

# Comparative Study of Minerals and Heavy Metal Content Among Different Natural Sources of Chitosan

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## Article Info

Volume 7, Issue 3

Page Number : 16-29

## Publication Issue :

May-June 2022

## Article History

Accepted : 15 May 2022

Published : 30 May 2022

## ABSTRACT

Chitosan is an amino polysaccharide made by partially deacetylating chitin in shrimp, crab, and grasshopper (shell) processing. Many biochemists have discovered that chitosan is biocompatible, biodegradable, and non-toxic, allowing it to be used in traditional pharmaceuticals. The current study focuses mostly on chitosan extraction. The crude chitin was extracted from the exoskeletons of shrimp, crabs, and grasshoppers, then processed using various ways to produce chitosan, which was then tested for mineral and heavy metal content.  $Ca > Zn > Na > K > Fe > Cu$  was the order of heavy metal concentration in diverse natural sources.

Keywords: Minerals and heavy metals, Chitosan, natural sources.

## I. INTRODUCTION

Chitin is a structural biopolymer that functions similarly to collagen in higher animals and cellulose in plants. It is mostly obtained from crustacean exoskeletons, particularly shrimp waste [1,2]. Chitosan is a polysaccharide made up of 1,4-linked 2-acetamido-D-glucose and 1,4-linked 2-amino-D-glucose units, with the chitin acetyl groups replaced by amino groups at the C-2 position in the carbon chain [3,4]. Chitosan deacetylation can be done chemically using intense alkaline solutions for long periods of time, resulting in structural changes in the chitosan [4,5]. A biological technique is also available that allows for less depolymerization due to better process control; nevertheless, the usage of chitin-deacetylase makes it a high-cost and time-consuming technology [5]. Chitin is a hydrophobic, rigid,

inelastic N-acetylated aminopolysaccharide that is insoluble in water and most organic solvents [6,7]. In this study, chitin isolated from natural sources was deacetylated and transformed into chitosan, which was then analysed for mineral and heavy metal levels in crab, shrimp, and grasshopper chitosan.

## II. METHODS AND MATERIAL

Fresh natural organism like shrimp, crab, and grasshopper was collected and collected material was thoroughly washed with fresh tap water to remove most of the impurities acquired during handling allow for sun dried for 3 days and subjected to grinding. Representative samples of dried were taken, pooled, ground in a grinder and analyzed for proximate composition [8]. Chitosan extraction involved demineralization, deproteinization and deacetylation.

Deprotonation and deacetylation in the conventional method is brought about by using NaOH. Deacetylation process based on removal of acetyl groups from the chitin was achieved by using 70% NaOH solution with a solid to solvent ratio of 1:14 (w/v) at room temperature for 72 hours. The residue was washed to neutrality in running tap water and the pH was checked by pH paper. The chitosan residue was dried overnight at 60°C and weighed [9]. The degree of deacetylation (DDA) was measured by the acid-base titration method [10]. The Solubility of chitosan powder was determined as percentage solubility [11,-13].

### III. RESULTS AND DISCUSSION

Mean±SD of sodium was evaluated with triplicates from different natural resources. Maximum sodium level was found in Shrimp's shell 1.973±0.114 whereas least amount was recorded 0.910±0.013 (Mg/Kg) in grasshopper's shell (Table 2 and Fig 2). Most mean with SD potassium level was established in Shrimp's Shell 0.082±0.008 whereas least was recorded 0.018±0.006 (Mg/Kg) in grasshopper's shell (Table 3 and Fig 3). Maximum Ca level was found in Shrimp's 0.082±0.008 while minimum was evidenced 0.018±0.006 (Mg/Kg) in grasshopper's (Table 4 and Fig. 4) Maximum mean±SD Iron level was found in Shrimp's 0.009±0.002 but same assessment of iron was found 0.007 ±0.001 (Mg/Kg) in grasshopper's and Crab's (Table 5 and Fig 5). Maximum Zn stage was found in shrimp's Shell 0.149±0.015, minimum was 0.087±0.009 in grasshopper's and 0.126 ±0.006 (Mg/Kg) Zinc was accounted in crab's shell (Table 6 and Fig 6).

In case of Ni, maximum Ni level was found in Shrimp's 0.0028±0.006, minimum was 0.0010±0.001 in grasshopper's and 0.0014±0.004 (Mg/Kg) Ni was accounted in crab's (Table 7 and Fig 7) Maximum As level was found in Shrimp's 0.674±0.010, minimum was 0.364±0.010 in grasshoppers and 0.446±0.013 (Mg/Kg) Arsenic was reported in crab's shell (Table 8

and Fig 8). Maximum Cu level was found in Shrimp's 0.246±0.010, minimum was 0.068±0.008 in grasshopper's and 0.170±0.009 (Mg/Kg) Arsenic was accounted in crab's (Table 9 and Fig 9). Maximum Cr level was found in Shrimp's 0.021±0.003, lowest amount was 0.068±0.008 in crab's and there was no As content was accounted in grasshopper's (Table 10 and Fig 10). Maximum Pb level was found in Shrimp's 0.039±0.004, minimum was 0.020±0.003 in crab's and there was no Lead (Pb) content was accounted in grasshopper's (Table 11 and Fig 11). [14-16].

Tin and Hg levels and standard deviations from grasshoppers, crabs, and shrimp were not found in any amounts (Table 12). Except for the heavy metal content, all heavy metal concentrations in shells from various natural sources of chitosan were below the standard standards. The amounts of heavy metals in different chitosan shells are compared (Fig 13). The creation of chitosan matrices and their in vitro evaluation were used to deliver drugs.

At room temperature, a heavy metal has metallic characteristics. In small amounts, they can be dangerous or poisonous. Despite the fact that heavy metals are inherent components of the earth's crust, mining and industrial operations, as well as geochemical processes, have boosted heavy metal concentrations in aquatic environments. Pesticides, batteries, mining operations, alloys, metal plating facilities, textile dyes, tanneries, and other industrial uses all employ heavy metals. Throughout chitosan action in mice on an HFD and chitosan action with carboxymethylated chitosan, fat storage was reduced [18,19].

Studies have demonstrated that heavy metals (iron, copper, and zinc) are required in trace amounts throughout life because they are integrated into metabolism. Because they cannot disintegrate or be removed, they can cause toxicity and other difficulties at higher quantities. Additionally, they are prone to

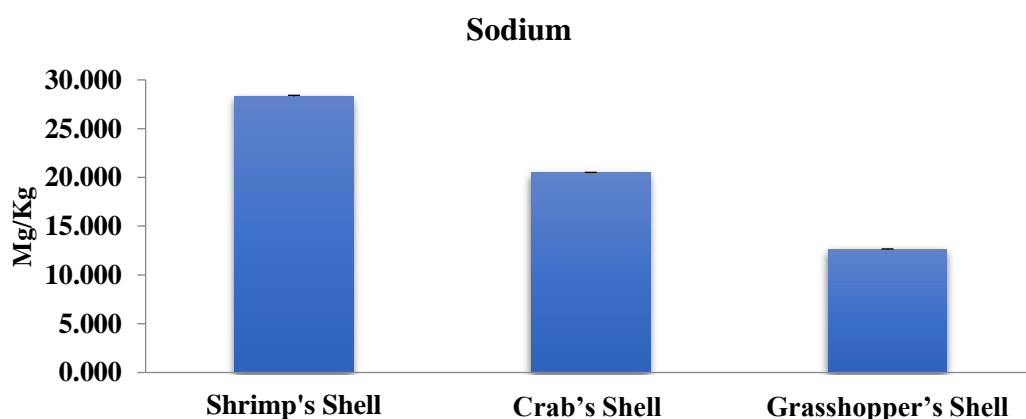
bioaccumulation [20,21]. In this method, the mineral and heavy metal content data from various common chitin sources were compared.  $\text{Ca} > \text{Zn} > \text{Na} > \text{K} > \text{Fe} > \text{Cu}$  was the order of heavy metal concentrate in various natural sources.

Because of their quantity and prevalence in natural water resources, the sources accounted for the majority of the Ca content, according to the data. Calcium is also required for the exoskeleton structure in naturally existent animals. The quantities of heavy metals in seafood were compared to worldwide standards set by the FDA, WHO, and FAO. Various

chitosan polymer dosage formulations have been examined as appealing and compatible for application [22,23]. Heavy metals can infect aquatic species in the food chain, according to our findings [24], which were compared to standard data. Heavy metal toxicity and their tendency to accumulate in biota pose serious threats to ecosystem health. Cu levels were discovered to be greater than expected. During the analysis of chitosan and the creation of related co-products with identical chemistry, Cu values were discovered to be higher than those of other metals (Pb, Ni, As, and others) [25].

**Table 1.** Level and standard deviation of Sodium from different natural resources (Mg/Kg). Values expressed as mean  $\pm$  SD, n=3.

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	1.891	2.104	1.925	1.973 $\pm$ 0.114	0.789
2.	Crab's Shell	1.458	1.469	1.398	1.442 $\pm$ 0.038	0.290
3.	Grasshopper's Shell	0.896	0.912	0.921	0.910 $\pm$ 0.013	0.112



**Fig 1.** Comparison of Sodium contents among different natural sources of chitin.

Table 2. Level and standard deviation of potassium mineral from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, (n=3).

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	0.089	0.084	0.074	<b>0.082<math>\pm</math>0.008</b>	27.11
2.	Crab's Shell	0.045	0.033	0.037	<b>0.038 <math>\pm</math>0.00</b>	12.44
3.	Grasshopper's Shell	0.011	0.019	0.023	<b>0.018<math>\pm</math>0.006</b>	5.56

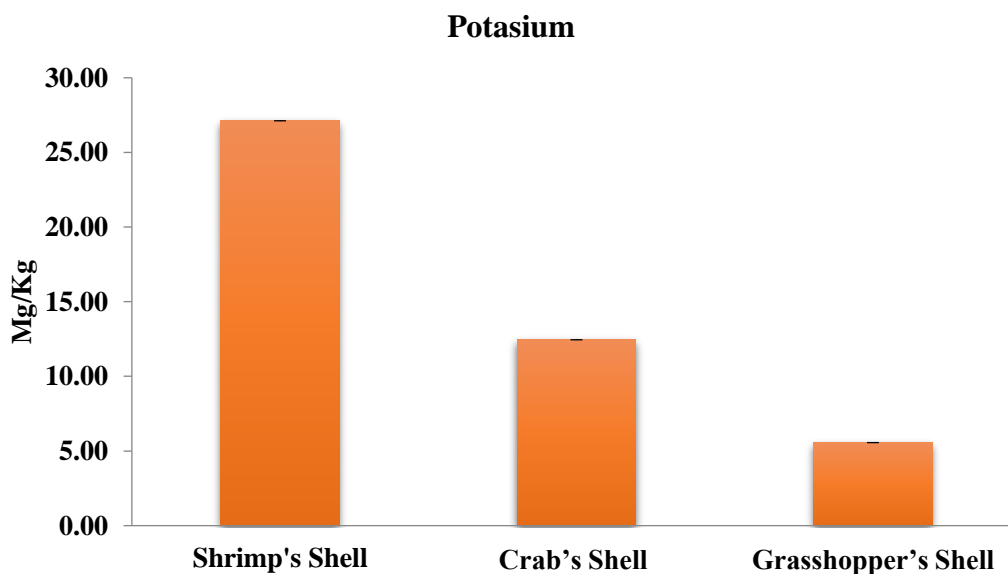


Fig 2. Comparison of potassium contents among different natural sources of chitin.

Table 3. Level and standard deviation of Calcium mineral from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, n=3.

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	0.142	0.138	0.157	<b>0.146<math>\pm</math>0.010</b>	48.222
2.	Crab's Shell	0.458	0.467	0.443	<b>0.456 <math>\pm</math>0.012</b>	151.667

3.	Grasshopper's Shell	0.083	0.091	0.075	<b>0.018±0.008</b>	27.333
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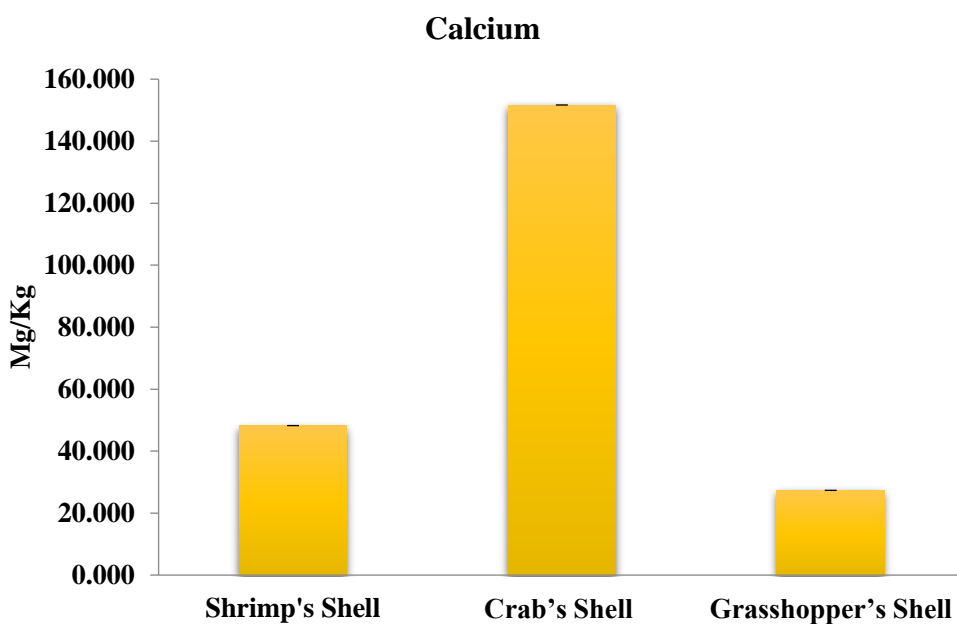


Fig. 3. Comparison of Calcium content among different natural sources of chitin.

Table 4. Level and standard deviation of Iron from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, (n=3).

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	0.011	0.009	0.008	<b>0.009±0.002</b>	2.778
2.	Crab's Shell	0.008	0.006	0.008	<b>0.007 <math>\pm</math>0.001</b>	2.111
3.	Grasshopper's Shell	0.007	0.008	0.005	<b>0.007±0.002</b>	1.889

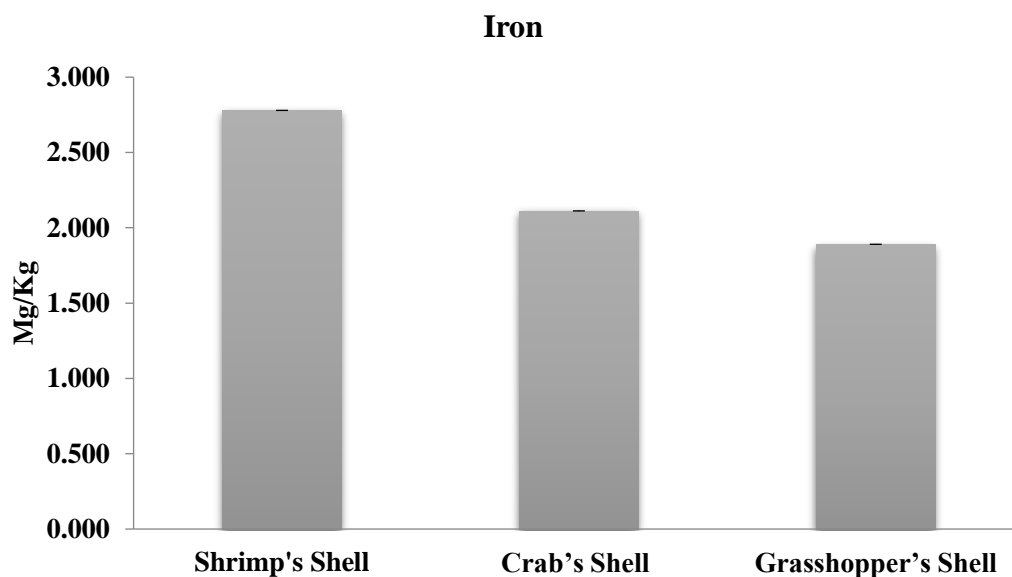


Fig 4. Comparison of iron contents among different natural sources of chitin.

Table 5. Level and standard deviation of zinc from different natural resources in (Mg/Kg). Values are presented as mean ± SD, (n=3).

S.No.	Natural sources	Absorbance			Mean ± SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	0.149	0.164	0.135	0.149±0.015	49.444
2.	Crab's Shell	0.132	0.124	0.121	0.126 ±0.006	41.556
3.	Grasshopper's Shell	0.089	0.076	0.093	0.087±0.009	28.333

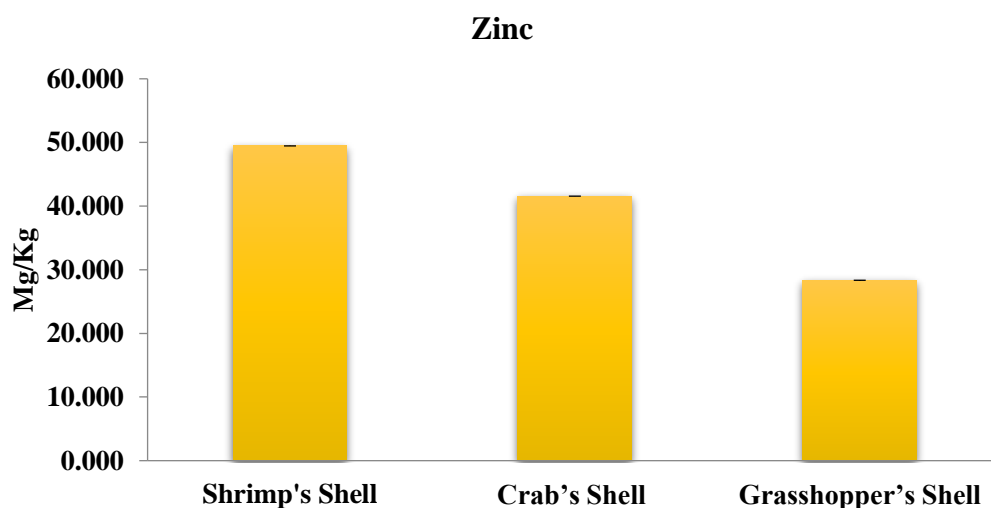


Fig 5. Comparison of zinc contents among different natural sources of chitin.

Table 6. Level and standard deviation of Nickel from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, (n=3).

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	0.0022	0.0029	0.0034	<b>0.0028<math>\pm</math>0.006</b>	0.611
2.	Crab's Shell	0.0018	0.0011	0.0014	<b>0.0014<math>\pm</math>0.004</b>	0.146
3.	Grasshopper's Shell	0.0008	0.0013	0.0010	<b>0.0010<math>\pm</math>0.001</b>	0.011

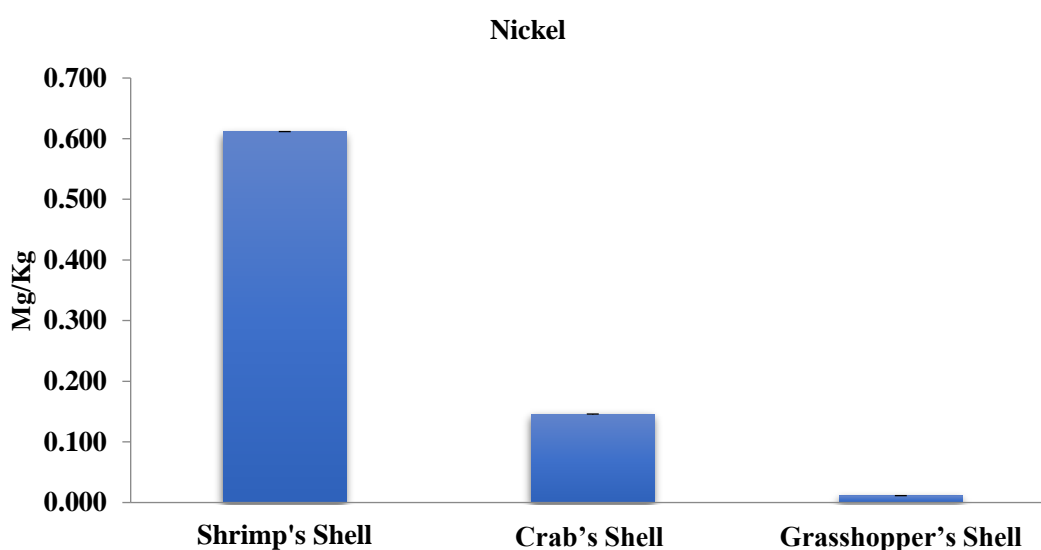


Fig 6. Comparison of Nickel contents among different natural sources of chitin.

Table 7. Level and standard deviation of Arsenic from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, n=3.

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	0.676	0.663	0.682	<b>0.674<math>\pm</math>0.010</b>	0.789
2.	Crab's Shell	0.432	0.448	0.457	<b>0.446<math>\pm</math>0.013</b>	0.290
3.	Grasshopper's Shell	0.368	0.353	0.371	<b>0.364<math>\pm</math>0.010</b>	0.112

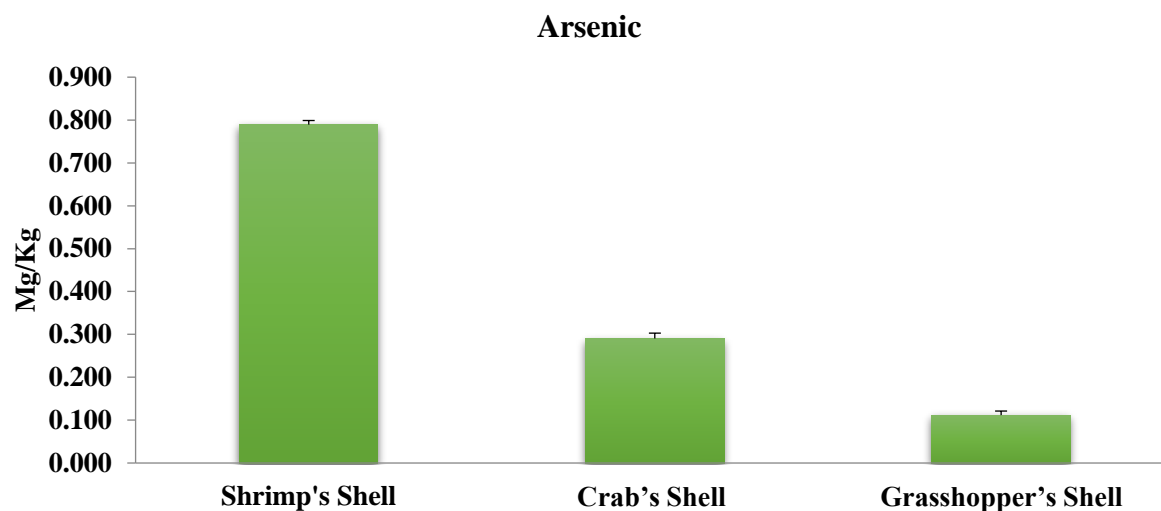


Fig 7. Comparison of Arsenic contents among different natural sources of chitin.

Table 8. Level and standard deviation of Copper from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, (n=3).

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	0.264	0.273	0.254	0.246 $\pm$ 0.010	3.190
2.	Crab's Shell	0.162	0.179	0.168	0.170 $\pm$ 0.009	1.970
3.	Grasshopper's Shell	0.083	0.077	0.068	0.068 $\pm$ 0.008	0.753

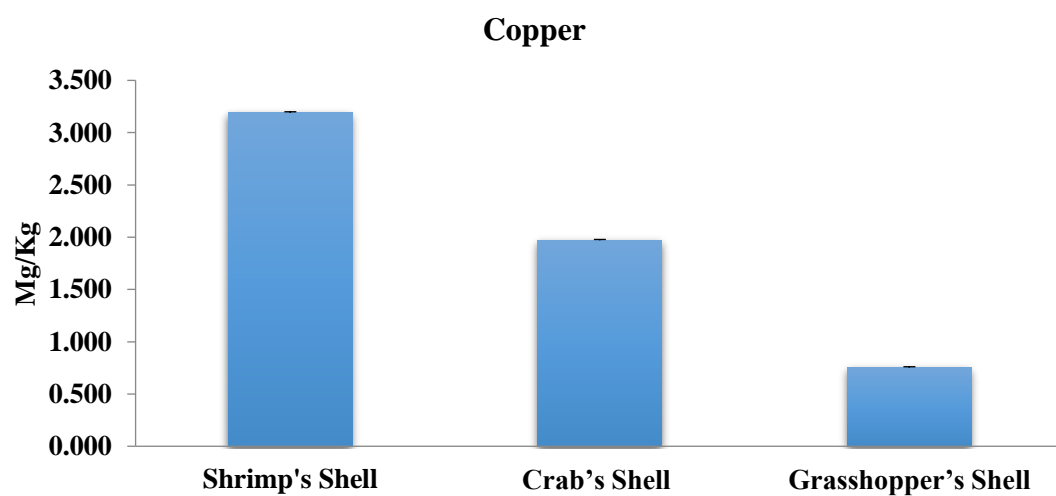


Fig 8. Comparison of Copper contents among different natural sources of chitin.



Table 9. Level and standard deviation of Chromium from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, (n=3).

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	0.021	0.023	0.018	0.021 $\pm$ 0.003	0.029
2.	Crab's Shell	0.019	0.022	0.020	0.020 $\pm$ 0.002	0.014
3.	Grasshopper's Shell	0.000	0.000	0.000	0.000 $\pm$ 0.000	NA

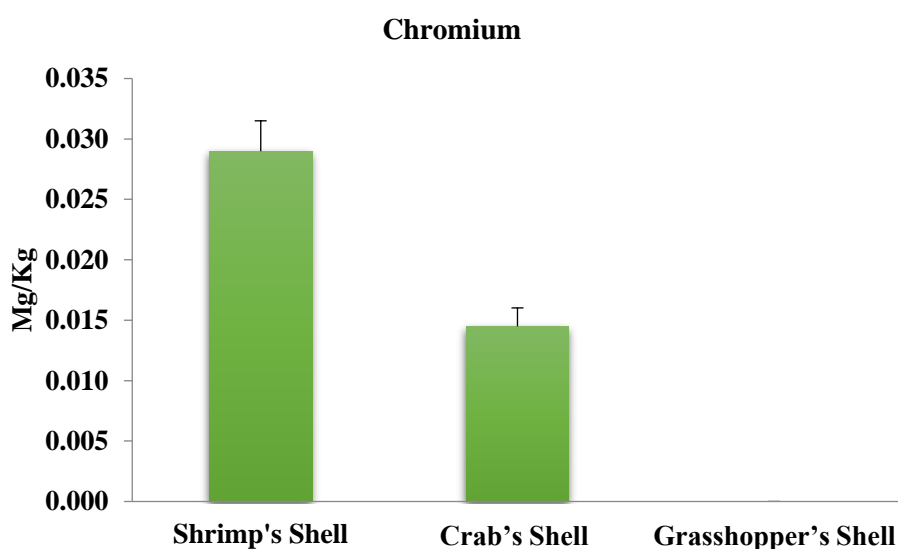


Fig 9. Comparison of Chromium (Cr) among different natural sources of chitin.

Table 10. Level and standard deviation of Lead (Pb) from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, (n=3).

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	0.036	0.039	0.043	0.039 $\pm$ 0.004	1.611
2.	Crab's Shell	0.025	0.021	0.019	0.020 $\pm$ 0.003	0.139
3.	Grasshopper's Shell	0.021	0.023	0.019	0.000 $\pm$ 0.002	0.083

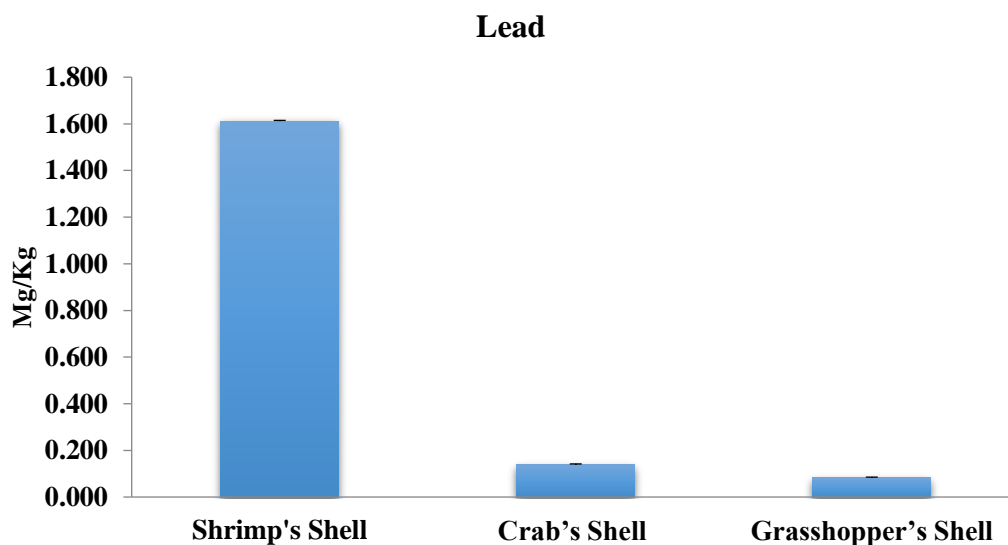


Fig 4.4.4.10. Comparison of lead (Pb) contents among different natural sources of chitin.

Table 11. Level and standard deviation of Mercury (Hg) from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, (n=3).

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	NA	NA	NA	NA	NA
2.	Crab's Shell	NA	NA	NA	NA	NA
3.	Grasshopper's Shell	NA	NA	NA	NA	NA

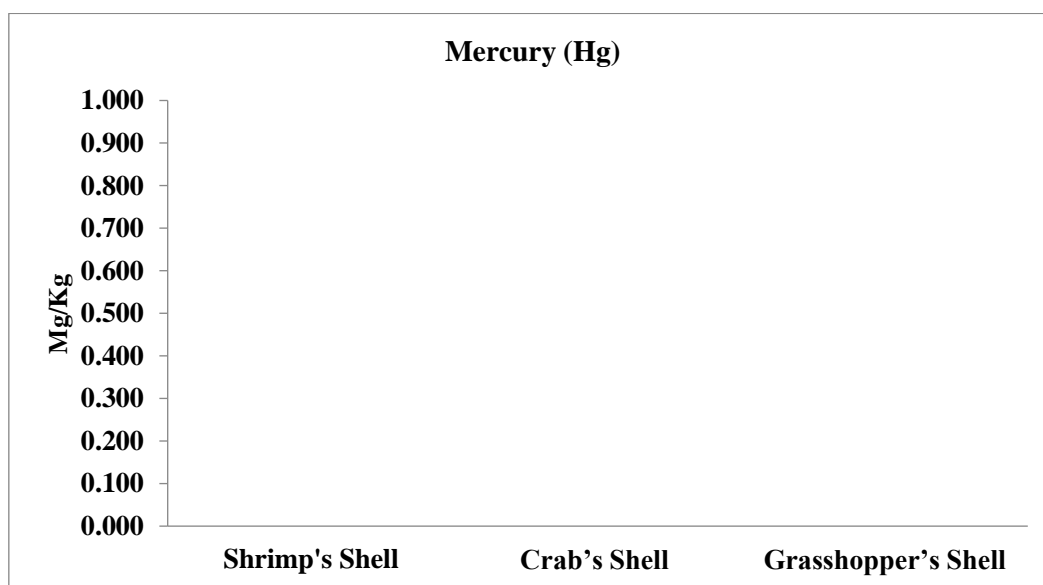
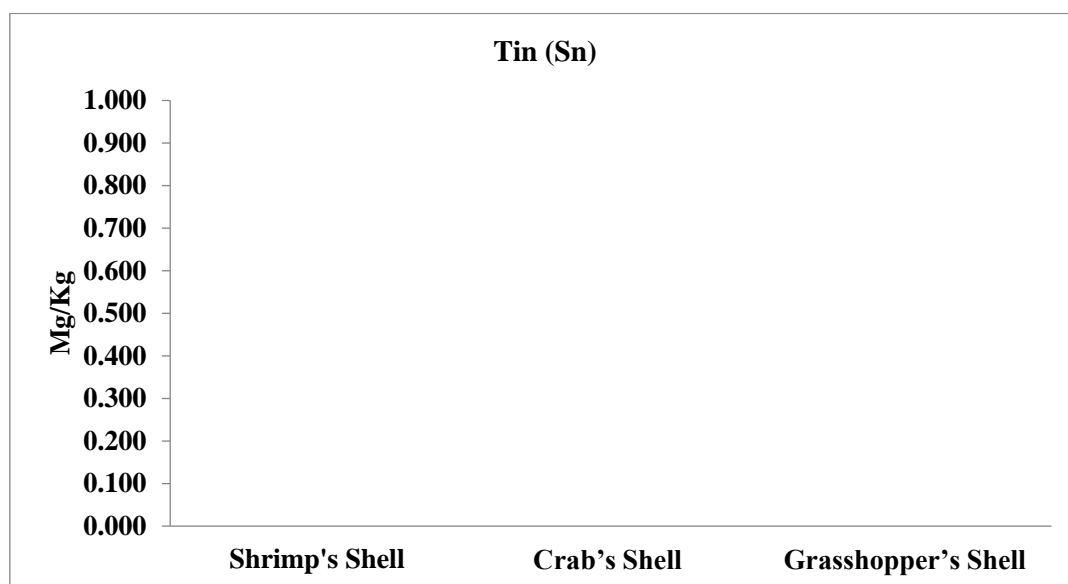


Fig 11. Comparison of Mercury (Hg) contents among different natural sources of chitin.

**Table 12. Level and standard deviation of tin from different natural resources (Mg/Kg). Values expressed as mean  $\pm$ SD, (n=3).**

S.No.	Natural sources	Absorbance			Mean $\pm$ SD	Mg/Kg
		1	2	3		
1.	Shrimp's Shell	NA	NA	NA	NA	NA
2.	Crab's Shell	NA	NA	NA	NA	NA
3.	Grasshopper's Shell	NA	NA	NA	NA	NA



**Fig 12. Comparison of Tin (Sn) contents among different natural sources of chitin.**

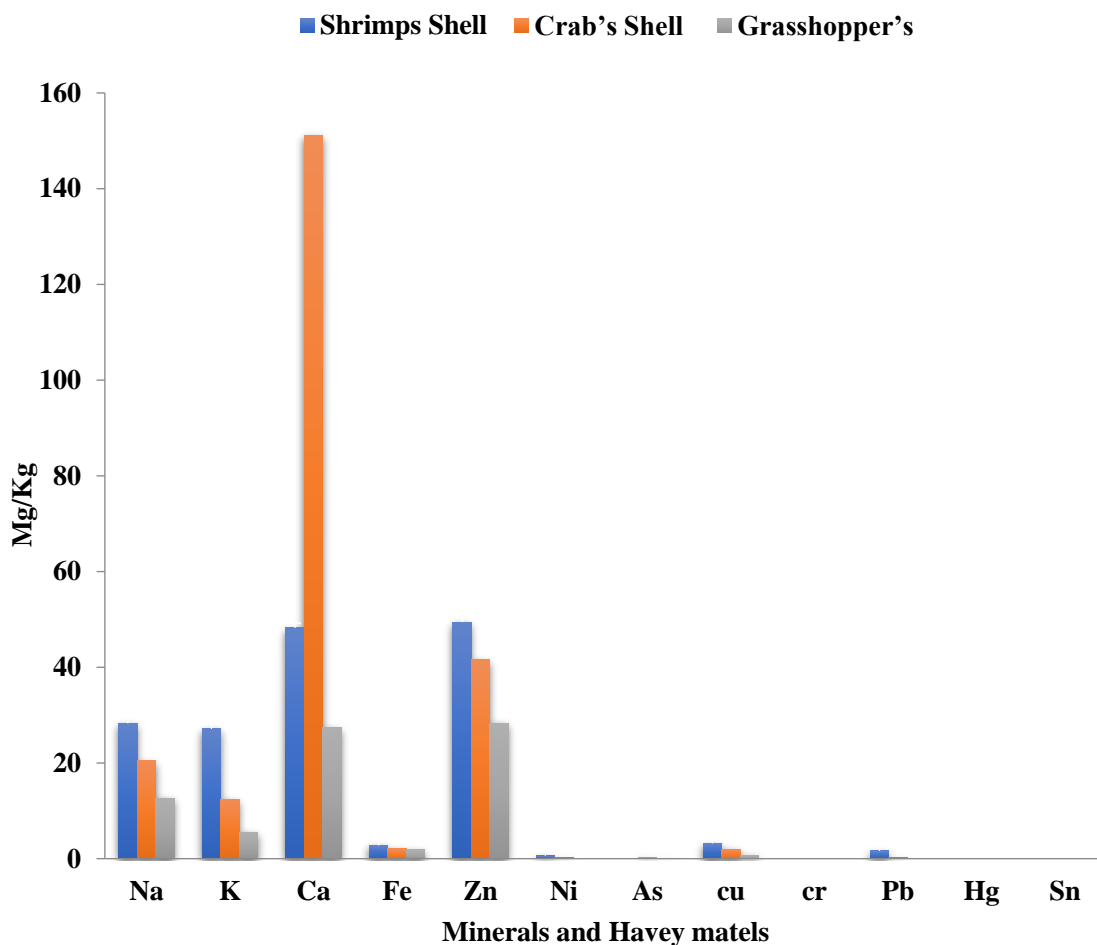


Fig. 13. Comparison of mineral and heavy metals contents.

#### IV. CONCLUSION

Comparative results suggested that appropriate amount of chitosan extraction from the natural resources. The decreasing order of extracted crude chitosan from exoskeleton was shrimp, crab, and Grasshopper. Comparative mineral and heavy metal content was nil in concern to Sn, Hg and Cr whereas the least was As and Ni. Results expressed as order of minerals and heavy metal level in various natural sources was  $Ca > Zn > Na > K > Fe > Cu > Pb$ . Maximum Ca minerals was exhibited in crab, shrimp represent descending order as Zn, Ca, Na, and K.

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**Cite this article as :**

Hemant Kumar, Bharat Kumar Vishwakarma, Nagmani Manikpuri, "Comparative Study of Minerals and Heavy Metal Content Among Different Natural Sources of Chitosan", International Journal of Scientific Research in Chemistry (IJSRCH), ISSN : 2456-8457, Volume 7 Issue 3, pp. 16-29, May-June 2022.

URL : <https://ijsrch.com/IJSRCH22732>