate was indicated. One gram of chitosan input increased removal rate to 50 mass %. Finally, Mn removal rate reached 90 mass % when 4 g of chitosan was applied to the adsorbent for 0.5 mM of Mn solution.

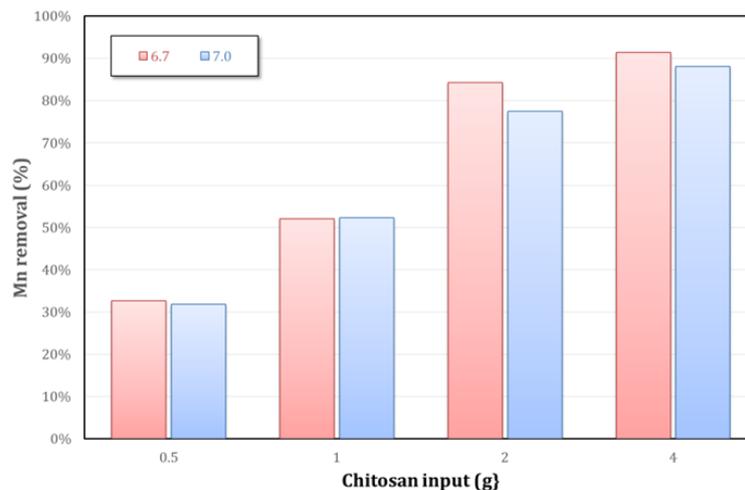


Figure 3. Effect of input amounts of chitosan on Mn removal from the simulated solutions at pH 6.7 and 7

Figure 4, shows change in Mn concentration and the amount of Mn removal as a function of input amount of chitosan. Due to adsorption removal using chitosan, the Mn concentration in solutions reduced from 25 mg L^{-1} (0.5 mM) to approx. 3 mg L^{-1} with an increase in chitosan input. This corresponded to about 1.5 mg of Mn removal from the solution (Figure 4). Chitosan input increased the solution pH to around 8. Chitosan, a cationic polymer obtained from chitin, has a lot of amino groups ($-\text{NH}_2$). Increase in the solution pH may be caused by consumption of H^+ due to transferring $-\text{NH}_2$ to $-\text{NH}_3^+$. Consequently, these results revealed that chitosan had good performance for Mn removal from the simulated solution. And then, application of chitosan to Mn removal from the real AMD water was attempted.

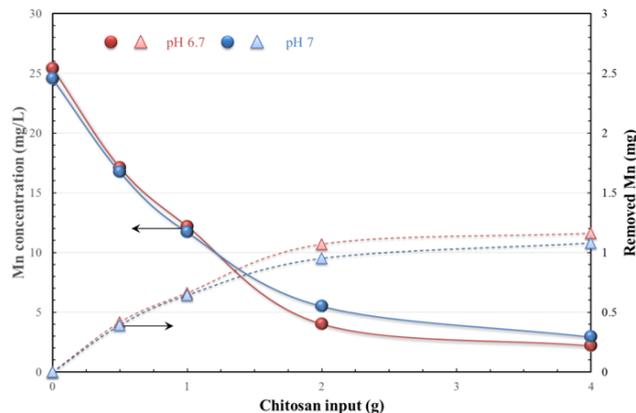


Figure 4. Change in Mn concentration and the amount of Mn removal at pH 6.7 and 7 as a function of input amount of chitosan

In this experiment, the real AMD water sample, which was derived from Robule Lake, was used. From this AMD sample, almost of Fe and Cu were already removed due to the two-step neutralization treatment at pH 4 and 8, respectively. The treated AMD sample had the pH value of 8, and finally contained about 53 mg L^{-1} of Mn. And then, Mn removal test with 5 g of chitosan as an adsorbent was conducted. Figure 5, shows change in Mn concentration before and after adsorption removal test using 5 g of chitosan. Results of Mn concentration in the simulated solutions are also presented in Figure 5. Due to adsorption into chitosan, Mn concentration in the simulated solutions reduced to around 2 mg L^{-1} . This result was equivalent to approx. 90 mass % of Mn removal. Then, chitosan was incubated in the real treated AMD water for 24 h. As a result, Mn concentration decreased to about 10 mg L^{-1} , higher than that in the simulated solutions (Figure 5.). From these results, it was indicated that the combination of neutralization and adsorption methods had a potential for effective treatment of AMD.

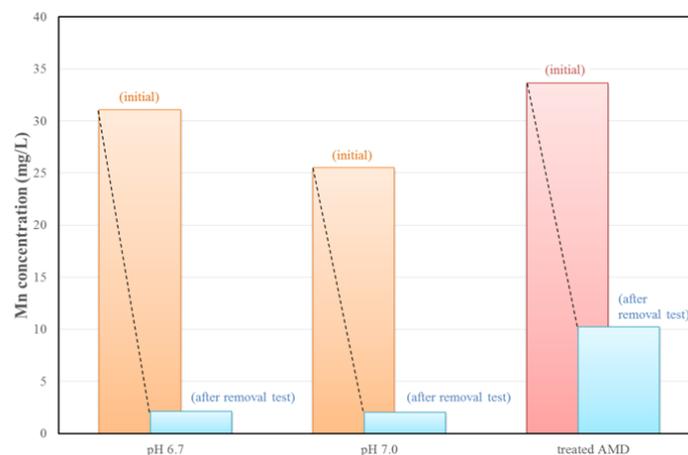


Figure 5. Change in Mn concentration before and after adsorption removal test using 5 g of chitosan

3.2. COPPER AND ZINC RECOVERY FROM SLUDGE OBTAINED AFTER AMD WATER NEUTRALIZATION

Chemical characterization of treated neutralization sludge is presented in Table 15.

Table 15. Chemical characterization of sludge obtained after neutralization with $\text{Ca}(\text{OH})_2$ at pH8

Element	Unit	Content	Element	Unit	Content
Al	%	7.58	Cr	ppm	4.60
Mg	%	3.19	Co	ppm	435.90
Mn	%	2.11	Ni	ppm	202.60
Fe	%	0.96	As	ppm	9.80
S	%	12.98	Cd	ppm	18.80
Zn	%	0.73	Cs	ppm	0.16
Cu	%	1.70	Pb	ppm	<10
Sr	%	0.0075	Mo	ppm	<10
Ca	%	14.88	-	-	-

In this study, copper and zinc recovery process from neutralization sludge included following technological phases:

- leaching of neutralization sludge;
- solvent extraction of copper;
- obtaining of copper sulphate;
- electrowinning of copper;
- obtaining of zinc carbonate and
- obtaining of zinc oxide.

Schematic representation of technological phases used for treatment of neutralization sludge is presented in Figure 6.

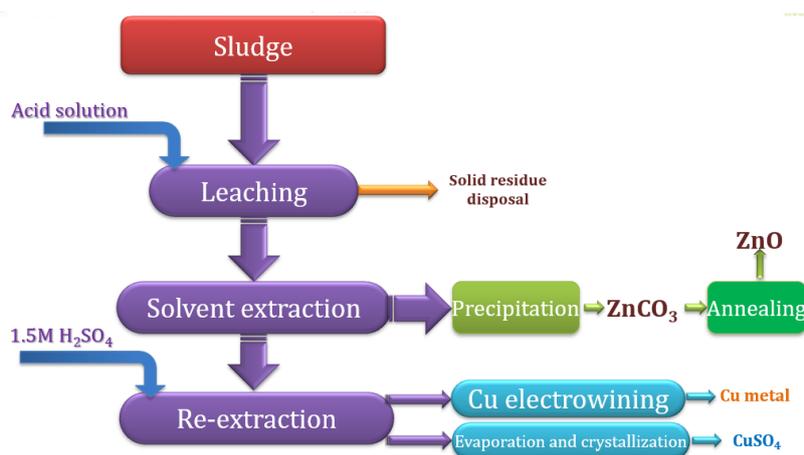


Figure 6. Schematic representation of the neutralization sludge treatment process

3.2.1. Leaching of neutralization sludge

For experimental testing of the leaching process, a magnetic stirrer with automatic regulation of temperature and speed was used, as well as a pH meter device.

Four samples of neutralization sludge in the amount of 20 g were used in an experimental study of the influence of the concentration of leaching agent (H_2SO_4 solution) on the leaching degree of copper and iron. The tests were performed at atmospheric pressure, under the following conditions:

- leaching agent: 0.5 M, 1.0 M, 1.2 M and 1.5 M H_2SO_4
- phase ratio: S:L=1:5
- mixing speed: 300 rpm
- leaching time: 60 min
- temperature: room ($\sim 25^\circ C$)

After the leaching process was completed, the samples were filtered, pregnant leaching solutions (PLS) were given for chemical analysis to determine the concentration of copper and iron, and the solid residues were dried in dryer. The obtained pregnant leaching solutions are shown in Figure 7.

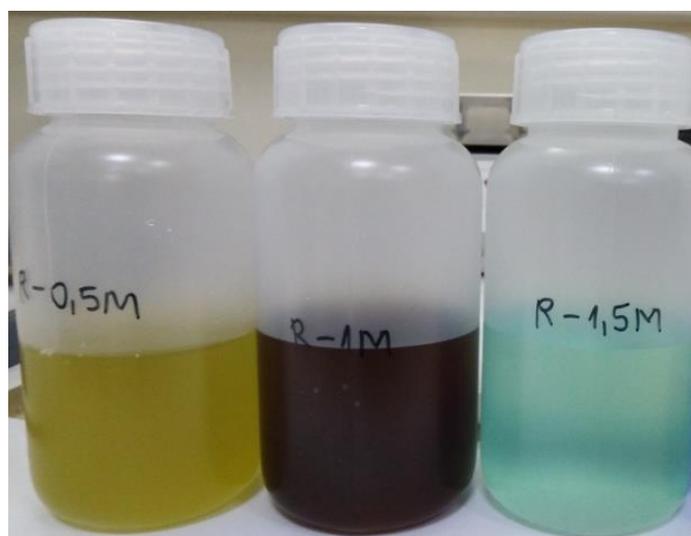


Figure 7. Pregnant leaching solutions obtained by leaching with 0,5 M, 1,0 M i 1,5 M H_2SO_4

Depending on the concentration of the leaching agent, the leaching degrees of copper and iron are as presented in Table 16:

Table 16. Leaching degrees of copper and iron depending on the concentration of leaching agent

Concentration of the leaching agent (H_2SO_4 solution)	Leaching degree (%)	
	Cu	Fe
0.5 M	58.74	0.69
1.0 M	87.35	10.39
1.2 M	99.30	84.79
1.5 M	97.17	100.00

The obtained results show that the highest leaching degree of copper was achieved by leaching with 1.2 M H₂SO₄ solution. Chemical analysis of this solution is presented in Table 17.

Table 17. Chemical analysis of pregnant leaching solution (PLS)

Element	Unit	Content
Ni	mg/L	22.7
Mo	mg/L	1.2
Mn	mg/L	1798.2
Fe	mg/L	1549.7
As	mg/L	<0.020
Zn	mg/L	1317.1
Cu	mg/L	3104.4
Se	mg/L	<0.033
Pb	mg/L	<0.020
Cr	mg/L	0.16

PLS was used to investigate the copper solvent extraction process.

Chemical characterization of solid residue obtained after leaching process with 1.2 M H₂SO₄ solution is presented in Table 18.

Table 18. Chemical analysis of solid residue formed after leaching with 1.2 M H₂SO₄ solution

Content (%)										
Fe	Mn	Cu	Mo	Zn	As	Ni	Pb	Cd	Cr	Se
0.37	2.74	0.026	<10	0.018	<10	33	16	<1	16	<40

Leachability test and the Toxicity Characteristic Leaching Procedure (TCLP test) of the solid residue obtained after leaching process were performed and presented in Part 3.3. of this Study.

3.2.2. Solvent extraction of copper

The pregnant leaching solution obtained after the neutralization sludge leaching process with 1.2 M H₂SO₄ solution, was treated in a solvent extraction process (SX process) in order to selectively remove Cu from the solution. The pH of the solution was adjusted from 0.94. to 1.49 using 10% Ca(OH)₂ solution, before starting the process.

For experimental solvent extraction and re-extraction tests, glass separating funnels and a shaker device with the possibility of regulating the process duration and phase mixing speed, shown in Figure 8, were used.

The influence of extractant type for the two-stage solvent extraction process was investigated. As solvent was used kerosene. For investigations of SX process were used 4 following extractants: LIX984N, LIX622N, M5910 and M5640 under conditions presented in Table 19. Two-stage re-extraction process was performed with 1.5 M H₂SO₄. The concentrations of metals in the aqueous phase after the extraction and re-extraction process are presented in Table 20.



Figure 8. Shaker device

Table 19. Conditions of process solvent extraction and re-extraction

No	Extractant	Stages EX+REX	pH	Contact time (min)	Mixing rate (rpm)	Phase ratio organic:aqua			
						EX I	EX II	REXI	REXII
1	5% LIX984N	2+2	1.5	15	300	1:1	1:1	5:1	5:1
2	5% LIX622N	2+2	1.5	15	300	1:1	1:1	5:1	5:1
3	5% M5910	2+2	1.5	15	300	1:1	1:1	5:1	5:1
4	5% M5640	2+2	1.5	15	300	1:1	1:1	5:1	5:1

Table 20. Metals concentrations in the aqueous phase after the extraction and re-extraction process

No	Extractant	Concentrations of elements (mg/L)								
				Cu	Fe	Mn	Zn	Ni	Cd	Cr
1	LIX984N	EX	I	1080.6	1564.1	1876.2	1329.3	22.2	2.1	0.43
			II	8077.9	52.1	57.1	48.7	1.4	0.077	<0.005
		REX	I	1626.9	2.4	2.0	4.0	0.083	<0.008	<0.005
			II	53.7	1548.57	1802.29	1298.9	22.4	2.1	0.41
		EX	I	2755.3	121.5	120.5	75.6	2.3	0.18	<0.005
			II	809.1	5.8	4.9	4.6	0.12	<0.008	<0.005
2	LIX622N	EX	I	968.7	1352.0	1698.3	1059.8	19.3	2.2	0.45
			II	6898.9	87.7	93.7	581	2.4	0.2	<0.005
		REX	I	1762.2	3.6	3.4	2.4	0.12	0.1	<0.005
			II	25.9	1464.4	1700.4	1072.1	18.5	2.1	0.44
		EX	I	2882.9	84.5	82.1	46.5	1.8	0.11	<0.005
			II	1061.1	7.5	6.2	4.3	0.12	0.013	<0.005
3	M5910	EX	I	922.1	1495.6	1824.4	1290.0	22.7	2.2	0.45
			II	8021.4	40.9	46.0	31.2	1.0	0.087	<0.005
		REX	I	1596.4	1.6	1.1	0.71	0.061	<0.008	<0.005
			II	66.7	1484.0	1781.6	1260.1	22.6	2.1	0.4
		EX	I	1624.2	71.7	79.5	54.3	1.7	0.14	<0.005
			II	718.9	6.4	5.7	4.2	0.039	<0.008	<0.005

4	M5640	EX	I	829.9	1351.6	1683.9	1095.2	19.8	2.0	0.42
			II	7887.1	44.3	51.2	34.0	1.3	0.1	<0.005
		REX	I	1817.8	2.2	1.6	1.0	0.077	<0.008	<0.005
			II	41.0	1253.3	1491.6	932.7	21.6	2.3	0.42
		EX	I	2697.0	84.0	87.8	56.5	2.1	0.18	<0.005
			II	949.3	4.0	3.8	2.8	0.1	<0.008	<0.005

In Table 21 are presented extraction and re-extraction degrees of Cu, Fe and Zn depending on the type of extractant.

Table 21. Extraction and re-extraction degrees of Cu, Fe and Zn

Extractant	Extraction degree I+II			Re-extraction degree I+II		
	Cu	Fe	Zn	Cu	Fe	Zn
LIX984N	98.55	14.38	15.69	73.31	13.98	10.99
LIX622N	99.30	19.03	30.41	68.65	10.62	1.44
M5910	98.17	17.95	18.21	65.87	7.43	6.44
M5640	98.89	69.29	40.00	73.01	5.19	1.22

Best selectivity for copper in relation to iron and zinc were achieved using LIX984N as extractant. In aim to investigate impact of concentration of extractant, experiments with 10% LIX984N were done. The solvent extraction process was carried out in two stages under the following conditions:

1st stage:

- Aqueous phase: (PLS) pH (1.5)
- Organic phase: 5% i 10% LIX984N
- Solvent: kerosene
- Contact time: 15 min
- Aq/org ratio: 1:1
- Mixing speed: 300 rpm

2nd stage:

- Aqueous phase: aqueous phase from the first extraction stage
- Organic phase: 5% i 10% LIX984N
- Solvent: kerosene
- Contact time: 15 min
- Aq/org ratio: 1:1
- Mixing speed: 300 rpm

Phase separation after a two-stage SX process using 5% LIX984N and 10% LIX984N is shown in Figure 9.

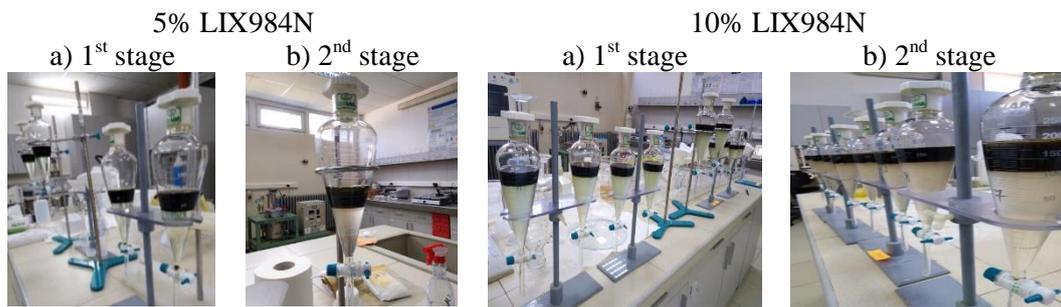


Figure 9. Two-stage solvent extraction process: a) 1st stage; b) 2nd stage

For the two-step re-extraction process, a 1.5 M sulfuric acid solution was used as the re-extraction agent, under the following conditions:

- Aqueous phase: 1.5 M H₂SO₄
- Organic phase: organic phase saturated with copper
- Contact time: 15 min
- Aq/org ratio: 1:5
- Mixing speed: 300 rpm

Figure 10 shows the phase separations after the two-step re-extraction process of the first and second phases of the solvent extraction process.

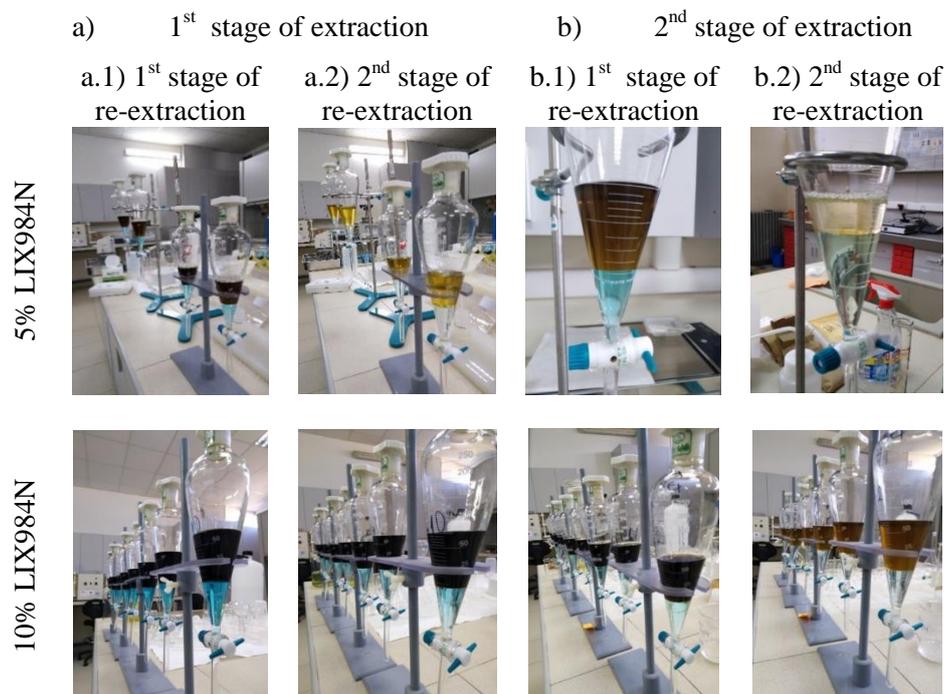


Figure 10. Two-step re-extraction processes after two-step extraction process

At the end of the two-stage extraction and two-stage re-extraction experiments, the separated aqueous phases were analysed by the ICP method to determine the metal concentration in the aqueous phases.

Table 22 shows comparative results for extraction and re-extraction degrees of Cu, Fe and Zn.

Table 22. Extraction and re-extraction degrees of Cu, Fe and Zn

Extractant	Extraction degree (%)			Re-extraction degree (%)		
	Cu	Fe	Zn	Cu	Fe	Zn
5% LIX984N	98.55	14.38	15.69	73.31	13.98	10.99
10% LIX984N	99.12	1.82	1.32	90.90	0.56	0.80

As can be seen from Table 19, the Cu extraction degree are very similar in both of cases, while the Cu re-extraction degree using 10% LIX984N is approximately 20% higher than when using 5% LIX984N as an extractant. A satisfactory degree of selective separation of copper in relation to zinc and iron has been achieved. The solution obtained after the SX process is suitable for further treatment in order to obtain a zinc-based products. Solution obtained after re-extraction process is suitable for further treatment in order to obtain a copper-based products.

3.2.3. Treatment of solution obtained after re-extraction process in order to obtain copper-based products

Experimental tests of the process of obtaining copper-based products (copper-sulphate and cathode copper) from sulfuric acid solution formed after the copper re-extraction process were performed.

3.2.3.1. Obtaining copper sulphate

The chemical characterization of the sulfuric acid solution obtained after a two-step solvent extraction process with 10% LIX984N extractant (BASF manufacturer) and a two-step re-extraction using 1.5 M H₂SO₄ is shown in Table 23.

Table 23. Chemical characterization of the solution obtained after the re-extraction process

Element	Cu	Fe	Mn	Zn	Ni	Cd	Cr
Concentration (mg/L)	13269.2	181.8	184.5	132.9	3.9	0.27	<0.005

The sulfuric acid solution, obtained after the re-extraction process, was evaporated on a sand bath at a temperature of 60°C in order to obtain copper sulfate. After the volume of the solution was reduced by 2/3, the evaporated solution was left in the cold to crystallize. Obtained CuSO₄ crystals by crystallization are shown in Figure 11.

After drying the obtained CuSO₄ crystals in a dryer at 105°C, the sample was ground in a mortar, the chemical characterization of the obtained copper sulfate on the impurity content was performed and is shown in Table 24.



Figure 11. CuSO₄ crystals

Table 24. Characterization of the obtained copper sulfate

Element	Content (ppm)
Al	290
Ba	<1
Be	<1
Ca	100
Co	10
Fe	478
Mg	500
Mn	270
Na	40
Si	6
Sr	<1
Ti	32
Zn	196

3.2.3.2. Obtaining cathodic copper

An experimental study of the process of electrochemical obtaining of cathode copper (electrowinning (EW) process) from sulfuric acid solution obtained after the re-extraction process depending on the process current density was performed. The re-extraction process was performed in two stages at an O:A = 5:1 ratio, using copper electrolytes with an H₂SO₄ content of 170 g/L.

A schematic representation of the process of electrochemical separation of copper from sulfuric acid solutions is shown in Figure 12. Electrowinning process was tested at the laboratory level on the apparatus shown in Figure 13.

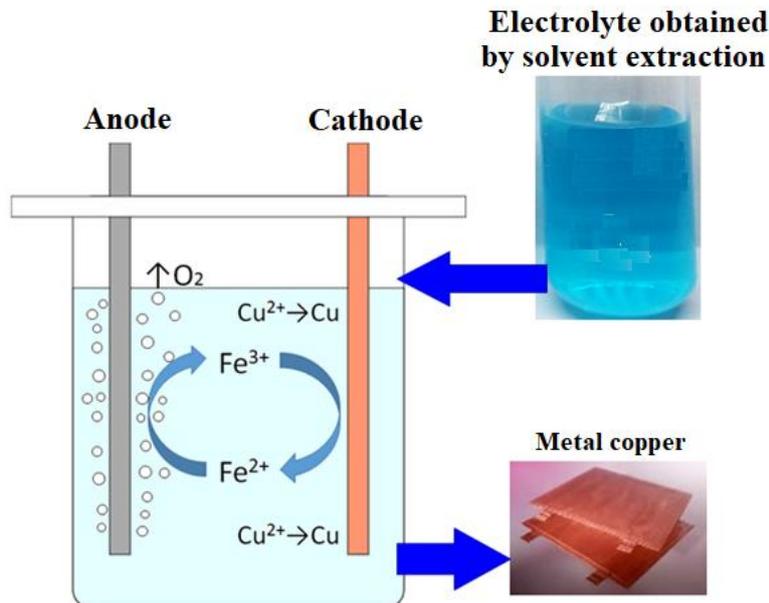


Figure 12. Schematic representation of electrochemical separation of copper from CuSO₄ solution



Figure 13. Apparatus for laboratory electrochemical tests

A series of experiments was performed in order to examine the influence of current density of 50-250 A/m² on current utilization. The experiments were performed under the following conditions:

- electrolyte volume: 1L,
- Cu concentration in electrolyte: 35 g/L,
- Fe concentration in electrolyte: 0.135 g/L,
- H₂SO₄ concentration in electrolyte: 170 g/L,
- temperature: 45°C,
- process time: 4h.

The results of laboratory experimental tests of EW process showed that by applying a current density of 250 A/m², a cathodic deposit of the best characteristics and a current efficiency of over 98% was obtained.

3.2.4. Treatment of solution obtained after extraction process in order to obtain zinc-based products

3.2.4.1. Obtaining of zinc carbonate

From the aqueous phase after the two-stage SX process, in aim to selectively separate copper from the solution, zinc precipitation was performed by neutralization (precipitation) process in two stages. The initial pH value of the solution used was 1.16.

The neutralization process was performed in the following order:

- 1st stage: precipitation of iron at pH 4.05 with 10% solution of $\text{Ca}(\text{OH})_2$;
- 2nd stage: precipitation of ZnCO_3 at pH 7.3 with Na_2CO_3 .

After the first stage of the neutralization process, the pulp was filtered, and then the second phase of the neutralization process was performed. After completion of the second phase of the process of neutralization and precipitation of zinc carbonate, the pulp was filtered. Samples of the solution after both neutralization stages were given for chemical analysis. Metal concentrations in solutions after the first and second neutralization stages are shown in Table 25.

Table 25. Metal concentrations in solutions after the first and second neutralization phases

Neutralization process	Concentration (mg/L)										
	Fe	Mn	Cu	Mo	Zn	As	Ni	Pb	Cd	Cr	Se
1 st stage	5.7	1489.4	15.6	<0.007	1014.2	<0.02	20.2	<0.02	1.6	0.075	<0.033
2 nd stage	<0.007	1284.6	0.054	<0.007	56.1	<0.02	8.1	<0.02	0.61	<0.005	<0.033

The dried sample of zinc carbonate obtained after the second stage of the neutralization process is shown in Figure 14.

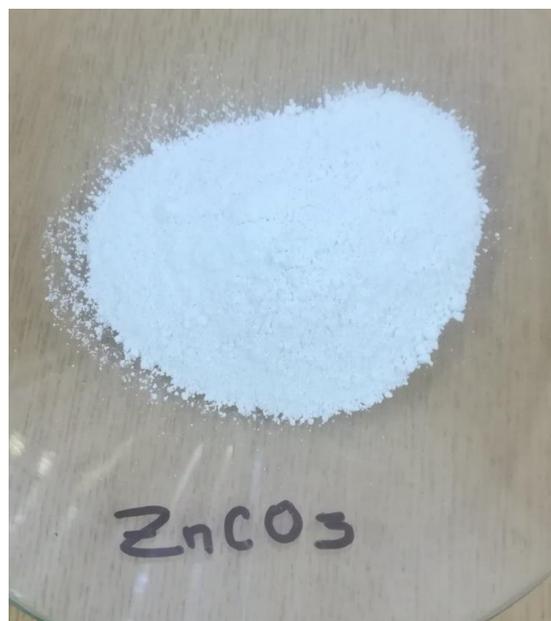


Figure 14. Obtained zinc carbonate

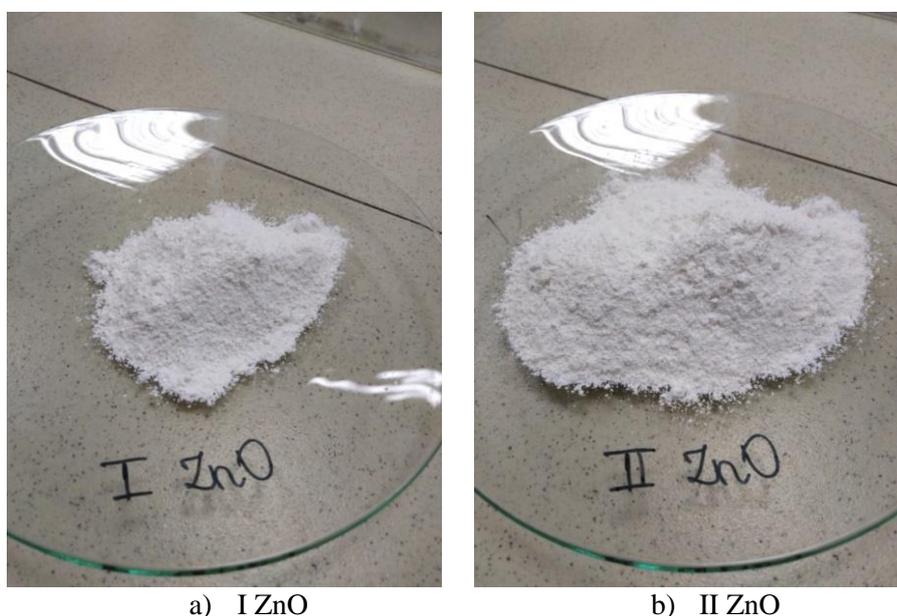
3.2.4.2. Obtaining of zinc oxide

Two samples of the obtained zinc carbonate were annealed in a chamber furnace at 450°C, first one for 2 hours (sample I ZnO) and the second one for 4 hours (sample II ZnO), in order to obtain zinc oxide.

The chemical characterizations of the obtained zinc oxides are shown in Table 26, while the samples of the obtained products are shown in Figure 15.

Table 26. Chemical characterizations of the obtained zinc oxides

Element	I ZnO	II ZnO
Zn (%)	80,9	81,32
Ag (ppm)	<10.0	<10.0
Al (ppm)	372.2	378.2
Ba (ppm)	<10.0	<10.0
Cr (ppm)	<10.0	<10.0
Cu (ppm)	21.3	<10.0
Fe (ppm)	76.6	65.4
K (ppm)	98.8	72.0
Li (ppm)	11.2	9.6
Mg (ppm)	747.1	772.7
Ni (ppm)	<10.0	<10.0
Pb (ppm)	11.0	<10.0
S (%)	3.77	3.05
Sr (ppm)	<10.0	<10.0
Ti (ppm)	<10.0	<10.0



a) I ZnO

b) II ZnO

Figure 15. Obtained zinc oxides

3.2.5. Sludge leaching, separation of Cu by solvent extraction process

Based on the results of experimental laboratory investigations in previous quarterly periods, the optimal conditions for the treatment of sludge from the neutralization of AMD water were defined. In the VII quarter period, the results of the process of leaching copper from sludge obtained after neutralization of AMD water on semi-industrial level and separation of copper from the pregnant leaching solution by the process of solvent extraction under defined optimal conditions are presented.

3.2.5.1. Leaching of sludge obtained after neutralization of AMD water with $\text{Ca}(\text{OH})_2$ on semi-industrial scale equipment

Chemical characterization of sludge obtained after neutralization of AMD water with $\text{Ca}(\text{OH})_2$ on semi-industrial scale equipment at pH8 is presented in Table 27.

Table 27. Chemical characterization of neutralization sludge

Element	Mn	Fe	Zn	Cu	Ni	As	Cd	Pb
Unit	%	%	%	%	ppm	ppm	ppm	ppm
Content	1.33	0.58	0.42	1.05	140	<20	<20	215

Leaching process of neutralization sludge on atmospheric pressure was carried out under previously defined conditions:

- Leaching reagent: 1.2 H_2SO_4
- Phase ratio: S:L=1:5
- Stirring speed: 300 rpm
- Leaching time: 60 min
- Temperature: room ($\sim 25^\circ\text{C}$)

After the leaching process was finished, pulp is filtered, and solid residue was washed with distilled water. Washing waters and pregnant leaching solution were analyzed separately. Chemical analysis of the pregnant leaching solution (PLS) and washing waters are presented in Table 28.

Table 28. Concentrations of metals in pregnant leaching solution and washing waters

Solution	Concentration (mg/L)							
	Fe	Mn	Cu	Cd	Zn	As	Ni	Pb
PLS	1085.16	1486.06	2440.88	2.00	970.58	<0.02	16.21	<0.002
Washing waters	48.48	63.84	108.64	0.19	44.73	<0.02	1.27	<0.002

Washed solid residue was dried and then chemical analyses were done (Table 29). Reduction in sample mass relative to input was 53%.

Table 29. Chemical analysis of solid residue formed after leaching at atmospheric pressure

Element	Fe	Mn	Cu	Cd	Zn	As	Ni	Pb
Content (%)	0.162	1.37	0.018	<0.002	0.006	<0.003	0.001	0.0004

3.2.5.2. Copper removal from pregnant leaching solution - Solvent extraction (SX) process

Experimental investigations in previous quarterly periods have defined the optimal parameters for the selective separation of copper from pregnant leaching solution by the solvent extraction process. Before SX process started, pH value of solution was set at 1.5 using 10% solution of calcium hydroxide. Extractant used in this experiment was LIX984N confirmed to be the most selective for copper in relation to iron and zinc, and re-extraction process was performed with 1.5 M H₂SO₄. For solvent extraction and re-extraction processes, glass separating funnels and a shaker device with the possibility of regulating the process duration and intensity of mixing the phases were used.

The extraction process was performed in two-stage with the following parameters:

1st stage:

- Aqueous phase: PLS (pH 1.5)
- Organic phase: 10% LIX984N (Acorga)
- Solvent: kerosene
- Contact time: 15 min
- Ratio of org/aq phases: 1:2
- Mixing rate: 300 rpm

2nd stage:

- Aqueous phase: aqueous phase from 1st stage extraction
- Organic phase: 10% LIX984N (Acorga)
- Solvent: kerosene
- Contact time: 15 min
- Ratio of org/aq phases: 1:2
- Mixing rate: 300 rpm

Re-extraction process was performed in two-stage with the following parameters:

- Aqueous phase: 1.5M H₂SO₄
- Organic phase: saturated organic phases from each step of extraction containing extracted copper
- Contact time: 15 min
- Ratio of org/aq phases: 5:1
- Mixing rate: 300 rpm

At the end of two-step extraction and re-extraction experiments, separated aqueous phases were analyzed by the ICP method in order to determine the metal concentration in the aqueous phases. Results of chemical analysis are presented in Table 30.

Table 30. Concentration of metals in solutions after extraction (EX) and re-extraction (REX)

Concentration (mg/L)	EX		REX (after I EX)		REX (after II EX)	
	I	II	I	II	I	II
Zn	967.87	992.75	47.12	1.89	73.71	2.89
Fe	1101.0	1128.1	68.64	2.76	127.56	4.68
Mn	1486.9	1521.9	76.78	2.68	108.47	4.09
Cu	500.2	21.58	11782.45	4147.7	3009.93	1311.68

As	<1.00	<1.00	0.28	0.34	0.23	0.46
Ni	18.36	18.56	1.75	0.15	2.14	0.12
Cd	2.60	2.60	0.19	0.012	0.28	0.016
Pb	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002

The treatment of the obtained solid residue and solution will be performed under already defined conditions in the previous quarter period using following processes:

- Solid residue after leaching will be characterized by TCLP method in aim to safely disposal as non-toxic waste.
- Separation of copper from aqueous solution after re-extraction process using copper electro-winning process.
- Precipitation of zinc in the $ZnCO_3$ form from aqueous solution after extraction process.

3.3. CHARACTERIZATION AND STABILIZATION OF SOLID WASTE GENERATED IN NEUTRALIZATION SLUDGE LEACHING PROCESS

3.3.1. Characterization of solid waste generated in neutralization sludge leaching process from first selected water

Sludge generated in neutralization process of selected AMD –W1 is leached with sulfuric acid in controlled conditions (temperature and stirring during the experiments are controlled). After leaching process some solid wastes are generated. Two type of investigations was carried out to determine the impact of generated waste on the environment after disposal:

First-investigation of waste leachability (LP procedure) by providing leaching test according to standard methods EN 12457-2 (Characterization of waste - Leaching - Compliance test for leaching of granular waste materials and sludges - Part 2: One stage batch test at a liquid-solid ratio of 10 l / kg for materials with a high content solids and particle size of less than 4 mm (with decreasing particle size, or without decreasing) and

Second -investigation of waste toxicity (TCLP procedure) by providing Toxicity Characteristic Leaching Procedure according to standard method EPA 1311.

The results of waste testing according to *LP procedure* are shown in Table 31, and the results of waste testing according to *TCLP procedure* are shown in Table 32.

Table 31. Results of physicochemical testing waste (solid waste remaining after leaching) investigation of waste leachability according with SRPS EN 12457-2:2008

Parameter	Unit	Measured value				Method
		Label of the sample				
		02.20.11 OR				
Moisture content	%	45.44				SRPS EN 12879
Solid content	%	54.56				P
Parameter	Unit	Measured value	The reference value for non-	The reference value for non-	The reference value for inert waste ⁴⁾	Method
		Label of the sample				



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		01.20.11 OR-10 L/kg	hazardous waste ²⁾	hazardous waste ³⁾		
Content in EP extract (neutral test, L/S = 10/1)						
Conductivity	µS/cm	2007	-	-	-	EPA 120.1*
pH (25 °C) ¹⁾	-	8.96	-	6-13	-	SRPS EN 12506*
Arsenic, As	mg/kg dm	<0.020	25	2	0.5	SRPS EN ISO 11885
Antimony, Sb	mg/kg dm	<0.5	5	0.7	0.06	VMK C.g.2
Copper, Cu	mg/kg dm	0.096	100	50	2	SRPS EN ISO 11885
Molybdenum, Mo	mg/kg dm	<0.007	30	10	0.5	SRPS EN ISO 11885
Cadmium, Cd	mg/kg dm	<0.008	5	1	0.04	SRPS EN ISO 11885
Selenium, Se	mg/kg dm	<0.033	7	0.5	0.1	SRPS EN ISO 11885
Nickel, Ni	mg/kg dm	<0.007	40	10	0.4	SRPS EN ISO 11885
Mercury, Hg	mg/kg dm	<0.005	2	0.2	0.01	SRPS EN ISO 11885
Lead, Pb	mg/kg dm	0.44	50	10	0.5	SRPS EN ISO 11885
Chromium, Cr	mg/kg dm	<0.020	70	10	0.5	SRPS EN ISO 11885
Zinc, Zn	mg/kg dm	0.097	200	50	4	SRPS EN ISO 11885
Sulphate, SO ₄ ²⁻	mg/kg dm	13 000	50000	20 000	1 000	SRPS EN ISO 11885
Fluoride, F ⁻	mg/kg dm	10.4	500	150	10	SRPS EN ISO 11885
Chloride, Cl ⁻	mg/kg dm	<10	25000	15 000	800	SRPS EN ISO 11885

1) Annex 7 of the Regulation on waste categories, testing, and classification (Official gazette RS 56/2010. and 93/2019), H15 waste characteristics

2), 3), 4) Annex 10 of the Rules on categories, testing and classification of waste (Official Gazette RS 56/2010 and 93/2019), Article 2, Parameters for testing waste and process water from hazardous waste landfill²⁾, non-hazardous waste landfill³⁾ and inert waste⁴⁾



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Table 32. Results of physicochemical testing of toxic waste characteristics for disposal -TCLP test (EPA 1311)

Parameter	Unit	Measured value	The reference value for non-hazardous waste ⁵⁾	Method
		Label of the sample		
		02.20.11 OR-TCLP		
Content in Eluate (TCLP test)				
Antimony, Sb	mg/L	<0.011	15	SRPS EN ISO 11885
Chromium, Cr	mg/L	0.018	5	SRPS EN ISO 11885
Molybdenum, Mo	mg/L	<0.007	350	SRPS EN ISO 11885
Nickel, Ni	mg/L	0.048	20	SRPS EN ISO 11885
Selenium, Se	mg/L	<0.033	1	SRPS EN ISO 11885
Zinc, Zn	mg/L	22.6	250	SRPS EN ISO 11885
Copper, Cu	mg/L	1.6	25	SRPS EN ISO 11885
Arsenic, As	mg/L	<0.020	5	SRPS EN ISO 11885
Cadmium, Cd	mg/L	0.77	1	SRPS EN ISO 11885
Lead, Pb	mg/L	4.6	5	SRPS EN ISO 11885
Mercury, Hg	mg/L	<0.0005	0.2	SRPS EN ISO 11885
Vanadium, V	mg/L	0.032	24	SRPS EN ISO 11885
Silver, Ag	mg/L	0.016	5	SRPS EN ISO 11885

5) Annex 10 of the Role Book on the categories, testing and classification of waste (Official Gazette RS 56/2010), Article 1, Parameters for testing the toxic characteristics of waste intended for disposal

After comparison with limits given in Annex 10 of the Regulation on categories, testing and classification of waste (Official Gazette RS 56/2010.) for LP and TCLP procedure comment are as follows:

- concentrations of sulfuric ions in LP test are near limit value for disposal on landfill for non-hazardous waste
- concentration of lead and cadmium in TCLP test are near limit value for waste toxicity criteria.

3.3.2. Stabilization of solid waste generated in neutralization sludge leaching process

Aim to ensure non-hazardous character of generated solid wastes after leaching for disposal on landfill for non-hazardous wastes, stabilization process is carried out.

Stabilization of waste includes the following steps:

- determine the homogeneity of the waste,
- determine the moisture of the waste,
- determination optimal ration lime and waste,
- determination of optimal contact time needed for stabilization process
- confirmation defined parameters of stabilization by minimum five times repeated tests of leachability and toxicity of waste

Optimal parameters for solid waste stabilization process are shown in Table 33.

Table 33. Optimal parameters for solid waste stabilization process

Homogeneity of waste	Range of moisture content (%)	Optimal ratio of lime: waste (m/m)	Optimal contact time (h)
high	42-46	1:10	12

3.3.3. Characterization of stabilized solid waste

Two types of investigations were carried out to determine the impact of generated waste on the environment after disposal, LP and TCLP procedure (same as for unsterilized waste).

The results of stabilized waste testing according to *LP procedure* are shown in Table 34, and the results of waste testing according to *TCLP procedure* is shown in Table 35.

Table 34. Results of physicochemical testing waste (residue after leaching) leachability according with SRPS EN 12457-2:2008

Parameter	Unit	Measured value				Method
		Label of the sample				
		02.20.11 ST				
Moisture content	%	42.30				SRPS EN 12879
Solid content	%	57.70				P
Parameter	Unit	Measured value	The reference value for non-hazardous waste ²⁾	The reference value for non-hazardous waste ³⁾	The reference value for inert waste ⁴⁾	Method
		Label of the sample				
		01.20.11 ST-10 L/kg				
Content in EP extract (neutral test, L/S = 10/1)						
Conductivity	μS/cm	6658	-	-	-	EPA 120.1*
pH (25 °C) ¹⁾	-	12.34	-	6-13	-	SRPS EN 12506*
Arsenic, As	mg/kg dm	<0.020	25	2	0.5	SRPS EN ISO 11885
Antimony, Sb	mg/kg	<0.5	5	0.7	0.06	VMK C.g.2

	dm					
Copper, Cu	mg/kg dm	<0.005	100	50	2	SRPS EN ISO 11885
Molybdenum, Mo	mg/kg dm	<0.007	30	10	0.5	SRPS EN ISO 11885
Cadmium, Cd	mg/kg dm	<0.008	5	1	0.04	SRPS EN ISO 11885
Selenium, Se	mg/kg dm	<0.033	7	0.5	0.1	SRPS EN ISO 11885
Nickel, Ni	mg/kg dm	<0.007	40	10	0.4	SRPS EN ISO 11885
Mercury, Hg	mg/kg dm	<0.005	2	0.2	0.01	SRPS EN ISO 11885
Lead, Pb	mg/kg dm	0.012	50	10	0.5	SRPS EN ISO 11885
Chromium, Cr	mg/kg dm	<0.020	70	10	0.5	SRPS EN ISO 11885
Zinc, Zn	mg/kg dm	0.015	200	50	4	SRPS EN ISO 11885
Sulphate, SO ₄ ²⁻	mg/kg dm	11400	50000	20 000	1 000	SRPS EN ISO 11885
Fluoride, F ⁻	mg/kg dm	12.3	500	150	10	SRPS EN ISO 11885
Chloride, Cl ⁻	mg/kg dm	<10	25000	15 000	800	SRPS EN ISO 11885

1) Annex 7 of the Regulation on waste categories, testing, and classification (Official Gazette RS 56/2010. and 93/2019), H15 waste characteristics

2), 3), 4) Annex 10 of the Rules on categories, testing and classification of waste (Official Gazette RS 56/2010 and 93/2019), Article 2, Parameters for testing waste and process water from hazardous waste landfill²⁾, non-hazardous waste landfill³⁾ and inert waste⁴⁾

Table 35. Results of physicochemical testing of toxic waste characteristics for disposal -TCLP test (EPA 1311)

Parameter	Unit	Measured value	The reference value for non-hazardous waste ⁵⁾	Method
		Label of the sample		
		02.20.11 ST-TCLP		
Content in Eluate (TCLP test)				
Antimony, Sb	mg/L	<0.011	15	SRPS EN ISO 11885
Chromium, Cr	mg/L	0.052	5	SRPS EN ISO 11885
Molybdenum, Mo	mg/L	<0.007	350	SRPS EN ISO 11885



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Nickel, Ni	mg/L	0.035	20	SRPS EN ISO 11885
Selenium, Se	mg/L	<0.033	1	SRPS EN ISO 11885
Zinc, Zn	mg/L	0.60	250	SRPS EN ISO 11885
Copper, Cu	mg/L	0.35	25	SRPS EN ISO 11885
Arsenic, As	mg/L	<0.020	5	SRPS EN ISO 11885
Cadmium, Cd	mg/L	0.017	1	SRPS EN ISO 11885
Lead, Pb	mg/L	0.16	5	SRPS EN ISO 11885
Mercury, Hg	mg/L	<0.0005	0.2	SRPS EN ISO 11885
Vanadium, V	mg/L	0.045	24	SRPS EN ISO 11885
Silver, Ag	mg/L	0.010	5	SRPS EN ISO 11885

⁵⁾ Annex 10 of the Role Book on the categories, testing and classification of waste (Official Gazette RS 56/2010), Article 1, Parameters for testing the toxic characteristics of waste intended for disposal

After comparison with limits given in Annex 10 of the Regulation on categories, testing and classification of waste (Office Gazette RS 56/2010.) for LP and TCLP procedure comment are as follows:

-concentration of sulphuric ions in LP test of stabilized waste is lower than in unsterilized waste, but influence of stabilization process on immobilization of sulfuric ion is not high.

-concentration of lead and cadmium ions in TCLP test are lower than in unsterilized waste, and influence of stabilization process on immobilization of lead and cadmium ions is very high.

Conclusion is that proposal stabilization process is applicable for stabilization of solid waste generated in neutralization sludge leaching process.

3.3.4. Characterization of solid waste generated in neutralization sludge leaching process from second selected water

1. Sample receiving date: 05.07.2020.

2. Type /code/The origin of the sample: Solid residue after leaching/07.21.05 OR/Solid residue after leaching stabilized/07.21.05 ST

3. Special conditions / additions / deviations related to sampling: The analysis was performed on a sample prepared during the research at the Mining and Metallurgy Institute Bor

4. Supplements, exclusions or deviations related to testing: -

Table 36. Results of physicochemical testing waste (residue after leaching) leachability according with SRPS EN 12457-2:2008



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Parameter	Unit	Measured value				Method
		Label of the sample				
		07.21.05 OR				
Moisture content	%	43.78				SRPS EN 12879
Solid content	%	56.22				P
Parameter	Unit	Measured value	The reference value for non-hazardous waste ²⁾	The reference value for non-hazardous waste ³⁾	The reference value for inert waste ⁴⁾	Method
		Label of the sample				
		07.21.05 OR-10 L/kg				
Content in EP extract (neutral test, L/S = 10/1)						
Conductivity	µS/cm	2221	-	-	-	EPA 120.1*
pH (25 °C) ¹⁾	-	8.62	-	6-13	-	SRPS EN 12506*
Arsenic, As	mg/kg dm	<0.020	25	2	0.5	SRPS EN ISO 11885
Antimony, Sb	mg/kg dm	<0.5	5	0.7	0.06	VMK C.g.2
Copper, Cu	mg/kg dm	0.116	100	50	2	SRPS EN ISO 11885
Molybdenum, Mo	mg/kg dm	<0.007	30	10	0.5	SRPS EN ISO 11885
Cadmium, Cd	mg/kg dm	<0.008	5	1	0.04	SRPS EN ISO 11885
Selenium, Se	mg/kg dm	<0.033	7	0.5	0.1	SRPS EN ISO 11885
Nickel, Ni	mg/kg dm	<0.007	40	10	0.4	SRPS EN ISO 11885
Mercury, Hg	mg/kg dm	<0.005	2	0.2	0.01	SRPS EN ISO 11885
Lead, Pb	mg/kg dm	0.12	50	10	0.5	SRPS EN ISO 11885
Chromium, Cr	mg/kg dm	<0.020	70	10	0.5	SRPS EN ISO 11885



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Zinc, Zn	mg/kg dm	0.151	200	50	4	SRPS EN ISO 11885
Sulphate, SO ₄ ²⁻	mg/kg dm	13 520	50000	20 000	1 000	SRPS EN ISO 11885
Fluoride, F ⁻	mg/kg dm	15.4	500	150	10	SRPS EN ISO 11885
Chloride, Cl ⁻	mg/kg dm	<10	25000	15 000	800	SRPS EN ISO 11885

1) Annex 7 of the Regulation on waste categories, testing, and classification (Official gazette RS 56/2010. and 93/2019), H15 waste characteristics 2), 3), 4) Annex 10 of the Rules on categories, testing and classification of waste (Official Gazette RS 56/2010 and 93/2019), Article 2, Parameters for testing waste and process water from hazardous waste landfill2), non-hazardous waste landfill3) and inert waste4)

Table 37. Results of physicochemical testing of toxic waste characteristics for disposal -TCLP test (EPA 1311)

Parameter	Unit	Measured value	The reference value for non-hazardous waste ⁵⁾	Method
		Label of the sample		
		07.21.05 OR-TCLP		
Content in Eluate (TCLP test)				
Antimony, Sb	mg/L	<0.011	15	SRPS EN ISO 11885
Chromium, Cr	mg/L	0.021	5	SRPS EN ISO 11885
Molybdenum, Mo	mg/L	<0.007	350	SRPS EN ISO 11885
Nickel, Ni	mg/L	0.056	20	SRPS EN ISO 11885
Selenium, Se	mg/L	<0.033	1	SRPS EN ISO 11885
Zinc, Zn	mg/L	43.7	250	SRPS EN ISO 11885
Copper, Cu	mg/L	2.9	25	SRPS EN ISO 11885
Arsenic, As	mg/L	<0.020	5	SRPS EN ISO 11885
Cadmium, Cd	mg/L	0.31	1	SRPS EN ISO 11885
Lead, Pb	mg/L	2.4	5	SRPS EN ISO 11885
Mercury, Hg	mg/L	<0.0005	0.2	SRPS EN ISO 11885

Vanadium, V	mg/L	<0.0005	24	SRPS EN ISO 11885
Silver, Ag	mg/L	<0.0005	5	SRPS EN ISO 11885

5) Annex 10 of the Role Book on the categories, testing and classification of waste (Official Gazette RS 56/2010), Article 1, Parameters for testing the toxic characteristics of waste intended for disposal

Table 38. Results of physicochemical testing waste (residue after leaching) leachability according with SRPS EN 12457-2:2008

Parameter	Unit	Measured value				Method
		Label of the sample				
		07.21.05 ST				
Moisture content	%	39.20				SRPS EN 12879
Solid content	%	60.80				P
Parameter	Unit	Measured value	The reference value for non-hazardous waste ²⁾	The reference value for non-hazardous waste ³⁾	The reference value for inert waste ⁴⁾	Method
		Label of the sample				
		07.21.05 ST-10 L/kg				
Content in EP extract (neutral test, L/S = 10/1)						
Conductivity	µS/cm	4850	-	-	-	EPA 120.1*
pH (25 °C) ¹⁾	-	12.25	-	6-13	-	SRPS EN 12506*
Arsenic, As	mg/kg dm	<0.020	25	2	0.5	SRPS EN ISO 11885
Antimony, Sb	mg/kg dm	<0.5	5	0.7	0.06	VMK C.g.2
Copper, Cu	mg/kg dm	<0.005	100	50	2	SRPS EN ISO 11885
Molybdenum, Mo	mg/kg dm	<0.007	30	10	0.5	SRPS EN ISO 11885
Cadmium, Cd	mg/kg dm	<0.008	5	1	0.04	SRPS EN ISO 11885
Selenium, Se	mg/kg	<0.033	7	0.5	0.1	SRPS EN

	dm					ISO 11885
Nickel, Ni	mg/kg dm	<0.007	40	10	0.4	SRPS EN ISO 11885
Mercury, Hg	mg/kg dm	<0.005	2	0.2	0.01	SRPS EN ISO 11885
Lead, Pb	mg/kg dm	0.021	50	10	0.5	SRPS EN ISO 11885
Chromium, Cr	mg/kg dm	<0.020	70	10	0.5	SRPS EN ISO 11885
Zinc, Zn	mg/kg dm	0.009	200	50	4	SRPS EN ISO 11885
Sulphate, SO ₄ ²⁻	mg/kg dm	11870	50000	20 000	1 000	SRPS EN ISO 11885
Fluoride, F ⁻	mg/kg dm	15.3	500	150	10	SRPS EN ISO 11885
Chloride, Cl ⁻	mg/kg dm	<10	25000	15 000	800	SRPS EN ISO 11885

1) Annex 7 of the Regulation on waste categories, testing, and classification (Official gazette RS 56/2010. and 93/2019), H15 waste characteristics. 2), 3), 4) Annex 10 of the Rules on categories, testing and classification of waste (Official Gazette RS 56/2010 and 93/2019), Article 2, Parameters for testing waste and process water from hazardous waste landfill2), non-hazardous waste landfill3) and inert waste4)

Table 39. Results of physicochemical testing of toxic waste characteristics for disposal -TCLP test (EPA 1311)

Parameter	Unit	Measured value	The reference value for non-hazardous waste ⁵⁾	Method
		Label of the sample		
		07.21.05 ST-TCLP		
Content in Eluate (TCLP test)				
Antimony, Sb	mg/L	<0.011	15	SRPS EN ISO 11885
Chromium, Cr	mg/L	0.038	5	SRPS EN ISO 11885
Molybdenum, Mo	mg/L	<0.007	350	SRPS EN ISO 11885
Nickel, Ni	mg/L	0.042	20	SRPS EN ISO 11885
Selenium, Se	mg/L	<0.033	1	SRPS EN ISO 11885
Zinc, Zn	mg/L	0.54	250	SRPS EN ISO 11885

Copper, Cu	mg/L	0.28	25	SRPS EN ISO 11885
Arsenic, As	mg/L	<0.020	5	SRPS EN ISO 11885
Cadmium, Cd	mg/L	0.011	1	SRPS EN ISO 11885
Lead, Pb	mg/L	0.09	5	SRPS EN ISO 11885
Mercury, Hg	mg/L	<0.0005	0.2	SRPS EN ISO 11885
Vanadium, V	mg/L	<0.0005	24	SRPS EN ISO 11885
Silver, Ag	mg/L	<0.0005	5	SRPS EN ISO 11885

5) Annex 10 of the Role Book on the categories, testing and classification of waste (Official Gazette RS 56/2010), Article 1, Parameters for testing the toxic characteristics of waste intended for disposal

All testing samples are on level of non-hazardous waste.

3.4. REMEDIATION OF METALS POLLUTED WATERS USING MICROORGANISMS

The accumulation of metals by microbial strains has received considerable attention in recent decades due to the potential use of microorganisms to clean water contaminated with metals. Heavy metals are natural elements in the Earth's crust that can pass into human food through industrial, agricultural or polluted mining waters. These elements are not biodegradable, some of them are known as pollutants and toxins, and their bioaccumulation in plant and animal tissues can cause unwanted effects for humans [9,10]. Therefore, their amount in water and food should always be under control. Bioremediation of mining water using microorganisms has generated interest for researchers, because of its advantages, such as the quality of being more accurate and environmentally friendly [11,12].

3.4.1 Cleaning of copper-enriched synthetic water using microorganisms

The aim of this research was to investigate the bioremediation conditions of copper in synthetic water. In our study, the efficiency of copper accumulation was determined using various genetically modified strains of *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Escherichia coli*. The growth rate of strains grown in specific liquid media containing a concentration of 1 mM copper sulphate was analyzed by measuring the optical density of the cultures at 600 nm. Optical emission spectrometry with inductively coupled plasma analysis was performed on supernatant samples in order to identify the concentration of copper accumulated into the cells. The results showed that the tolerance of bacteria strains on copper-containing media 1 mM was lower than the tolerance of yeast strains with the same concentration of copper. ICP-OES analysis showed that *E. coli* BL21 (DE3) RIL strain had the best copper accumulation efficiency. *S. cerevisiae* BJ5465 yeast strain was most efficient in the accumulation of copper from a total of six yeast strains used. Furthermore, *Escherichia coli* cells that internally expressed a recombinant peroxidase were able to accumulate more copper than the cells which produced periplasmic recombinant peroxidases [13]. The results are presented in the further figures.



Cooperation beyond borders.

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METHODS

Assay of copper tolerance of yeast and bacteria strains

Different bacteria and yeast strains were cultivated on LB and YPD solid media comprising different concentration of copper sulphate: 0 (negative control), 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0 mM. Plates were incubated for two days at 37°C and 30°C, respectively. Tolerance to copper was given by observations related to the growth of bacterial and yeast colonies on plates.

Transformation of E. coli with peroxidase recombinant plasmids

The recombinant plasmids containing peroxidase genes were transformed in E. coli BL21 (DE3) OverExpress strain according to the protocol described by Miller and collaborators [14].

Organism growth conditions

The bacteria and yeast strains were cultured in the described media. The growth of microorganisms was repeated starting from an optical density at 600 nm (OD 600 nm) around 0.1 and cultures were incubated under the same conditions for 24 hours. Simultaneously, a volume of 200 µL of recombinant strains precultures containing plasmids with peroxidase genes was first inoculated in 10 mL LB containing 100 µg/mL ampicillin and then incubated for 2 h at 35°C, 300 rpm. In the exponential phase (OD 600 nm around 1.0) the expression of recombinant peroxidases was induced by adding 1mM IPTG into the cultures. These were further incubated at 35°C, 300 rpm for 7 hours. For each 3 mL from all diluted cultures 3 mL of specific media, LB, YPD, and LB-ampicillin-IPTG, containing 2 mM copper sulphate were added. All cultures were incubated for 48 h, 300 rpm, 35°C (bacteria), and 30°C (yeast). The OD 600 was measured after 48 h of incubation, as well. The series of cultures that have not contained copper were used as sample controls.

Cell samples preparation

After 48 h of growth, 3 mL from every culture were subjected to centrifugation for 2 min at 11000 g. The collected cells were resuspended two times in 1.5 mL distilled water and centrifuged, under the same conditions. After removing the supernatant, the cells were resuspended in 500 µL 2% HNO₃ solution and left at room temperature for 72 hours. Cell debris were additional subjected to homogenization for 30 s, at 20000 rpm, using a Micra D-1 homogenizer, Germany. The samples were centrifuged for 10 min at 11000 g. The supernatant from each cell sample was subjected to optical emission spectrometry with inductively coupled plasma (ICP-OES) analysis.

ICP-OES analysis of the cell samples

A volume of 300 µL of each sample was analyzed using an optic emission spectrometer with inductively plasma (ICP-OES) model Spectro Arcos, Germany. The plasma was stabilized and optic profiled according to the manufacturer recommendations [14]. Copper accumulated in the cells was determined at 324.754 nm wavelength. The operating conditions were the following: forward power - 1450 W, coolant flow - 13.0 L/min, nebulizer flow - 0.75 L/min, an auxiliary flow - 1.0 L/min, sample aspiration rate - 2 mL/min. All used chemicals were of high purity grade. Certified reference materials (VHG Labs, QWPTM-15) was used for quality control of chemical analysis.

RESULTS AND DISCUSSIONS

Tolerance of yeast and bacteria strains on copper containing media

Tolerance on copper of five *Saccharomyces* strains, four *Pichia* strains, and nine *Escherichia* strains was first assessed by a drop test on YPD and LB solid media supplemented with increasing



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concentrations of CuSO₄. As seen in Figure 17A, *Pichia* yeast strains are viable up to a copper concentration of 10 mM. Nevertheless, *S. cerevisiae* INVSc was viable up to 5 mM copper concentration, while the brewing yeast was viable at a lower concentration (1 mM), with only a few colonies growing at 2.5 and 5 mM copper concentrations. Plates containing only specific solid medium without copper, were used as the control for the growth of the strains. The bacterial strains are more sensitive at high concentration of copper, than yeast. All bacteria strains used are viable up to a concentration of 2.5 mM of copper, higher concentrations being fatal for them (17B).

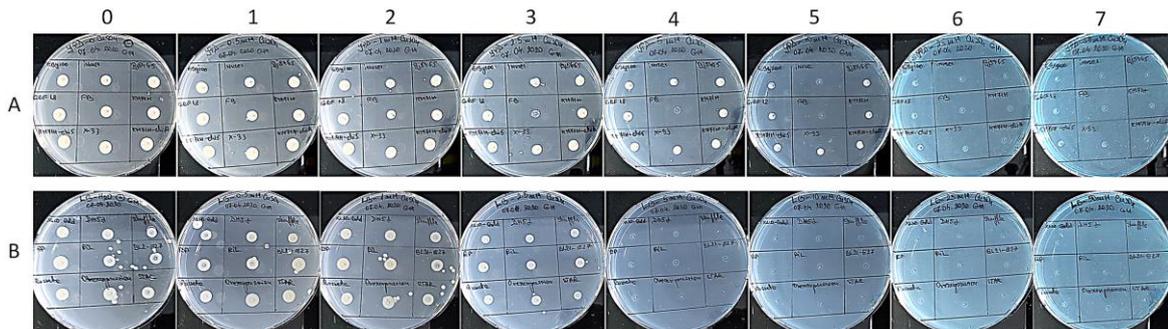


Figure 17. Tolerance test of different strains of yeast (A) and bacteria (B), on different concentrations of copper sulphate media. Plates from 0 to 7 contains different concentrations of CuSO₄ media (mM): line 0 = 0 (negative control), line 1 = 0.5, line 2 = 1.0, line 3 = 2.5, line 4 = 5.0, line 5 = 10.0, line 6 = 25.0, line 7 = 50.0

The growth rate of bacteria and yeast was determined by the cultivation of the strains in specific copper-containing liquid media. The chosen concentration of copper was 1 mM, that concentration at which all strains were viable on plates. The results obtained regarding the growth rate of bacteria and yeast by comparing the optical density after 48 h incubation at 30°C, and 35°C, in media with and without copper, are presented in Figure 18, Figure 19, and Figure 20.

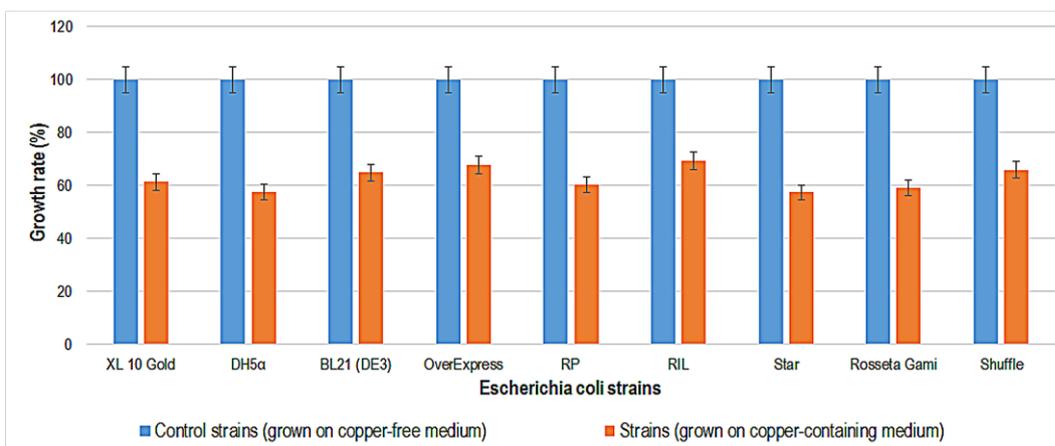


Figure 18. Growth rate (%) for different *Escherichia coli* strains, after 48 h of incubation, at 35°C, in LB medium without and with copper 1mM.

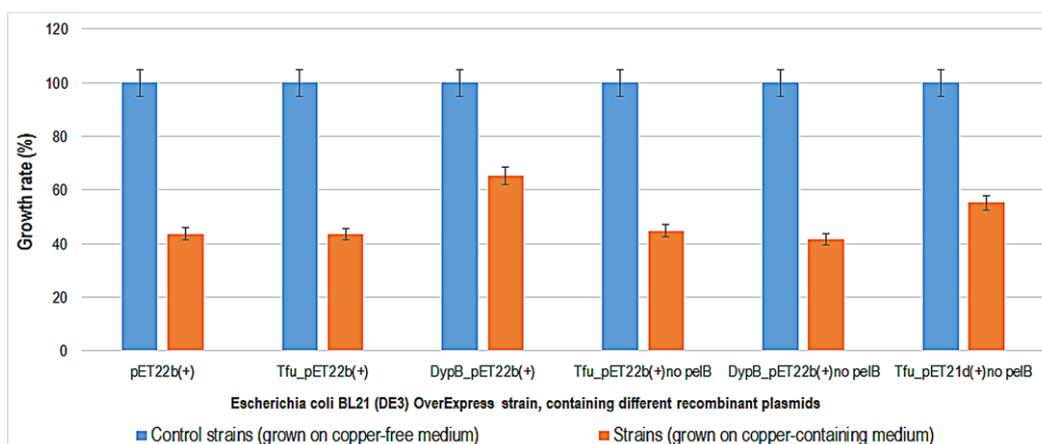


Figure 19. Growth rate (%) for *Escherichia coli* OverExpress containing different recombinant plasmids, after 48 h of expression in LB-IPTG-ampicillin medium without and with copper (1mM).

The growth rate values show that the bacterial strains are around 40% inhibited on media containing 1 mM copper sulfate, after 48 h of incubation at 35°C. These results were obtained against reference cultures grown on media without copper that were considered 100%. Growth inhibition of *Escherichia coli* OverExpress strain expressing two types of recombinant peroxidase was between 40-60%. Interestingly, there is almost no difference between the growth rate of the yeast cultures grown on media with and without copper (Figure 20). This result leads us to the conclusion that the used yeast strains are more resistant on media containing copper, than the bacterial strains. In the previous experiment it was also confirmed that the yeast cells are viable up to a concentration of 10 mM of copper, while the bacterial cells are viable only up to a concentration of 2.5 mM, therefore the growth rate in copper-containing liquid media is in accordance with the results obtained for copper tolerance assay.

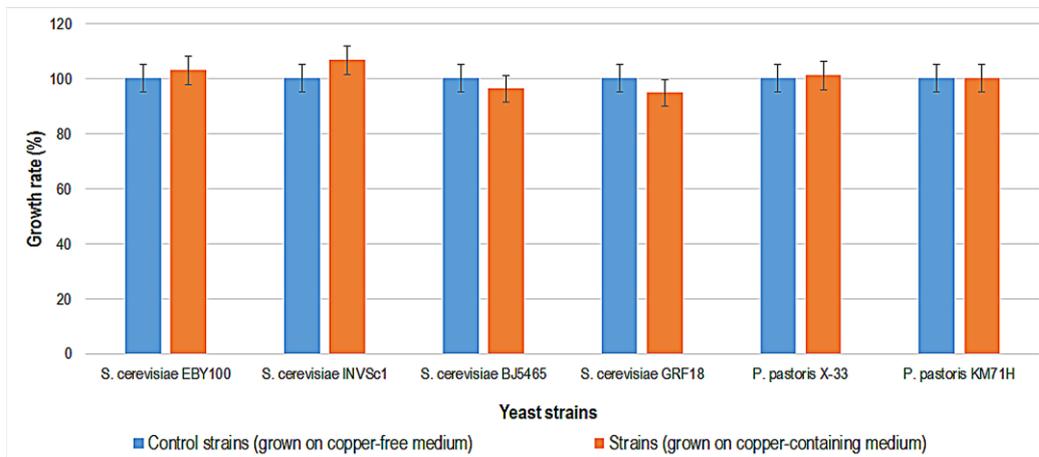


Figure 20. Growth rate (%) for different yeast strains, after 48 h of incubation, at 30°C, in YPD medium without or with copper (1mM).

Copper accumulation efficiency

ICP-OES analysis gives us more information about the capacity of the cells to accumulate copper, in every strain, including the E. coli strains, which produced peroxidase enzymes. Cells from 3 mL of cultures, collected by centrifugation and washed two times with distilled water, were subjected to cell disruption in 500 μ L 2% HNO₃ and homogenized. A volume of 300 μ L of supernatant was subjected to ICP-OES analysis. Nine strains of E. coli were tested for copper bioaccumulation characteristics.

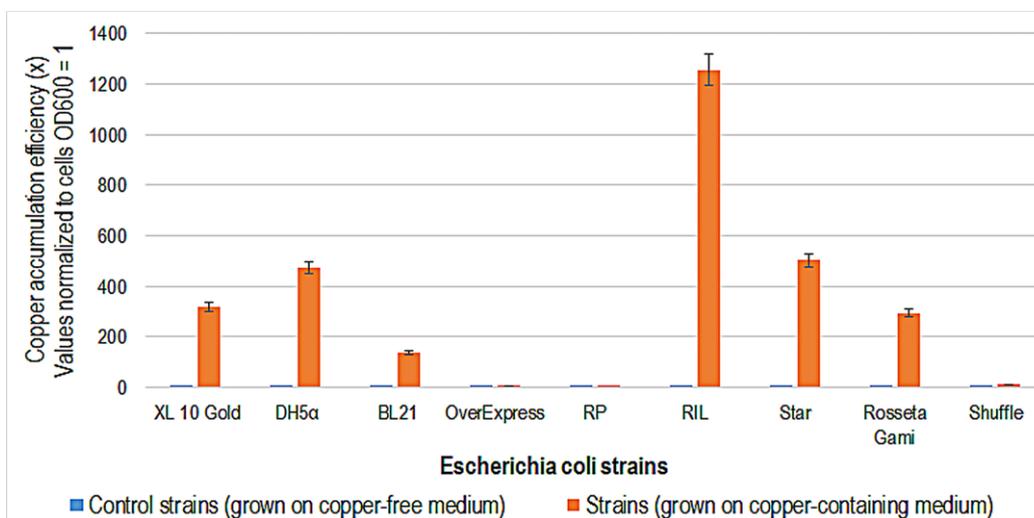


Figure 21. Copper accumulation efficiency (x) in different strains of Escherichia coli, after 48 h of growing in LB copper containing-medium, at 35°C. Control strains were grown on copper-free medium.

In Figure 22, *E. coli* RIL strain has the best copper accumulation efficiency (1250 times more effective than the control strain), while DH α and STAR strains are over 450 times more efficient in retaining copper ions. The cultures of *E. coli* BL21 (DE3) OverExpress containing different recombinant plasmids that carry genes for peroxidase were expressed for 7 h under IPTG inductor. The enzymes were periplasmic and internally expressed, and cultures were further exposed by dilution to LB-IPTG-copper-containing medium for another 48 hours. Only *E. coli* BL21 (DE3) OverExpress cells that contained the recombinant plasmids for internal expression of peroxidase (Tfu_pET22b no pelB and Tfu_pET21d no pelB) had efficiency in the accumulation of copper (Figure 22) [16,17].

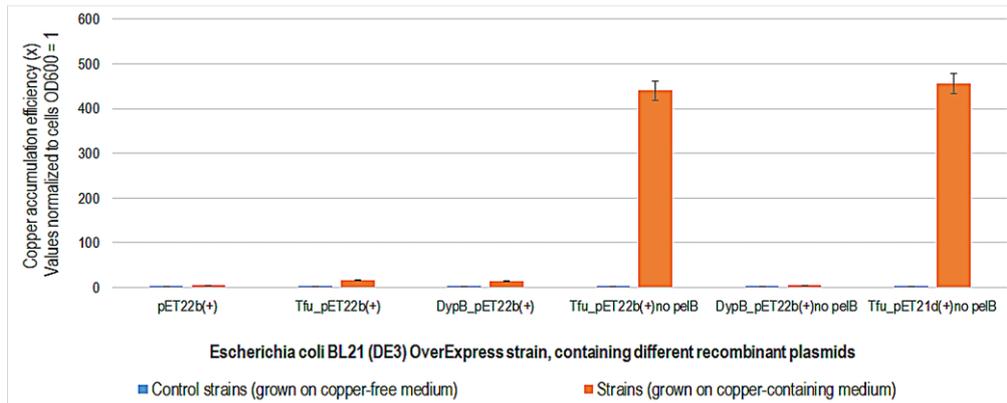


Figure 22. Copper accumulation efficiency (x) in *Escherichia coli* BL21 (DE3) containing different recombinant plasmids, expressing peroxidase, after 48 h of expression in LB-IPTG-ampicillin copper containing-medium, at 35°C. Control

In Figure 23, *S. cerevisiae* BJ5465 strain shows the highest copper accumulation efficiency, followed by *S. cerevisiae* INVSc1 strain. *S. cerevisiae* yeast seems to have a higher capacity of copper accumulation than *P. pastoris*.

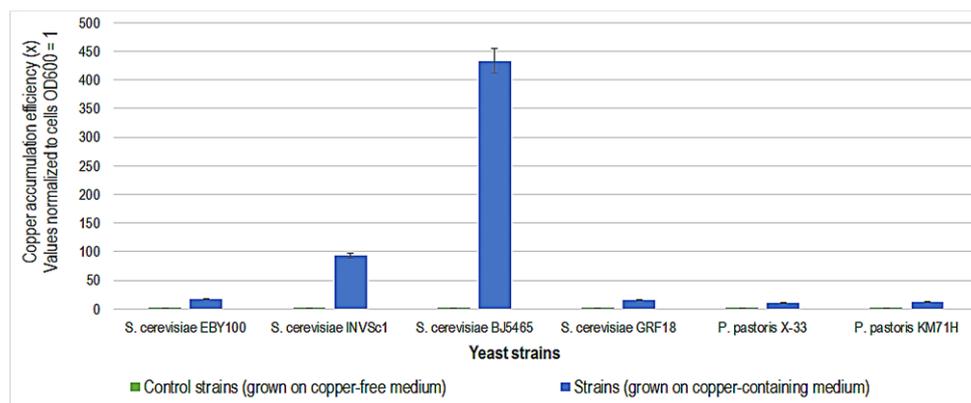


Figure 23. Copper accumulation efficiency (x) in different yeast strains, after 48 h of incubation at 30°C, in YPD copper containing-medium. Control strains were grown on copper-free medium.

The growth of bacteria strains, used in these experiments, was inhibited on 1 mM copper exposure, while yeast cultures were not affected at this concentration. These results were in accordance with the rapid test on YPD and LB solid media regarding tolerance on copper. ICP-OES analysis showed that *E. coli* RIL strain has the best copper accumulation efficiency (1250 times more effective than the control strain), while DH α and STAR strains is over 450 times more efficient. *E. coli* BL21 (DE3) OverExpress cells that internal expressed peroxidase had the best efficiency in the accumulation of copper. *S. cerevisiae* BJ5465 yeast strain is most efficient in the accumulation of copper among the six yeast strains used. The common genetic characteristics of the strains, that showed efficiency on copper accumulation, involve the protease deficiency and resistance to toxic protein production, which is a possible key for the successful accumulation of heavy metals. Genetic modified bacteria and yeasts can be successfully use for copper accumulation.

3.4.2. Isolation of bacterial strains resistant to copper

The tolerance to heavy metals (copper) was investigated using microbial strains isolated from soil samples collected from Tăușani – Boșneag tailing pond (Table 41 and Figure 24), during the second thematic excursion in Moldova Nouă area, on 02 July 2021 and also from samples collected in February. Herein, isolates from glycerol stock from -80°C were used (Table 42 and Figure 25).

METHOD

Soil samples collected from Tăușani – Boșneag tailing pond were suspended in distilled water and aliquots of the supernatant was inoculated on solid non-selective media containing 100 mg/L CuSO₄ in order to screen for microbial strains tolerant to copper.

RESULTS

As seen in Figure 24 and Table 41, from the samples collected in July, from Tăușani – Boșneag tailing pond soil, only one copper-tolerant strain was isolated.

Table 41. Screening from Cu²⁺ tolerance – samples collected from Tăușani – Boșneag tailing pond in July 2021

Sample ID	Location of the sample	Colony morphology	Bacterial strain code
S85	Soil near Boșneag tailings pond	No growth	-
S86	Soil at 200 m from Boșneag tailings pond (N-W direction)	White mucilaginous colony	WM1
S87	Soil at 400 m from Boșneag tailings pond (N-W direction)	No growth	-
S88	Soil at 600 m from Boșneag tailings pond (N-W direction)	No growth	-



Figure 24. Screening from microbial strain resistant to Cu^{2+} 100mg/L – isolates from samples collected in July 2021

Microbial strain isolated from Tăușani – Boșneag tailing pond soil in February 2021 and kept on glycerol stocks at -80°C were also tested for tolerance to copper, as same as described above.

As shown in Figure 2 and Table 2, only two of the strains tested were tolerant to 100 mg/L copper.

Table 42. Screening from Cu^{2+} tolerance – samples collected from Tăușani – Boșneag tailing pond in February 2021 (glycerol stocks kept at -80°C)

Sample ID	Location of the sample	Colony morphology	Bacterial strain code
S85 M2 1	Soil near Boșneag tailings pond	Yellow colony	Y1
S86 M2 1	Soil at 200 m from Boșneag tailings pond (N-W direction)	White colony	W1
S87 M2 1	Soil at 400 m from Boșneag tailings pond (N-W direction)	No growth	-
S87 M2 2		No growth	-
S88 1	Soil at 600 m from Boșneag tailings pond (N-W direction)	No growth	-
S88 2		No growth	-
S88 3		No growth	-

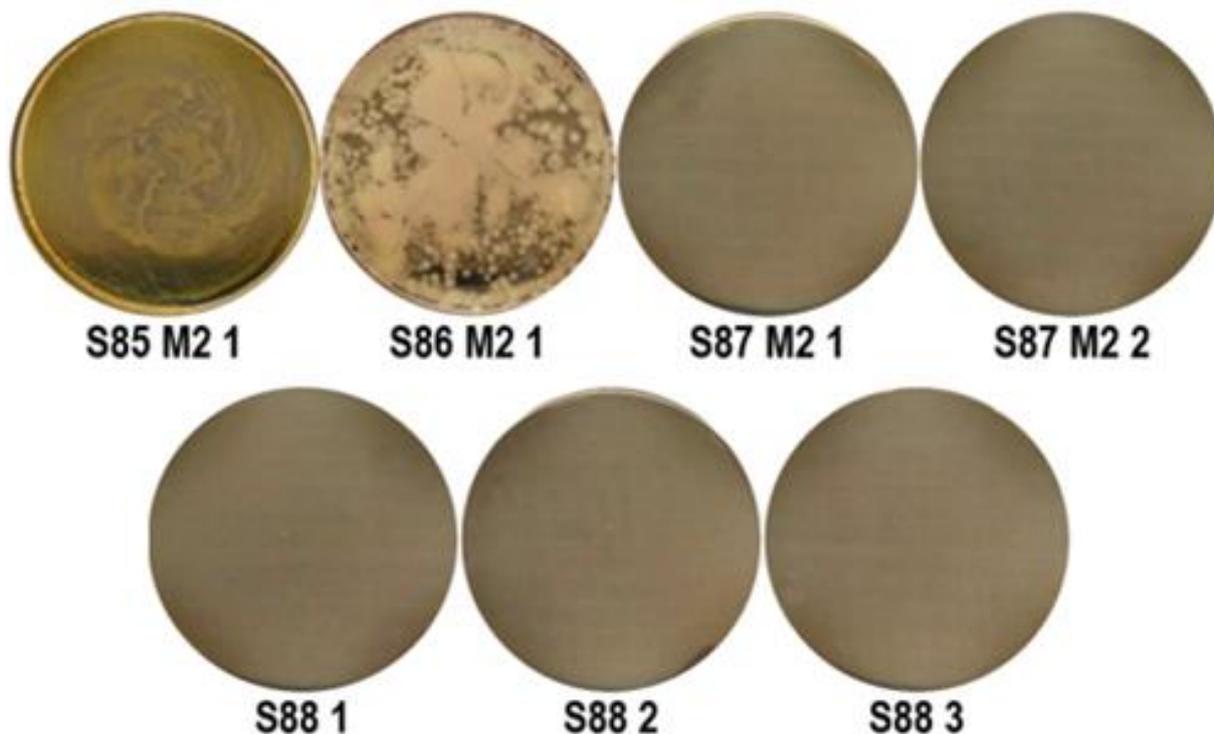


Figure 25. Screening from microbial strain resistant to Cu^{2+} 100mg/L – glycerol stocks with isolates from soil samples collected in February 2021

Environmental adaptability of bacterial strains

The adaptability to pH and salinity and the tolerance to heavy metal of the three bacterial strains (listed under the code WM1, Y1, W1) was investigated.

Tolerance concentrations to heavy metals

METHOD

The isolated strains were inoculated in LB solid media containing different concentrations of copper (100, 150, 200, 250, 300, 350, 400, 600 mg/L) and LB liquid media containing 100, 150, 200, 250, 300, 350, 400, 600, 800 mg/L CuSO_4 , and incubated at 25°C for 24 hours.

The healthy and well grown colonies were regarded as bacteria capable of resisting to the heavy metals (copper). For the liquid cultures, the optical density measured at 620 nm indicated the survival and the growth of bacteria in liquid media containing copper.

Adaptability to pH and salinity

METHOD

The pHs of LB liquid mediums were adjusted to pH 4.0, pH 5.0, pH 6.0, pH 7.0, and pH 9.0 with the solutions of 2 mol/L HCl and 2 mol/L NaOH.

The activated bacteria were inoculated in the liquid mediums at OD_{620nm} of 0.1 a.u., culturing under 28°C, 250 rpm for 18 h under linear agitation. The OD values of bacteria suspensions with different pHs were determined with automated microplate reader (Biotek Synergy H1) under 620 nm (OD₆₀₀). The same density of bacteria was inoculated in the LB liquid mediums containing 1, 2, 3, 5, and 7% of NaCl, culturing under 28°C, 250 rpm for 18 h in shaker.

The OD values of bacteria suspensions with different salinities were determined with spectrophotometer under 620 nm (OD₆₂₀).

RESULTS

As seen in Table 43, all bacterial strains survived at high concentration of copper. The growth of bacteria on solid media containing copper at different concentrations was possible up to 300mg/L for the Y1 strain, while WM1 and W1 strains were able to grow up to a copper concentrations of 250 mg/L.

Regarding the survival of bacteria in liquid media containing copper, the results showed that bacteria survived at even higher copper concentrations (400 mg/L for Y1 and W1 and 600 mg/L for WM1).

Table 43. Copper tolerance for bacterial strains isolated from Tăușani – Boșneag tailing pond samples

Sample ID	Bacterial strain code	Growth on solid media – copper concentration (mg/L)	Survival on liquid media – copper concentration (mg/L)
S 85 M2 1	Y1	300	400
S 86 M2 1	W1	250	400
S 86 1	WM1	250	600

Given the adaptability of bacteria to different environmental conditions (different pH values and salinity concentrations) [18], the results showed that all bacterial strains were able to grow even at high salinity concentrations and survived at all pH values tested (Table 44).

Table 44. Environmental adaptability of bacterial strains isolated from Tăușani – Boșneag tailing pond samples

Sample ID	Bacterial strain code	Survival in liquid media of different pH (4, 5, 6, 7, 9)	Survival on liquid media of different NaCl concentration (%)
S 85 M2 1	Y1	4, 5, 6, 7, 9	1, 2, 3, 5 and 7
S 86 M2 1	W1	4, 5, 6, 7, 9	1, 2, 3, 5 and 7
S 86 1	WM1	4, 5, 6, 7, 9	1, 2, 3, 5 and 7

3.4.3 Analysis of remediation samples collected from Moldova Nouă area

According to the Sample Collection Procedure (SCP) remediation samples were collected from Romania, from the Moldova Nouă area, although the collection and analysis of this kind of

samples is not a mandatory part of the A.T1.6 activity of this projects, “Treatment of wasted waters and acid mine drainage streams to remove copper and other pollutant metals”. In the SCP, it is mentioned that “The organisms that represent an interest in the remediation process are “inferior” organisms such as cyanobacteria, microalgae, other protozoa, nematodes or other organisms smaller than 1-2 mm, both aquatic and terrestrial. This procedure describes the methods used in the sampling of these organisms, but it is not mandatory that each of these organisms to be found in the samples”.

The organisms identified in the collected samples were not used in any bioremediation process due to the fact that these samples were not heavily polluted by mining activities. Even if some samples had a higher concentration of heavy metals, these did not represent a significant model for heavy mining pollution, thus these organisms were not adapted to such extreme environmental conditions.

The samples collected in the four sampling campaigns were analyzed under the microscope and the present organisms were photographed and identified if possible. Then samples were analyzed with the Lionheart™ FX automated microscope in order to identify organisms that might be of interest for bioremediation as described above. The water samples were pipetted directly on the microscope slide and the cover glass was placed on the drop of sample. For the soil and sediment samples, a drop of sterile distilled water was pipetted on the slide and with another pipette a small quantity of sample was smeared into the water drop. Then the drop was covered with a cover glass. The prepared slides were analyzed with the automated microscope.

It was observed that from the four sample types (surface water, groundwater, sediment and soil) only in the groundwater samples there were no identified organisms. In the soil samples only nematodes were observed, but in a very small number (Figure 26). The surface water and sediment samples had the most abundant organisms of a possible interest. The surface water samples (Figure 27) contained organisms such as green algae, protozoa and diatoms, while the sediment samples (Figure 28) contained diatoms preponderantly.

The surface water samples were most abundant in autumn, while the sediment samples were most abundant in spring. The organism type that was the most abundant in all samples are the diatoms.



Figure 26. Nematode observed in soil samples



Figure 27. Organisms observed in surface water samples

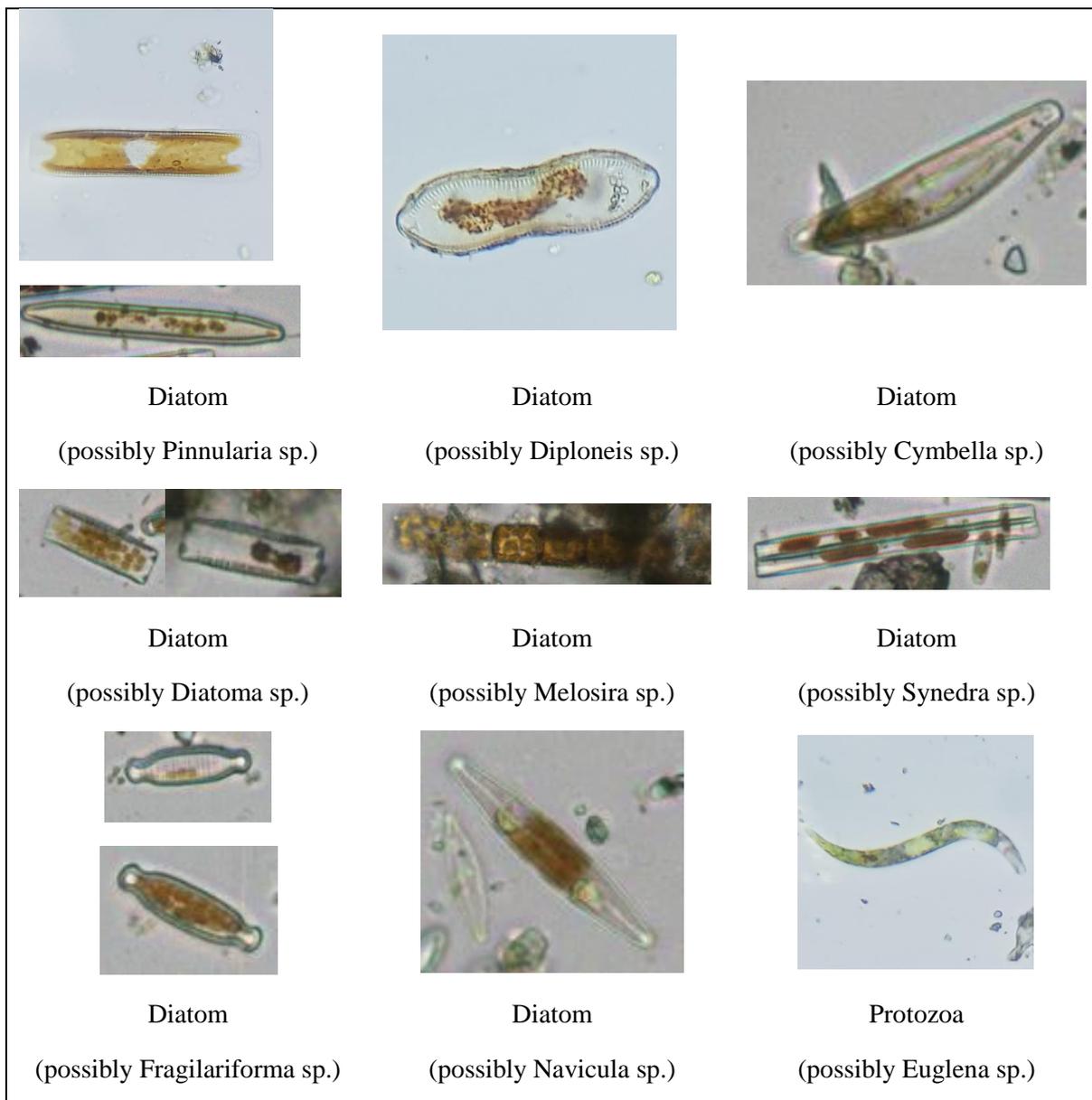


Figure 28. Organisms observed in sediment samples

3.4.4 Remedial solutions for waters polluted with heavy metals

Adsorption is an efficient method for removing heavy metals from polluted waters due to its affordable cost, simplicity of installation, the possibility of adsorbent regeneration and metal recovery and the lack of sediment that is formed in other methods. The process is suitable even when metal ions are present in a concentration of less than 1 mg / L. Adsorption is influenced by several factors, such as contact time, amount of adsorbent, temperature, adsorbent concentration, pH and the efficiency of the method increases with the increase of these factors [19].

Bioadsorption is an alternative to removing heavy metals from water using cheap and highly efficient biological materials. Biosorption is an ecological method allowing the use of inactive raw materials that can come from agricultural, industrial or plant waste [19,20].

Modified biopolymers, such as nanocellulose, chitin and chitosan, starch derivatives, alginate, gelatin, can significantly reduce the concentration of copper in water, are environmentally friendly, biodegradable, renewable and are easily found in nature.

Chitosan, a deacetylated derivative of chitin, is a natural, polysaccharide heteropolymer consisting of D-glucosamine and N-acetyl-D-glucosamine. It is a cheap and non-toxic biopolymer obtained from chitin, the second most abundant biopolymer in nature, after cellulose. Its adsorption capacity is due to the ability to chelate heavy metals such as copper. Thus, chitosan can be used for the removal of heavy metals from wastewater, the recovery of precious metals or the adsorption of waste metals from drinking water [20-23].

Alginate is a linear copolymer of α -L-guluronate (G) and α -D-mannuronate (M), which constitutes 10–40% of the dry weight of all species of brown algae. Due to its ability to form stable structures, cross-linked alginate has been used for the removal of heavy metal from wastewater [24].

Several analytical methods such as atomic absorption spectrometry (AAS), UV-Vis spectrometry, inductively coupled plasma atomic emission spectrometry (ICP-AES) and high performance liquid chromatography (HPLC) have been used to determine the concentration of copper or other metals in the environment. Although these methods are very sensitive, they have certain disadvantages such as the need for expensive instruments, qualified personnel and complex preparation of samples. Moreover, their sampling and transport to the laboratory to perform the analysis can lead to contamination of the sample and a long time for analysis.

Potentiometry is an electrochemical analysis technique that can be used to determine the concentration of an analyte in a solution, and in the case of this work, copper in wastewater. The advantages of this method are the wide range of concentrations that can be determined (10^{-7} - 1 M), high accuracy, easy to handle equipment, low cost, selectivity, low detection limit, the fact that colored or opaque solutions can be used, and its applicability in situ. In addition, the potentiometric method allows the simultaneous determination of heavy metals in a sample.

The aim of this experimental study was to determine the ability of alginate and chitosan microspheres in removing copper (II) ion from aqueous medium. The concentration of copper ions (Cu^{2+}) in different solutions, after their adsorption on different amounts of dehydrated microspheres, was determined by potentiometry. The influence of the contact time between the biopolymers microspheres and the $\text{Cu}(\text{NO}_3)_2$ solutions and the effect of the amount of adsorbent on the adsorption process were studied.

For this purpose, $\text{Cu}(\text{NO}_3)_2$ solutions with a concentration between $1.5 \cdot 10^{-4}$ M and $6 \cdot 10^{-4}$ M were prepared. For each concentration, 0.050 and 0.025 g of chitosan and 0.01 g and 0.02 g of alginate dehydrated microspheres, weighed to the analytical balance to 5 decimal places, were used as adsorbent. The potentiometric method was chosen due to its ease, high accuracy and short time required for analysis.



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MATERIALS AND METHODS

MATERIALS

Sodium alginate, low molecular weight chitosan (LMW), calcium chloride (CaCl_2), glacial acetic acid (CH_3COOH) and copper (II) hemi nitrate (pentahydrate) ($\text{Cu}(\text{NO}_3)_2 \cdot 2,5 \text{H}_2\text{O}$) were purchased from Sigma Aldrich.

Preparation of biosorbent beads

Calcium alginate beads

A sodium alginate solution was dropped into 2% calcium chloride solution through the tip of the syringe. The drops of sodium alginate solution gelled into beads upon contact with calcium chloride solution. The beads were kept in contact with calcium chloride solution till the formation of insoluble and stable beads. Water soluble sodium alginate was converted to water insoluble calcium alginate beads using CaCl_2 solution. The beads were rinsed with distilled water and dried at room temperature till reach constant weight. It was observed that the size of the beads decreases on drying (Figure 29).

Chitosan beads

The microspheres were formed by dripping the chitosan solution, using a syringe, into the 0.5 M NaOH solution, under continuous stirring. The formed microspheres were washed with distilled water and allowed to dry at room temperature. It was observed that the chitosan microspheres significantly decreased after drying, and their color changed from white to yellow (Figure 30).

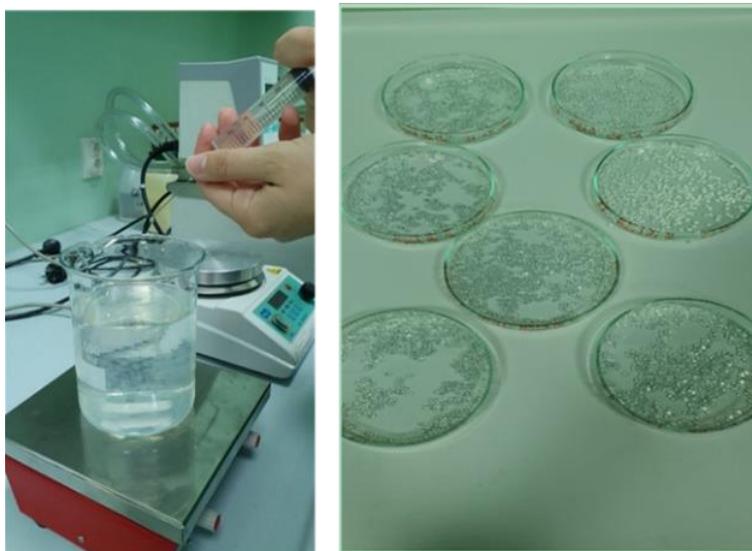


Figure 29. Preparation of alginate beads

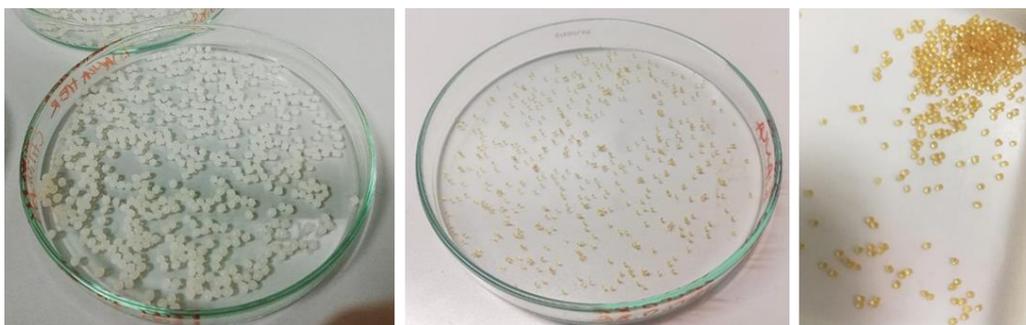


Figure 30 Chitosan beads

Potentiometric determination of Cu (II) ions

The potential of the copper solutions was measured using the Mettler Toledo SevenCompact™ S220 millivoltmeter at a constant temperature of $25 \pm 0,1$ (Figure 31). The equipment was purchased with funds from project RORS 337.



Figure 11. Mettler Toledo SevenCompact™ S220 Millivoltmeter

The potentiometric measurements were performed to determine the concentration of copper in synthetic solutions, before and after adsorption on biopolymer beads. $\text{Cu}(\text{NO}_3)_2$ solutions of concentrations between $1.5 \cdot 10^{-4}$ and $6 \cdot 10^{-4}$ M were prepared by diluting the stock solution with a concentration of 10-1 M. In neutral solutions, the concentration of copper ions can be measured up to 10^{-8} mol / L (6×10^{-4} mg / L). Also, at determinations below 10^{-5} mol / L (0.6 mg / L) great care is required not to contaminate the sample or to avoid adsorption of copper ions on the vessel walls.

Copper ion selective electrode

The copper selective ion electrode consists of a detection element protected by an epoxy material. When the sensing element is in contact with a solution containing copper ions, the electrode potential varies along the sensing element. The potential, which depends on the level of free copper ions in the solution, is measured in relation to a reference potential with a digital pH / mV-meter or an ISE-meter [25].

The copper electrode measures the activity of copper ions in the same way that a pH electrode measures the activity of hydrogen ions. To measure the activity of copper ions, copper standards are assigned activity values without adjusting the pH or ISE in the samples.

The coefficients of ionic activity are variable and largely depend on the total ionic strength. The ionic strength of a solution is determined by all the ions present. The specifications of the electrode used are:

Concentration range is 6×10^{-4} mg / L to 6354 mg / L (10^{-8} mol / L to 0.1 mol / L);

pH range is from 2 to 12;

Temperature range is from 0 to 80°C;

Electrode resistance is less than 1MΩ;

Calibration curve

In order to attain the calibration curve, $\text{Cu}(\text{NO}_3)_2$ solutions of concentrations in the range $2 \cdot 10^{-5}$ - 10^{-3} M were prepared and their potential was measured. The values of the obtained potentials were represented graphically according to $\log C$.

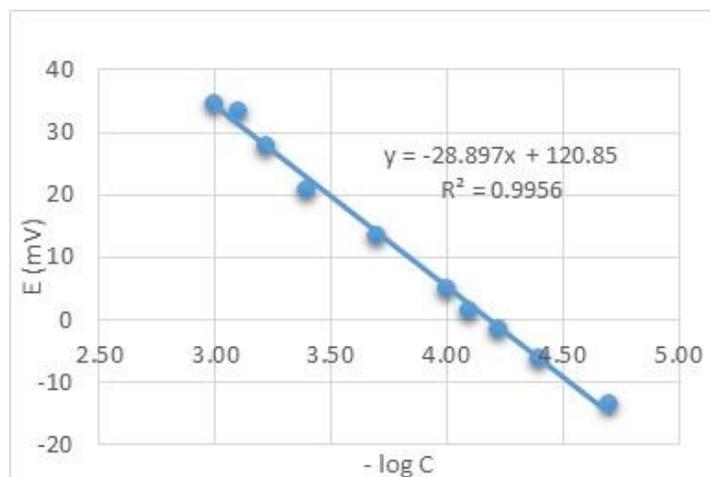


Figure 32. Calibration curve of Cu (II) solutions

RESULTS AND DISCUSSIONS

The adsorption study was performed in 50 mL Cu (II) solutions of different concentrations and 1mL ISA (solution for adjusting the ionic strength). The amounts of microspheres used for adsorption were 0.01 g and 0.02 g - alginate beads and 0,025 g and 0,050 g - chitosan beads. After the addition of the biosorbent, the mixture was stirred for various periods of time. The concentration of Cu(II) ions was determined potentiometrically using the Mettler Toledo SevenCompact™ S220 millivoltmeter. The study was performed at room temperature at a stirring speed of 300 rpm.

Adsorption capacity

The amount of Cu (II) ions adsorbed at equilibrium was calculated using the equation:

$$\text{Adsorption capacity (q}_e) = (C_0 - C_e) V / W$$

where C_0 - the initial concentration of Cu (II) ions (ppm); C_e - final concentration of Cu (II) ions (ppm), V - volume of Cu (II) solution (mL); W - mass of chitosan microspheres (g)

The effect of contact time

Stirring time is the time required for adsorption to reach equilibrium. Figures 34 and 35 show that in the first 20 minutes a rapid adsorption took place, and the adsorption equilibrium was reached after 150 minutes. Therefore, the optimum stirring time for both biosorbents is 150 minutes.

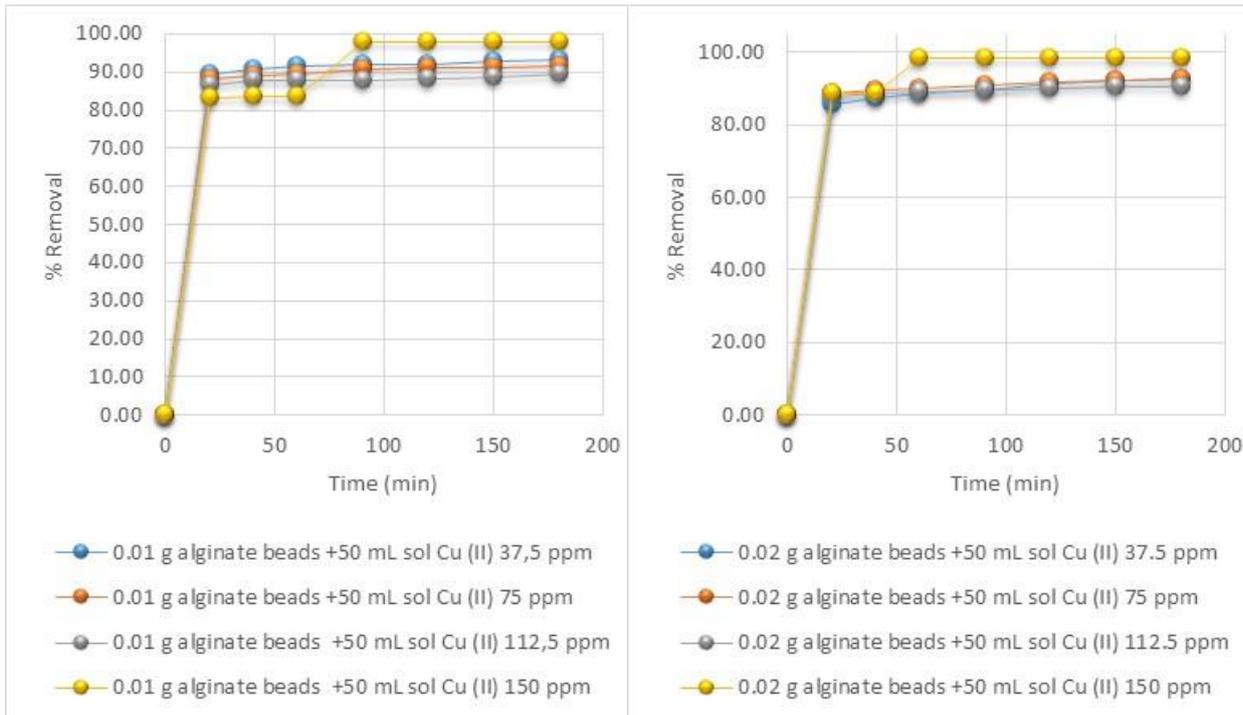


Figure 33. Yields of adsorption processes when using 0.01 g of algininate microspheres and 50 mL of Cu (II) solution of different concentrations

Figure 34. Yields of adsorption processes when using 0.02 g of algininate microspheres and 50 mL of Cu (II) solution of different concentrations

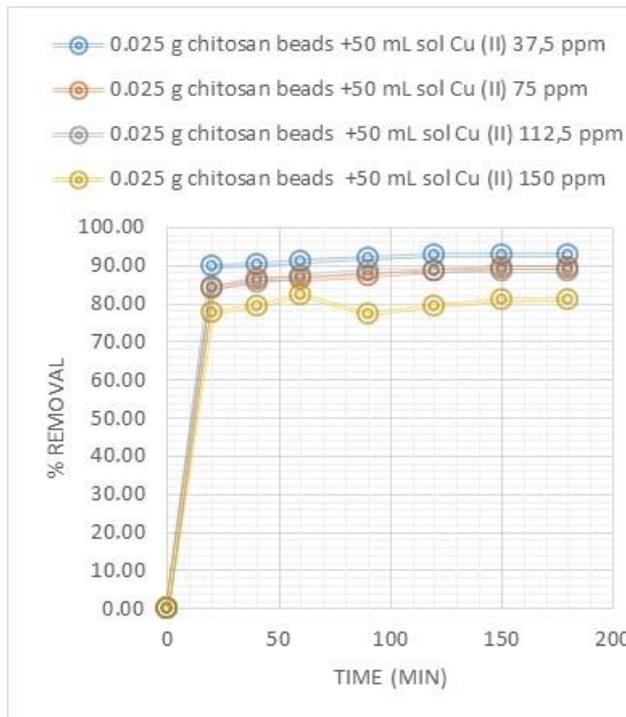


Figure 35. Effect of contact time on the yield of Cu (II) ion adsorption process on 0.025 g of chitosan microspheres

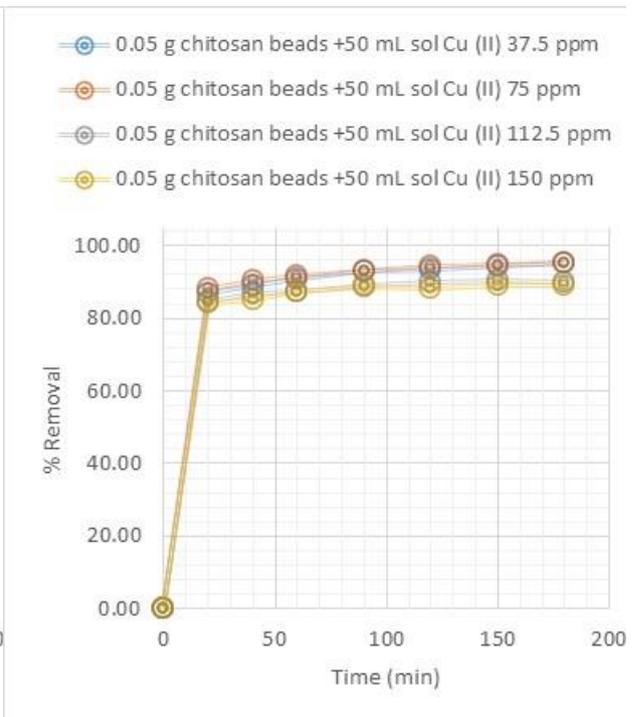


Figure 36. Effect of contact time on the yield of Cu (II) ion adsorption process on 0.05 g of chitosan microspheres

The influence of the amount of adsorbent

The dependence of the adsorption of Cu (II) ions on the amount of adsorbent used was also studied and the results are shown in Figures 9 - 12. Different amounts of biosorbent (0.01 g and 0.02 g of alginate microspheres, 0.025 and 0.05 g chitosan beads) were used for the adsorption of Cu (II) ions, and the rest of the experimental parameters were kept constant (stirring time, stirring speed, etc.). Figures 37 - 40 show that when the amount of adsorbent is doubled, the adsorption capacity decreases. This result can be explained by the aggregation of biosorbent and the unsaturation of the adsorption sites. In addition, an equilibrium is established between the Cu (II) ions in the solution and those on the adsorbent surface.

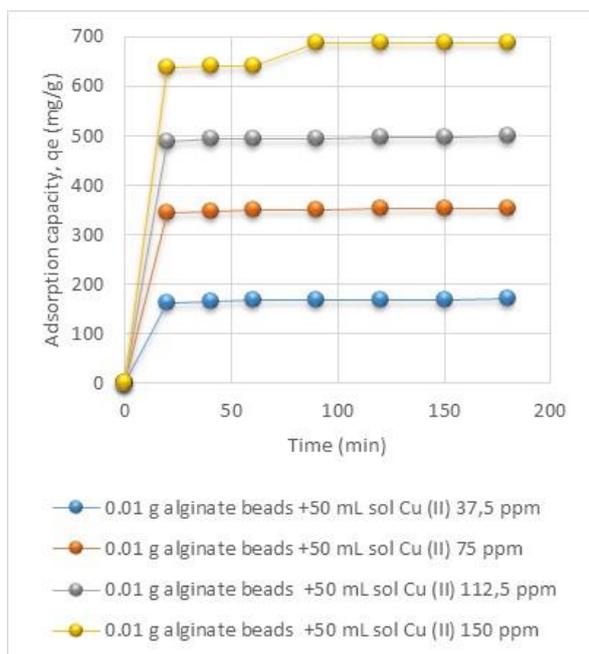


Figure 37. Adsorption capacity of alginate microspheres when using 0.01 g alginate microspheres and 50 mL Cu (II) solution of different concentrations

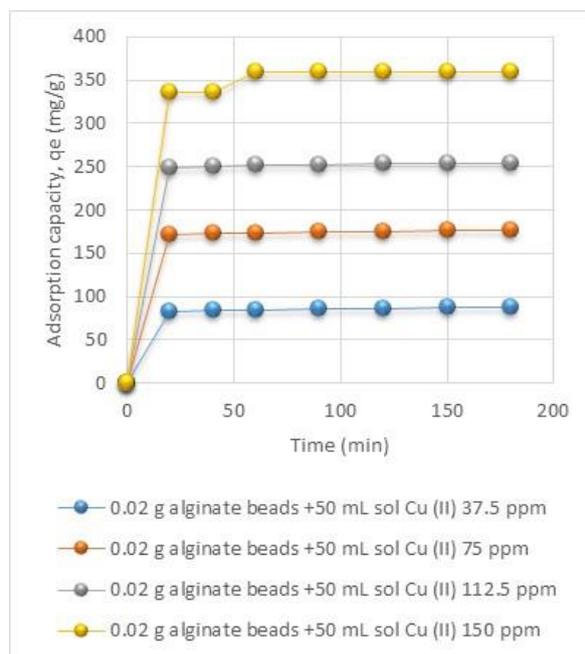


Figure 38. Adsorption capacity of alginate microspheres when using 0.02 g alginate microspheres and 50 mL Cu (II) solution of different concentrations

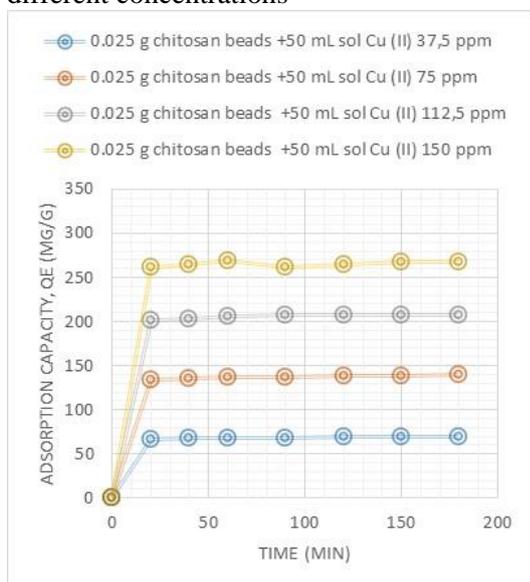


Figure 39. Adsorption capacity of chitosan microspheres when using 0.025 g chitosan microspheres and 50 mL Cu (II) solution of different concentrations

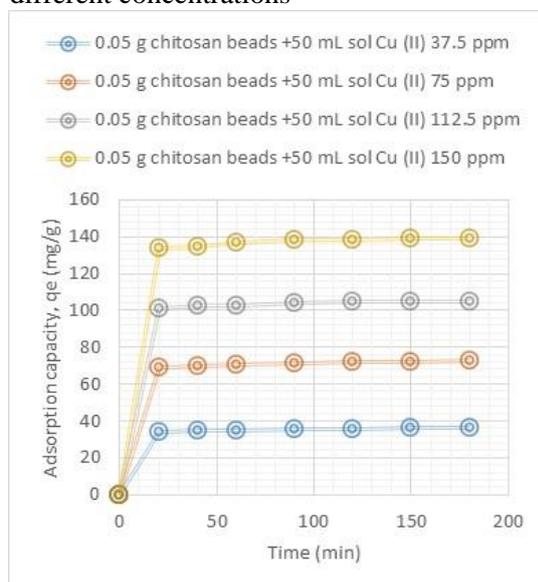


Figure 40. Adsorption capacity of chitosan microspheres when using 0.050 g chitosan microspheres and 50 mL Cu (II) solution of different concentrations

Adsorption isotherms

Equilibrium isotherms are essential in describing the interactions between adsorbent and solute and are also essential in the design of the adsorption system. Two of the most widely used theories were used in this study, namely Langmuir and Freundlich. The linearized form of the Langmuir equation is

$$C_e / q_e = 1 / (Q_0 b) + C_e / Q_0$$

where C_e - the equilibrium concentration of metal ions in the solution (ppm); q_e - is the amount of metal ions adsorbed per unit mass of adsorbent at equilibrium (mg / g); Q_0 - is the amount of metal ions that form the saturated monolayer (mg / g) and b - the Langmuir constant correlated with the affinity for the binding sites (mL / mg) and which is a measure of the adsorption energy.

This theory is based on the assumption that the binding sites are homogeneously distributed on the surface of the adsorbent and have the same adsorption affinity, forming a saturated monolayer of adsorbed molecules. The binding to these adsorption sites may be chemical or physical but must be strong enough to prevent desorption of the adsorbed molecules [26,27]. The linearized form of the Freundlich equation is:

$$\log q_e = \log KF + 1 / n \log C_e$$

where KF and n are Freundlich constants indicating the adsorption capacity (mg / g) and its intensity, respectively. In Freundlich's theory it is considered that the adsorbent has a heterogeneous surface and has different affinities for the adsorbed molecules [26]. The values of the Langmuir and Freundlich constants and the correlation coefficients for the adsorption of Cu(II) ions on alginate beads are presented in Table 45.

Table 45. Parameters of adsorption isotherms for Cu (II) on alginate microspheres

Weight of the alginate microspheres (g)	Concentration of Cu(II) solution	Langmuir				Freundlich		
		R2	Q (mg/g)	b (L/mg)	RL	R2	KF	n
0.01 g	37.5 ppm	0.999	151.515	3.667	0.007	0.982	183.738	12.019
	75 ppm	1	322.581	3.100	0.004	0.988	387.704	15.625
	112.5 ppm	0.999	454.545	1.467	0.006	0.993	583.848	13.175
0.02 g	37.5 ppm	0.999	84.034	4.958	0.005	0.989	91.939	13.245
	75 ppm	0.999	163.934	3.389	0.004	0.995	192.752	15.128
	112.5 ppm	1	232.558	1.792	0.005	0.998	294.509	13.210

Based on the correlation coefficients, it can be stated that the adsorption process on alginate microspheres is well described by both mathematical models (Langmuir and Freundlich), indicating that monolayer and heterogeneous adsorption processes coexist, but monolayer adsorption is probably dominant.

The RL factor ($1/(Q_0b)$) indicates whether the adsorption is favourable ($0 < RL < 1$), unfavourable ($RL > 1$), linear ($RL = 1$) or irreversible ($RL = 0$). The RL values are between 0 and 1, therefore the adsorption of Cu (II) ions on alginate microspheres is favourable.

Experimental data obtained on the adsorption of Cu (II) ions on chitosan microspheres were processed and plotted. The correlation coefficients obtained for Langmuir model are 0.999 and even 1 which demonstrates that the adsorption process of Cu (II) ions on the chitosan microspheres proceeds according to this model.

The correlation coefficients obtained for Freundlich model are over 0.97 which shows that the adsorption process of Cu (II) ions on the chitosan microspheres is well described also by this model. Since both models are able to represent the data satisfactorily, but the Langmuir model offers a better representation, it can be stated that monolayer adsorption is dominant but there is also a heterogeneous adsorption.

The values of the Langmuir and Freundlich constants are presented in Table 46. In the case of adsorption of Cu (II) ions on chitosan microspheres, the RL factors obtained are between 0 and 1, therefore the process is favourable.

Table 46 Langmuir, Freundlich constants and correlation coefficients (R2) for the adsorption of Cu (II) ions on chitosan microspheres

Amount of chitosan beads (g)	Concentration of Cu(II) solution	Langmuir				Freundlich		
		R2	Q (mg/g)	b (L/mg)	RL	R2	KF	n
0.025 g	37.5 ppm	0.999	625	0.372	0.067	0.982	74.816	12.610
	75 ppm	0.999	125	1.778	0.007	0.982	159.147	12.300
	112.5 ppm	1	188.679	1.178	0.007	0.998	254.038	10.570
	150 ppm	0.999	238.095	0.677	0.009	0.997	353.671	9.389
0.050 g	37.5 ppm	0.999	33.003	8.189	0.003	0.979	36.702	17.006
	75 ppm	0.999	66.667	5	0.003	0.978	76.067	18.726
	112.5 ppm	0.999	95.234	1.418	0.006	0.995	124.394	11.682
	150 ppm	0.999	123.456	0.870	0.008	0.997	174.783	10.384

The results showed that the contact time required to reach equilibrium is about 150 minutes. The adsorption of copper ions on the alginate microspheres is well described by both the Langmuir isotherm and the Freundlich, so it can be stated that the adsorption takes place predominantly in the monolayer but there is also a heterogeneous adsorption.

The obtained results showed that both chitosan and alginate can be used as economical, ecological and efficient adsorbents in the removal of Cu (II) ions from wastewater and their processing in the form of dehydrated microspheres ensures a larger area of adsorption surface and therefore, a higher adsorption capacity.

4. CONCLUSIONS

In this study, the possibility for a new approach of metal ion removal was investigated. This approach included the two-step neutralization and adsorption methods and was applied to the real AMD water generated from the copper mines in the southeast Serbia. As for the neutralization process, the experiment results revealed that two-step pH control neutralization and precipitation method was effective and recovered Fe and Cu separately in the sludge generated along the process. It was confirmed that the AMD water quality was drastically improved. Manganese ions still remained in the treated AMD water after two-step neutralization. In the adsorption removal tests. Mn was removed at a higher pH. Thus, it is expected that the combination process with the two-step neutralization and adsorption methods will contribute to not only the AMD purification but also to Cu recycling. From the remediation experiments result that microorganism as indigenous bacteria found in mining waters, as well as genetically modified bacteria and yeast, are efficient candidates for removing copper from metal polluted waters. Chitosan and alginate polymers were also successfully used as adsorbents of copper ions from wastewaters.

All the collected data regarding the possible remediation methods to be used for cleaning water contaminated by mining pollution are publicly available within the knowledgebase that was created in the project [27] following the link: <http://www.elearning-chemistry.ro/rosnet2/knowledge-base/>.

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