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Study on Biosorption of Lead (II) and Manganese (II) from Aqueous Solutions Using Sodium Alginate and Pleurotus ostreatus (Oyster Mushroom) Beads

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Abstract

The present study investigated the development of beads from alginate and mushroom powder for the removal of lead (Pb) and manganese (Mn) from water. Batch biosorption experiments were conducted, varying pH and the composition of alginate and mushroom powder. Results indicated that the combination of alginate and mushrooms achieved over 86.8% reduction in Pb and 65.9% reduction in Mn concentrations, with higher reductions observed for both metals at pH 5. These findings suggest that the beads effectively reduced Pb and Mn concentrations, with the mushroom content playing a significant role in their efficacy. The analysis of the FTIR spectrum showed that the uptake of metal ions by mushrooms involves interactions of ions with hydroxyl, carboxyl, and amide groups. This study underscored the potential applications of these beads in addressing heavy metal pollution in water sources. By providing a sustainable and effective method for heavy metal removal, the use of alginate and mushroom-based beads could offer a valuable solution for environmental remediation efforts.

Keywords: AAS, Biosorption, FTIR, Heavy metal, Mushroom, Alginate bead

Introduction

Heavy metals are naturally occurring elements in the Earth's crust since its formation, posing serious risks to human health and the environment. Groundwater and surface water contamination by heavy metals is a global concern, particularly in low-income or developing countries. Most heavy metals are non-biodegradable and tend to accumulate in living organisms [1; 2]. In Myanmar, heavy metal pollution has been reported in various regions, including Mandalay, where population growth has exacerbated environmental challenges. Studies have identified the presence of calcium (Ca), copper (Cu), magnesium (Mg), mercury (Hg), arsenic (As), manganese (Mn), lead (Pb), iron (Fe), and zinc (Zn) in water samples, with concentrations sometimes exceeding WHO standards [1; 2; 3; 4; 5].

The removal or reduction of these heavy metals is crucial for human health and environmental protection. Researchers have investigated various techniques, including physical, chemical, and biological methods, to address this issue. While these methods have shown promise, challenges such as high costs, sludge production, and technical constraints remain [6]. Water, essential for all life forms, supports plants, animals, and human life. However, heavy metal contamination in groundwater is a serious issue globally. In Myanmar, Mandalay has seen a rise in population, leading to increased studies on heavy metals since 2014. Samples from regions like Aung-Myay-Tharzan, Chan-Aye-Tharzan, Chan Mya-Tharzi, Mahar-Aung-Myay, Pyi-Gyi-Tagon, and Amarapura townships have shown levels of calcium (Ca), copper (Cu), magnesium (Mg), mercury (Hg), arsenic (As), manganese (Mn), lead (Pb), iron (Fe), and zinc (Zn). Lead is particularly toxic to every organ and system in the human body, with the maximum acceptable lead limits in water set at 0.01 mg/L (10 ţg/L) by the World Health Organization [4; 7]. Similarly, excessive manganese (Mn) in the environment can cause health issues. The WHO suggests a concentration of manganese in drinking water should be less than 0.05 mg/L [3; 6; 8].

In 2015, it was reported that the concentrations of manganese and other metals were found unsafe in many wells in Myanmar [5]. In a study conducted by Maw et al. in 2020, manganese was found to exceed permissible limits in 3 out of 6 townships, and lead in 1 out of 6 townships in the Mandalay region [7]. Recent studies have explored biosorption and adsorption methods for heavy metal removal, utilizing materials like chitosan, bentonite, zeolite, and alginate as biosorbents. Alginate, extracted from brown marine algae and bacterial sources, has shown promise for heavy metal removal. Its high surface area, biocompatibility, and abundance make it an attractive option. Alginate works by forming complexes with metal ions, effectively sequestering them from aqueous solutions. Studies have demonstrated its effectiveness in removing various heavy metals, including lead, copper, cadmium, and nickel. Alginate-based adsorbents are relatively easy to prepare and can be tailored to specific metal removal requirements, making them a promising candidate for future research in heavy metal remediation [1; 9].

Mushrooms, including *Pleurotus ostreatus*, have shown excellent binding properties and tolerance to metals and adverse environments, such as various pH and temperature conditions [10; 11]. Oyster mushrooms, such as *Pleurotus ostreatus*, are environmentally friendly and can decompose organic or lignocellulosic wastes within 34 weeks [12]. *Pleurotus ostreatus* mushroom, in particular, has shown promise in absorbing heavy metals from its surroundings. In combination with other materials, such as alginate, mushrooms have demonstrated synergistic effects, enhancing heavy metal removal efficiency. This study aims to reduce heavy metal concentrations using a biosorption method with *Pleurotus ostreatus* mushroom as an inexpensive biosorbent, mixed with sodium alginate to form beads for its low cost, ease of use, and excellent removal efficiency. The objective of this work is to evaluate the effect of *Pleurotus ostreatus* powder and alginate mixture beads on removing lead and manganese from aqueous solutions.

Materials and Methods

Instrumentation

The Atomic Absorption Spectrophotometer (AA-7000, Shimadzu, Japan) with an air-acetylene flame system was utilized for precise metal determination. It is capable of measuring heavy metals and other elements at parts per billion (ppb or μg/l) concentrations with minimal sample volumes. A shaker (SK 2000, Thermo Fisher, USA) was used for mixing solutions, while a pH meter (Serial NO. 600719039029) was employed to measure acidity or alkalinity in water-based suspensions. Glassware was sterilized and dried using an air oven (SH-DO-100FG, Serial No. 200530DO-100FG, Korea). An analytical balance (AY120, Serial No. D432712541, Shimadzu, Japan) was used for measuring object mass or chemical weight. Biomass spectra were analyzed using a Fourier Transform Infrared Spectrophotometer (FTIR) (IRTracer-100, Shimadzu, USA) with spectral scanning from 500 to 3500 cm^{-1} to identify functional groups responsible for heavy metal adsorption and provide information on oxidation state, bonding, and morphology of dispersed clusters of heavy metal ions. A digital hotplate stirrer (Hotplate Stirrer, HSD-330, Korea) was used to homogenize alginate and mushroom powder for bead formation.

Chemicals and Reagents

Sodium alginate ($C_6H_7O_6Na$), anhydrous calcium chloride ($CaCl_2$), lead (II) acetate, manganese (II) solution, hydrogen peroxide (H_2O_2), nitric acid (HNO_3), and hydrochloric acid (HCl) were purchased as analytical-grade chemicals from well-established scientific supply stores in Myanmar.

Collection and Preparation of Mushroom Powders

The fresh mushrooms (*Pleurotus ostreatus*) samples were purchased from the local market in Kyaukse, Myanmar. The mushroom samples were thoroughly cleaned with tissue paper to remove dust, cut into pieces with a knife, and dried at room temperature for 3 days. After drying, they were ground with a blender and stored in plastic bags at room temperature for later use.

Preparation of Bead Formation

All glassware (tubes, conical flasks, beakers, etc.) was soaked in HNO_3 for several hours in acidic water (10% nitric acid) to remove any residual organic contaminants and oxidize any trace metal ions present on the surface before undergoing a regular washing procedure with deionized (DI) water. Then, they were dried in an oven at 60řC for 24 hours. Based on preliminary experiments assessing the structural integrity of formulated beads, concentrations of 4% and 6% for both sodium alginate and mushroom powders were selected to investigate their effects on bead efficacy for heavy metal removal. Firstly, sodium alginate (4% and 6%) was added to 100 ml of DI water and stirred with a magnetic stirrer at 60řC for 35 minutes. Mushroom powders (4% and 6%) were then added to the sodium alginate solutions and stirred with a magnetic stirrer for 35 minutes. Different concentrations of sodium alginate and mushroom powders were used to investigate their effects on bead properties and efficacy for heavy metal removal. This approach helps understand the influence of these parameters on bead performance, providing insights for potential optimization of bead synthesis. After cooling, the solution was dropped using a 60 ml CC syringe without a needle into 1M calcium chloride solution and left for 5 hours to solidify. Fresh bead diameters ranged from 2 to 4.5 mm. Beads were washed with DI water and dried at room temperature overnight. Blank samples were included to account for background contamination. Figure 1 illustrates the bead formation process.

Preparation of Lead (II) and Manganese (II) Solutions

Lead (II) acetate and manganese (II) solution were purchased from the local market in Myanmar. Pb (II) and Mn (II) solutions were prepared separately by dissolving 100 ppm of Pb (II) and Mn (II) in deionized water. The solutions were stirred with a magnetic stirrer for 30 minutes.

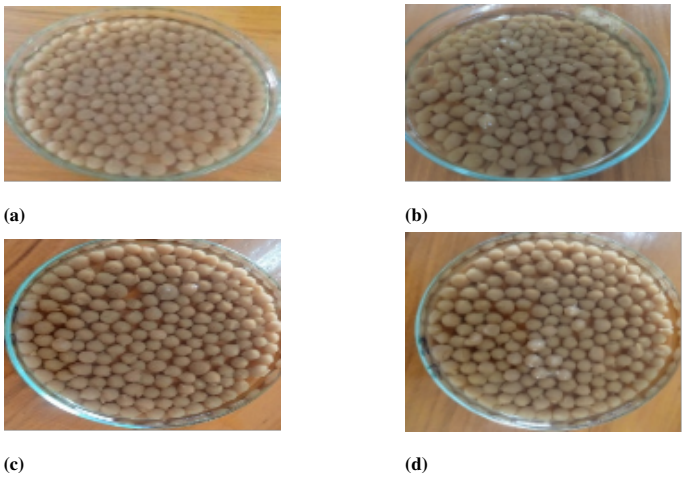

Figure 1. Bead formation of (a) 4% Alginate and 4% Mushroom Powders, (b) 4% Alginate and 6% Mushroom Powders, (c) 6% Alginate and 4% Mushroom Powders, and (d) 6% Alginate and 6% Mushroom Powders.

pH Adjustment

The original pH of Pb (II) and Mn (II) solutions was adjusted using 1 M NaOH solution and 1 M HCl solution to achieve pH 3 and pH 5. After adjusting the pH, the mixture of alginate and mushroom powder beads was added to the Pb (II) and Mn (II) solutions.

Biosorption Experiments

A bead dosage of 2 g was added to flasks containing 20 ml of lead (II) and Mn (II)-bearing solutions adjusted to pH 3 and pH 5. Flasks were shaken at 100 rpm at room temperature for 45 minutes, during which the equilibrium stage of Pb (II) concentration removal was reached [11]. Samples were filtered using cellulose membrane papers. The filtrate of lead (II) was digested using closed digestion (microwave), and that of Mn (II) using the open digestion method. Metal concentrations in the filtrate were determined by AAS (AA-7000) for each treatment in duplicate.

FTIR Characterization

Dried metal-loaded and metal-free (control) beads were separately prepared for FTIR analysis. Firstly, the dried bead sample was ground with a mortar and pestle to a fine powder. Equal amounts (2.0 mg) from each sample were taken and mixed with approximately 200.0 mg of potassium bromide (KBr). Each potassium bromide-treated control and sample were thoroughly mixed with a mortar and pestle and pressed under vacuum into pellets, which were then analyzed using FTIR.

Statistical Analysis

Statistical analysis was conducted in R (version 4.3.0) using several packages. Data were read from an Excel file using the `read_excel` function from the `readxl` package. Variable renaming was performed using the `str_c` function from the `stringr` package to ensure consistent naming conventions. For data manipulation and variable coding, the `mutate` function from the `dplyr` package was utilized. Linear regression analysis was conducted using the `lm` function from the `stats` package to model the relationship between treatment variables and the reduction percentage of Pb and Mn. Data visualization was performed using the `ggplot2` and `ggpattern` packages to create plots illustrating the relationships between variables. The data were trimmed to only include treatment variables and calculated as the percent reduction relative to the initial concentrations of Pb and Mn. A 2k factorial design was employed, with lower values coded as -1 and higher values as 1 for all three treatment variables, allowing evaluation of main effects (individual variables) and interaction effects (combined effects of variables) on the sorption capacity of the biosorbent. Separate regression models were conducted for each metal (Pb and Mn) to assess the impact of pH, alginate concentration, and mushroom powder concentration on the reduction percentage. The coefficients of the regression models were interpreted to determine the magnitude and direction of the effects of each variable. Additionally, p-values were calculated to assess the statistical significance of each variable's effect on the reduction percentage.

Results and discussions

The reduction of lead

Across all pH, *alginate*, and mushroom powder combinations, an average reduction of 82.1% of Pb (II) was achieved, with the maximum reduction reaching 86.8% at 4% *alginate*, 6% mushroom powder, and pH 5, indicating the effectiveness of the biosorbent across various conditions in Figure 2:a. The effect of pH was stronger at pH 5 compared to pH 3 in Pb (II) reduction, aligning with findings by [8], who observed increased biosorption of Pb (II) at pH 5 due to increased negative charge on the biosorbent surface.

The regression analysis of Pb (II) reduction is shown in Table 1 along with the respective probability values. The residual error for the model was 1.889, the multiple R-squared was 0.7514, and the adjusted R-squared was 0.5857. The F-statistic of the model for Pb (II) reduction was 4.535 on 6 and 9 degrees of freedom and the probability value of the model was 0.02164. Although the model was significantly better than the default model, the adjusted R-squared value showed that the model explained only over half of the variations in the experiment. The analysis showed that the mushroom had a significant positive effect on the reduction of Pb (II) with a p-value of 0.00276. This might mean that the higher the percentage of mushroom in the beads, the better the removal would be. Our findings are consistent with [13], who found that increased mushroom biomass enhanced Pb (II) biosorption because increasing the biomass dosage progressively increases the adsorption sites for the metal ions [13].

However, the mentioned fact is entangled with the interaction effect of mushrooms and *alginate*. The interaction graph was depicted in Figure 2:b showing that 4% *alginate* inclusion in the

Table 1. Linear regression analysis of the effect of mushroom, alginate, and pH on Pb reduction

	Estimates	t-value	Pr(>|t|)
Intercept	82.1177	173.861	< 2e-16 ***
pH	1.0054	2.129	0.06216.
Alginate	-0.5710	-1.209	0.25750
Mushroom	1.9265	4.079	0.00276 **
pH:Alginate	0.3775	0.799	0.44473
pH:Mushroom	0.2902	0.614	0.55416
Alginate:Mushroom	-0.8914	-1.887	0.09173

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Residual standard error: 1.889 on 9 degrees of freedom.

Multiple R-squared: 0.7514, Adjusted R-squared: 0.5857.

F-statistic: 4.535 on 6 and 9 DF, p-value: 0.0216

beads increased the chance of removing the metal at the higher percentage of mushroom powder in the beads. The change in pH effect in the range of 3 and 5 also showed a positive term for reducing the Pb (II) ions with a p-value of 0.06216. It has been known that pH has a strong influence on metal ion removal. The appearance of the weak effect in this experiment might be due to the fact that the range chosen was narrow and its effect showed a low value.

The reduction of manganese

Similar to Pb (II) reduction, all combinations of pH, *alginate*, and mushroom showed an average reduction of 59.2% of Mn (II) from the mixture, with the maximum reduction of 65.9% observed at 4% *alginate*, 6% mushroom powder, and pH 5 (Figure 2:c). The effect of pH was more pronounced at pH 5 than at pH 3 in the removal of Mn (II), consistent with findings by [14] regarding optimal biosorption at pH above 5. The mushroom showed the most distinct effect on the reduction of Mn (II). In general, the overall graph for both Pb (II) and Mn (II) reduction experiment showed that the inclusion of mushroom increased the percentage of the metals removed from the tested mixture (Figure 2:d).

Table 2 shows the regression analysis for the Mn (II) reduction from the mixture. The linear model had a residual error of 1.224. The multiple R-squared for the model was 0.9515, and the adjusted R-squared was 0.909. F-statistic for the model was 22.4 on 7 and 8 degrees of freedom, and the model was significantly better than the default model with a p-value less than 0.001. The adjusted R-squared value indicated that the model explained more than 90% of the variation of the data in the experiment. The regression estimates for most of the variables and their interaction effects showed significant terms. The estimate for the mushroom effect on the Mn (II) reduction was distinct, with a magnitude of 2.4624 and a P-value of 4.18e-05, and it had a positive effect, meaning the more mushroom inclusion in the bead might increase the reduction percentage in removing Mn (II) from the mixture. [15] also revealed a marked increase in the removal of Mn (II) by increasing of biosorbent mass of *Aspergillus spp.*.

However, this interpretation might not be complete because there was an interaction term among all three variables with a magnitude of -1.7290 and a P-value of 0.0005. The analysis of interaction was visualized in Figure 2:d. While the effect of mushroom on Mn (II) reduction increased at both pH values of 3 and 5 in 4% *alginate*, its effect showed a higher degree of slope at pH 5. The mushroom effect in 6% *alginate* seemed lower than in 4% at both pH values, although the effect at pH 5 showed a higher reduction percentage.

Table 2. Linear regression analysis of the effect of mushroom, alginate, and pH on Mn reduction

	Estimates	t-value	Pr(>|t|)
Intercept	59.1987	193.511	5.69e-16 ***
pH	0.7100	2.321	0.048852 *
Alginate	-1.1167	-3.650	0.006492 **
Mushroom	2.4624	8.049	4.18e-05 ***
pH:Alginate	1.1577	3.784	0.005355 **
pH:Mushroom	0.2896	0.947	0.371461
Alginate:Mushroom	-1.5648	-5.115	0.000913 ***
pH:Alginate:Mushroom	-1.7290	-5.652	0.000480 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' .

Residual standard error: 1.224 on 8 degrees of freedom.

Multiple R-squared: 0.9515, Adjusted R-squared: 0.909.

F-statistic: 22.4 on 7 and 8 DF, p-value: 0.0001179

The Mn (II) reduction model appears to be more robust, with a higher Adjusted R-squared and more significant predictors, especially the three-way interaction term. Mushroom is consistently a significant positive predictor for both Mn (II) and Pb (II) reduction, suggesting its importance in promoting metal reduction. These results prompt further investigation into the specific mechanisms by which mushroom, pH, *alginate*, and their interactions influence Mn (II) and Pb (II) reduction. The significance of the three-way interaction in the Mn (II) reduction model underscores the complexity of these relationships, necessitating a nuanced understanding of the variables involved. Replication studies with a larger sample size and under varying environmental conditions, such as temperature, initial concentration, and contact time, are essential to validate these findings and enhance their reliability and generalizability. While this study focused on pH, *alginate*, and mushroom powder, exploring alternative explanations could

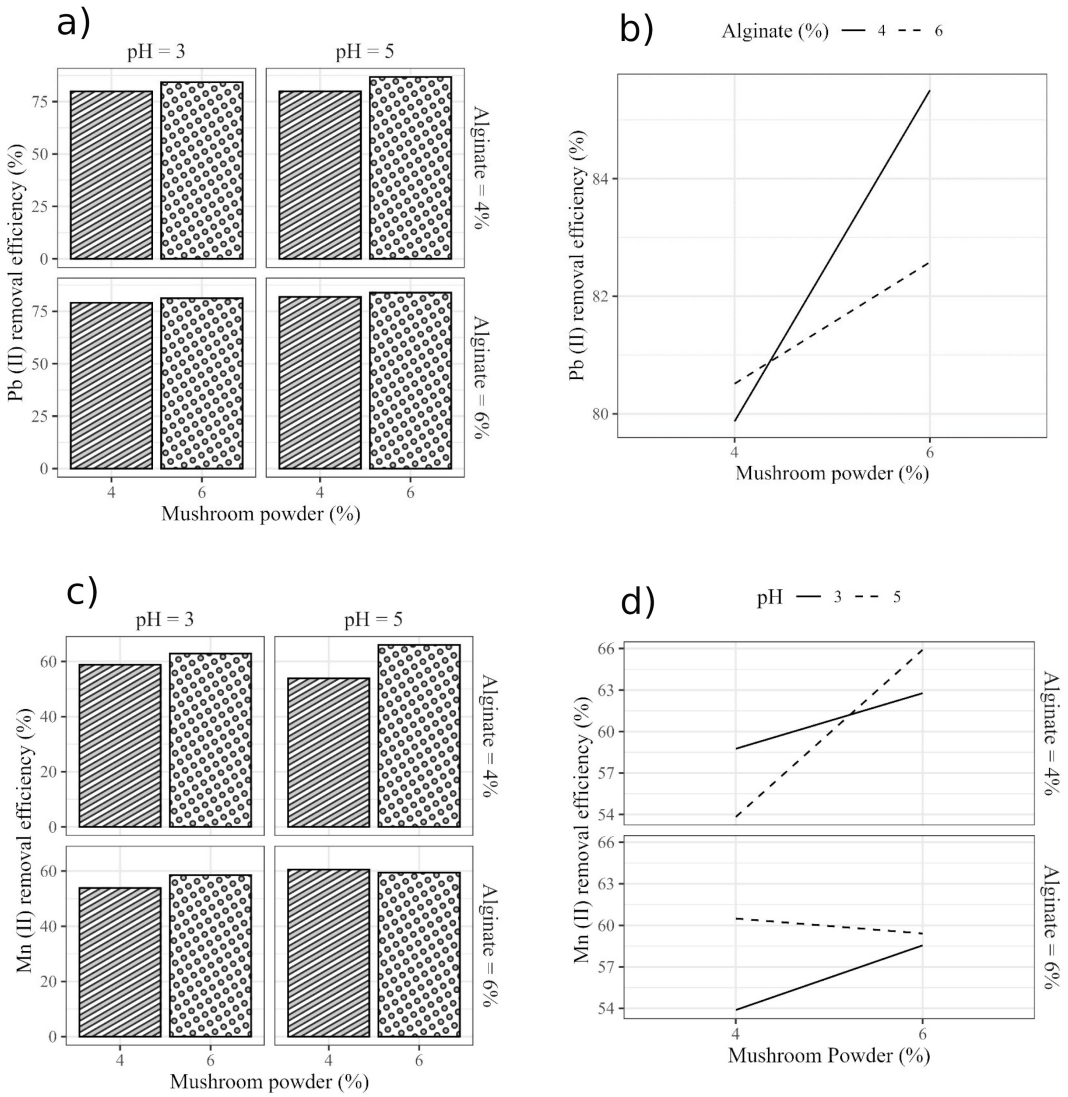

Figure 2. (a) The effect of mushroom, alginate, and pH on Pb (II) reduction. (b) Interaction effect between mushroom and alginate on Pb (II) reduction. (c) The effect of mushroom, alginate, and pH on Mn reduction. (d) Interaction effect between mushroom and alginate on Mn (II) reduction.

strengthen future analysis. Factors like organic matter or other ions in water might interact with these variables, affecting adsorption. Metal ion properties, such as oxidation state, could also interact with treatment variables. Investigating these could enhance our understanding and improve biosorption material design.

FTIR characterization

The characteristic spectra of *alginate* and mushroom bead exhibit several notable features: a broad band centered at approximately 3313 cm^{-1} corresponding to hydroxyl group stretching, low-intensity bands around 2926 cm^{-1} attributed to CH_2 groups, a distinct peak at 2364 cm^{-1} representing stretching of the (CN) group, a peak at 1612 cm^{-1} indicating C=O stretching vibration, peaks at 1533 cm^{-1} and 1450 cm^{-1} representing asymmetric and symmetric stretching modes, respectively, of carboxylate salt groups (-COONa), and various vibrations between 11381093 cm^{-1} associated with glycoside bonds in the polysaccharide (C-O-C stretching). FTIR spectra before and after adsorption are shown in Figure 3 and Table 3.

After the adsorption of Pb (II) ions on a mixture of *alginate* and mushroom bead, notable spectral changes are observed: There is a shift of nearly 20 cm^{-1} towards lower wavenumbers in the peak assigned to C-H stretching vibration. The peak associated with O-H stretching mode at over 3300 cm^{-1} disappears. Two peaks related to C=O stretching vibration and C=C stretching mode shift towards higher wavenumbers by approximately 20 cm^{-1}. The C=N vibration mode experiences a downward shift of 10 cm^{-1}. A new peak is observed at 1344 cm^{-1}, attributed to C-H bending. Peaks at 1081 cm^{-1} and 1045 cm^{-1} correspond

Table 3. Position of the main peaks in FTIR spectra of sodium alginate and mushroom bead before adsorption, after adsorption of Pb (II), and Mn (II)

Wave length before adsorption (cm^{-1})	Wave length after adsorption (cm^{-1})		Assignment
	Pb (II)	Mn (II)	
3313		3435	O-H stretching
2926	2902	2924	asymmetric CH_3 and CH_2 stretching
2364	2394		(CN) stretching
1612	1637	1639	C=O stretching
1533	1550		C=N stretching
1450	1423	1385	C-H bending
1138	1344	1269	C-N stretching
1093	1081	1153	C-N stretching
756	1045	1045	C-H bending
657	1022	893	C-metal stretching
	935	819	

to C-O stretching vibrations in carbohydrates and polysaccharides, potentially indicating changes due to metal-ion interactions. Furthermore, the peak at 1022 cm^{-1} could be associated with C-O or C-C stretching vibrations in the context of carbohydrates or polysaccharides, suggesting possible alterations post-Pb (II) adsorption.

After the adsorption of Mn (II) ions on a mixture of *alginate* and mushroom bead, distinct changes are observed in the spectrum. The O-H stretching vibration peak shifts to a nearly 10 cm^{-1} higher wavelength compared to the pure bead spectrum. However, the C-H stretching mode peak remains at the same wavelength as in the pure bead spectrum. The stretching mode peak of the (CN) group disappears. The C-H bending mode experiences a significant shift of 60 cm^{-1} to lower wavelengths compared to the pure bead IR spectrum. Similarly, peaks related to C-O stretching, C-N stretching, and C-H bending vibration modes also shift to higher wavelengths. These shifts or alterations in the peaks post-Mn (II) adsorption likely signify interactions between Mn (II) ions and the functional groups present in *alginate* and mushrooms.

The infrared (IR) spectra of *alginate* and mushroom beads, along with the alterations observed following the adsorption of Pb (II) and Mn (II) ions onto the mixture of *alginate* and mushroom beads, offer insights into changes in the molecular composition induced by metal-ion interactions. The observed shifts and variations in peak intensities post-metal ion adsorption (Pb (II) and Mn (II)) on the *alginate* and mushroom bead mixture imply modifications in the bead's molecular structure due to interactions with the metal ions. These modifications potentially denote the coordination of metal ions with specific functional groups present in *alginate* and mushrooms. Such alterations provide crucial insights into the adsorption mechanism and the nature of interactions between metal ions and the components of the beads. [1; 2; 12].

Various methods for removing heavy metals, including precipitation, filtration, ion exchange, carbon adsorption, evaporation, membrane technology, reverse osmosis pre-concentration, redox reactions, electrowinning, chelation, wastewater coagulation, and electrochemical processes, have been demonstrated to be ineffectual according to studies by Ali et al. (2019) and Taha et al. (2023) [16; 17]. Under metal stress, a notable decline in protein and carbohydrate content was observed, as evidenced by a reduction in the intensity of absorption bands, particularly within the 1800 to 800 cm^{-1} regions. This region is known to be characteristic of proteins and carbohydrates, as highlighted in studies by Dumas and Miller (2003), Walkers et al. (2004), and Yee et al. (2004) [18; 19; 20]. Future research should investigate the impact of contact time and explore pre-treatment methods for the mushroom powder to further optimize lead and manganese removal efficiency.

Comparison of different adsorbents for Pb (II) and Mn (II) removal efficiency

The removal efficiency of Pb and Mn by various adsorbents in recent studies is summarized in Table 4. The *alginate* + mushroom powder beads in this work exhibited a Pb removal efficiency of 86.8% and a Mn removal efficiency of 65.9%. While these results demonstrate the competitive nature of the *alginate* + mushroom powder beads in terms of Pb removal efficiency, they also indicate a slightly lower efficiency for Mn removal compared to some other adsorbents. Further optimization efforts could potentially lead to an enhancement in the effectiveness of the beads, thereby improving their performance in removing Pb and Mn from water.

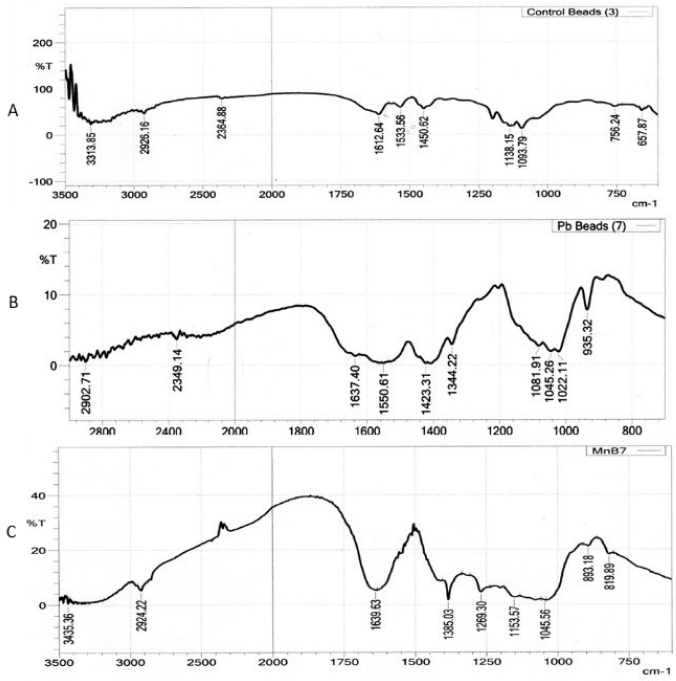

Figure 3. FTIR spectrum of (A) pure beads, (B) biomass after Pb (II) adsorption, and (C) biomass after Mn (II) adsorption

Table 4. Comparison of Pb and Mn removal efficiency (RE) of different adsorbents.

Biosorbent	RE (%)	Pb	Mn
Aguapé	63.00		[21]
Moringa oleifera leaves	97.00		[22]
Azadirachta indica (neem leaves)	93.50		[23]
raw tea factory waste	94.07		[24]
modified tea factory waste	97.73		[24]
sugarcane bagasse	89.31		[25]
Milled olive stones	94.50		[26]
Dried Biomass Microalgae *Aphanothece sp*	99.90		[27]
waste fungal biomass	87.00		[28]
waste fungal biomass with pre-treatment	93.00		[28]
Moringa oleifera seed		92.00	[29]
modified sugarcane bagasse biochar		65.80	[30]
date palm biochar		73.20	[31]
polyvinyl alcohol/sodium alginate		72.34	[32]
Alginate + mushroom powder beads	86.80	65.90	This work

Practical implications

The high reduction percentages observed for both lead (Pb) and manganese (Mn) highlight the potential of these biosorbent materials in treating contaminated water sources. Implementing such biosorption techniques could offer a cost-effective and environmentally friendly approach to reducing heavy metal pollution in water bodies. Furthermore, understanding the factors that influence the efficiency of these biosorbents, such as pH, *alginate*, and mushroom powder concentrations, can inform the design of optimized remediation strategies tailored to specific environmental conditions. This research provides a foundation for further studies and the development of practical applications for the sustainable management of heavy metal contamination in aquatic environments.

Conclusions

The analyses of Mn (II) and Pb (II) reduction dynamics reveal relationships among key variables in biological metal reduction processes. In Mn (II) reduction, the model demonstrates a good fit (Adjusted R-squared: 0.909), emphasizing the role of mushroom in promoting Mn (II) reduction. The negative impact of *alginate* hints at potential inhibitory effects, while the three-way interaction (pH, *alginate*, and mushroom) underscores the complexity of their contributions. Further research should explore the biochemical pathways underlying these effects, enhancing our understanding of Mn (II) reduction. In Pb (II) reduction, the model, though statistically significant (F-statistic: 4.535, p-value: 0.02164), exhibits a slightly lower goodness of fit (Adjusted R-squared: 0.5857). Mushroom remains a significant positive predictor, aligning with its role in Mn (II) reduction. The marginal significance of pH and the non-significant effect of *alginate* suggest a nuanced relationship with Pb (II) reduction. FTIR results suggested carboxyl, hydroxyl, and amide were the potential functional groups that may be involved in the Mn (II) biosorption. In conclusion, the mixture of mushrooms (*Pleurotus ostreatus*) and *alginate* beads has been more effective for the removal of Pb (II) than Mn (II) from the aqueous solution. Future research could focus on replicating the study with larger sample sizes and in different environmental conditions to validate the findings. Additionally, exploring the long-term stability and reusability of the beads in real-world applications would provide valuable insights into their practicality for environmental remediation. Moreover, to further elucidate the mechanisms underlying the observed effects of mushroom, pH, and *alginate* interactions on metal reduction, future investigations could employ advanced analytical techniques, such as surface characterization or scanning electron microscopy.

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