Comparative Analysis between Crustacean - derived Chitosan and Mollusc - derived Chitosan With Respect To Their Metal Adsorption Property.

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Abstract : Chitosan is a well-known and efficient by-product derived from shells of aquatic species. It is a bio polymer composed of a linear polysaccharide of alpha (1 - 4) linked 2- amino -2- deoxy beta-D glucopyranose synthesized from chitin present in the shells. Chitosan shows an excellent quality of adsorption of metal ions leading to it's application in treatment of contaminated water. Since the chitosan is derived from chitin, which is derived from shells which are waste products mostly, it's greatly contributing in a sustainable solution for environment pollution. Different sources of chitosan yield different types and amounts of chitosan, and this information can be used to choose the best source for various applications. In the present work crustacean derived chitosan and molluscan derived chitosan were observed for their adsorption properties on copper metal, using different concentrations of solutions. Metapaeneus affinis_ prawns shells were collected for crustacean chitosan and Meretrix casta for mollusc derived chitosan. Upon deproteinization and demineralisation, the chitin was subjected to deacetylation to produce chitosan. Copper solutions were treated with both the chitosans and tested for their adsorption properties. Using ammonia complex method, the treated solution was tested for copper ion residues. Upon testing the results showed a higher percentage removal of copper ions in the solution treated with crustacean derived chitosan compared to the molluscan derived chitosan. Therefore, crustacean derived chitosan and molluscan derived chitosan differ by a percentage of 2-3.5 in their adsorption property of metal ions.
Keywords: **Chitosan, Metal Adsorption, Sustainable, Water pollution.**

INTRODUCTION

Chitosan is a well-known and efficient by-product derived from shells of aquatic species. It is a bio polymer composed of a linear polysaccharide of alpha (1 - 4) linked 2- amino -2- deoxy beta-D glucopyranose synthesized from chitin present in the shells. The figure shows the structure of chitosan ((Mohanasrinivasan et al., 2014).). Chitosan being a by-product and modified biopolymer is used in various ways like Nanoparticles, nanocomposites, hydrogels, biofilms, chitosan membranes, chitosan fibres, chitosan fabric, cross linked chitosan, etc. Among these, the composites made of 1,2 cyclohextlenedinitrilotetraaceric acid, graphene, and chitosan were used for heavy metal removal from aqueous solution. This implied its use in chemically treated water form industries such as gold mining and electronic and so on ((Zia et al., 2018).). The different forms of chitosan allows to reduce its limitations which involves solubility in acidic medium and lower pH, reduced activity at certain temperatures, setc. The nanoparticles and nanosorbents of chitosan have reportedly tested for the removal of pollutants from soil and not only water (Abirami et al., 2025) .It's application as an adsorbent is due to the presence of NH3 group along with hydroxyl group which binds to the metal ions. It acts as a successful biosorbent of heavy metals in water upon modification. Chitosan polymer is obtained mainly from waste materials that are shells and hence, is sustainable and environment friendly, promoting sustainability while helping curb water pollution which is caused by metals, dyes and more. With its successful application in dye removal as an adsorbent, it can be used to treat water and prevent harmful dyes from entering the ecosystem.(Crini et al.,2008).

The prepared Chitosan was tested to remove metal ions (copper) which is a potential heavy metal that pollutes the water. One of the major causes of water pollution is metal toxicity. Metals like nickel, copper and lead along with copper can be harmful and cause damage to living beings if the ecological safety limit exceeds. Due to growing industries and anthropogenic activities, there has been a reported rise in these toxic metals leading to water pollution. Metal ions through water gets absorb in the blood stream of human and other animals dysfunctioning normal body functions and get accumulated in large amounts. Some metals are also proven carcinogens. Since these metal ions don't break down in organisms, they are not excreted but instead get accumulated in fat muscles and bones and joints. This not only possesses a threat to human and other terrestrial living organisms but also affects aquatic life. Copper is a heavy metal present in water which can be harmful [Cu(II)] to human system. Copper is mainly discharged by electronic industries as a trash device and needs proper treatment before it's release in water bodies.

This work aims to achieve a comparative analysis between molluscan and crustacean derived chitosan with respect to it's ability to remove metal ions from aqueous solution. Crustacean derived chitosan and molluscan shells derived chitosan were observed for their adsorption properties on copper metal, using different concentrations of solution. The shells were sourced from waste in fish market and carefully cleaned and dried before subjecting it to - deproteinization and demineralisation using sodium hydroxide and hydrochloric acid respectively, followed by deacetylation. Metapaeneus affinis prawns' shells were collected for crustacean chitosan and Meretrix casta for mollusc derived chitosan. Both species are abundantly found in Mumbai from Versova Coast to Malad Coast. Clams and prawns are widely consumed delicacy among the population of Mumbai and therefore, were easily available.

The obtained chitosan after drying and grinding was tested for copper adsorption using ammonia complex method. Upon treatment of more than 40 hours the chitosan powder actively adsorbed copper ions from the solution. Crustacean chitosan showed 99% removal of copper ions while molluscan chitosan showed 96-98% removal.

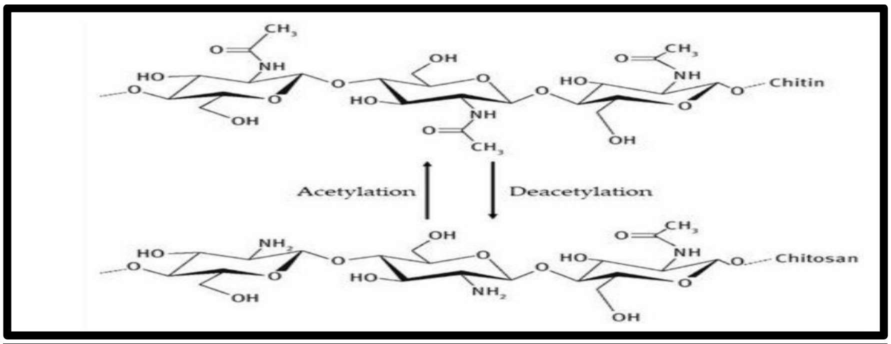

Fig. 1 : Structure of Chitosan

RESEARCH METHODOLOGY

Chitin and chitosan are both polymers and differ in presence of amino groups and the percentage of deacetylation. One with less than 50% deacetylation is chitin whereas a percentage more than 50% is chitosan. Both polymers can easily be obtained from waste materials such as shells of prawns and other crustaceans, bone of molluscs such as cuttlefish and some fungi and insects. The shells of prawns and that of clams are waste products and are easily accessible from fish markets. Different species of prawns and clams vary in colour, composition, features and hence need different amounts and concentrations of chemicals for breakdown and processing. Chitosan particularly from crab and shrimp shells has high molecular weight (500–1000 kDa), elevated degrees of deacetylation and extraction yields ranging between 60–75%. Whereas fungal-based chitosan offers lower molecular weight (50–300 kDa), with yields generally between 50–70%. Insect-derived chitosan (100-400 kDa), which has a moderate molecular weight is gaining prominence nowadays (Salman and Zedain, 2025). Whereas Crustacean-derived chitosan has been analysed to remove Cu(II) from aqueous solutions. ((Chalakkal et al., 2025) (Wahyuni et al., 2016) .

Chitosan has been extracted by different methods over the past years, from biotechnological extraction which involves enzymatic chitin extraction followed by microbial fermentation and finally biotechnological deacetylation of chitin to chemical extraction using different solvents. The standard and general procedure involves extraction based on acids and alkaline solvents for demineralisation and deproteinization. The chemicals used in this method often are known to be toxic and hence are being replaced by DES - deep eutectics solvents (Nagarajan et al., 2026). For more sustainable practising, greener methods for chitosan extraction have also been found which involves using graviola leaf extract as a powerful solvent to remove proteins and minerals due to presence of acetogenin in them (Rajendran et al., 2025). In the work presented in this paper, chemical extraction is used to obtain chitosan form two sources - crustacean and mollusc.

(A) Chitosan Extraction :

Prawns shells and Molluscan shells were collected as waste material from local fish market.
Crustacean source collected: Metapaeneus affinis
Mollusc source collected: Meretrix casta
Chemicals used: 5% NaOH, 4% HCl, 70% NaOH
The shells were washed thoroughly and dried in sun for a day and then grounded using mortar and pestle. Grinded powder of shells were weighed for estimation of yield of prawn shell chitosan and clamp shell chitosan.Approximately 3 g of prawn shells were taken and 3 g of clam shells, for preparation.

–The Chitosan Extraction Process:

1) Deproteinization*:* Shell powder was deproteinized in 50% NaOH for 5 hours at 100 degree Celsius. Later, the solution was filtered and washed to remove NaOH.

2) Demineralisation: The filtered shell powder was now put in 4 % HCl for 3-5 hours at 20 degree Celsius. Later, the powder was filtered and washed for further processing. This process gives chitin, which is again a biopolymer which upon deacetylation will become chitosan.

3) Deacetylation: This process involves the removal of acetyl groups from chitin obtained in the previous process. This was done by adding the grinded chitin to 70 % NaOH for around 24 hours at room temperature.

4) Chitosan: The solution was filtered and the final grinded chitosan was obtained. The chitosan obtained was washed and dried followed by grinding it in fine powder using mortar and pestle. Now the chitosan was measured for its weight after drying for 24 hours.The obtained Chitosan from prawns' shells and clamp shells was weighed and calculated for their yield percentages.

(B) Testing for Heavy Metal – Copper ions using CuSO4 solution

The obtained chitosans were tested for their absorbance efficiency using the ammonia complex method colorimetrically (Mehligs et al., 2002). Metals like copper have been estimated using spectrophotometry for which different methods have been developed that could be applied to environmental and industrial laboratories. Such methods have been affirmative in monitoring copper levels in industrial effluents, biological fluids and tap water too. Though rapid and more sensitive methods for copper estimation are used which mostly include spectrophotometry, chromatography, sorbent extraction, colorimetric methods are often preferred (Dalman et al., 2002). Due to its low instrumentation cost and it's sensitivity towards chromogenic agents, colometric estimation using the ammonia complex method was used to estimate the presence and amount of copper metal in a solution. Copper upon reaction with ammonia forms tetraamine complex of blue colour, that can be used to estimate the presence of copper in the solution.

For this different concentrations of CuSO4 were made to obtain a standard curve graph.

1) Standard Curve: 10%, 20%, 30%, 40%, 50% solutions of copper sulphate were made and added with 1-2 ml ammonia to test the presence of metal and it's concentration. This involved colorimetric analysis, and plotting graphs using the readings.

a) Stock Solution: 100 mg in 100 ml
b) Working Solution: 10 ml Stock solution + 90 ml Distilled water.
c) Table
d)The obtained graph was used to calculate the unknown concentration of CUSO4 in test.

Table 1: Treatment of Copper sulphate with Chitosan

Conical flask	Copper Solution	Prepared Chitosan
A	50 ml	0.0 g
B	50 ml	0.3 g
C	50 ml	0.3 g

2) Treatment with Chitosan

Two conical flasks of 250 ml each were labelled "Control" and "Test". Both had a 50 ml CuSO4 solution (250 ml + 0.25 g CuSO4 powder). Another conical flask of the same volume of CuSO4 solution was labelled as "CONTROL".

FLASK A: Control
FLASK B: Prawns Shell Chitosan
FLASK C: Clamp shell chitosan.

Flask B and Flask C were treated with 0.3 g chitosan powder of prawn shell chitosan powder and clamp shell chitosan powder respectively. Both the flasks were kept in a shaker for 1 hour and later incubated for over 40 hours. After 40 - 42 hours the mixture solution was filtered using Whatmann Filter paper. For estimation of copper 10 ml filtrate was tested each of the conical flask B and C. The filtrate was added with 2 ml ammonia solution (25%) at a pH 7 and allowed to settle for 10 minutes . After 10 minutes absorbance was measured at 640 nm.

OBSERVATION

Following Deacetylation of Crustacean and Mollusc shells that is a prawn shells and clamp shells yield of the Chitosan was measured

Chitosan derived from prawn shells: 0.2 g

Chitosan derived from clam shells: 0.9 g

Figure 2 : Obtained Chitosan:
Upper Plate : Prawns Shell Chitosan
Lower Plate : Clam Shell Chitosan

Solubility: The prawns dissolved in 1:1 acetic acid solution while was not soluble in normal water. However, the clamp derived chitosan and dissolved in a higher concentration of acetic acid 1:2 and remained insoluble in water. After an incubation period of nearly 48 hours the mixture (Chitosan +copper sulphate solution) was filtered and tested for presence of metal. The filtrate obtained was clear with the Flask C showing a little blue tint to the solution.The filter papers showed tiny particles coloured in blue copper sulphate absorbed by chitosan's particles. Upon testing for estimation of metals using ammonia complex method the filtrate showed no deep blue colour formation in Flask B and hence no amount of copper sulphate was present after chitosan treatment. However, Flask C showed a little blue tint upon adding ammonia.

(1) Standard curve for copper sulphate

Table 2: Standard curve for copper sulphate

Concentrations	O.D Absorbance
10%	0.12
20%	0.26
30%	0.32
40%	0.4
50%	0.48

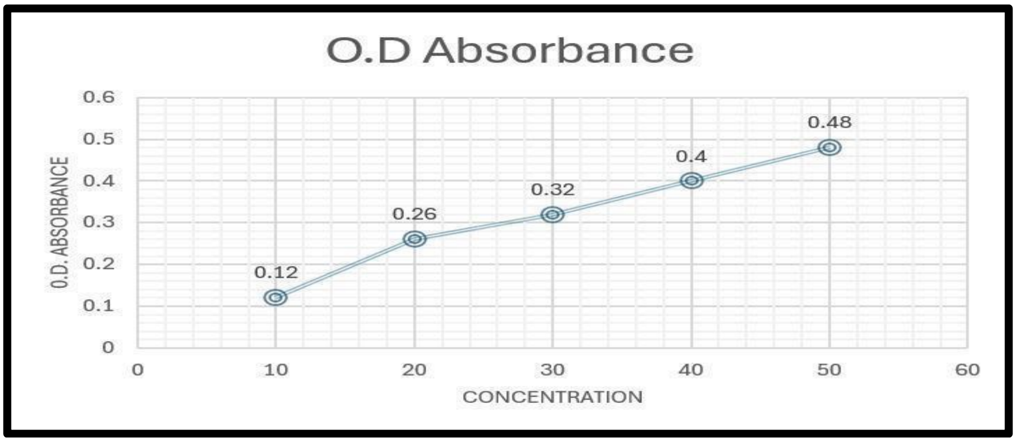

Fig.3 Standard Graph of Concentrations for CUSO4

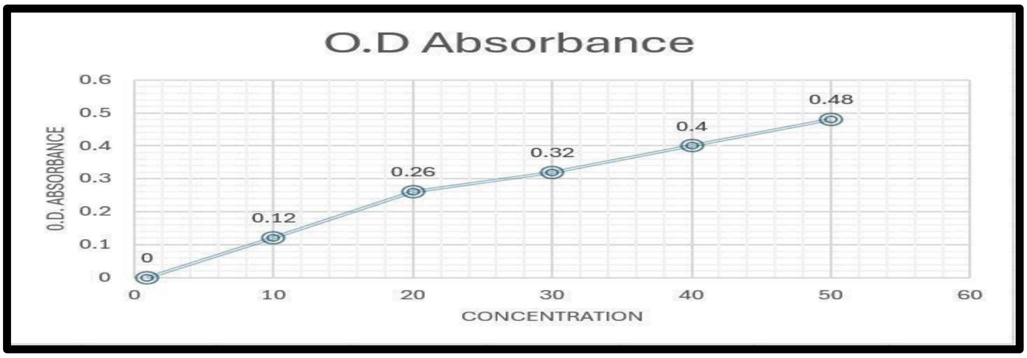

Fig 4. Standard Graph with Unknown Concentration in Flask B

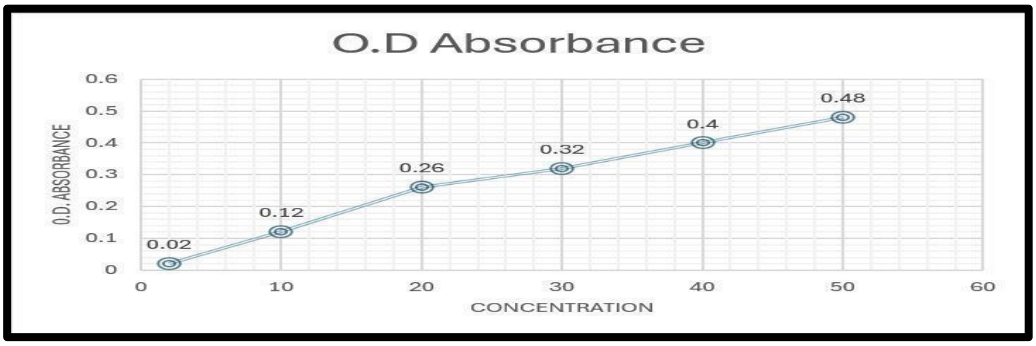

Fig 5. Standard Graph with Unknown Concentration in Flask C

The concentration is found using the formula:

Concentration = O.D ÷ slope

= 0.02 ÷ 0.0105 = ~1.9 mg /L (clam shell)

= 0.00 ÷ 0.0105 = 0.0 mg / L (prawn shell)

(2) Ammonia Complex Method

Table 3: Ammonia complex method

Test tubes	Filtrate	Ammonia Solution	Absorbance
A	10 ml	2 ml	0.01
B	10 ml	2 ml	0
C	10 ml	2 ml	0.02

The concentration of CuSO4.5H2O and Cu2+ ions in the test solutions were :
By Graph: 0.0 mg/L - prawn shell, Flask B
1.9mg/L - clam shell, Flask C

Percentage Removal (%):
[Co - Ce ÷ Co] ×100
=100 % for Prawns Shell, Flask B
=99.25% for Clam Shell, Flask C
Where Co = Initial concentration
Ce = Final concentration

Adsorption Capacity (qe) = Co - Ce × V ÷ M (g)
= 41.66 g/g
= 38.33 g/g
Where Co = Initial concentration
Ce = Final concentration
M = Mass of Chitosan
V = Volume of solution

Adsorption is a physio-chemical process involves when one substance binds to another. When biological substances are in use as an adsorbent, the process is known as bio adsorption. During biosorption between a liquid and a solid, the solid acts and an adsorbent while in case of liquid and gas, the liquid adsorbs the gas.
The high initial concentration of CuSO4 in test solution quickly gets absorbs over a period of 40 hours. This time may act as an advantage to the test and help the chitosan bind to Cu^{2+} ions efficiently. The obtained filtrate was transparent in observation and hence the absorbance was less than 1, (OD< 1) at even 640 nm. This indicates nearly 99 - 100% removal of copper from solution given the time and pH of solution in which the chitosan acts. The alkaline pH of the test solution allows chitosan to remain insoluble and adsorb over it the metal effectively. Hence when chitosan is added to metal solutions like that of copper it adsorbs Cu^{2+} ions over time and not quickly or rapidly. With a percentage removal of 100 % the prawns chitosan removes more effectively than clamp chitosan which has a percentage removal of 99.25%. Hence with respect to comparing the two sources and types of chitosan the Flask C slightly differ from flask B which can be negligible. With an adsorption capacity of 41.66 g/g in crustacean chitosan, it is ahead by a fraction of 03.33 from mollusc chitosan which has 38.33 g/g adsorption capacity. The yield of prawns shell chitosan is more than that of clam shell chitosan. Also, the solubility difference between the two helps understand their structure complexity and applications. The prawns she'll chitosan dissolves at a faster pace as compared to clam shell chitosan that would need a higher concentration of acetic acid and more time. This difference is due to their difference in degree of deacetylation. Prawns' shells have a higher degree of deacetylation as compared to the clamp shells. Higher the degree of deacetylation more the number of NH_2 groups present for binding metal ions. Therefore, due to presence of higher degree of deacetylation the prawns shell chitosan dissolves in acetic acid. And so, the prawns shell was seen to be more effective in adsorbing Cu^{2+} ions than clam shells, with a slight difference of 2 -3 %.

RESULTS

Among the various sources of Chitosan, prawn has been studied earlier to remove Cu(II) evaluated as best with high yielding capacity and adsorption quality. This work evaluated the adsorption of Cu 2+ ions from aqueous solution using Chitosan derived from prawn's shells and clam shells, using ammonia complex method at 640 nm.

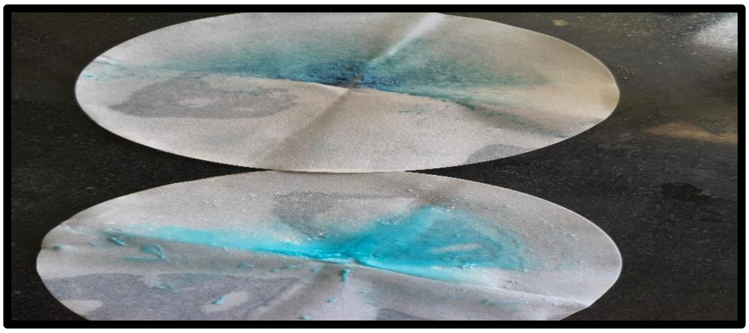

Fig 6. $CuSO_4$ adsorbed to Chitosan Particles

Chitosan extracted from both sources was applied under identical experimental conditions, including equal adsorbent dosage, contact time (40-42 hours), and initial copper concentration. The filtrates obtained after treatment were analyzed colorimetrically. The absorbance values of treated samples were found to be negligible (approaching zero), indicating significant removal of Cu^{2+} ions. Based on calibration data, both adsorbents demonstrated high adsorption efficiency. Quantitative comparison revealed that:

Prawn shell-derived chitosan exhibited slightly higher adsorption efficiency. Mollusc shell-derived chitosan showed comparable performance with only a 2–3% lower removal efficiency. The percentage yield of chitosan was also determined:

Mollusc shells: ~30%

Prawn shells: ~40%

This indicates that prawn shells not only provide higher yield but also marginally better adsorption performance. Therefore, chitosan derived from prawns and chitosan derived from clamps have little to no difference in their absorption properties given that the conditions of preparation, extraction and estimation of metals were same for both. Moreover, the prawns shell chitosan shows an adsorption capacity of 41.66 g/g whereas the clam shell chitosan has 38.33 g/g adsorption capacity. Hence there is no major difference in adsorption ability, observed between prawn shell chitosan and clamp shells derived chitosan.This helps us to know that there is no significant difference but still can help choose right source for Chitosan production.

Fig 7. Clear filtrate obtained upon treatment using Chitosan.

CONCLUSION

Water pollution has become a serious issue in recent years. The development of environmental friendly and sustainable water treatment technology is necessary. Due to chitosan's unique qualities, including affordability, superior environmental friendliness, adsorption performance, biocompatibility and biodegradability, and bioactivity, it has attracted a lot of attention from researchers as an adsorbent. The only limitation chitosan has is it's activity in a certain pH. Chitosan is known to be ineffective in pH lower than 4 i.e. acidic pH. In acidic medium the chitosan easily dissolves and hence no metal would adsorb to it. Hence to prevent this waste water or contaminated water has to be treated with a pH buffer or added with a base to maintain a suitable pH for it to work.Though the physical form of chitosan may affect its function and efficiency in adsorbing metal ions it is a reliable solution to reduce concentration of heavy metals with least effort and machinery required. Modifications such as cross-linking, grafting, and impregnation techniques improved the adsorption capacity of chitosan. This also includes creating hydrogels and biofilms for various uses. Its has been used in fields such as bio engineering, textile products, agriculture, food industry and paper industry, and wastewater treatment. The ability of chitosan to form a chelating agent and it's solubility in acids (ex: acetic acid) helps in formation of fibres that are used in sutures and fabrics for wound healing. Recent in-vitro studies show that the removal efficiency of chitosan also depends on contact time with the metal ions. The contact time is directly proportional to the amount and efficient adsorption of metal ions. Lead being a toxic heavy metal possesses a higher density than water and is easily transported to the bodies of living organisms. Therefore, chitosan can be used as an effective and sustainable remedy for large-scale water treatments.

The present study demonstrated the comparison between chitosan derived from both prawn and mollusc shells as an effective biosorbent for the removal of copper ions from aqueous solutions. Although prawn shell-derived chitosan exhibited slightly higher adsorption efficiency, the difference between the two sources was minimal (approximately 2–3%), indicating that both materials possess comparable adsorption capacities. This suggests that the availability of functional amino groups responsible for metal binding is sufficiently high in both types of chitosan .However, prawn shells yielded a greater amount of chitosan, likely due to higher chitin content and lower mineral composition, making them a more efficient raw material for large-scale production. The negligible absorbance observed in treated samples indicates that chitosan is highly effective in reducing copper concentration to below detectable limits under the given conditions. This highlights its potential application in wastewater treatment and bioremediation .Overall, while prawn shell chitosan may be slightly superior in terms of yield and adsorption efficiency, mollusc shell chitosan remains a viable, cost-effective, and sustainable alternative for heavy metal removal.

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