# CHITOSAN-ASSISTED SOIL WASHING: INFLUENCE OF CONCENTRATION AND LIQUID-TO-SOLID RATIO ON HEAVY METAL REMOVAL

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***ABSTRACT:*** *The process of mining has led to metal contamination of soils, which is an environmental concern in Romania, and especially in the Apuseni Mountains region, where considerable extractive activity has been conducted that has left behind large amounts of tailings and waste materials. The purpose of this study was to investigate the effect of chitosan-based washing solutions on the removal of copper (Cu) and lead (Pb) from contaminated soil collected from the Larga de Sus mining site (Alba County, Romania). Soil washing was conducted using chitosan solutions with various chitosan content (0%, 2% and 5% w/v) and at a liquid-to-solid ratios (mL:g) of 5:1 and 8:1. The washing process was done using a mechanical stirrer for up to 40 hours, and the concentration of metals was measured by Atomic Absorption Spectrometry (AAS). Results demonstrated that both chitosan concentration and washing solution volume strongly influenced metal removal efficiency. The maximum rate of metal removal was obtained using the 2% chitosan solution and an 8:1 liquid-to-solid ratio, resulting in the removal of Cu and Pb by 74.5% and 79.6%, respectively. Copper concentration after soil washing dropped below the Romanian regulatory intervention limit while Pb concentration remained slightly over that limit. Overall, the results indicate that chitosan is a natural, green, and effective chelating agent for soil remediation, with a potential for large scale and field applications to remediate heavy metal contaminated sites.*

***Keywords:*** *chitosan; soil washing; sustainable remediation; heavy metal removal;*

## Introduction

The use of outdated technologies in the extractive industry, combined with limited concern for environmental protection in Romania, has led to soil pollution with metals across approximately 200,000 hectares [1]. Among other regions from Romania, the Apuseni Mountains are well known for their mining activities, which have been ongoing for more than two millennia, mainly for the extraction of non-ferrous and particularly gold–silver ores. Mining operations at Pârâul Fericel, Dealul Ungurilor, Techereu, Stănija, and Alma ul Mare date back to the Roman period [2].

As a result, more than 550 tailings dumps have been constructed across Romania to date, covering an area of about 800 hectares and storing over 200 million m³ of waste materials. The environmental consequences of these dumps' facilities are significant and occur during and after mining. These consequences include soil erosion, changes in groundwater flow, acid mine drainage generation, reduced fertility of agricultural land, and reduced soil quality because of heavy metal contamination [3]. Due to their toxicity, persistence, and bioaccumulation, heavy metals have received increasing attention from scientists and regulators over the past few decades [4,5]. Heavy metals will have limited effect on soil biota and the environment as long as they remain strongly bound to soil particles and have low mobility. However, when certain soil conditions favor the release of heavy metals into the soil solution, there is a direct risk of plant and food product contamination and subsequent transfer of these metals along the food chain [6].

Given the wide extent and long-term implications of heavy metal pollution on the environment and human health, the implementation of suitable soil decontamination strategies is imperative. According to Mathew (2005), four main approaches can be applied to heavy metal contaminated soils: i) restricting land use without applying any decontamination process; ii) complete or partial encapsulation of the contaminated soil; iii) excavation and relocation of the contaminated soil to a controlled landfill; iv) soil remediation (either in-situ or ex-situ treatment) [7].

To avoid land-use restrictions, as is the case of the first three approaches, the removal of heavy metals from polluted soil is necessary. Different technologies can be used to remove heavy metals applicable both ex-situ and in-situ (e.g. immobilization, soil washing, phytoremediation, electrokinetic remediation, vitrification). Currently, soil washing has become one of the most widely adopted techniques worldwide for remediating metal-contaminated soils, with numerous pilot- and full-scale applications reported in the literature [8]. There are many reasons for the widespread application of this technology, including high removal efficiency; relatively short treatment times; applicability for many soil types; and relatively low operating costs. This method is also considered a permanent remediation solution because once the soil is treated, it can then be re-deposited on land at a low cost [9].

Nevertheless, large-scale, pilot-scale, and laboratory-scale applications developed so far, although effective, still face challenges. Most rely on rudimentary technologies and chemical washing agents that are expensive and may cause secondary environmental impacts. These agents can alter the soil's physicochemical and microbiological properties, significantly hindering subsequent soil recultivation [10-13]. Therefore, soil washing remains a promising and viable remediation technique if the washing solution used is natural, efficient, readily available, environmentally safe, and does not adversely affect soil characteristics [14].

Accordingly, this research aimed to evaluate the effectiveness of a washing solution derived from a natural material—chitosan extracted from shrimp shells—in removing lead (Pb) and copper (Cu) from real contaminated soil collected from the "Larga de Sus" mine (Alba County, Romania). The influence of several operational parameters, including the washing solution concentration, liquid-to-solid ratio (mL:g), and washing time, was investigated.

## Materials and methods

The soil used in the washing experiments was collected from a pasture area located downstream of the "Larga de Sus" gallery situated in Alba County, Romania. The "Larga de Sus" mining gallery, was established in the early 1960s and is currently in the closure stage. Although mining operations ceased in 2006, the area is highly polluted with metals. Two nearby tailings deposits (Figure 1), covering approximately 3.2 ha and containing around 50 tons of waste rock, contribute to metal dispersion in the environment [15]. Also, the proximity of these waste deposits to residential and agricultural areas amplifies the environmental and health risks.

After the closure of the "Larga de Sus" mining gallery, various materials and wastes were improperly deposited on the site. The materials, which resulted from the closure works or previous extraction activities, were not properly disposed of and included, among others, several kinds of disassembled batteries casings, wood waste, metal parts, and other tech rubbish. They all contribute to environmental pollution, primarily soil pollution.

As well, the site still has various abandoned pieces of equipment, dilapidated buildings, and closed platforms that give the place a very unattractive visual impact (Fig. 1).

Fig.1. Tailings deposit at the "Larga de Sus" mining site

Heavy metal concentration in the collected sample was determined by Atomic Absorption Spectrometry (AAS), and the corresponding results are detailed in a previously published work [16].

Chitosan from shrimp shells (Sigma-Aldrich) was used to prepare 2% and 5% solutions for the soil washing experiments. Chitosan is chemically classified as (1-4)-2 amino-2-deoxy-β-D glucan and is derived from chitin (the second most abundant polysaccharide behind cellulose). Natural sources of chitin include crustacean shells, insect cuticles and algal tissues [17].

Soil washing tests were carried out under continuous stirring at room temperature, using a liquid-to-solid ratio of 5:1 and 8:1 (mL:g) and washing solutions containing 0%, 2%, and 5% chitosan. Contact times extended up to 40 hours. After each treatment, soil was collected from the washing solution using filtration, rinsed in distilled water and dried at 95°C. Soil samples were digested with HCl and HNO and analyzed for Pb and Cu using Atomic Absorption Spectrometry (AAS). Each analytical sample was analyzed in duplicates and increments of the mean of the duplicates were reported.

The metal removal rate was calculated using Equation (1):

$$\textit{Metal removal rate}\ (\%) = \frac{C_{initial} - C_{final}}{C_{initial}} \cdot 100 \quad (1)$$

where $C_{initial}$ and $C_{final}$ represent the initial and final concentration of metals in the soil, respectively [18].

## Results and discussion

The efficiency of chitosan-based washing solutions in removing Cu and Pb from contaminated soil sample collected from the Larga de Sus mining area was evaluated at two liquid-to-solid ratios (5:1 and 8:1) and at three chitosan concentration (0%, 2%, and 5%). The results are presented in Figures 2–5 and discussed below.

At a liquid-to-solid ratio of 5:1, the addition of chitosan to the washing solution was effective for removing Cu from the soil (see Figure 2). Increasing the chitosan concentration from 2% to 5% resulted in a consistent and significant decrease in Cu content after 4 hours of agitation, thereby confirming the strong effect of chitosan dosage on metal mobility in contaminated soil samples. A substantial increase in copper mobilization was observed after the 6 hours of contact time, whereby the Cu concentration in soil (223.58 mg·kg$^{1}$) decreased by 99.47 mg·kg$^{1}$ as compared to the 2% washing solution. After 24 hours, Cu concentration decreased with increasing chitosan concentration from 424.81 mg·kg$^{1}$ to 301 mg·kg$^{1}$ for the 2% solution, and to 201 mg·kg$^{1}$ for the 5% solution. This steady decrease confirms that Cu removal was time- and concentration-dependent. When the 5% chitosan-based solution was used, a removal rate of nearly 53% from the initial total Cu content was observed after 24 hours (Fig. 2).

Importantly, at the end of the experiment, the Cu concentration fell slightly below the intervention threshold (200 mg·kg$^{1}$) established by the Romanian regulation [19], confirming the effectiveness of the 5% chitosan solution in remediating the contaminated soil under the investigated conditions.

This represents a removal rate of about 55% of the initial Cu content and this finding is particularly significant because it demonstrates that the treatment successfully brought Cu concentration to safe levels for soil quality restoration.

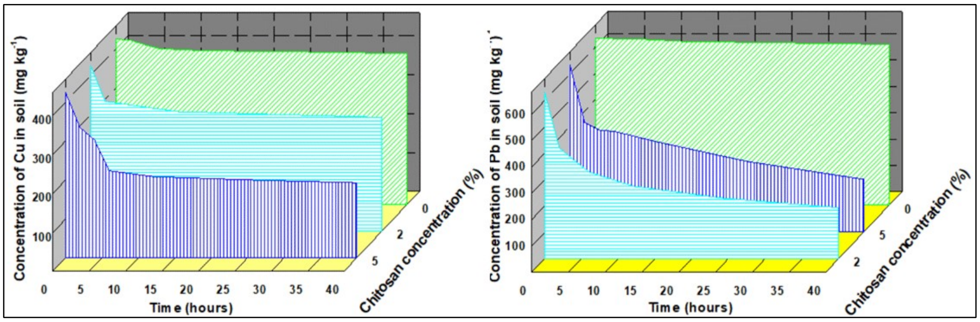


Fig. 2. Effect of chitosan concentration in the washing solution on Cu and Pb content in soil (liquid-to-solid ratio of 5:1)

The observed enhancement in Cu desorption with increasing chitosan concentration could be attributed to the presence of a greater number of amino and hydroxyl groups available for metal binding, which promote complexation and chelation reactions [20].

In contrast, lead exhibited a different pattern of behavior compared to copper. Although the presence of chitosan exhibited greater enhancement of Pb mobilization in comparison to the control (0% chitosan), the greatest lead removal occurred when the 2% solution was used, rather than the 5% solution. After 24 hours, concentration of Pb decreased from 633.05 mg·kg $^{-1}$ to 231 mg·kg$^{-1}$ when the 2% solution was used and decreased to 268 mg·kg$^{-1}$ in the case of 5% solution, representing removals of nearly 64% and 58% respectively. These removal rates demonstrate substantial Pb desorption, but lead concentration remains above the intervention threshold (100 mg·kg$^{-1}$), suggesting lead is more strongly retained in soil than copper and thus more difficult to desorb.

With an increase in the volume of washing solutions to liquid-to-solid ratio of 8:1, the total removal efficiencies for both metals were significantly improved (Fig.3).

After 24 hours, the Cu concentration decreased from 424.81 mg·kg$^{-1}$ in soil, to 190 mg·kg $^{1}$ in the case of 5% chitosan solution, and to 209 mg·kg $^{1}$ in the case of a 2% chitosan solution. After 40 hours of washing, Cu concentration decreased more to 169 mg·kg$^{-1}$ (5% chitosan) and 108 mg·kg$^{-1}$ (2% chitosan), both below the intervention threshold (200 mg·kg$^{-1}$). The value obtained with 2% chitosan solutions was close to the alert threshold (100 mg·kg$^{-1}$), suggesting an efficient Cu removal process.

At this higher washing volume, 2% chitosan solution proved more effective than 5%. This behavior may arise from competition for binding sites, where at a higher chitosan concentration, some polymer chains may form complexes with other ions in soil (e.g. Fe, Ca, Al) and limit the availability of active sites for Cu [20].

The amount of lead that was removed also increased with washing solution volume, but less significantly than Cu. After 24 hours had passed, lead concentration decreased from 633.05 mg·kg$^{-1}$ to 196 mg·kg$^{-1}$ (2%) and 241 mg·kg$^{-1}$ (5%). Following a 40-hour period, Pb levels were reduced to 128 mg·kg $^{1}$ with 2% chitosan and 218 mg·kg$^{-1}$ with 5% chitosan. While this is an impressive result, it was still above acceptable limits. This supports our hypothesis that lead forms stronger bonds with organic soil matter.

Taking into account that better results were obtained in the case of using 2% chitosan based solution, the effect of the washing solution volume was further analyzed at a constant chitosan concentration of 2% (Fig. 4).

Increasing the liquid-to-solid ratio from 5:1 to 8:1 led to a marked enhancement in copper desorption from the soil. After 24 hours, Cu concentration decreased to 301 mg·kg $^{1}$ (5:1) and to 209 mg·kg$^{-1}$ (8:1), indicating an increase of more than 20% in the removal rate. At the end of the experiment, the final Cu concentration at a liquid-to-solid ratio of 8:1 was well below the intervention threshold (200 mg·kg$^{-1}$) and close to the alert threshold (100 mg·kg$^{-1}$) coresponding to a reduction of 74.5% of Cu from the soil. The improvement can be attributed to better contact between the washing solution and soil particles, which enhances mass transfer and diffusion processes, in line with previous studies [13,21, 22].

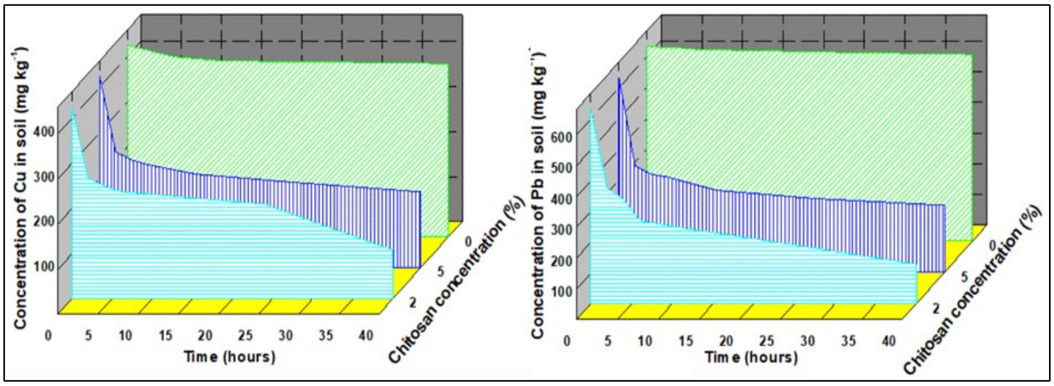


Fig. 3. Effect of chitosan concentration in the washing solution on Cu and Pb content in soil (liquid-to-solid ratio of 8:1)

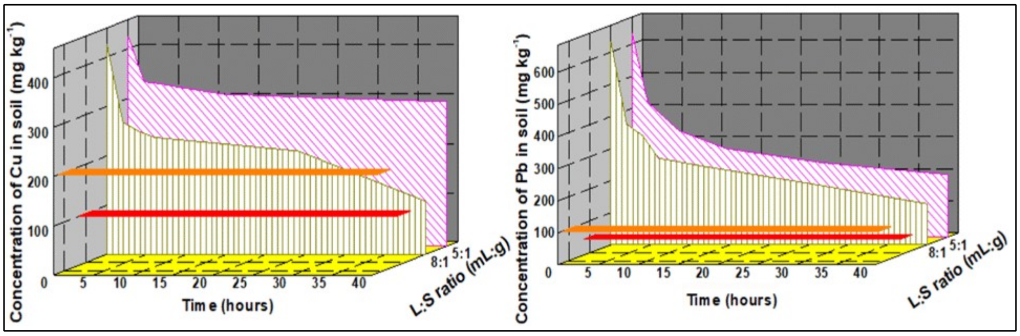


Fig. 4. Effect of the liquid-to-solid ratio on Cu and Pb concentration in soil when 2% chitosan-based washing solution was used.

For Pb, a moderate improvement was observed with increasing washing solution volume. The Pb concentration decreased from 633.05 to 194 mg·kg$^{-1}$ (5:1) and 128.8 mg·kg$^{-1}$ (8:1) during the agitation period. Thus, Pb decreased more than 3-fold with respect to its original concentration. Final Pb levels remained, however, higher than the threshold for intervention, suggesting Pb is less mobile for all conditions evaluated. The persistence of Pb in soil, even under the conditions that gave the best results, highlights the need for extended treatment times or the use of combined remediation strategies.

Figure 5 shows the Cu and Pb removal rates after agitation for 40 hours for all variations of chitosan concentration and liquid-to-solid ratio. The results indicated that both metals were more mobile in the washing solution mixed with chitosan than without. The best removal rates were achieved with the 8:1 liquid-to-solid ratio and 2% chitosan (74.5% and 79.6% for Cu and Pb respectively).

The control treatment (0% chitosan) did not result in a significant amount of copper (≈ 8-10%) and lead (3-4%) being removed. This demonstrates that without chitosan in the washing solution the ability to desorb the two metals was limited. Overall, the following efficiency order was recorded: Cu: (8:1, 2%) > (8:1, 5%) > (5:1, 5%) > (5:1, 2%) > control; Pb: (8:1, 2%) > (5:1, 2%) >(5:1, 5%) >(8:1, 5%) > control.

These results suggest that using a moderate concentration of chitosan with the largest volume of washing water promotes desorption of metals from the soil. The application of more chitosan may instead cause more metal to be re-adsorbed, while also potentially forming more soluble metal–chitosan complexes to wash out of the soil, resulting in less efficient removal. The differences seen between metal removal efficiencies indicate the metals may bind differently to soil. Cu is more readily exchanged and Pb may be more strongly bound to organic fractions.

Overall, these results show that washing solutions with chitosan can safely and effectively

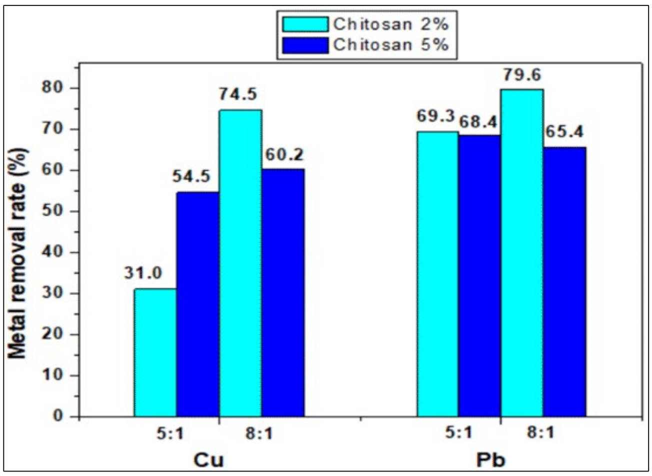


Fig. 5. Removal efficiency of Cu and Pb from soil after the investigated time

remove heavy metals from contaminated soil in an environmentally friendly way. By using a 2% chitosan concentration and an 8:1 washing solution to soil ratio the highest removal efficiencies were observed for both metals. The washing solutions removed Cu to below the intervention level while substantially removing Pb. This experiment shows that chitosan may be used as a safe and sustainable substitution for synthetic chelating agents.

## Conclusions

The potential of using chitosan-based washing solutions to remediate heavy metal-contaminated soil from the Larga de Sus mining site (Romania) was illustrated in this study.

The results showed that both chitosan concentration and liquid-to-solid ratio impacted the removal performance of Cu and Pb from contaminated soil.

The use of 2% chitosan along with an 8:1 liquid-to-solid ratio provided the best removal performance, achieving copper concentration below the intervention threshold (200 mg·kg $^{-1}$) with lead concentration removed by close to 80%, but still above the threshold.

Collectively, these findings highlight the potential of chitosan to act as a green and sustainable alternative to synthetic chelating agents for soil washing applications. Further research is warranted to assess its potential scalability in field conditions and its reapplication potential of washing solutions.

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