CIBTech Journal of Zoology ISSN: 2319–3883
Online, International Journal, Available at http://www.cibtech.org/cjz.htm
2024 Vol.13, pp.350-359/Amreen et al.
***Research Article** (Open Access)*

ISOLATION OF CHITOSAN FROM PRAWN SHELL AND ITS POTENTIAL APPLICATION AS A HEAVY METAL SEQUESTERING AGENT FOR INDUSTRIAL EFFLUENTS AND WASTEWATER TREATMENT

Amreen Husna. I, Lucky. R* and Hiba. M.
P. G. and Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women (Autonomous), Chennai- 600018, Tamil Nadu, India
**Author for Correspondence: lucky.r@jbascollege.edu.in*

ABSTRACT
Industrial activities often generate effluents containing elevated levels of heavy metals, posing significant environmental hazards. Conventional methods for heavy metal removal from industrial wastewater often prove costly and environmentally unfriendly. In recent years, chitosan-based adsorption has emerged as a promising alternative for mitigating heavy metal pollution in industrial effluents. Chitosan, a biopolymer derived from chitin, exhibits excellent adsorption properties due to its high surface area and abundant functional groups. This study reviews recent advances in chitosan-based adsorbents for heavy metal sequestration, highlighting their efficacy, mechanisms of action, and potential applications in industrial settings. Moreover, the paper also discusses the challenges and future directions for the practical implementation of chitosan-based adsorption in industrial effluent treatment, emphasizing the need for further research to optimize adsorbent performance, enhance cost-effectiveness, and ensure environmental sustainability. Overall, chitosan-based adsorption holds great promise as a viable strategy for addressing heavy metal contamination in industrial effluents, offering a pathway towards cleaner and safer industrial wastewater management practices.

Keywords: *Environmental Hazards, Heavy Metal Sequestration, Chitosan-Based Adsorption, Industrial Effluent Treatment, Environmental Sustainability*

INTRODUCTION
A wide range of human activities result in the production of huge quantities of wastewater containing heavy metals. The Potential sources of the heavy metals release into environment are the industries such as mining, electroplating, fabrication, leather tanning, fertilizer, textile dyes, printing, and acid battery manufacturing. Other causes for the metal release include natural weathering processes, waste emissions, atmospheric depositions, landfills, pesticide application to crops and additional anthropogenic activities (Monier *et al*., 2010; Wang and Chen 2009). The harmful effect of metals is due to enzyme inhibition or activation, damage of subcellular organelles, carcinogenicity, and effects on kidneys, nervous system, endocrine system, reproduction and respiratory system (Hodgson, 2004).
It is obligatory on the industries to remove the metal pollutants from effluents before disposing them. The methods commonly employed for eliminating the metal ions from liquid streams are the following: chemical precipitation, solvent extraction, membrane process, ion exchange, electrochemical method and adsorption (Kanamadi *et al*., 2006).
Heavy metals removal from wastewater is the most important because they not only contaminate the water bodies but are also toxic to the ecosystem. As the majority of the heavy metals are non-degradable and highly toxic in nature (Chio *et al*., 2004), their concentration has to be reduced to acceptable levels before discharging into the environment, or else these can pose a threat to human as well as animal health. Heavy metals such as lead, zinc, nickel, mercury, cadmium, copper, arsenic, cobalt, chromium, bismuth, and ferrous have been recognized as poisonous to the environment and human health, even when present in

traces. Their presence in the waste water of several industrial processes has brought about more environmental concerns due to their toxicity even at low concentrations. Recently in the heavy metal sequestration of wastewaters, the trend to use different bio-sorbents is observed. Chitosan-based adsorption for heavy metal sequestration holds promise in waste water treatment of industrial effluents. Chitosan is a biopolymer derived from chitin, found in the exoskeletons of crustaceans like shrimp and crab. Its unique properties, such as high surface area, abundant functional groups (like amino and hydroxyl groups), biodegradability, and low toxicity, make it a favourable material for heavy metal adsorption.

Several studies have shown that chitosan can effectively adsorb heavy metals from aqueous solutions through mechanisms like complexation, ion exchange, and electrostatic attraction. Moreover, chitosan can be modified to enhance its adsorption capacity and selectivity for specific heavy metals.

Chitosan is an abundantly available low-cost bio-polymer for dye removal that can be obtained from natural resources. As compared with other commercial adsorbents, it has received a lot of focus due to its specific properties such as cationicity, high adsorption capacity, macromolecular structure, abundance, and low price. Different dye, metal, and other pollutants have been reported to be effectively removed by a chitosan or different modifications of this biopolymer (Gupta *et al.*, 2009). Chitosan is a very promising adsorbent, which can be modified in many ways (grafting, cross linking, functionalisation for forming composites, etc.). Since chitosan is very sensitive to pH, forming either gel or dissolve depends on pH values. Some cross-linking reagents such as glyoxal, formaldehyde, glutaraldehyde, epichlorohydrin, ethylene glycon diglycidyl ether and isocyanates have been used to improve its performance as adsorbent. This process of cross linking stabilises chitosan in acid solutions becoming insoluble and enhances its mechanical properties (Gupta *et al*., 2009). Recently, chitosan-based metal particle composites have been studied increasingly as an alternative adsorbent in water treatment, using metals oxides, magnetite and bimetals to adsorb heavy metals and dyes from wastewater. For example, chitosan-coated magnetite nanoparticles (CMNP) were prepared and used as bactericidal agent to remove organic contaminants and bacteria from water (Moradi *et al*., 2014).

MATERIALS AND METHODS

Sample collection of shrimp shell:

Shrimp shell waste was collected from Koyambedu fish market, Chennai district, Tamil Nadu, India. Samples were taken and washed properly with flowing tap water to remove the soil and extragenous matter. Thoroughly cleaned sample was kept for drying at 80°C in hot air oven for 2-3 days. Dried sample was finely ground and kept in an air tight container.

Extraction of chitin and chitosan from shrimp shell:

For extraction of chitin, the conventional chemical method was followed. Chitin extraction was done following three major steps, i.e., demineralization, deproteination, deacetylation. For demineralization, 10 g of sample was treated with 2N Hydrochloric acid at solid to solvent ratio of 1:15 for 2 hours with constant stirring at 150 rpm in incubator shaker at room temperature (No *et al*., 1999).

Acid was slowly added to avoid frothing due to gas formation occurring because of calcium carbonate content of shell which reacts with the acid and form carbon dioxide. After demineralization, the sample was washed with tap water till the sample reaches neutral pH. Final wash was given with hot distilled water and sample was kept for drying at 80°C overnight (Seo *et al*.,2006).

For deproteination, demineralised shrimp shell powder was treated with 2N NaOH at solid to solvent ratio 1:20 for 2 hours with constant stirring at 150 rpm at 50°C in an incubator shaker followed by thorough washing and drying as mentioned above. After this step, the end product was chitin. (Seo *et al*.,2006).

For deacetylation, chitin was treated with strong alkali, i.e., 1 g of chitin was added to 50% NaOH for 1 hour at 121°C, 15 psi followed by washing till it reaches neutral pH. After drying, the final product recovered was chitosan. Chitosan was further dried at room temperature and stored (Renata *et al*., 2012).

Estimation of chitosan yield sample preparation:

The weight of chitosan produced is measured and the yield is calculated.

Chitosan solution:
A stock solution of chitosan (5 mg/ml (w/v) was prepared in 1% (v/v) acetic acid. Suitable dilutions were prepared from the stock using distilled water (1 mg/ml, 2 mg/ml, 3 mg/ml, and 4 mg/ml).
Sodium nitrite reagent:
An aqueous solution of 0.5 M $NaNO_2$ was prepared and stored at 4 °C
Dinitrosalicylic (dnsa) acid reagent:
DNSA reagent (50 ml) was prepared by dissolving 15 g of sodium potassium tartrate, 10 ml of 2 N NaOH and 0.5 g of DNSA and the volume was made up to 50 ml using distilled water.
Preparation of a standard curve for mannose:
A standard curve of commercially available mannose was prepared for a concentration range of 100 µg/ml to 1000 µg/ml by following standard assay protocol using DNSA reagent.
Preparation of a standard curve for 2,5-anhydromannose:
(Deamination product of chitosan after treatment)
A standard curve for 2, 5-Anhydromannose was prepared by using commercially available chitosan for a concentration range of 100 µg/ml to 1000 µg/ml followed by treatment of the same with $NaNO_2$. The end product was assayed with standard assay protocol using DNSA reagent.
Chitosan preparation by DNSA method-assay procedure
0.1 ml of $NaNO_2$ (0.5 M) was added to the tubes containing sample, blank and standard (1 ml each). The reaction mixtures were then heated at 80 °C for 45 min in a water bath to complete the depolymerization deamination reaction and cooled under tap water. I ml of DNSA was then added to the reaction mix after adjusting the pH to 4 by adding 1 N HCI. The tubes were then placed in water bath at 75 °C for 15 min. The tubes were cooled under tap water and absorbance was measured at 540 nm. The method was also conducted at semi-micro scale with a final proportionally. In brief, 250 µl of the sample was mixed with 25 µl of 0.5 M NaNO2 followed by incubation at 10 °C for 45 min in a water bath 250 µl of DNSA was then added to the reaction mix alter adjusting the pH to 4 by adding I N HCI. The tubes were then placed in water bath at 75°C and the colour development was observed. Optical value was measured by 540 nm (Abhik *et al*.,2019).
Column chromatography
Column chromatography is suitable for the physical separation of gram quantities of material. A Solvent acts as the mobile phase while a finely divided solid surface acts as the stationary phase. The stationary phase will absorb the components of the mixture to varying degrees. As the solution containing the mixture passes over the adsorbent, the components are distributed S compete for positions on the solid adsorbent, the solvent displacing the sample reversibly and continuously in the direction of the solvent flow (Chandana kumara *et al*., 2022). Consequently, a weakly adsorbed compound will spend more time in the solvent, and will therefore be eluted first. Adsorption column chromatography involves the affinity if a compound to bind to metal ions in a solution. The chitosan obtained was mixed with silica gel 60-120 mesh using hexane as a solvent for dispersion. Aqueous solutions of Sodium, Iron and Magnesium were prepared in the following composition.
Adsorption chromatography
Organic matter in sample is digested by wet digestion or dry digestion or high pressure microwave digestion and determine the amount of heavy metals, i.e. arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) by using graphite furnace atomic absorption spectrophotometer (GF-AAS) and flow injection analysis system -atomic absorption spectrophotometer (FIAS-AAS).
Prepare As standard calibration solutions concentration of 5, 10, 20, 30 and 50 g/L in 0.5% v/v nitric acid, respectively.

- For FIAS-AAS (Hydride generation technique)
- Prepare As standard calibration solutions concentration of 1 µg/ml.
- Pipette 200, 400, 600, 800 µl from standard calibration solutions concentration into separate 100 ml. volumetric flask.

- Prepare Cd standard calibration solutions concentration of 0.5, 1, 2, 3 and 5 g/L. in 0.5% v/v nitric acid, respectively.
- Pb: Prepare Pb standard calibration solutions concentration of 5, 10, 20, 30 and 50 g/L in 0.5% v/v nitric acid, respectively.
- Hg: Prepare Hg standard calibration solutions concentration of 0. 5. 1, 2, 3 and 5 g/L in 3% v/v hydrochloric acid, respectively.
- Mg: Prepare Mg standard calibration solutions of 0. 5, 1, 2, 3 and 5 g/L in 3% v/v hydrochloric acid, respectively.
- Na: Prepare Na standard calibration solutions of 0. 5, 1, 2, 3 and 5 g/L. in 2% v/v hydrochloric acid, respectively.
- Fe Prepare Fe standard calibration solutions of 0. 5, 1, 2, 3 and 5 g/l. in 3% viv nitric acid, respectively.
- Modifier for GF-AAS (graphite furnace atomic absorption spectrophotometer) For As 1,000 g/ml.
- For Pb and Cd: Mix 1:1 of 0.2% w/v Mg(NO3)2.6H2O in 0.5% v/v nitric acid and 0.2% w/v NH4H2PO4 in 0.5% v/v nitric acid
- Reagent for pretreatment of As (Arsenic) Mix 1:1 of 10% w/v potassium iodide and 10% w/v ascorbic acid.

Flow Injection Analysis System- Atomic Absorption Spectrophotometer (Hydride generation Technique)

Condition:

Element wavelength (nm) reducing agent carrier atomization temp. (°C) Injection volume (µL) As 193.7 0.2% w/v NaBH4 10% v/v HCI 900 500

Flow Injection Analysis System Vapour Technique) Atomic Absorption Spectrophotometer (Cold

Condition:

Element wavelength (nm) reducing agent carrier atomization temp. (ºC) injection volume (µL) Hg 253.7 1.1% w/v SnCl2 or 0.2% w/v NaBH4 3% v/v HCI 300 500

Electrodeless Discharge Lamp or Hollow Cathode Lamp: As, Cd, Pb, Hg.

PROCEDURE:

Sample Preparation:

Reagent Blank was prepared as in sample preparation but without adding the sample. Sample preparation can be carried out by either one of the following methods.

- Microwave digestion (for As, Cd, Pb, Hg).
- Accurately weighed to the nearest mg in duplicate, 0.15-0.20 gm of sample was added into a high-pressure resistance 50 ml. quartz or TFM vessel. 3 ml of concentrated nitric acid and 30% hydrogen peroxide of 1 ml was added by using graduated Pipette. If sample has talcum or pigment, 1 ml of hydrochloric acid can be added.
- After the lid was closed it was left for about 15 minutes to ensure complete reaction. Digested in microwave digestion system at the specified program.
- After cooling to room temperature, deionised water 20 ml was added to the digested solution, rinse the inner wall and lid thoroughly. Filtered through Whatman paper no.1 into 50 ml volumetric flask and diluted to volume with deionised water.
- Dry ashing (for As, Cd, Pb).
- Accurately weighed 2.5 g sample was taken in a silica dish and added 3 ml. of 50% w/v magnesium nitrate.
- Dried on the water bath and ash was residue first in the heating mantle until no more fume and then in the muffle furnace at 500°C for 3 hours.
- After cooling, 25 ml of 6M hydrochloric acid was added, filtered into a 50 ml volumetric flask and diluted to volume with water.

Wet digestion (for Hg)

- Accurately weighed 0.5 g sample was taken in a digestion tube with screw cap and 7 ml of concentrated nitric acid was added.
- The sample solution was heated in a block heater at 60°C maximum for at least 3 hours.
- Cooled and diluted to volume (50 mL) with water. Kept for 24 hours in the refrigerator. The solution was filtered through Whatman paper No. 40.
- The digested solutions are used for analyses by FIAS-AAS (cold vapour mercury technique).

Pretreatment for As

- 10 mL was pipetted out to each of deionised water (as standard blank), the reagent blank. The standard solutions and the sample solution into separate 100 ml. volumetric flasks.
- 10 ml of concentrated hydrochloric acid was added and 10 ml of reagent for pretreatment of As(3.9) to each of the solutions and allow them to stand for 45 minutes at ambient temperature. Dilute to volume with water. The final concentrations of the standard solutions are 2.0, 4.0, 6.0 and 8.0 g/L respectively.
- These solutions are used for analyses by FIAS-AAS (Hydride Generation Technique).

Calibration curve

Standard calibration solutions were injected into the GF-AAS or FIAS-AAS (Cold vapour Technique) or FIAS-AAS (Hydride Generation System) at the specified condition. Response was plotted (absorbance or peak height or area) versus concentration of each standard solution.

Sample solutions were injected into GF-AAS or FIAS-AAS (Cold vapour Technique) or FIASAAS (Hydride Generation System). Record the response and concentration (g/L) of As, Cd, Pb, Hg in sample solution, calculate g/g of As, Cd, Pb, Hg in sample (Pavithra *et al*., 2021).

RESULTS

Extraction of chitosan:

Chitosan was extracted by three major steps which are demineralisation, deproteination and deacetylation**.** After extraction the final product chitosan was obtained.

Estimation of chitosan yield sample preparation:

Stock preparation: 250mg starch with 100ml of distilled water

Working standard: 10 ml stock solution and 100 ml of distilled water.

***Chitosan preparation by DNSA methods*:** By stock and working standard solution concentration of chitosan is done through adding DNSA solution to five test tubes.

Adsorption chromatography:

The solution containing the heavy metals were passed through the columns. The eluents of heavy metals solution were passed through silica gel, silica gel +isolate Chitosan and the heavy metals concentation of these ions were determined using absorption spectroscopy.

Optical density (OD value) chitosan: Standard concentration increasing while the absorbance of chitosan simultaneously increases.

Table No. 1: Absorbance of standard chitosan

Standard concentration mg/ml	Absorbance at 580nm
200	0.0298
400	0.0371
600	0.0788
800	0.1172
1000	0.1591

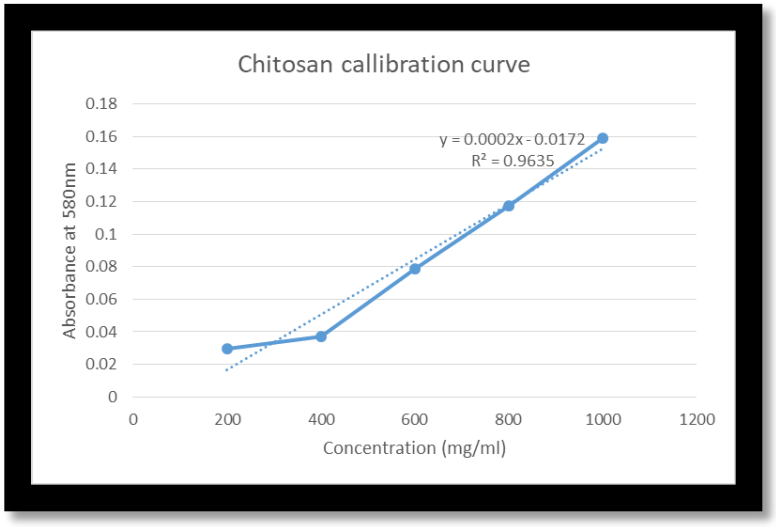

Figure 1: standard chitosan calibration curve

Calculation of percentage of chitosan:

The five standard concentration increase shows the percentage equivalence of chitosan quality

Table No. 2: Percentange equivalence of chitosan quality

Standard concentration mg/ml	Percentage equivalence	Percentage
200	235	2.35
400	271.5	2.715
600	480	4.8
800	672	6.72
1000	881.5	8.815

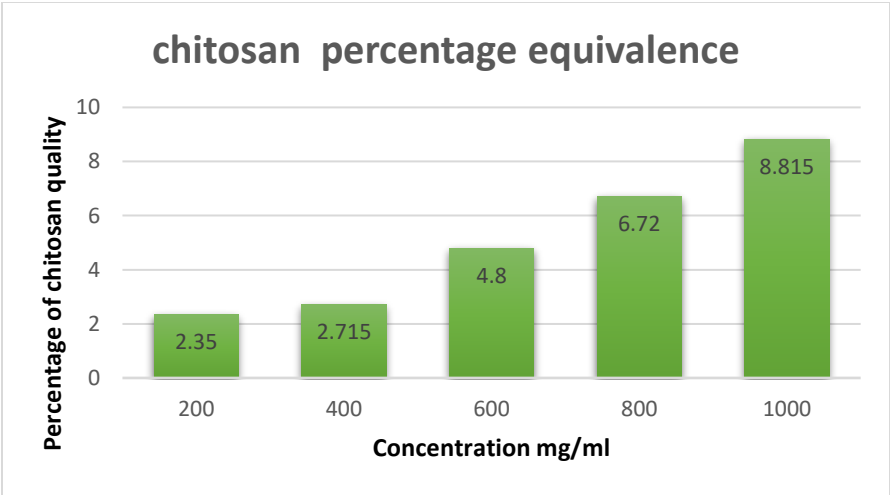

Figure 2: Percentage of chitosan

Heavy metal concentration in different chemicals:
Heavy metals like arsenic cadmium zinc iron sodium show the absorbance in different chemicals silica gel+chitosan, silica gel.

Table 3: concentration of the heavy metals in solution, silica gel eluate and silica + chitosan eluate

Samples	Heavy metal solution	Silica gel eluate	Si + Chitosan eluate
Heavy metals	**concentration of heavy metals in ppm**		
arsenic	2	2	2
cadmium	2.3	1.2	1.3
zinc	1.1	1	1
magnesium	160	134	67
iron	324	307	218
sodium	255	227	140

Heavy metal adsorption:
This chart show that these two metal ion arsenic and cadmium show low absorption concentration because of different affinity to chitosan.

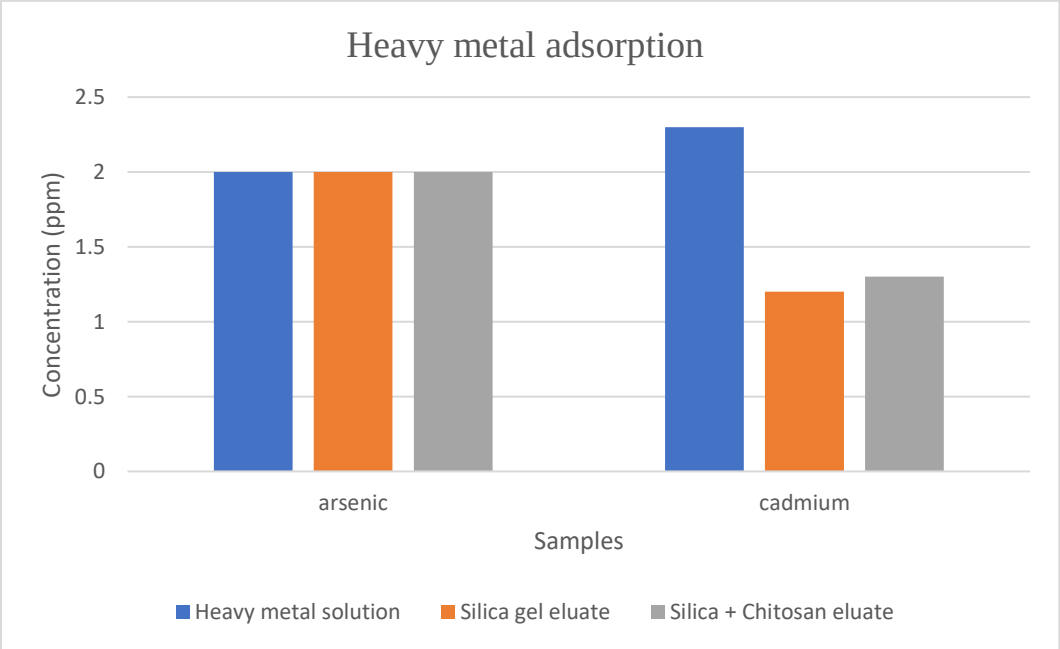

Figure 3: Heavy metals adsorption of arsenic and cadmium

This chart shows silica+chitosan eluate has significant high absorption concentration while comparing to other heavy metals. Initially magnesium has 150ppm silica gel was able to adsorp 130ppm from 150ppm.Silica+chitosan was able to adsorption more than 100ppm from 150ppm only 50ppm left.

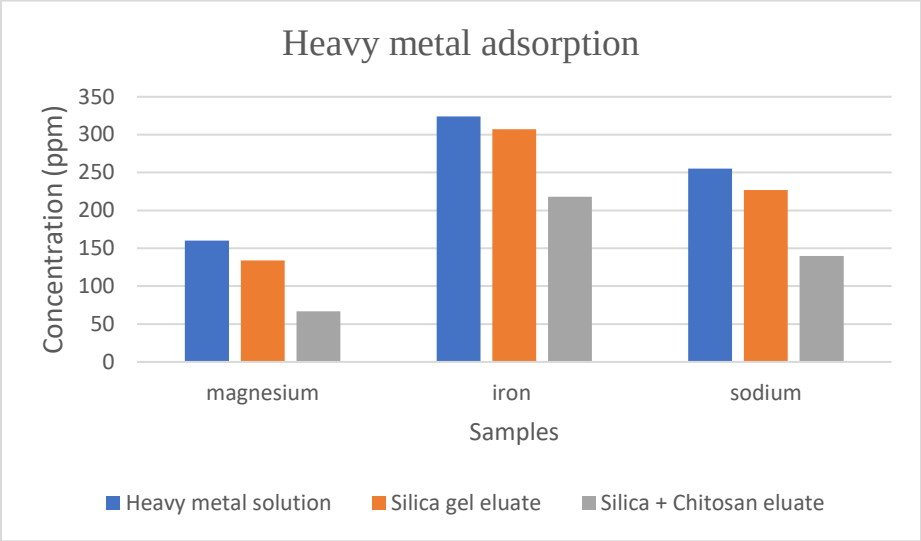

Figure 4: Concentration of the heavy metals in solution, silica gel eluate and silica + chitosan eluate

Relative adsorption: In silica+chitosan absorbs arsenic and cadmium have adsorped only 1times where low absorption. In case of magnesium and sodium the adsorption is 2 and 1.7 times the absorption was more in comparing with just silica absorption

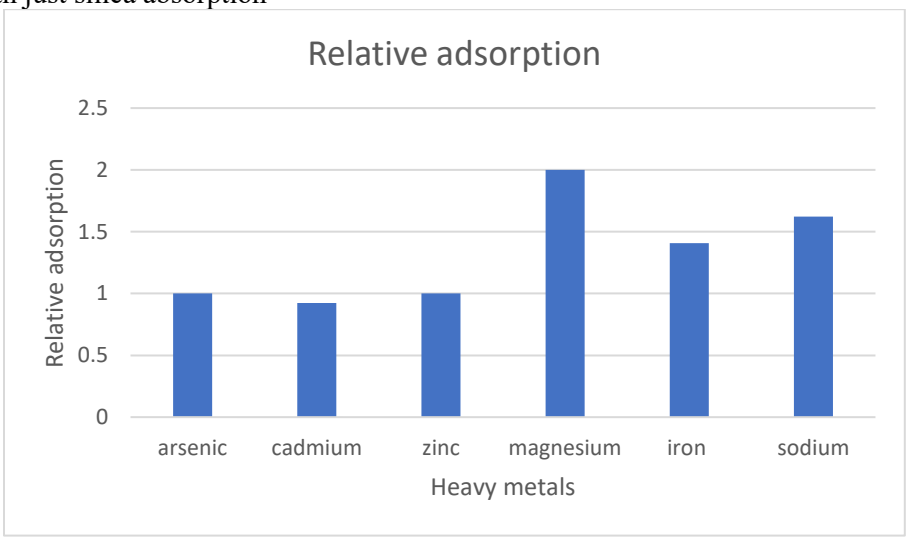

Figure 5: Relative adsorption of Chitosan + Silica gel compared to Silica gel

DISCUSSION

Chitin and chitosan, are typical marine polysaccharides as well as abundant biomass resources. Chitosan is a natural biopolymer material produced from chitin biopolymer which is the second most abundant natural biopolymer found in nature after cellulose. Chitosan apart from being used for various applications in the pharma industry such as adjuvants, carriers for vaccines, etc., is least exploited for wastewater treatment owing to its bio-absorption properties (Carmen and Juan, 2020).

The chemical methods used in the isolation of chitosan from prawn shell wastes in this study involved simple and scalable approaches that can be reproduced in industrial manufacturing processes. The chemical treatment does not include the usage of toxic bleaching substances that could potentially leach out to the environment. Chitosan estimation by the DNSA method revealed high concentrations and yield of the

purified chitosan (Abhik *et al*., 2019). Adsorption column chromatography revealed that chitosan mixed silica gel was able to sequestrate the heavy metal ions with a relative increase of several times in adsorption. Even in low concentrations, heavy metals (Cu, Ni, Zn, Pb, Cr, Cd, Co, As, Fe) can provoke several life-threatening effects in animals and human beings (Danuta Witkowska *et al*., 2021).

Hence, heavy metal contamination has evolved into a severe challenge for both the ecological system and public health, on a global basis. The removal of heavy metals from water bodies is necessary for the safe consumption of water and human activities. The effective removal of heavy metals from wastewater has witnessed vast developments in recent years. Adsorption processes possess superior advantages, such as simple design and low initial cost, over other methods such as membrane filtration and photocatalysis. An effective and worthwhile approach to mention for boosting the adsorption rate of several polymeric structures is their modification and addition of several new functional groups in the polymeric macromolecular chains. The demand for seafood has considerably increased, and millions of tons of crustacean waste are discarded every year. These waste products are rich in natural biopolymer chitin. The deacetylated form of chitosan has attracted attention as an adsorbent and it is a biocompatible and biodegradable polymer that can be modified and converted to various derivatives (Ngah *et al*., 2006). The synergetic effect of chitosan and PVA shows great potential for heavy metal removal from aqueous solutions.

REFERENCES

Abhik M, Anjani KU and Vishakha R (2019). A Simple and rapid colorimetric method for the estimation of chitosan produced by microbial degradation of chitin waste. *Microbial Metabolism* 66-70

Anubhav S, Anuj SR and Rushikesh (2022). Heavy metals contamination of water and toxic on living organisms. *The toxicity of Environmental Pollution*, 321-322

Carmen and Juan (2020). Chitosan: A natural Biopolymer with a wide and varied range of application. *Molecules*, 43-44

Chang YK, Chang JE, Lin TT and Hsu YM (2002). —Integrated copper containing wastewater treatment using xanthate process. *Journal of Hazardous Materials*, **94**(1), 89-99.

Chiou MS, and Li HY (2004) Adsorption of anionic dyes in acid solutions using chemically cross-linked chitosan beads. *Dyes Pigment*, **60** 69-84.

Clark L, and Smith A (1936). X-ray diffraction studies of chitin, chitosan and derivatives. *Chitin chemistry*; 1-53

Fu F, and Wang Q (2011). —Removal of heavy metal ions from wastewaters: a review. *J. Environmental Manage*, **92**(3), 407-418.

Gomez R, Barrera RU and Rios-Estepa R (2017). Comparison of process technologies for chitosan production from shrimp shell waste: *A techn-oeconomic Approach Using Aspenplus*, 335-337

Gupta A, ChauhanVS and Sankararamakrishnan (2009). Preparation and evaluation of iron-chitosan composites for removal of As(III) and As(V) from arsenic contaminated real life groundwater. *Water Residue*, 3862-3870.

HMilne E, crevette BD and indes (1837). Indian white prawn- penaeus indicus. *Aquaculture feed and Fertilizer Resources Information System*, **2**(1) 1-3

Joe M, Regenstein, and Regenstein (1984). In Food Protein Chemistry. *Lntroduction for food Scientists*, 1-4.

Kanamadi RD, Ahalya NU and Ramachandra T (2006). Biosorption of Heavy Metals by Low cost Adsorbents. *heavy metal*, 978-979.

Khor E, & Lim LY (2003). Implantable applications of chitin and Chitosan. *Biomaterials*, ***24***(13), 2339–2349.

Sureshkumar V, Kiruba Daniel SC, Ruckmani K and Sivakumar M (2015). Fabrication of chitosan–magnetite nanocomposite strip for Chromium Removal. *Applied Nanoscience*, **6**(2), 277–285.

Kim E, Xiong Y, Cheng Y, Wu HC, Liu Y, Morrow B, Ben-Yoav H, Ghodssi R, Rubloff G, Shen J, Bentley W, Shi X and Payne G (2014). Chitosan to connect biology to electronics: Fabricating the bio-device interface and communicating across this interface. *Polymers*, **7**(1), 1–46.
D'Abramo L, Silva J, & Kim T (2009). Optimization of crustacean quality through husbandry and adherence to post-harvest standards for processing. *Shellfish Safety and Quality*.
Moradi Dehaghi S, Rahmanifar B, Moradi AM, and Azar PA (2014). Removal of permethrin pesticide from water by chitosan–zinc oxide nanoparticles composite as an adsorbent. *Journal of Saudi Chemical Society*, **18**(4), 348–355.
Monier M, Ayad DM, Wei Y, and Sarhan AA (2010). Adsorption of cu(ii), co(ii), and Ni(ii) ions by modified magnetic chitosan chelating resin. *Journal of Hazardous Materials*, **177**(1–3), 962–970.
Ngah WW, and Ilanatiah MAKM (2008). Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: a review. *J Bioresource Technology* ,**99**(10). 3935-3948.
Nordtveit R (1996). Degradation of partially N-acetylated chitosans with hen egg white and human lysozyme. *Carbohydrate Polymers*, **29**(2), 163–167.
Osman Z and Arof AK (2003). FTIR studies of chitosan acetate based polymer electrolytes. *Electrochimica Acta*, **48**(8), 993–999.
Özverdi A and Erdem M (2006). Cu2+, CD2+ and PB2+ adsorption from aqueous solutions by Pyrite and Synthetic Iron Sulphide. *Journal of Hazardous Materials*, **137**(1), 626–632.
Pavithra S, Thandapani G, S, S., P.N., S., Alkhamis HH, Alrefaei AF and Almutairi MH (2021). Batch adsorption studies on surface tailored chitosan/orange peel hydrogel composite for the removal of Cr(vi) and Cu(ii) ions from synthetic wastewater. *Chemosphere*, 271, 129415.
Mutreja R, Thakur A and Goyal A (2020). Chitin and Chitosan: Current status and future opportunities. *Handbook of Chitin and Chitosan*, 401–417.
Seo, Seung-wook (2006). Depolymerization and decolorization of chitosan by ozone treatment. LSU Master's Theses. 3833.
Shariful MI, Sharif SB, Lee JJ, Habiba U, Ang BC and Amalina MA (2017). Adsorption of divalent heavy metal ion by mesoporous-high surface area chitosan/poly (ethylene oxide) nanofibrous membrane. *Carbohydrate Polymers*, **157** 57–64.
Sonawanw S, settee YP and SapavCattu SN, (2015) Chemical and bioprocess engineering: *Trends and developments* (CRC press).
Strand SP, Tømmeraas K, Vårum KM and Østgaard K (2001a). Electrophoretic light scattering studies of chitosans with different degrees of *n*-acetylation. *Biomacromolecules*, **2**(4), 1310–1314.
Izah SC, Aigberua AO and Srivastav AL (2022). Factors influencing the alteration of microbial and heavy metal characteristics of river systems in the Niger Delta region of Nigeria. *Ecological Significance of River Ecosystems*, 51–78.
Vårum KM, Kristiansen Holme H, Izume M, Torger Stokke B and Smidsrød O (1996). Determination of enzymatic hydrolysis specificity of partially N-acetylated chitosans. *Biochimica et Biophysica Acta (BBA) - General Subjects*, **1291**(1), 5–15.
Wang LK, Chen JP, Hung YT and Shammas NK eds. (2009). Heavy metals in the environment. CRC Press.
Xu T and Huang C (2008). Electrodialysis-based Separation Technologies: A critical review. *AIChE Journal*, **54**(12), 3147–3159.