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Environmental Contamination and Assessment of Heavy Metals in Water, Sediments and Shrimp of Red Sea Coast of Jizan, Saudi Arabia

Abstract

The study was conducted to assess heavy metals concentrations in water, sediment and white shrimp (*Liptopenaeus vannamei*) of Red Sea, Jizan, Saudi Arabia, using ICP-MS. The concentrations of heavy metals in water were above the recommended drinking water standards setup by the WHO/USEPA (World Health Organization/United States Environmental Protection Agency). However the concentrations of heavy metals in sediment and white leg shrimp were lower than the recommended levels proposed by WHO/USEPA, except Cr level in shrimp muscle. The level of contamination degree (C_d) and modified contamination degree (mC_d) indicated 'low' and 'very low' degree of contamination respectively. Pollution load index (PLI) of the studied area was lower than unity, indicated no pollution. Furthermore, a toxic response factor was applied to determine the potential ecological risk index (RI) of these heavy metals into the water body. The results of this study exhibited a low potential ecological risk of heavy metals.

Keywords: Environment; Contamination; Heavy metals; Sediment; Pollution and risk assessment

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Introduction

The marine environment has become contaminated with a wide range of pollutants, causing worldwide attention over the last few decades [1]. The petrochemical and oil industries are the major sources of pollution. Due to rapid industrial development, everyday a large amount of industrial wastage discharge into nature without proper treatment. This causes contamination of the marine biota of fishes, shrimps, oysters and crabs [2]. Heavy metals in water and sediment pose potential threats to the environment and can damage human health through food chains [3-5].

Sediments may provide useful information for the environmental pollution status [6]. Heavy metals are inert in the sediment, and are often considered to be conservative pollutants although they may be released into the water in response to certain disturbances, causing potential threat to ecosystems [7-12].

Despite the importance of marine life and seafood resources in the red sea, little is known of the flux and distribution of pollutants

and their subsequent effects on marine organisms. To quantify the heavy metals and the health risks associated with heavy metals in water, sediments and shrimps of Jezan coast were evaluated. To assess the influence of pollution by heavy metals, the following approaches were employed, contamination factor ($C_{\rm F}^{\rm i}$), degree of contamination ($C_{\rm d}$), modified degree of contamination ($mC_{\rm d}$), pollution load index (PLI), potential ecological risk factors ($E_{\rm r}^{\rm i}$), risk index and pollution degree.

Materials and Methods

Study area

The coastline of Saudi Arabia is about 1,840 km in length, accounting for 79% of the eastern seaboard of the Red Sea (MEPA/IUCN 1987). The Province of Jizan lies in the south west section of the Kingdom of Saudi Arabia. The area is in an average up to 50 m water depth and 125 km in width with reefs and low-lying limestone islands. The area has a subtropical desert climate, and drainage is principally westward. Several ephemeral wadi systems drain to the shelf, like Jizan, Mais, Bish and others [13].

Annual rainfall in this area is higher than most other parts of the coastal plain; it ranges from 50-100 mm at the coast to as much as 500-600 mm inland [14]. Jizan is a key industrial center, a port and a center of farming.

The present study focused on the coastal area of Jizan, Saudi Arabia extending from 16°52′34.73" to 16°55′03.49" N and from 42°32′43.69" to 42°32′50.13" E, to evaluate different contamination sources that affect this area. Three distinctive areas, namely, Corniche North Park (spot-1), Fish landing center (spot-2) and Sea Port (spot-3), that have commercial, industrial and agricultural facilities, were included in this study (Figure 1).

Sample collection and preparation

Water and surface sediment samples were collected from three sampling spots of Red sea of Jezan coast, those were around 2 km away from each other. Water samples were collected in precleaned plastic bottles following filtration through Whatmann No. 541 filter paper and kept in a refrigerator at 4°C with addition of 2 mg/I HNO₃ before laboratory analysis [15] (Figure 1).

Sediment samples from the surface bed were collected with a stainless steel Ekman grab sampler, which allows free water through the sampler during descent penetration. The sediment samples were collected from the same sampling spots, which were previously indicated for water sampling. The sediment samples were first air dried for several days over Pyrex petri dishes and then samples were dried in an oven at 105°C in laboratory. White leg shrimps (Litopenaeus vannamei) were collected from a fish market near the fish landing center of red sea, Jizan. The shrimps were killed with percussive stunning [16]. Then shrimps were transferred in a cooler packed with ice blocks in order to maintain the freshness and later brought to the laboratory. A total of 30 shrimps were collected with an average length of 15.47 ± 2.93 cm (length range 13.5-19.3 cm) and an average weight of 13.80 ± 5.62 g (weight range 2.74-22.29 g). Each sample was carefully dissected for its muscle. To prevent metal contamination, special care was taken, and tissues were dissected with special ceramic knife, scissors and plastic forceps (Miyako, California, USA). The samples were then washed with distilled water and cut into small pieces (2-3 cm). Then the tissues were oven dried at 65°C overnight and allowed to cool at room temperature. The dried samples were powdered using a glass mortar, sieved through 1 mm mesh and stored in airtight plastic vials inside desiccators.

Analytical procedure and analysis

Dried soil samples (0.2 g) were digested according to the method of Wade et al. [17]. The dried shrimp muscles were digested according to the method of Hanson as described by Rahman et al. [18]. 0.5 g of dried powdered shrimp muscle tissues (three replications) were taken in a digestion apparatus and 2.5 ml conc. H_2SO_4 and 4 ml conc. HNO_3 were added. The mixture was slowly heated using a hotplate for 20 min. at 130°C and allow cooling at room temperature [19]. The content was double distilled water and filtered quantitatively into a 50 ml volumetric flask.





Detection of heavy metals (Cr, Mn, Ni, Cu, Zn, Cd, Pb and As) in all samples (water, sediments and shrimp tissues) were carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin Elmer, NexION 300D). Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared on the basis of mono element certified reference solution ICP Standard (Merck). All laboratory plastics and glassware were cleaned by soaking overnight in a 10% nitric acid solution and then rinsing with deionized water.

All samples were collected and analyzed in triplicates and the average results were used to represent the data. All calculations were performed by Microsoft Excel 2010.

Results and Discussion

Heavy metals in water

The results of heavy metal concentrations in water, surface sediment and shrimp samples collected from the Red Sea of Jizan, Saudi Arabia are shown in **Table 1** and compared with WHO/USEPA maximum limit, presented in **Table 2**. The mean concentrations of studied heavy metals in water followed a decreasing order of Cu>Ni>Zn>Cr>Mn>Pb>As>Cd. The average concentrations of metals in water samples more or less exceeded the WHO/USEPA guidelines, with the exception of arsenic (**Table 1**). The concentration of heavy metals in sediment and shrimp tissues were found to be lower than maximum permissible limits recommended by the World Health Organization and US Environmental Protection Agency, except Cr in shrimp muscle [20, 21].

The average Cr concentration in surface was 1.36 mg/l which is higher than the recommended maximum concentration of Cr for drinking water (0.1 mg/l) set by WHO [22]. In aquatic environment, Cr is a biochemically active transition metal. Weathering of the earth crust is the primary and natural source of Cr in the surface water. Anthropogenic sources of emission of Cr in the surface water are from municipal wastes, chemical industries, paints, leather, road run off due to tire wear, corrosion of bushings, brake wires, radiators [23]. Most of the tannery industries used "Chrome tanning method" and the waste discharged by this method exerts toxicity to the fish and aquatic life. Some tannery industries are located along the coast of Red Sea Jizan might have effects on Cr pollution. Therefore, the data findings from this study suggest that the industrial effluents and power plant wastes might be the principal source of Cr contamination in the study area. The data obtained for Cr in water from the study varied more or less regularly with the findings of the other authors [24-26]. It was apparent that Cr concentration of the present investigation was higher than the recommended value, indicating Cr pollution of Red Sea coast, Jizan. Manganese (Mn) concentration was 1.25 mg/l. The concentration of Mn in water of Red Sea, Jizan was higher than the maximum permissible limit of Mn concentration in drinking water which is 0.5 mg/l, set by WHO [22]. Mn is an element of low toxicity having considerable biological significance. It is one of the more biogeochemical and active transition metals in aquatic environment [27] (Table 1).

The mean nickel (Ni) concentration was 6.37 mg/l found in Red sea, Jizan. Ni concentrations were above the WHO recommended value (0.02 mg/l) for drinking water. Nickel is quite abundant in the Earth's crust, enters surface waters from the dissolution of rocks and soils, from biological cycles, atmospheric fallout, and especially from industrial processes and waste disposal [28]. The concentration of Copper (Cu) in water samples was 7.85 mg/l, the observed value of Cu was much higher than the permissible concentration of 0.05 mg/l, prescribed by WHO [22]. This is an indication of the fact that the presence of Cu in studied area received water mostly from the industrial and power plant effluents. Cu enters the aquatic environment through industrial effluents and also from river run offs domestic waters, etc. [29]. Copper is an essential substance to human life, however, in high concentrations, it can cause anemia, liver and kidney damage, stomach and intestinal irritation [30]. Zinc (Zn) concentration was 3.58 mg/l, which was higher than the WHO recommended guideline value (3 mg/l) [22]. Zn is a naturally abundant element present as a common contaminant in agricultural, food wastes, manufacturing of pesticides as well as antifouling paints [25]. The average cadmium (Cd) concentration was 0.17 mg/l. The maximum permitted concentration of Cd in drinking water is 0.01 mg/l, set by WHO [22]. Cd concentration in water samples of Red sea, Jizan was higher than the above-recommended value. It might have happened due to flushing of the metal from immobilized deposits. Cd as a transition element behaves in the environment as a cumulative poison [31]. It is listed by EPA as one

Table 1 International guideline for heavy metals in water, sediment and fish [21, 22].

	Wat	er	Sedin	nent	Shrimp Tissues		
Heavy Metals	Mean concentrations (mg/l)	Maximum Limit WHO/USEPA (mg/l)	Mean concentrations (mg/kg)	Maximum Limit WHO/USEPA (mg/kg)	Mean concentrations (mg/kg)	Maximum Limit WHO/USEPA (mg/kg)	
Cr	1.36	0.1	5.64	25	4.35	1	
Mn	1.25	0.5	9.58	30	0.90	1	
Ni	6.37	0.02	14.32	20	0.19	0.5-1	
Cu	7.85	0.05	16.39	25	3.82	30	
Zn	3.58	3	24.74	123	14.05	100	
Cd	0.17	0.01	0.48	6	0.63	1	
Pb	0.56	0.05	3.86	10	1.06	2	
As	0.28	0.03	0.34	3	ND	_	

of the 129 priority pollutants and listed among the 25 hazardous substances. Moreover, there is an international agreement for cadmium not being dumped into the sea, since it is included in the black list [32]. The lead (Pb) concentration (0.56 mg/l) in water of the Red sea, Jezan remained close to the maximum safe limit of 0.05 mg/l as recommended by WHO [22]. It was apprehended from the observation that high level of Pb in the sea water was indicative of the fact that the source of higher Pb content was not only the industrial effluents but also some other outlets. The mean value of arsenic (As) concentration found in the Red sea, Jizan (0.28 mg/l) was higher than the limits given by WHO (0.01 mg/l) for potable water quality standard.

The higher standard deviation observed for heavy metals As, Ni, Cr, and Zn in water suggests that these metals were not uniformly distributed in the study area which is reported by Sharma et al. [33]. The data obtained for heavy metals in water from the present study varied more or less regularly with the findings of the other authors [34, 35]. This variation might be described to a variety of industries discharging their treated and untreated waste water into the Red Sea. The concentrations of heavy metals in industrial and power plant effluents also depend on the process of product manufacturing and raw materials used in the industries [33].

Heavy metals in sediment

The concentration of heavy metals in the surface sediment of Red Sea, Jezan is presented in Table 1. The concentrations of heavy metals in sediment were observed in the order Zn>Cu>Ni>Mn>Cr>Pb>As>Cd. The data indicated that Zn accumulation in the sediment was the most whereas at the least. All heavy metal concentrations in the sediment samples did not exceed the maximum limits set by WHO/USEPA. Comparisons of heavy metal concentrations with the other observed values by different authors around the world. Compared to the heavy metal concentrations in sediment of other coast of Red Sea, the metal levels in surface sediment of the present study were found to be lower in all investigated metals [25, 36, 37]. Compared with other than Red Sea, the lowest ranged concentrations of Cr, Mn, Ni, Cu, Zn and Pb were found 3 mg/l, 12.5 mg/l, 0.66 mg/l, 3.82 mg/l, 0.04 mg/l and 0.22 mg/l, respectively in Gulf of Mexico [38]. The maximum concentration for Cr, Mn, Cu, Zn and Pb were 233.93 mg/l, 658.87 mg/l, 111 mg/l, 263.49 mg/l and 138.06 mg/l, respectively found in Gulf of Aden and only highest concentration was reported in Mn (76.9 mg/l) from Gulf of Mexico [38, 39]. The low concentrations of metals observed in sediments in the present investigation did not exceed the permissible levels reported by WHO/USEPA. This indicated that

Table 2 Mean metal concentrations in water (mg/l) sediment (mg/kg) and shrimp tissue (mg/kg).

Туре	Cr	Mn	Ni	Cu	Zn	Cd	Pb	As
Water	1.36 ± 0.37	1.25 ± 0.29	6.37 ± 1.88	7.85 ± 1.52	3.58 ± 0.94	0.17 ± 0.04	0.56 ± 0.13	0.28 ± 0.13
Sediment	5.64 ± 1.51	9.58 ± 2.31	14.32 ± 3.87	16.39 ± 3.81	24.74 ± 6.47	0.48 ± 0.12	3.86 ± 0.82	0.34 ± 0.76
Shrimp	4.35 ± 1.02	0.90 ± 0.09	0.19 ± 0.04	3.82 ± 0.07	14.05 ± 2.58	0.63 ± 0.79	1.06 ± 0.03	ND

Table 3 Concentration of heavy metals (mg/kg) in surface sediment compared to other studied areas [25, 36, 37, 39].

Location	Cr	Mn	Ni	Cu	Zn	Cd	Pb	As	References
Red Sea, Jezan	9.64	39.58	44.32	16.39	24.74	0.48	3.86	0.02	Present work
Red Sea, Jedda	12.98-22.81	33.71-205.06	67.78-85.50	17.47-23.77	52.74-76.36	3.08-3.51	80.30-98.77	-	Badr et al. (2009)
Red Sea, Egypt	-	235.7	-	21.43	51.4	-	-	-	Okbah et al. (2005)
Red Sea, Yemen	15.9-24.5	20.7-65	9.3-14.7	24.8-39.3	88.6-138	-	5.12-8.7	-	Hassan and Nadia (2000)
Gulf of Aden	17-233.93	138.23-658.87	16.17-48.07	8.09-111	21.85-263.49	-	14.8-138.06	-	Saleh (2006)
Gulf of Mannar, IndiaAden	148-195	290-301	22.63-24.5		71-74.06	-	15.97-16	-	Jonathan and Mohan (2003)
Gulf of Mexico	3-100	12.5-448.9	0.66-76.9	3.82-18.7	0.04-79.6	-	0.22-20.2	-	Macias-Zanora et al. (1999)
Florida Bay	57-347	211-1140	4.9-54	-	-	-		-	Caccia et al. (2003)

Table 4 Heavy metal concentration in white shrimp (μ /g wet weight) and different international and national safe limits.

Heavy metal	Present study	FAO/WHO ^a	SASO ^b	SEPA ^c
Cr	4.35	1	2	0.5
Mn	0.90	1	-	-
Ni	0.19	20	15	10
Cu	3.82	30	20	20
Zn	14.05	100	50	100
Cd	0.63	1	0.5	0.2
Pb	1.06	2	2	9
As	ND	-	1	-

^a Codex alimentarious commission 1984

^bSaudi Arabian standards organization, SASO (1977)

the sediments of Red sea coast of Jezan were low contaminated with heavy metals (Tables 2 and 3).

Heavy metals in shrimp

The average concentrations of heavy metals in the muscle of white shrimp are shown in Table 1. The analysis of the data depicted an order of heavy metal accumulation in shrimp muscle that was Zn>Cr>Cu>Pb>Mn>Cd>Ni>As. The order of bioaccumulations of these metals might be as a result of the fact that different metals tend to accumulate differently in tissues. Bioaccumulation is also related with feeding, swimming and metabolic activity of the individual and species. These heavy metal concentrations were compared to sea food standards set by the world health organization, India, China and Saudi standard. The concentrations of Zn is higher than other metals in shrimp muscles, however, Zn concentration does not exceed any sea food standard in Table 4. The levels of heavy metals in shrimp muscles in this report were relatively lower than those of other sea food standard, except the Cr concentration in shrimp muscle. Mn concentration in white shrimp muscle is higher than China, India and Saudi standard but lower than that of WHO standard. Ni, Cu, Zn and Pb had the lowest concentrations than that of sea food standard. Arsenic was not detected in any shrimp muscles; this may be explained by both low arsenic (As) levels in water, sediments and biological metabolism of this element [40] (Tables 2 and 4).

The concentrations of heavy metal may be dependent on size, feeding habits, the bio concentration capacity of each species or ecological zone [41]. The concentrations of heavy metals in shrimp muscle were generally lower than those in current world standards except Cr [42, 43]. This study shows new information on the distribution of metals in white shrimp along the Red Sea coast of Jizan. The present findings showed different levels of metals compared to previous studies in the red sea of the other coasts [25, 36, 37]. The measurement of heavy metals in aquatic organism as a water quality indicator could be more reliable than water chemical analysis [44-48]. Therefore, it is strongly recommended that a monitoring system be deployed, especially in polluted areas. Stricter regulations for shrimp harvesting and farming could satisfy health and safety considerations in the shrimp market. The high, hazardous levels of Cr pollution in the shrimp of Red sea coast Jizan is alarming, since Cr in the human body tend to cause deadly diseases.

Contamination degree of heavy metals

The contamination factor (C_{f}^{i}) is the ratio of measured concentration of the heavy metals in sediment and the preindustrial reference value for the same metal [49]. The degree of contamination is calculated as the sum of all contamination factors. The computing equation for contamination factor (C_{i}^{i}) and the degree of contamination (C₂) are as follows Cif=Ci/Cin (i)

$$Cd = \sum_{i=1}^{n} c_{f}^{i}$$
(ii)

Here, Cⁱ is the measured concentration of the heavy metals

(Table 5). Modified degree of contamination (mC_d) and pollution load index (PLI) The modified degree of contamination means the sum of all contamination factors (C_{i}) divided by the number (n) of analyzed contaminants. The equation for modified degree of contamination (mC₄) can be expressed as described below by Abrahim and Parker (2008). $mCd = \frac{\sum_{i=1}^{n} c_{f}^{i}}{n} \dots$

It was suggested the values of moderate degree of contamination described as mC_d<1.5, 1.5 \leq mC_d<2, 2 \leq mC_d<4, 4 \leq mC_d<8, 8 \leq mC_d<16, 16 \leq mC_d<32 and mC_d \geq 32 indicate nil to very low, low, moderate, high, very high, extremely high and ultra-high degree of contamination, respectively. This study demonstrated 'nil to very low' degree of sediment contamination with respect to the analyzed eight heavy metals (Table 5).

in sediment and C_n^i is the standard pre-industrial reference level (mg/kg). 90 for Cr, 850 for Mn, 68 for Ni, 50 for Cu, 175

for Zn, 1 for Cd, 70 for Pb and 15 for As [49, 50]. The calculated

contamination factor (C_{4}^{i}) existed in the following order, Cd>Cu>Ni>Zn>Cr>Pb>Mn>as in the sediment of red sea coast of

Jezan. The contamination factor and degree of contamination

were classified into four groups by Hakanson. The values of

contamination factor are characterized as $C_{f}^{i} < 1$, $1 \le C_{f}^{i} < 3$, $3 \le C_{f}^{i} < 6$

and $C_{i}^{i} \ge 6$ indicated as low, moderate, considerable, and very high

contamination factor respectively. The degree of contamination

defines the quality of the environment in the following manner C_d <8, 8 ≤ C_d <16, 16 ≤ C_d <32 and C_d ≥ 32 indicate low, moderate,

considerable and very high degree of contamination accordingly.

The (C_{i}) of studied heavy metals were viewed to be low and the

low degree of contamination (C_d<8) was indicated for all metals

The calculation of the extents of pollution of heavy metals was used in the method based on pollution load index (PLI) introduced by Tomlinson et al. and the equation is shown as:

Here, n=8 (number of metals in this study) and C_{f}^{i} is the contamination factor. PLI provides a simple, comparative means for assessing a site or estuarine quality, a value of zero indicates perfection, a value of one indicates only baseline levels of pollutants present and values higher than one would indicate progressive deterioration of the site and estuarine quality [51]. In this study, PLI values very lower than one, means no pollution (Table 5).

Assessment of potential ecological risk

The assessment of the potential ecological risk of the heavy metal contamination was proposed as a diagnostic tool for water pollution control purposes, as a result of the increasing content of heavy metals in sediments and their subsequent release into the water, which could threaten ecological health. Hakanson described a method to measure the potential ecological risk index for aquatic pollution control purposes, to sort out which

(iii)

Concer	ntration f	factors (C	i _f)					Degree of	Modified degree of	Pollution load index
Cr	Mn	Ni	Cu	Zn	Cd	Pb	As	contamination (C _d)	contamination (mCd)	nCd) (PLI)
0.06	0.01	0.21	0.33	0.14	0.48	0.05	0.001	1.28	0.16	0.06

 Table 5 Contamination factors Cif, degree of contamination (Cd), modified degree of contamination (mCd) and pollution load index (PLI) of heavy

 metals in surface sediments from Red Sea, Jezan.

Table 6 Potential ecological risk factors and potential ecological risk indexes (RI) of heavy metals in sediments from Red, Jezan, Saudi Arabia

Pollution degree	Risk index (RI)	Potential ecological risk factors (E ⁱ ,)								
Low	17.00	As	Pb	Cd	Zn	Cu	Ni	Mn	Cr	
	17.05	0.01	0.25	14.4	0.14	1.65	1.05	0.01	0.12	

water bodies and substances should be given special attention. According to this method, the potential ecological risk factor (E_r^i) of single element and the potential ecological risk index (RI) of multi-element can be computed by the following equations

$$RI = \sum_{i=1}^{n} E_{r}^{i}$$
(vi)

Here C_{f}^{i} is the contamination factor for the element of 'i'; T_{f}^{i} is the toxic-response factor for the given element of 'I', which accounts for the toxic requirement and the sensitivity requirement. The toxic-response factors for Cr, Mn, Ni, Cu, Zn, Cd, Pb and as were 2, 1, 5, 5, 1, 30, 5 and 10, respectively [49, 52]. According to Hakanson, the indices and grades of potential ecological risk were calculated by using equations 1, 2, 5 and 6. The results of evaluation on potential ecological risk factor Eir and the potential ecological risk index (RI) are summarized in Table 6. The order of potential ecological risk factor of heavy metal in sediments of red sea coast of Jizan was Cd>Cu>Ni>Pb>Zn>Cr>Mn>As. The potential ecological risk factors (Eⁱ,) of Cr, Mn, Ni, Cu, Zn, Cd, Pb and As were lower than 40, which indicated low ecological risk. The ecological risk index (RI) values lower than 150 is regarded as "low" pollution degree [49]. The results indicated that there was low potential ecological risk posed by the heavy metals based on the value of RI in red sea of Jizan coast of Saudi Arabia (Table 6).

Conclusion

The result indicated that the concentration of seven heavy metals (Cr, Mn, Ni, Cu, Zn, Cd and Pb) out of eight in water samples, are

higher than the WHO recommended guideline for drinking water [22]. The elevated levels of these heavy metals could ultimately contaminate the coastal aquaculture and thus making them toxic for human consumption. However, the average heavy metal concentrations in sediments and shrimp muscle are lower than the WHO standard and the others, except Cr concentration in shrimp muscle [53]. Factor analysis in this study, $C_{d'}$, $mC_{d'}$, PLI, E^{i}_{f} and RI recorded values were low indicating low pollution that is safe for environment and human consumption except Cr level. Regular monitoring program of the heavy metals is recommended to protect these water bodies and also to reduce environmental risk.

This study shows new information on the distribution of metals in white shrimp along Red Sea coast of Jizan. The shrimp market in Saudi Arabia is dependent upon shrimp farming and harvesting from these regions. Therefore, it is strongly recommended that a monitoring system be deployed, especially in polluted areas. Stricter regulations for shrimp harvesting and farming could satisfy health and safety considerations in the shrimp market. The high, hazardous levels of Cr pollution in the shrimp of Red Sea, Jizan is alarming for authorities, since Cr in the human body tend to cause deadly diseases.

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