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BIOADSORBENT POTENTIAL OF CHITOSAN FROM *PORTUNUS SANGUINOLENTUS* AND *FENNEROPENAEUS INDICUS* SHELLS FOR PROTEIN AND HEAVY METAL REMOVAL

V. RATNA BHARATHI*[1], J. LAXMI MANGAMMA[2], B. SANDHYA SRI[3]

*[1]*Senior Lecturer, Department of Zoology, Govt. Degree College Chodavaram, Anakapalli District, Andhra Pradesh, India*
[2]Senior Lecturer, Department of Chemistry, Govt. Degree College, Chodavaram, Anakapalli District, Andhra Pradesh, India
[3]Lecturer, Department of Botany, Govt. Degree College, Chodavaram, Anakapalli District, Andhra Pradesh, India

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ABSTRACT

Industrialisation causes a tremendous amount of water pollution, especially from the addition of heavy metals and organic pollutants to aquatic ecosystems. The present study is focused on the extraction and characterization of chitosan from the shell waste of two marine species, *Portunus sanguinolentus* and *Fenneropenaeus indicus*. The effectiveness of this chitosan as bioadsorbents for the removal of proteins and heavy metals from the industrial wastewater was also evaluated. Chitosan was extracted by chemical treatments and characterized for yield, moisture, ash content, solubility, viscosity, degree of deacetylation and water and fat binding capacities. The results showed that the chitosan content was higher in F. indicus shells, while the chitosan from *P. sanguinolentus* showed higher solubility and water binding capacity. The protein flocculation and heavy metal chelation assays proved that chitosan obtained from both species has significant flocculants and chelating properties with higher efficiency in protein flocculation and metal adsorption for chitosan obtained from F. indicus especially for Zn and Fe ions. The findings emphasize the possibility of utilizing shell waste derived chitosan as an inexpensive, environmentally friendly, and efficient approach for the remediation of industrial effluents, promoting the advancement of sustainable and environmentally sound wastewater treatment solutions.

Keywords: *Portunus sanguinolentus, Fenneropenaeus indicus, Chitosan, Bioadsorption, Heavy metals, Protein flocculation.*

***Corresponding Author**
Dr. V. Ratna Bharathi

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INTRODUCTION

Industerialisation over the past decade has both polluted and degraded water quality, and this issue is significant in emerging and industrialised nations [1]. Industrial trash thrown into waterways contains heavy metals that harm aquatic life. Industrial effluent contains arsenic, mercury, lead, copper, chromium, nickel, cobalt, etc. Because of their poisonous nature, several of these metals have harmful effect even at low concentrations [2]. For example, exposure to mercury increases accumulation in blood and organs that leads to the cardiovascular, kidney, bone, and neurological disorders [3]. Arsenic causes lung, skin, bladder, and kidney cancers [4]. Copper is the most common heavy metal found in waste water, originating from metal cleaning and plating, textiles, paper mills, as well as wood and pulp industries [5]. Accumulation of copper in liver causes gastrointestinal issues and long-term damage to liver and kidneys [6]. Therefore, removal of heavy metals from polluted waters and soils are very crucial for aquatic and terrestrial life. Despite the difficulty, a number of technologies exist for removing metal ions from wastewater. These include ion exchange, precipitation, membrane filtration, and adsorption [7].

Additionally, the food, textile, and leather, vegetable oil, oil and gas industries, as well as home sewage represent significant pollution sources typically containing diverse organic compounds. Among these, proteins, lipids, and other lipophilic pollutants featuring functional groups are rare in nature, persistent, and highly hazardous. These can inflict significant harm on the flora and fauna of the impacted ecosystems, in addition to being possibly carcinogenic to humans [8]. Consequently, the pursuit of unconventional, cost-effective, and eco-friendly remediation has heightened interest in natural-based materials. Ali et al. [9] assert that alternative that alternative materials should possess certain common qualities, including abundance and ease of acquisition from nature or as by-products

from other industries, economic viability, minimal or no processing requirements, and non-toxicity. Among all the pollutant removal methods, adsorption is the most advantageous technique, owing to its high adsorption efficacy, cost-effective, and ease of handling [10].

Recently, the utilisation of natural biopolymers for various applications in life sciences has significantly increased. The numerous benefits of natural biopolymers, including their accessibility from renewable agricultural or marine sources, biocompatibility, biodegradability, and adaptability could expanded their applications. Polysaccharides, a category of natural macromolecules, exhibit significant bioactivity and are often sourced from agricultural feedstocks or crustacean shell byproducts [11]. Consequently, biopolymer-based adsorbents, including chitosan, cellulose, alginate, and lignin, are significant in adsorption due to their biocompatibility and reabsorption capacity [12]. Chitosan plays a crucial role in the adsorption process due to its hydroxyl and amino groups, which are involved in metal adsorption. Thus, biopolymers like chitosan represent a sustainable alternative, as they originate from renewable and biodegradable sources.

Chitosan is a linear heteropolysaccharide composed of β(1-4) linked N-acetylglucosamine and D-glucosamine units. It is synthesised through the deacetylation of chitin, which is found in shrimp and crab shell, cuttlefish pens, insect cuticles, fungal cell walls, and mushrooms [13]. Numerous reports indicate that crabs and shrimps are exceptional sources of chitin among marine organisms. Hence, this study aims to extract chitosan from *Portunus sanguinolentus* and *Fenneropenaeus indicus* to investigate their bioadsorption capabilities for the removal of protein and heavy metals contaminants from industrial wastewater.

MATERIALS AND METHODS

Sample Collection

The shell waste of *P. sanguinolentus* and *F. indicus* were collected from a local sea food processor fisherman in the fishing harbor(Long: 83°18' 9.01" E; Lat: 17° 41' 43.63" N) have been situated on the North East coast of Andhra Pradesh, adjoining the Bay of Bengal, Visakhapatnam, India.The collected shell waste was transferred to the sterile zip pouches and stored in iceboxes till transported to the laboratory where they were kept in refrigerator at -20^{0}C until further analysis. The collected shell waste was washed under running tap water, and then with distilled water. After the cleaning, the excess water was removed with blotting paper. Then the shell waste was dried in hot air oven 60°C for 24 hrs. Dried shell waste were packed in polyethylene bag and stored in dry place. Dried shells were pulverized manually.

Extraction of Chitosan

Extraction of chitosan from the shell waste of *P. sanguinolentus* and *F. indicus* was performed by the chemical treatments that includes demineralization, deproteinization, decoloration [14], and deacetylation of chitin [15].

Characterisation of Chitosan

The characterisation of extracted chitosan from *P. sanguinolentus* and *F. indicus* shell was evaluated by determination of parameters such as yield, moisture [16], ash content [16], solubility [17], degree of deacetylation [18], viscosity, water and fat binding capacities [19].

Figure 01:Extraction of chitosan from shell waste of *P. sanguinolentus* and *F. indicus.*

Determination of Protein Flocculation

The protein flocculation potency of extracted chitosan from *P. sanguinolentus* and *F. indicus* was determined by using a column running with bovine serum albumin. In this experiment, three glass columns with the dimensions of 15 cm x 0.9 cm were taken, and these columns were washed, sterilized, and dried before packing. Then, the columns were packed with chitosan. To accomplish column packing, 10 gms of extracted chitosan from each of the two samples, such as *P. sanguinolentus* and *F. indicus*, were placed in 500 ml beaker containing 200 ml of 2% acetic acid, and the pH of the solution was adjusted to 6.5. Then, the chitosan solution was allowed to soak with constant stirring at room temperature for 24 hours. The pre-soaked each chitosan solution from the respective two samples was poured into two separates columns by constant stirring to avoid air cracks in the column. Then the column was allowed to stand for 12 hours to achieve proper packing by sedimentation of chitosan without any air cracks. After packing of the column, the protein flocculation capacity was determined by running the column with known concentrations of bovine serum albumin (BSA). The known concentrations of BSA were prepared by dissolving a series of concentrations from 2 to 10 mg/ml in HPLC grade distilled water. The prepared BSA solutions were passed through the columns containing respective chitosan at a flow rate of 12 ml/minute, and subsequently, 5 ml of eluted fraction was collected from the three columns. Finally, the protein concentration in the eluted fractions was determined by using the Bradford [20] method.

Determination of Heavy Metal Chelation Ability

The determination of metal ion removal and recovery potency of chitosan extracted from the *P.*

sanguinolentus and *F. indicus* was done according to the methodology of Chui et al. [21] by using a column packed with chitosan as described in previous method. In this experiment, the metal chelation activity of chitosan was determined by using heavy metals such as Cobalt (Co), Copper (Cu), Iron (Fe), and Zinc (Zn). After packing of the column, the metal chelation activity was assessed by running the column with known concentrations of above-mentioned metal solutions. To prepare Cobalt, Copper, Iron, and Zinc solutions, 100 mg of respective salts, such as Cobalt chloride ($CoCl_2$), Copper sulphate ($CuSO_4$), Iron (III) sulphate ($Fe\ (SO_4)_3$), and Zinc sulphate ($ZnSO_4$) were dissolved in 1 liter of HPLC grade deionized water. The prepared metal solutions were passed through the columns containing respective chitosan at a flow rate of 2ml/minute at room temperature until the saturation of volume matrix reached. The eluted fractions were collected in a clean and sterile tubes. Then, distilled water contain pH-7 was passed several times through the column to wash the remaining metals, and the elutes were collected. Finally, the concentration of metal ions in the respective elutes were measured by using an atomic adsorption spectroscopy.

RESULTS AND DISCUSSION

Yield of Chitosan

The yield of chitosan extract from the shells of *P. sanguinolentus* and *F. indicus* was presented in Table 1. From these results, it was observed that both the species possess significant quantity of chitosan and the chitosan yield from the *P. sanguinolentus* and *F. indicus* was reported as 15.51±0.81 and 19.23±0.63 gm respectively. The percentage of chitosan yield obtained from the dried shell powder of *P. sanguinolentus* and *F. indicus* were measured as 31.02±1.2 and 38.461.6 %. According to these results, it was observed that the shells of *F. indicus* considered as a good source of chitosan than the *P. sanguinolentus.*

In the present study, significant chitosan yield justifies the potential of mud crab and shrimps' chitosan usage as an economic source for the production of chitosan on an industrial scale due to the availability of shell waste from mud crabs and shrimps the low cost of the source. However, the yield obtained is also affected by the loss of sample mass from excessive removal of acetyl groups from the polymer during deacetylation [17].

Moisture Content

The moisture content in the chitosan extracted from the *P. sanguinolentus* and *F. indicus* was reported as 2.92±0.6 and 4.760.3 gm respectively (Table 1). The percentage of moisture content in the chitosan from the dried shell powder of *P. sanguinolentus* and *F. indicus* were calculated as 18.83±0.9 and 24.75±1.2%. According to these results, it was observed that the chitosan extracted from the shells of *F. indicus* possess high water content than the *P. sanguinolentus.*Ocloo et al. [19] has been reported that, lower moisture content of chitosan indicates better shelf stability and enhances the quality. The standard moisture content of chitosan for used in application ranged from 5.0 %–15.0 % changing with humidity and form of chitosan whether it in the form of flake or powder [22].

Ash Content

The ash content in the chitosan was significantly varied in both the species and the ash content in the chitosan extracted from the *P. sanguinolentus* and *F. indicus* was reported as 0.65±0.01 (4.19±0.1%) and 0.92±0.02 (4.78±0.1%) gm respectively (Table 1). According to these results, it was observed that the chitosan from the shells of *F. indicus* possess high ash content than the *P. sanguinolentus.* In evident with the present study, Kucukgulmez et al. [23] showed that the snow crab possessed lower ash content (0.59–0.61%). However, No and Meyers [24] reported that a high-quality grade of chitosan should have an ash content of less than 1%. Fernandez-Kim [17] has been stated that the ash content in chitosan is an important parameter as some ash residual of chitosan may affect its solubility and consequently, contribute to lower viscosity, or can affect other more important characteristics of the final product.

Table 01:The yield of chitosan, moisture and ash content extracted from the shells of *Portunus sanguinolentus* and *Fenneropenaeus indicus*.

Species	Yield		Moisture		Ash	
	(gm)	(%)	(gm)	(%)	(gm)	(%)
Portunus sanguinolentus	15.51±0.81	31.02±1.2	2.92±0.6	18.83±0.9	0.65±0.01	4.19±0.1
Fenneropenaeus indicus	19.23±0.63	38.46±1.6	4.76±0.3	24.75±1.2	0.92±0.02	4.78±0.1

Solubility

The solubility percentages of chitosan extracted from the shells of *P. sanguinolentus* and *F. indicus* was presented in Table 2. The solubilities of chitosan obtained from the extraction of dried shell powder of *Portunus sanguinolentus* and *Fenneropenaeus indicus* were measured as 64±2.56 and 44±1.32 % respectively. According to these results, it was observed that the chitosan extracted from the shells of *P. sanguinolentus* possess highest solubility than the *F. indicus.* These results show collinearity with the results of Fernandez-Kim [17] who observed the extracted chitosan from mud crabs had low solubility compared to the solubility of the commercial chitosan, which may be due to the incomplete removal of protein. The study by Fernandez-Kim [17] found that the solubility of

crawfish chitosan was in the range of 93.3–94.3 %. The insolubility of chitosan in aqueous and organic solvents is a result of its crystalline structure, which is attributed to extensive intramolecular and intermolecular hydrogen bonding between the chains and sheets, respectively. Rinaudo [25] has been reported that the chitosan can be dissolved easily in HCl, lactic acid, mild acetic acid, and some organic acids.

Viscosity

The viscosity of the chitosan from the shells of *P. sanguinolentus* and *F. indicus* were measured as 0.9241±0.001 cP and 0.959±0.001 cP, respectively (Table 2). There is a significant difference ($p<0.05$) in the viscosity value between the chitosan extracted from both species. According to these results, the extracted chitosan from crab shells exhibits a lower viscosity in the 1%acetic acid solution compared to the *F. indicus*. These results are evident with the studies of Moorjani et al. [26] who showed that an increase in demineralization time resulted in a decrease in the viscosity of chitosan. Chitin, following the complete removal of protein during the deproteinization process, results in higher chitosan viscosity values, depending on the reagent and shell ratio.

Degree of Deacetylation

The degree of deacetylation of chitosan extracted from the shells of *P. sanguinolentus* and *F. indicus* were determined as 36.91±2.6 and 56.89±3.1% respectively (Table 2). According to these results, the chitosan extracted from the shells of *F. indicus* was found to be significantly higher ($p<0.05$) than the degree of deacetylation of *P. sanguinolentus* chitosan. These results exhibit similarity with studies of Martino et al. [27] who reported that the degree of deacetylation (DD) may range from 30 to 95%depending on the source and preparation procedure. The degree of deacetylation of the extracted chitosan from snow crabs which determined by potentiometric titration was reported as being about 92.19 % [23].

Table 02:Solubility, Viscosity, and Degree of deacetylation of chitosan extracted from the shells of *P. sanguinolentus* and *F. indicus*.

Species	Solubility (%)	Viscosity (Cp)	Degree of deacetylation (%)
Portunus sanguinolentus	64±2.56	0.9241±0.001	36.91±2.6
Fenneropenaeus indicus	44±1.32	0.959±0.001	56.89±3.1

Water Binding Capacity (WBC)

The water binding capacity of the extracted chitosan from the shells of *P. sanguinolentus* and *F. indicus* were measured as 325±5.2 and 219.64±4.6% respectively (Table 3). There was a significant difference ($p<0.05$) in the water binding capacity between the chitosan extracted from both the species. According to these results, it was observed that the chitosan extracted from the shells of *P. sanguinolentus* possess highest WBC. The extracted chitosan from the shrimp shells showed a lower water binding capacity compared to the crab chitosan. Kucukgulmez et al. [23] reported that the chitosan extracted from *M. stebbingi* shells had a water binding capacity of 712.99 %. Similarly, Ocloo et al. [19] and Fernandez-kim [17] found that the water binding capacity of chitosan extracted from shrimp and crawfish are 582.40 and 660.6% respectively.

Fat Binding Capacity (FBC)

The fat binding capacity of the extracted chitosan from the shells of *P. sanguinolentus* and *F. indicus* were measured as 119.04±6.2 and 121.86±5.8% respectively (Table 3). There was a significant difference ($p<0.05$) in the fat binding capacity between the chitosan extracted from *P. sanguinolentus* and *F. indicus*. According to these results, it was observed that the chitosan extracted from the shells of *F. indicus* possess highest FBC. In the present study, it was observed that the fat binding capacity analysis conducted showed that the chitosan exhibited decreased FBC. A study by Cho et al. [28] presented that the FBC of different commercial chitosans were reported to range from 314 to 535 %. However, when the sequence of steps was changed so that demineralization was conducted prior to deproteinization and finally deacetylation, it resulted in an increase in FBC compared to when deproteinization is conducted prior to demineralization and finally deacetylation [26].

Table 03:Water and Fat binding capacities of chitosan extracted from the shells of *Portunus sanguinolentus* and *Fenneropenaeus indicus*.

Species	Water binding capacity (%)	Fat binding capacity (%)
Portunus sanguinolentus	325±5.2	119.04±6.2
Fenneropenaeus indicus	219.64±4.6	121.86±5.8

Protein flocculation capacity

The results of protein flocculation by the two chitosan extracts such as *P. sanguinolentus* and *F. indicus*shell were shown in Table 4. From these results, the chitosan extract from *F. indicus* exhibit greater protein flocculation ability. The protein flocculation capacities of *P. sanguinolentus*chitosan with increasing concentrations of BSA such as 1, 2, 3, 4, and 5 mg/ml were reported as 0.82±0.02, 1.76±0.03, 2.73±0.02, 3.68±0.01, and 4.29±0.01 respectively, and their

corresponding protein flocculation percentages were calculated as 82±0.01, 88±0.01, 91±0.03, 92±0.01, and 85.8±0.01%. As well as, the protein flocculation capacities of *F. indicus* chitosan with increasing concentrations of BSA such as 1, 2, 3, 4, and 5 mg/ml were reported as 0.92±0.02, 1.8±0.01, 2.9±0.01, 3.91±0.01, and 4.59±0.02 respectively, and their corresponding protein flocculation percentages were calculated as 92±0.02, 90±0.02, 96.6±0.01, 97.75±0.01, and 91.8±0.02%.

In the present results, it was observed that the chitosan extracts from *P. sanguinolentus* and *F. indicus* exhibit significant (<.0005) protein flocculation abilities. Furthermore, the protein flocculation abilities are increased with increasing BSA concentration. The protein flocculation abilities of chitosan from *P. sanguinolentus* and *F. indicus* shows less positive correlation of r=0.346567 and r=0.45307 with respect to increasing concentration of BSA. When compared to the protein flocculation abilities of *P. sanguinolentus*chitosan, the *F. indicus*chitosan shows more positive correlation such as r = 0.721853. The results were shown in Table 5.

The present results demonstrated that the chitosan extracted from the *Portunus sanguinolentus* and *Fenneropenaeus indicus* exhibit significant bioflocculant activity. Also, these results are evident by previous works of Fernandez and Fox [29] who reported that the chitosan has been shown to be an effective coagulating agent in wastewater treatment and recovery of lipids and proteins from plant processing food wastes. Furthermore, Selmer Olsen et al. [30] has been reported that chitosan removes proteins and lipids from diary waste water, as well as Ausar et al. [31] observed that the chitosan induces the formation casein micelles. In the dairy industry, chitosan has been used to remove milk fat, proteins, and peptides from cheese whey [32]. According to the study of Yang et al. [33], the flocculation mechanism of long chain chitosan leads to destabilization of proteins as a result of the charge neutralization of their surfaces, followed by their aggregation into the large flocs through the bridging effect.

Table 04:Protein flocculation abilities of chitosan extracted from the shells of *P. sanguinolentus* and *F. indicus.*

BSA Concentration (mg/ml)	**Protein flocculation (Mean ± SD)**			
	Portunus sanguinolentus		***Fenneropenaeus indicus***	
	(mg)	**(%)**	**(mg)**	**(%)**
1	0.92±0.02	92±0.02	0.82±0.01	82±0.01
2	1.8±0.01	90±0.02	1.76±0.03	88±0.01
3	2.9±0.01	96.6±0.01	2.73±0.02	91±0.03
4	3.91±0.01	97.75±0.01	3.68±0.01	92±0.01
5	4.59±0.02	91.8±0.02	4.29±0.01	85.8±0.01

Table 05:Correlation analysis of the protein flocculation abilities of chitosan extracted from the shells of *P. sanguinolentus* and *F. indicus* with increasing BSA concentration.

Chitosan	***BSA Concentration***	***Portunus sanguinolentus***	***Fenneropenaeus indicus***
BSA Concentration	1		
Portunus sanguinolentus	0.346567	1	
Fenneropenaeus indicus	0.45307	0.721853	1

Determination of Heavy Metal Chelation Ability

The results of heavy metal chelation ability demonstrated that the chitosan extract from *F. indicus* exhibit greater metal chelation ability than the *P. sanguinolentus* chitosan. The metal chelation percentages of *P. sanguinolentus*chitosan for Co, Cu, Fe, and Zn were reported as 67.13±5, 64.83±4, 72.35±5, and 73.84±5% respectively. As well as, the metal chelation percentages of *F. indicus* chitosan for Co, Cu, Fe, and Zn were reported as 62.14±4.6, 72.64±4, 79.82±6, and 81.29±5 respectively (Table 6). In the present results, it was observed that the chitosan extracts from the two species exhibit significant (<.0005) metal chelation flocculation abilities. Furthermore, the chitosan of *P. sanguinolentus*chelates Zn ions in higher percentages, followed by Fe, Co, and Cu. As well as the chitosan of *F. indicus* chelates Zn ions in higher percentages, followed by Fe, Cu, and Co. The metal chelation abilities of the two chitosan extracts from *P. sanguinolentus* and *F. indicus* with tested heavy metals were respectively found as Zn>Fe>Co>Cu, Zn>Fe>Cu>Co. From the present results, it was found that both the chitosan extracts show good chelation activity of metal ions Zn and Fe.

The present results evident with the reports of Farhan et al. [34] who reported the average removal percentages of heavy metals such as Pb, Fe, and Mn by chitosan are 55.77, 69.65, and 69.6% respectively. Gamage and Shahidi [35] reported that the removal of toxic heavy metals from waste water is significantly done by using chitosan as a bioadsorbent. Similarly, Shanmugapriya et al. [36] observed that the heavy metal contaminants in organic polluted waste water from industries are effectively recovered by using chitosan as a nontoxic flocculant. De Silva et al. [37] have been reported that the chitosan can effectively adsorb trace metals from waste water contain very minute quantities of metals.

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Table 06:Metal chelation abilities of chitosan extracted from the shells of *Portunus sanguinolentus* and *Fenneropenaeus indicus.*

Metal ion	**% of metal chelation ability (Mean ± SD)**	
	Portunus sanguinolentus	***Fenneropenaeus indicus***
Cobalt (Co)	67.13±5	62.14±4.6
Copper (Cu)	64.83±4	72.64±4
Iron (Fe)	72.35±5	79.82±6
Zinc (Zn)	73.84±5	81.29±5

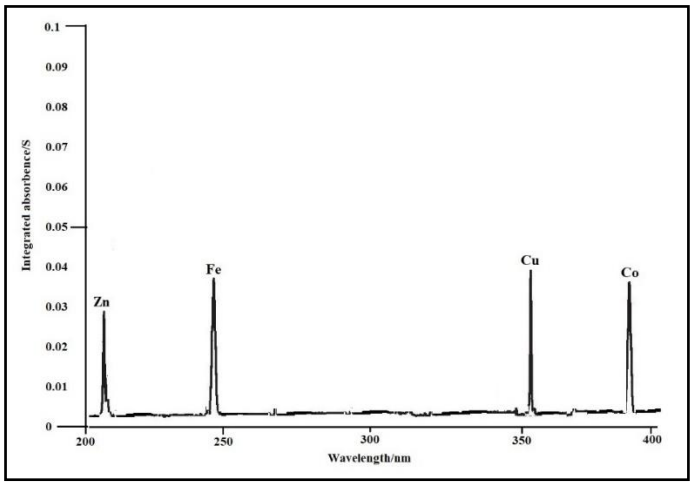

Figure 02:Atomic absorption spectrum of heavy metals recovered from the water passed through the *Portunus sanguinolentus*chitosan.

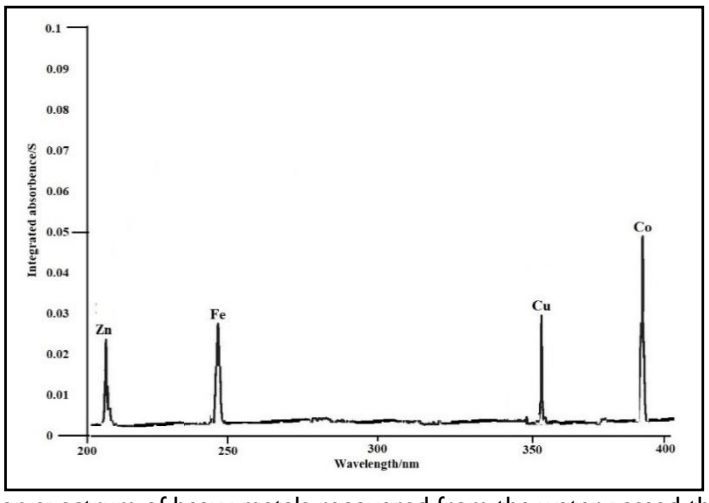

Figure 03:Atomic absorption spectrum of heavy metals recovered from the water passed through the *Fenneropenaeus indicus* chitosan.

CONCLUSION

The present study demonstrated that chitosan was successfully extracted and characterized from waste shells of *Portunus sanguinolentus* and *Fenneropenaeus indicus* which have potentials for bioadsorbent applications in the treatment of industrial wastewater. *F. indicus* chitosan showed better yield, protein flocculation and metal chelation particularly for Zn and Fe ions. The results confirm the efficiency and sustainability of chitosan from marine shell waste for the removal of hazardous proteins and heavy metals from polluted water. The application of these natural biopolymers allows for waste valorisation and promotes green remediation approaches for the increasing need for sustainable and cost-effective solutions in environmental management.

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AUTHOR CONTRIBUTIONS

V. Ratna Bharathi: Conceptualization, supervision, study design, validation, manuscript review and editing, and final approval of the manuscript.
J. Laxmi Mangamma: Methodology, chemical analysis, data interpretation, and manuscript review.
B. Sandhya Sri: Investigation, data collection, laboratory experiments, formal analysis, manuscript preparation, and data compilation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

ETHICAL STATEMENT

The present study involved shell waste materials obtained from commercially available marine species and did not involve experiments on live animals or human participants. Therefore, ethical approval was not required.

FUNDING

This research received no external funding.

INFORMED CONSENT STATEMENT

Not applicable. This study did not involve human participants or animals requiring informed consent.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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