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Removal of Chromium (Cr) and Copper (Cu) from Domestic Wastewater Using Chitosan Flakes from Seafood Waste

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Abstract

Heavy metals in wastewater from various sources pose significant risks to ecosystems and human health. This study investigates the effectiveness of chitosan, a biopolymer derived from seafood waste, in removing chromium (Cr) and copper (Cu) from domestic wastewater. The adsorption efficiency of chitosan was evaluated based on adsorbent dosage and contact time. Chitosan was synthesized through demineralization, deproteination, and deacetylation of seafood waste. Adsorption experiments were conducted using chitosan dosages of 2 g, 4 g, and 6 g, with contact times of 5, 30, and 60 minutes. The results indicate that increasing the adsorbent dosage enhances the removal efficiency of both Cr and Cu ions. Specifically, Cr removal improved from 34.73% at 2 g to 56.25% at 4 g and further to 78.40% at 6 g. Similarly, Cu removal efficiency increased from 28.21% at 2 g to 56.41% at 4 g and reached 82.05% at 6 g. Additionally, prolonged contact time significantly enhanced metal ion removal. Cr removal increased from 18.06% after 5 minutes to 50.00% after 30 minutes and 72.92% after 60 minutes. A similar trend was observed for Cu, with removal efficiency rising from 17.95% at 5 minutes to 55.13% at 30 minutes and 75.64% at 60 minutes. These findings suggest that extended contact time provides greater opportunities for adsorption, improving the removal efficiencies of Cr and Cu ions. Overall, this study demonstrates that chitosan is an effective adsorbent for Cr and Cu removal from wastewater, with adsorption efficiency influenced by both adsorbent dosage and contact time.

Keywords: Wastewater treatment; Heavy Metals; Chitosan; Seafood Waste; Adsorbent

1. Introduction

Heavy metals are a class of metallic elements that are harmful to both humans and the environment. They can occur naturally in the environment or be introduced by humans through activities such as industrial processes, mining, and pesticide and fertilizer use. Heavy metals in domestic wastewater can endanger human health and the environment. According to Ramirez Calderón, *et al.* (2020), several municipal, industrial, and agricultural activities use heavy metals-containing wastewater and discharge it into the environment. These heavy metal mixtures have a variety of hazardous and toxic effects on the environment and human health.

Domestic wastewater contaminated with heavy metals poses a risk to human health if not properly treated before being discharged into the environment (Mitra *et al.,* 2022).

Heavy metals can accumulate in the food chain through surface or groundwater, eventually reaching humans through consumption, potentially leading to health issues. These metals are also detrimental to the environment, as they accumulate in plant and animal tissues, disrupting ecological balance. Unlike organic pollutants, heavy metals cannot be broken down by microorganisms; once released into the environment, they persist and bioaccumulate through the food chain, posing long-term risks to both ecosystems and human health (Zeng *et al.,* 2020). Furthermore, heavy metals can travel and disperse in the environment via various routes, one of which is through water bodies. Extreme stress on water bodies has resulted in severe contamination of both groundwater and surface water, increasing human exposure.

Although certain heavy metals, such as iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn), are essential for normal physiological functions, they are only required in trace amounts as micronutrients to support enzymatic activities, immune function, and cellular metabolism (Prasad, 2008; Bost & Favier, 1999). These metals play critical roles in various biochemical processes, including oxygen transport (iron in hemoglobin), antioxidant defense (zinc in superoxide dismutase), and nerve signal transmission (copper in neurotransmitter synthesis). However, when present in excessive concentrations, heavy metals can become toxic and disrupt biological functions, leading to severe health complications.

Exposure to elevated levels of heavy metals has been linked to a range of adverse health effects, depending on the type of metal ion and its concentration in the body. Acute exposure to heavy metals such as lead (Pb), cadmium (Cd), and mercury (Hg) can result in symptoms like indigestion, nausea, and diarrhea due to their interference with digestive enzymes and gut microbiota balance. Long-term exposure may lead to chronic conditions, including neurological disorders, kidney and liver damage, cardiovascular diseases, and even carcinogenesis. For instance, prolonged ingestion of fluoride-contaminated water can cause dental fluorosis, characterized by discoloration and structural damage to tooth enamel (Ahmad *et al.,* 2022). Similarly, excessive chromium (Cr) exposure has been associated with skin allergies and dermatitis, while cadmium and arsenic are known carcinogens that increase the risk of lung, kidney, and bladder cancers (Ramirez Calderón *et al.,* 2020).

To prevent heavy metal contamination in domestic wastewater, it is critical to properly treat and dispose of heavy metal-containing household products, as well as to implement agricultural practices that minimize their release into the environment. Additionally, industries should ensure proper wastewater treatment before discharge. Heavy metals are released from various industrial activities, including mining, iron and steel production, glass manufacturing, tanning, energy and fuel processing, pipe corrosion, agro-industrial processes, municipal waste management, and wastewater treatment plants (Ramirez Calderón *et al.,* 2020).

Various adsorbent materials have been widely explored for their ability to remove heavy metals from wastewater, aiming to develop cost-effective and environmentally sustainable treatment methods. Among these, activated carbon has been the most extensively studied and utilized adsorbent due to its high surface area, well-developed porous structure, and exceptional adsorption capacity. Activated carbon efficiently removes a broad range of heavy metals, including lead (Pb), cadmium (Cd), chromium (Cr), and copper (Cu), primarily through mechanisms such as ion exchange, electrostatic attraction, and surface complexation. However, despite its effectiveness, the high production cost and regeneration challenges associated with activated carbon have driven the search for alternative adsorbents derived from natural, low-cost, and renewable materials.

Numerous studies have investigated the adsorption potential of agricultural and industrial waste materials as eco-friendly alternatives to activated carbon. For instance, coconut shell waste has demonstrated promising adsorption capabilities, particularly for removing heavy metals like Pb and Cu due to its high lignin and cellulose content, which provides abundant functional groups for metal binding

(Anirudhan & Sreekumari, 2011). Similarly, agricultural by products such as banana peels and orange peels contain pectin, lignin, and carboxyl functional groups that enhance their ability to capture metal ions from aqueous solutions (Baharudin *et al.,* 2018; Elangovan *et al.,* 2023). Other studies have explored durian rinds, which possess a fibrous structure and functional groups like hydroxyl and carboxyl that facilitate heavy metal adsorption (Baharudin *et al.,* 2021). Additionally, silica-based adsorbents have been investigated for their tunable surface properties, chemical stability, and potential for modification to enhance adsorption efficiency (Mostafa & El-Latif, 2020). The growing interest in these alternative adsorbents highlights the potential of low-cost, biodegradable materials for wastewater treatment, offering an environmentally sustainable solution for mitigating heavy metal contamination.

Thus, this study examined adsorption, which is considered one of the most effective methods for removing heavy metals from domestic wastewater, using chitosan a polymer derived from seafood waste. The specific objectives of this study were: (1) to identify the major components of chitosan flakes using XRF spectrometry and (2) to determine Cr and Cu removal efficiency across different doses of chitosan flakes and varying contact times.

2. Methodology

A laboratory experiment was conducted to evaluate the effectiveness of chitosan (CS) in removing the heavy metals chromium (Cr) and copper (Cu) from domestic wastewater through the adsorption process. The study focused on key parameters that influence adsorption, specifically adsorbent dosage and contact time. A review of previous studies highlighted the effectiveness of chitosan as an adsorptive material for heavy metal removal from wastewater. Prior research has consistently demonstrated that variations in adsorbent dosage and contact time significantly affect the adsorption efficiency of Cr and Cu.

2.1 Study area

The location of wastewater sample for this study was collected in one of sewage treatment plant (STP) in Universiti Teknologi MARA, Shah Alam between Mawar College, and UiTM Health Centre. The seafood waste used in this study was collected from Abah Seafood and Grill, Petaling Jaya as they serve many types of seafood with exoskeleton shells such as giant tiger prawn (*Penaeus Monodon*), mud crab (*Scylla Serrata*), and red king crab (*Paralithodes Camtschaticus*) which both rich with chitin sources that can produce chitosan through deacetylation process.

2.2 Chitosan synthesis from seafood waste

Generally, chitosan synthesis from seafood waste consists of three stages which are demineralization, deproteination and deacetylation. Before the demineralization process started, the seafood waste needs to be cleaned, dried and ground into fine particles which size ranging from 100 microns to 1 millimeter (i.e., a fine powder). Deproteination process is for separation of protein from the shell waste while demineralization process for separation of calcium carbonate ($CaCO_3$) and calcium phosphate ($Ca_3(PO_4)_2$). After deproteination and demineralization stage, chitin is produced. To produce chitosan, chitin needs to go through deacetylation process for removing acetyl group in the composition. The process for chitosan synthesis was referred to in (Fatnah *et al.,* 2020). The apparatus used in the study were glassware from the laboratory, filter paper, desiccator, ovens, magnetic stirrers, and hot plate stirrers while the chemicals used in the study were 3.5% sodium hydroxide (NaOH), 1N hydrochloric acid (HCL) solution, and 50% NaOH (deacetylation).

2.2.1 Deproteination

The shell flakes were mixed with 3.5% NaOH in a 1:10 weight over volume (w/v) ratio. The mixture was heated at 65 ºC and stirred for 2 hours. Afterward, it was rinsed using distilled water and separated by decantation. The mixture then was washed until the pH became neutral and filtered using filter paper to separate the powder from the mixture. The filtered flakes were dried in an oven at 90 ºC for 24 hours.

2.2.2 Demineralization

The flakes obtained from deproteination process were removed from the oven and 1 N HCl solution with ratio 1:15 (w/v) added little by little and the mixture was stirred at room temperature for 30 minutes. The mixture then was rinsed using distilled water and needs to be separated by decantation. Same goes for deproteination, the mixture needs to be washed until the pH neutral and filter it using filter paper. The chitin produced from deproteination, and demineralization then was dried in an oven at 90 ºC for 24 hours. Lastly, the weight of chitin was measured and recorded.

2.2.3 Deacetylation

Chitin obtained from mineral removal process was added with 50% NaOH solution with ratio 1:10 (w/v) then the mixture was heated at 90 ºC while stirring it for 2 hours. It was rinsed using distilled water and separated by decantation. pH of the mixture needs to be neutral by washing it continuously and then filtering it. Next, it was dried in an oven at a temperature of 90 ºC for 24 hours and the weight of chitosan produced was measured. Chitosan was then stored in a desiccator to keep it cool with lower temperature.

2.3 Chitosan composition using XRF Spectrometry

Composition of chitosan was determined using X-Ray Fluorescence (XRF) Spectrometer for characterized chemical composition of raw chitosan. According to Gao *et al.* (2021), X-ray fluorescence spectrometry (XRF) is a chemical analysis technique that uses X-Rays to excite atoms or molecules. These absorbed rays will be immediately followed by spontaneous secondary emission of another type of energy known as fluorescence of X-Rays. The spectrometer in the apparatus collects all of the secondary emissions and creates the XRF spectrum, with each peak corresponding to the quantitative concentration of the chemical elements within the materials. This is a non-destructive analysis of all elements ranging from sodium to uranium. In this study, Bruker S1 Titan from Bruker Elemental Company is used to determine the chemical composition in chitosan. To use this XRF Spectrometry, the device needs to be set according to the material being tested and the trigger needs to be pulled to emit X-rays from the device. When the ray already been radiating to the materials, the screen on the device will show the composition of the material.

2.4 Adsorption test (batch adsorption)

In this stage, the method was referred from (Ramirez Calderón, *et al.,* 2020), (Pavithra *et al.,* 2021), and (Shahwan *et. al.,* 2005) and these methods were adjusted in this study due to suitability of chitosan powder adsorption capacity. Wastewater sample volume in this study is 500 mL for each beaker (Jiang *et. al.,* 2019) and stirring rate was set to 160 rpm (Ramírez Calderón, *et al.,* 2020). Each parameter test was conducted three times with different day of sampling to get the average result for each parameter. The materials used in this test were domestic wastewater sample, extracted chitosan flakes from seafood waste, CuVer® 1 Copper Reagent, and Chromaver® 3 Chromium Reagent. Apparatus used in this test were beaker, Jar Test Apparatus, weighing scale, Whatmann filter paper, conical flask, filter funnel, and sampling bottle for DR 2800 Spectrophotometer test. Before the mixing process of extracted chitosan flakes and wastewater sample, the initial Cr and Cu concentration for each sample was taken.

2.4.1 Effect of adsorbent dosage

In these experiments, chitosan dosage from 2 g, 4 g (Ramírez Calderón, *et al.,* 2020), to 6 g (Pietrelli *et al.,* 2020) were used with a constant contact time of 60 minutes. The sample was distributed into three beakers and labelled them accordingly to each adsorbent dosage. According to study conducted by Hegazy *et al.,* (2021), adsorbent dose is a critical parameter in adsorption because it determines the amount of removal as well as the process economics. The sample was then mixed using a jar test apparatus.

2.4.2 Effect of contact time

The effect of contact time on the uptake of Cr and Cu ions on chitosan was tested using different contact times. These experiments were carried out using different contact times from 5 minutes, 30 minutes (Pavithra *et al.,* 2021), to 60 minutes (Pietrelli *et al.,* 2020), while the other parameters such as adsorbent dosage is constant about 4g (Ramírez Calderón, *et al.,* 2020).

2.5 Final Cr and Cu concentration

Experiments to investigate the effects of adsorbent dosage and contact time were conducted. The samples from each beaker were then filtered to separate the metal ions that had been adsorbed by the chitosan. The concentration of unadsorbed ions in the lasting solution was determined. The concentration of unadsorbed ions was determined by mixing the filtered sample after adsorption using CuVer® 1 Copper Reagent for copper (Cu) concentration (Method 8026 - Bicinchoninate Method) and Chromaver® 3 Chromium Reagent for chromium (Cr) concentration (Method 8023 (Diphenylcarbohydrazide Method for Chromium) and the mixture was tested using DR 2800 Spectrophotometer (HACH, Loveland, USA). Final concentration of Cr and Cu ions were compared with the initial concentration of Cr and Cu ions to calculate percentage of ions removal in the wastewater sample.

2.6 Data analysis

The removal efficiency for Cr and Cu is obtained using the following equation. Removal Efficiency, RE

$$\frac{C_0 - C_f}{C_0} \times 100 \qquad (1)$$

where;
C_o is initial concentration
C_f is final concentration for Cr and Cu ions in mg/L.

3. Results and Discussion

3.1 XRF Spectrometry analysis

Based on Figure 1, the major chemical components in the adsorbent were magnesium carbonate ($MgCO_3$) about 39.64% and calcium carbonate ($CaCO_3$) about 34.34%, and aluminium (III) oxide (Al_2O_3) about 21.11% while minor chemical components in chitosan flakes from seafood waste were, titanium dioxide (TiO_2), potassium oxide (K_2O), sulphur trioxide (SO_3), iron (III) oxide (Fe_2O_3), silica oxide (SiO_2), and manganese (II) oxide (MnO) about 0.038%, 0.226%, 0.738%, 0.965%, 1.393%, and 1.551% respectively. From two major components of chitosan flakes, $CaCO_3$ and $MgCO_3$ which both elements under carbonate group can potentially contribute to adsorption of Cr and Cu in domestic wastewater sample. $MgCO_3$ is one of the most prevalent carbonate minerals in the crust of the Earth, and as a result of precipitation, dissolution, and sorption reactions, it was crucial for controlling aquatic habitats. Among the carbonate minerals with a calcite structure, magnesite was projected to have the biggest trace metal partition coefficients based on a linear free energy correlation model (Soliman & Moustafa, 2020). The effectiveness of removing heavy metal cations from aqueous solutions by biosorption techniques that include calcium carbonate addition has been proven to be significantly enhanced. This effect was brought about by OH-anions that are released into the solution as a result of the dissolution of calcium carbonate and the subsequent hydrolysis reaction binding protons that were released into the solution during the ion exchange process (Yang *et al.,* 2019).

3.2 Domestic wastewater comparison with standards

In order to determine whether domestic wastewater sample from STP between Mawar College and Health Centre in UiTM Shah Alam was safe for discharge to the environment or not, the sample was compared with safe limits in wastewater from World Health Organization and Standard A from Environmental Quality (Industrial Effluents) Regulations 2009 under Environmental Quality Act 1974 (Table 1).

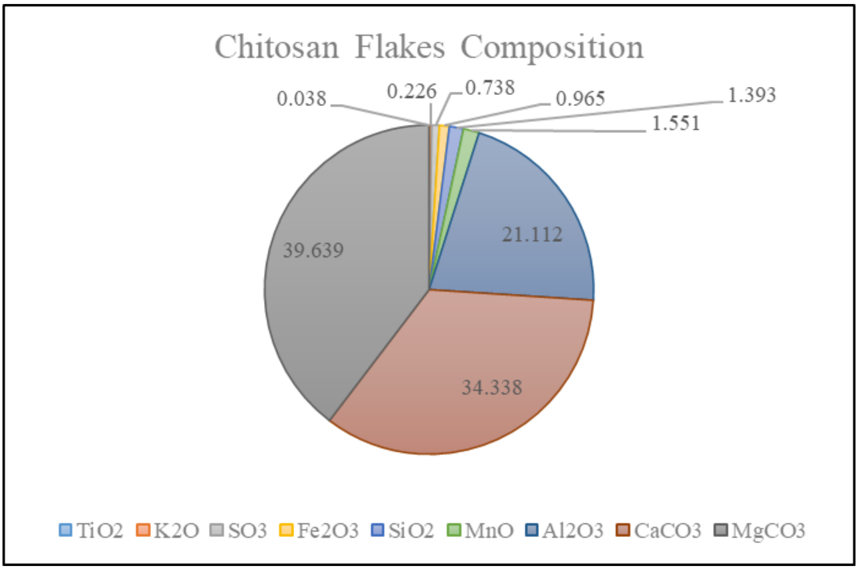

Figure 1. XRF analysis of chitosan flakes composition

Table 1. STP sample comparison with safe limits from WHO and Standard A

Cu	0.23	1.00	0.2	Below the safe limit by WHO. However, it is still higher than Standard A
Zn	0.16	0.03	2	Wastewater sample does not comply with safe limits from WHO but still acceptable for Standard A
Mn	0.122	0.2	0.2	Within acceptable limits as the concentration of Mn in the sample is below safe limits by WHO and Standard A
Cd	0.124	0.003	0.01	Concentration of Cd is higher than safe limits set by WHO and Standard A
Cr	0.05	0.05	0.05	Cr concentration in wastewater sample is still under acceptable safe limits set by WHO and Standard A
Pb	0.139	0.01	0.1	Pb concentration does not comply with safe limits from WHO and Standard A which indicates high pollution level

3.3 Batch adsorption test using different effects of parameter

The desired outcome of a batch adsorption test with varied parameter effects were knowledge of how various factors, such as adsorbent dosage and contact time, influence the adsorption. The experimental data may indicate how changes in these parameters affect the rate and amount of adsorption, as well as the overall efficacy of the adsorbent. The information may be utilized to adjust the adsorption process and increase the system's overall performance.

3.3.1 Effect of adsorption dosage

According to the data that has been provided in Figure 2 and Table 2, as the adsorbent dosage of the material increases, correspondingly rises the percentage of chromium (Cr) removal. 34.73% of the Cr was removed at a dosage of 2g for the adsorbent. When the adsorbent dosage was increased to 4 g, this removal efficiency increased significantly to 56.25%. A dosage increases to 6 g of adsorbent produced a greater removal effectiveness of 78.4%. This pattern suggests that increasing the dosage of the adsorbent has an advantageous impact on removing Cr from the solution. The increasing removal efficiency with increasing adsorbent dosage suggests that having more adsorbent available gives more surface area for chromium ions to bind to. More adsorbent molecules are available to interact with the chromium in the solution, leading to more effective removal.

Besides that, from the data given, the percentage of copper (Cu) removal also improves as the chitosan dosage increases. At a dosage of 2 g for the adsorbent, 28.21% of the Cu was removed. When the adsorbent dosage was increase to 4g, this removal efficiency significantly enhanced to 56.41%. A dosage increases of 6g of adsorbent produced an even higher removal efficiency of 82.05%. The data demonstrates that chitosan flakes removal efficiency for Cu was greater than Cr. This indicates that, although the difference is not great, the adsorbent may be slightly better at removing Cu than Cr.

Furthermore, based on the experimental data, it has been proven that Cu is more dosage – dependant than Cr. Increasing the adsorbent dosage in a constant liquid volume result in a reduction in the number of available adsorption sites because the effective surface area for adsorption is likely to decrease, even though the amount of active adsorption sites per unit mass of an adsorbent should remain constant and independent of total adsorbent mass. It could be explained that particle-particle interactions were believed to be the cause of the adsorbent dose effect (Cherono *et al.,* 2021). More readily available functional groups and adsorption sites for metal ions were the reason of this increase. The adsorption process reaches the equilibrium point due to the overcrowding of adsorbent particles caused by the overlapping of adsorption sites after an extended increase in the percentage removal of metal ions from the wastewater solution (Pietrelli *et al.,* 2020).

3.3.2 Effect of contact time

Based on the data provided in Table 3 and Figure 3, chromium (Cr) removal effectiveness improves with increased contact times. A 5-minute contact time removed 18.06% of the Cr, a 30-minute contact time increased removal efficiency to 50%, and a 60-minute contact time enhanced removal efficiency to 72.92%. This pattern suggests that increasing

Table 2. Effect of adsorbent dosage average result

Adsorbent dosage (g)	Initial concentration average (mg/L)		Final concentration average (mg/L)		Removal efficiency (%)		Standard deviation	
	Chromium (Cr)	Copper (Cu)	Chromium (Cr)	Copper (Cu)	Chromium (Cr)	Copper (Cu)	Chromium (Cr)	Copper (Cu)
2	0.048	0.26	0.031	0.187	34.72	28.21	1.76	1.41
4			0.021	0.113	56.25	56.41	2.81	2.82
6			0.010	0.047	78.40	82.05	3.92	4.10

the contact time of the mixing process has an advantageous impact on removing Cr from the solution. The pattern of change that has been observed indicated that the adsorption of Cr was time-dependent, with longer times allowing for higher adsorption to took place.

Additionally, based on the data shown, longer contact times result in greater copper (Cu) removal efficiency. Cu was eliminated with an efficiency of 55.13% after 30 minutes of contact time compared to 17.95% after 5 minutes. The removal efficiency was greatly enhanced to 75.64% by extending the contact time to 60 minutes. Longer contact time provide the adsorbent and Cu ions more chances to interact favourably, increasing adsorption capacity and increasing removal effectiveness.

It was significant to notice that Cr and Cu had different rates of increase in removal efficiency. When the contact time was increased from 5 to 60 minutes, the removal efficiency of Cr increases by around 54.86% (from 18.06% to 72.92%). But throughout the same contact time range, Cu showed a higher rate of improvement, with the removal efficiency increasing by roughly 57.69% (from 17.95% to 75.64%). This showed that in terms of an increase in removal efficiency, Cu was more responsive to longer contact times than Cr. These findings similar with experimental studies conducted by Eddya *et al.,* (2020) which used waste rubber tires to remove heavy metal in aqueous solution. Based on their findings, the removal effectiveness of heavy metal from aqueous solution increases rapidly up to 90% while after 60 minutes the removal efficiency starts to constant which already achieve equilibrium state. With longer contact times, more metal ions were removed on a percentage basis. After some time, there were no noticeable differences in the recorded percentage of removal, indicating that the adsorption sites had become saturated as a result of intense competition between the heavy metals under observation.

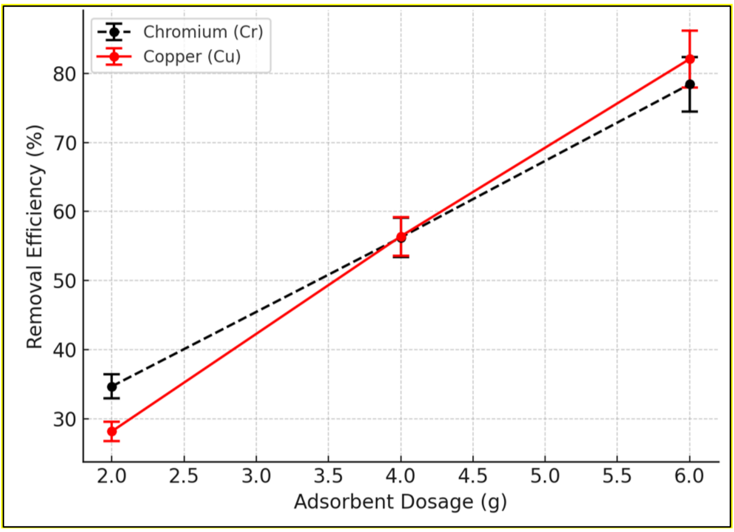

Figure 2. Removal efficiency for various adsorbent dosage

Table 3. Effect of contact time

Contact time (min)	Initial concentration average (mg/l)		Final concentration average (mg/l)		Removal efficiency (%)		Standard deviation	
	Chromium (Cr)	Copper (Cu)	Chromium (Cr)	Copper (Cu)	Chromium (Cr)	Copper (Cu)	Chromium (Cr)	Copper (Cu)
5	0.048	0.26	0.039	0.213	18.06	17.95	0.90	0.89
30			0.024	0.117	50.00	55.13	2.50	2.76
60			0.013	0.063	72.92	75.64	3.65	3.78

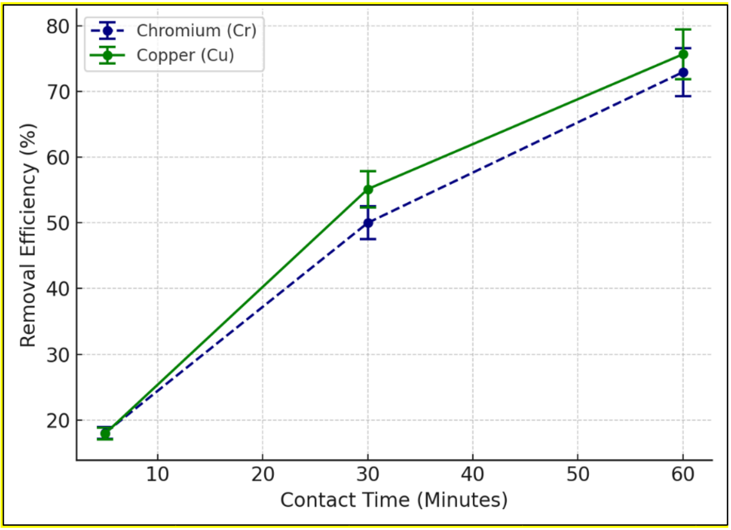

Figure 3. Removal efficiency for various contact times

4. Conclusion

This study explores the potential of chitosan synthesized from seafood waste for removing heavy metals, particularly chromium (Cr) and copper (Cu), from domestic wastewater. Seafood waste collected from restaurants was processed into chitosan flakes through deproteination, demineralization, and deacetylation, with structural analysis conducted using X-Ray Fluorescence Spectrometry. The resulting chitosan contained high calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$), enhancing its adsorption efficiency.

Experimental results showed that a 6 g dosage of chitosan flakes removed 78.40% of Cr and 82.05% of Cu. The increase in active sites and surface area contributed to greater metal adsorption. Additionally, extending the contact time from 5 to 60 minutes improved metal removal, with Cr adsorption increasing from 18.06% to 72.92% and Cu from 17.95% to 75.64%. These findings reinforce chitosan's suitability as an effective adsorbent for wastewater treatment.

However, further optimization is required to balance adsorption efficiency and affordability. While increasing the adsorbent dose and contact time improved removal rates, refining these parameters is essential for cost-effective application. Additional research should explore the effects of different dosage ranges and varying contact times to optimize removal efficiency with minimal adsorbent use. It is also recommended to consider other operating parameters that may have an impact on the adsorption process, even though this study concentrated on the effects of adsorbent dosage and contact time. The performance of the adsorbent can be considerably impacted by variables like pH, temperature, and the presence of competing ions. A thorough understanding of the adsorption system may be obtained by examining the effects of various parameters on metal removal, which can also help with process optimization.

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