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Shrimp, Crabs and Squids as bio-indicators for heavy metals in Arabian Gulf, Saudi Arabia

Mostafa Abdel Mohsen El Gammal, Ali Al-madan and Nabil Fita

Abstract
Measurement of heavy metals in bio-indicators (shrimp, crabs and squids) has diagnosed excessive relevant in eco toxicological terms; reflect the bioavailability in the ecosystem that reasonable measurement of public health standards for the animals' health. So, the aim of the present study is to find the levels of heavy metals (Pb, Cu, Cd, As, Zn, Ni, Hg, Fe and Cr) in shrimp, crabs and squids from the Arabian Gulf. The results showed that the heavy metals in shrimp, squids and crabs recorded no significant differences between them for the most metals, and no significant differences between muscles, exoskeleton and whole body metals for shrimp and crabs. Pb, Cu and Cd heavy metals were not detectable in water, sediment, shrimp, crabs and squids. The bio concentration (BCF) for squids recorded higher values than shrimp and crabs for As, Zn and Hg metals, but BCF for shrimp recorded high values for Fe and Cr. The accumulate of Zn, Hg, As in squids recorded more values than crabs, shrimp, sediment and water, but sediment recorded more values for Fe, Ni, and Cr than others. This study offers baseline information on the impact of trace metals contamination on these species and aquatic environments.

Keywords: water, sediment, shrimp, crabs, squids, heavy metals, Arabian Gulf

1. Introduction
It is known that some shrimp, crabs and squids may give a useful means of monitoring such elemental concentration (Pb, Cu, Cd, As, Zn, Ni, Hg, Fe and Cr) levels and their impact on the aquatic environment. Heavy metal pollutions are particularly hazardous contaminants aquatic food and the environment. In general, they are not biodegradable and have long biological half-lives. The heavy metals must be controlled in aquatic food sources to assure public safety [1]. An excessive amount of the attention of meals heavy metals is related to the etiology diseases, specially cardiovascular, renal, neurological, and bone diseases [2].
The highest trace metal levels in benthic molluscs and annelids of Kuwait Bay in the Persian Gulf compared to other regions of the word [3]. Zn and Cu pollution swimmer crabs and attributed this to the 1991 Gulf War oil spill into Kuwait's marine environment [4]. The concentrations of Cd, Pb, Cu, and Zn in fresh parts of the clam (*M. meretrix*) in the Persian Gulf near the Saudi Arabia coast line were within the acceptable standard range [5]. To the extent of the author's knowledge, few studies have been reported on heavy metals pollution of shrimp and fish in the Persian Gulf Water of Iran [6, 7]. Trace elements in cephalopods have received increasing attention in recent decades, in Europe and in Japan as these mollusks play a major role both as predator and prey in marine ecosystems [8] Hence, they are the major interest for monitoring variations of pollutant concentrations in the cephalopod tissues reflect the bioavailability and metal variations in their immediate environment over relatively short time scale [8, 9]. Squid are themselves important prey items for large fish, sea birds, and marine mammals [10, 11]. Squid and other cephalopod are very efficient accumulators of various trace elements [12-14]. Toxic metals as cadmium and mercury are bio accumulated and retained in squid [15, 16] and so passed on to predators, therefore potentially increasing the contaminant load in higher trophic levels, including humans [15, 17-19]. Bioaccumulation means an increase in metals concentration in biological organisms compared to their concentration in the environment [6]. Metals accumulation in living organisms at any time are taken up and stored faster than they are broken down (metabolized or excreted). Bioaccumulation indicates the pollution level in organisms which live in polluted environments. It changes among organisms based on the uptake, detoxification and the outside environment [20].

The use of marine organisms as bio indicators of metal pollution of aquatic environments and suitability for human use of toxicological point has been documented [21-24]. Continuous monitoring of aquatic animals is very important in a polluted environment to check the possible risk of human consumption [25].
The present study is important not only from the safety point of view of human health, but also from the health quality for shrimp, crabs and squids and water quality environment.

Material and Methods

Collection and preparation of samples

Water temperature, salinity, dissolved oxygen (DO) and pH were measured in situ at each site using a pH/ISE/conductivity/RDO/DO Meter Thermo Scientific Orion Star A329 Portable. Duplicate water samples for water quality variables were collected (surface samples) 0.25-m depth (below surface water) and above sediment 0.25-m (bottom samples), using a PVC Niskin bottle. NH_3-N concentrations were determined according to [26]. NO_2-N, NO_3-N, PO_4-P Total-P and SiO_4-Si concentrations were determined at pre-filtered seawater samples, (Whatman GF=C) following the techniques described by [27, 28]. For chlorophyll-a (Chl-a) determination, additional water samples were collected and filtered on 0.45 mm filters. Chl-a was extracted by using 90% acetone and measured spectrophotometrically according to [28]. Two water samples were taken from three sites in 250 ml polyethylene container, and acidified by addition of 2 ml of HNO_3 in 1 liter of water, to prevent the absorption of heavy metals on the vessel walls. The samples were then transported to the laboratory at (4 $^{\circ C}$) and heavy metals were analyzed on the same day, using the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES**;** Varian 720-ES) and mercury was measured by the Direct Mercury Analyzer (DMA-80), Milestone Company, Model Unit DMA-80ICP [29]. Three samples of sediment were taken from the surface of sediment every month (July - December 2015) deep (0-10 cm) of each site. They dried in air and an oven at (60 °C for 72 h) grinding and sieve mesh size 120 (125µ) take part for chemical analyses and other part (0.5 g) digests with 8 ml HNO_3 (65%) in a microwave mineralization (microwave Milestone Ethos one) (ISO 16729:2013 Soil quality - Digestion of soluble elements in nitric acid). The heavy metals were determined by the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES**;** Varian 720-ES) and mercury was measured by the Direct Mercury Analyzer (DMA-80), Milestone Company, Model Unit DMA-80ICP and they were quantified in ppm. Crab (*Portunus pelagicus*) shrimp (*Penaeus semisulcatus*) and squids (*Sepia Pharaonis*) were captured from three sites in the Arabian Gulf (Tarut Bay) by using cast nets every month during the period (July - December 2015). At once, sampling crabs squids and shrimp were stored in a container, preserved in crushed ice and transferred to the laboratory. The authors used 30 same size from shrimp, 15 Crabs and 10 squids and frozen at -20 °C until analyzed. Samples from shrimp and crabs were divided into three groups whole body**,** exoskeleton and muscle**;** they were dried in an oven (60 °C for 72 h) until fixed weight and calculated the moisture %**,** grind and sieve through a 2 mm screen for subsequent. The 500-600 mg of dried samples was accurate weighed into polyethylene tube, then digest with 8 ml HNO_3 (65%) in a microwave mineralization (microwave Milestone Ethos one). The heavy metals were determined by the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES**;** Varian 720-ES) and mercury was measured by the Direct Mercury Analyzer (DMA-80), Milestone Company, Model Unit DMA-80ICP and they were quantified in ppm.

Bio concentration Factor

The bio concentration factor (BCF) was calculated for each metals, as the relation between the metal concentrations in whole fish (crabs squids and shrimp) ((FC) and its concentration in the water (WC) according to the equation: BCF = FC/WC
Statistical analysis was run using the Statistical Package for the Social Science (SPSS 22). One and two way ANOVA were employed to find the significant differences of physicochemical parameters and heavy metals between sites and months**,** also, means ± standard errors and Duncan were derived from all data.

Results and Discussions

Results tabulated in Table (1) showed that no significant differences between three sites for water physicochemical parameters between surface water samples, also bottom water samples, but more significant differences between bottom water and surface water; bottom water were more increased in chlorophyll, Po_4, NH_3, No_2, Co_2 and So_2 in all sites in other hand the DO, DO saturated, and pH more significant in surface water samples than bottom samples. Salinity, alkalinity, TDS and heavy metals were recorded no significant differences between sites. This increase in chlorophyll a in bottom water because water samples collected at night where the algae move to bottom and more detritus of phytoplankton precipitate; this explains the increased CO_2 NH_3 NO_2 and SO_2 where the increased the organic matter in sediment which produced from fish metabolic activity and macro and micro flora in bottom exhaust the DO and release CO_2, organic compound and decreased the pH in the bottom. There is a negative correlation between the concentration of DO and degradation of organic matter [30]. The pH values showed a little variation at sites, similar results of pH concentrations were recorded in the Arabian Gulf, Saudi Arabia Eastern Region Coast [31-33]. The highest values of phosphorus and nitrate were recorded at bottom sites, it may be attributed to the high quantity of drainage water in this area; other reported data are in agreement with this result in Eastern Region Coast, SA [33]. The high concentration of nutrients may lead to the eutrophication problem while the lack of phosphorus and nitrogen may be a limiting factor for biological growth [30, 34]. Higher values of most physical-chemical parameters during summer than winter is related to the high amount of sewage and other anthropogenic activities during summer and increase in temperature. There is a high concentration of phosphorus and nitrate at sediment sites, these may be related to different sources of pollutants (sewage discharges and other effluents) in water at the same sites, it may lead to increase of nitrate, organic carbon and phosphorus in the Arabian Gulf [33]. It is found that correlated phosphorus and nitrate with the organic detritus and with carbonate materials [35]. The chemical analyses of sediment were recorded the significant difference between sites where site2>site3>site1 for salinity, electric conductivity and sulfide while was not significant differences between three sites in pH, NH_3, NO_2 and NO_3 parameters. The salinity and TDS increased when water evaporation increased and water column decreased. The heavy metals for three sites showed no significant differences for Hg, AS, Zn, Ni, Fe and Cr. The heavy metals in shrimp, squids and crabs recorded no significant differences between elements, and higher than sediments Table (4). No significant differences between muscle, exoskeleton and whole body elements for shrimp and crabs; where the mean values ± SE in shrimp muscle, shrimp exoskeleton, shrimp whole body, Crabs whole body, crabs muscle, crabs exoskeleton recorded in Table (3). The results showed no detectable in all samples for Pb, Cu and Cd. This result showed that Zn>As>Cr>Ni>Fe>Hg the same trend in these elements for shrimp, and crabs, but squids were Zn>As>Cr>Ni>Hg>Fe tabulated in table (2). BCF for squids recorded higher values than shrimp and crabs for As, Zn and Hg elements, but the shrimp BCF recoded high values for Fe, Ni and Cr elements figures (2) and Table (4). The average concentrations metals in crustaceans (shrimp and crabs) and squids were relatively higher compared with the sediments, this can be related to several factors such as habitat, dietary uptake, pollutant bioavailability as well as, Crustaceans is mainly fed through sediments which consider the main reservoir of pollutions [36, 37]. Crustaceans and squids are being as a good indicator for the long-term monitoring of metal pollution in the marine environment.

Zn toxicity is rare, yet it can be toxic above the limit of 50 μg·g−1 wet weight in muscle. It appears to have a protective effect against the toxicities of both cadmium and lead [24, 38]. Hence, the relatively high levels of these metals compared to other metals studied can be attributed to their essentiality [6]. The levels of Zn in shrimp, crabs and squids were under the allowed limits (7.38, 7.99, 15.84) ppm, respectively as (Table 5). In the edible tissue of Crustaceans and squids investigated the mean values were remarkably higher values when compared to the sediments and water; the observed high BCF indicates that these species have a high potential to concentrate heavy metals in their organs [39]. The extent of occurrence or accumulation of trace metals by organisms in different tissues is dependent on the route of entry. The accumulation process involves the biological sequestering of metals that enter the organism through respiration and epidermal. It has been indicated that BCFs from environment to fish tissue changes according to the species, the chemicals, the metabolite properties of the tissues and the pollution degree of the environment [40]. The investigated species are commonly consumed as seafood in many countries. Therefore, the investigation of heavy metals concentration in the tissues of these species may provide useful information on the transfer of potentially toxic elements from abiotic compartments (water, sediment) to higher consumers, including man [41]. So, from the results authors can conclude that, Zn, Cr, Ni, Hg and Fe concentrations of all edible tissues of the species were considerably relatively high but lower than the permissible levels set by FAO and WHO. The accumulation of Zn, Hg, As in squids recorded more values than crabs, shrimp, sediment and water, but sediment recorded more values for Fe, Ni, and Cr than others figures (1). The heavy metal levels in water sample were as follows Zn > Cu > Pb > Cd > Hg (Figure 1). The results of metal concentrations in water were used to calculate BCFs. The bioaccumulation factors (BCFs) of the tissues of the investigated fish species were presented in (Table 3). BCF is a number that describes the bioaccumulation as the ratio between the accumulated concentrations of a given pollutant in any species to the concentration in the surrounding environment according to [41].

The heavy metals concentration in water and sediment recorded during present result may be related to the nutrients, sewage and agricultural discharges at this area, which might be due to anthropogenic inputs from the coastal area. It is found that there is a relationship between heavy metal concentrations in water and sediment, where the concentrations of heavy metals in sediment were higher than that recorded in water agreement with [33] in the Arabian Gulf. Some heavy metals concentrations in sediment are duplicated (10-100 times) than that recorded in water, while the bioaccumulation in shrimp, crabs and squids are duplicated hundreds folds them in water and sediment (Table 4,5); bioaccumulation factors of fish were higher than water and sediment. These values are considered a part of a risk for shrimp, crabs and squids consumers and human health, especially for Arsenic.

The concentration of heavy metals in crabs, squids and shrimp showed detectable levels in this study; the values were lower in other reported data in Qatar [42], while this result was in agreement with other studies in Bahrain [43] and in the Eastern Region coast of the Arabian Gulf, SA [33]. Therefore, monitoring programs should continue to maintain the quality of the aquatic environment in Eastern Region coast and cut the risk of pollution in living fish health and human health.

Table 1: Average means of physico-chemical parameters and heavy metals for three water sites inside the gulf upper surface (under surface 0.25 m) and bottom (upper sediment 0.25 m)

Parameters	Site 1				Site 2				Site 3				Average sites	
	Upper 1		Bottom 1		Upper 2		Bottom 2		Upper3		Bottome3		Total	
	M	±SE	M	±SE	M	±SE	M	±SE	M	±SE	M	±SE	M	±SE
Chlorophyll a (µg/l)	8.73	3.03	46.81	14.34	6.35	4.21	55.37	18.83	9.84	1.68	27.93	18.72	25.84	6.36
NH_3 mg/l	0.018	0.009	0.023	0.006	0.008	0.008	0.018	0.005	0.023	0.014	0.033	0.014	0.020	0.004
NO_2 mg/l	0.004	0.001	0.005	0.002	0.004	0.002	0.003	0.002	0.003	0.000	0.003	0.001	0.004	0.001
NO_3 mg/l	0.48	0.08	0.38	0.14	0.53	0.10	0.45	0.13	0.55	0.14	0.53	0.18	0.49	0.05
Sulfide (µg/l)	3.00	2.68	4.50	2.96	3.00	2.35	4.25	2.98	11.25	7.70	4.50	2.40	5.08	1.56
Active phosphorus mg/l	0.10	0.02	0.42	0.27	0.74	0.45	0.28	0.17	0.60	0.54	1.34	0.70	0.58	0.17
Total phosphorus l mg/l	0.89	0.75	0.63	0.48	0.11	0.03	0.24	0.09	0.55	0.42	0.11	0.03	0.42	0.16
Total chloride mg/l	0.01	0.00	0.02	0.00	0.02	0.00	0.01	0.01	0.03	0.01	0.02	0.01	0.02	0.00
Total Alkalinity mg/l	152.25	23.20	153.75	9.44	156.50	14.80	156.25	12.48	144.75	8.52	172.50	13.92	156.00	5.53
CO_2 mg/l	1.33	0.24	2.43	0.35	1.73	0.27	2.50	0.50	1.50	0.00	2.17	0.88	1.94	0.19
DO. mg/l	7.59	0.28	5.98	0.59	6.98	0.33	5.20	1.38	7.22	0.41	6.21	0.86	6.53	0.32
DO %	90.65	7.65	73.75	10.75	82.55	4.15	71.10	24.30	85.45	11.45	74.70	16.70	79.70	4.75
PH	8.16	0.02	8.03	0.02	8.14	0.03	8.04	0.07	8.16	0.04	8.08	0.07	8.10	0.02
Electric conductivity mg/l	64.66	1.08	64.85	1.10	65.28	1.34	65.30	1.38	64.01	0.76	64.07	0.76	64.69	0.41
Salinity ‰	43.60	0.78	43.92	0.67	44.02	0.97	44.08	0.97	43.13	0.49	43.12	0.53	43.64	0.29
Total dissolves salts	31.73	0.51	31.90	0.47	31.92	0.57	32.01	0.67	31.38	0.37	31.38	0.38	31.72	0.19
Hg (ppm)	0.02346	0.02239	0.00066	0.00026	0.02363	0.02309	0.00050	0.00003	0.00090	0.00017	0.00054	0.00022	0.00828	0.00527
As (ppm)	0.00315	0.00212	0.00336	0.00210	0.00329	0.00201	0.00337	0.00201	0.00208	0.00128	0.00136	0.00133	0.00277	0.00070
Zn(ppm)	0.01440	0.00896	0.01380	0.01059	0.03506	0.03203	0.03320	0.02854	0.01004	0.00632	0.00990	0.00529	0.01940	0.00722
Ni (ppm)	0.00092	0.00078	0.00082	0.00068	0.00068	0.00068	0.00094	0.00064	0.00092	0.00062	0.00080	0.00060	0.00085	0.00025
Fe (ppm)	0.00008	0.00008	0.00006	0.00006	0.00006	0.00006	0.00008	0.00008	0.00024	0.00022	0.00006	0.00006	0.00010	0.00004
Cr(ppm)	0.00600	0.00600	0.00538	0.00492	0.00480	0.00480	0.00460	0.00460	0.00588	0.00446	0.00500	0.00475	0.00528	0.00184

Table 2: Chemical and heavy metals parameters for sediments in three sites in Arabian Gulf

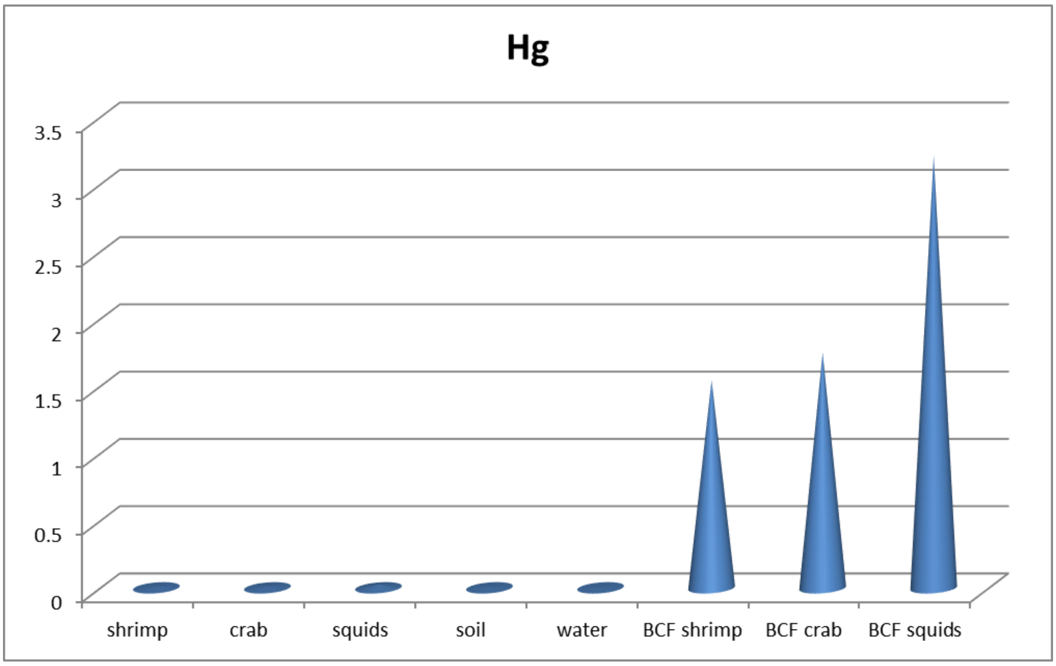
parameters	Site 1		Site 2		Site 3	
	M	±SE	M	±SE	M	±SE
PH	8.13	0.06	8.23	0.06	8.18	0.07
NH_3 mg/l	0.19	0.11	0.26	0.12	0.76	0.26
NO_2 mg/l	0.19	0.16	0.04	0.01	0.03	0.01
NO_3 mg/l	1.67	0.52	4.17	0.62	2.83	0.90
Sulfide (µg/l)	7.00	1.73	43.33	7.86	30.67	8.25
Active phosphorus mg/l	0.27	0.09	3.37	0.08	0.35	0.11
Organic Carbon	4.11	0.70	3.17	0.46	3.14	0.38
Hg (ppm)	0.01875	0.00482	0.01400	0.00058	0.10300	0.08361
As (ppm)	0.83898	0.57816	1.29850	0.75541	7.68968	7.24202
Zn(ppm)	2.70829	0.49314	3.72253	0.78553	3.77558	0.57917
Ni (ppm)	1.12373	0.42519	1.95596	0.59499	1.75700	0.49503
Fe (ppm)	0.93777	0.32368	1.25768	0.45715	1.14207	0.42832
Cr(ppm)	5.53658	1.72299	6.68460	0.93220	5.88421	1.28636

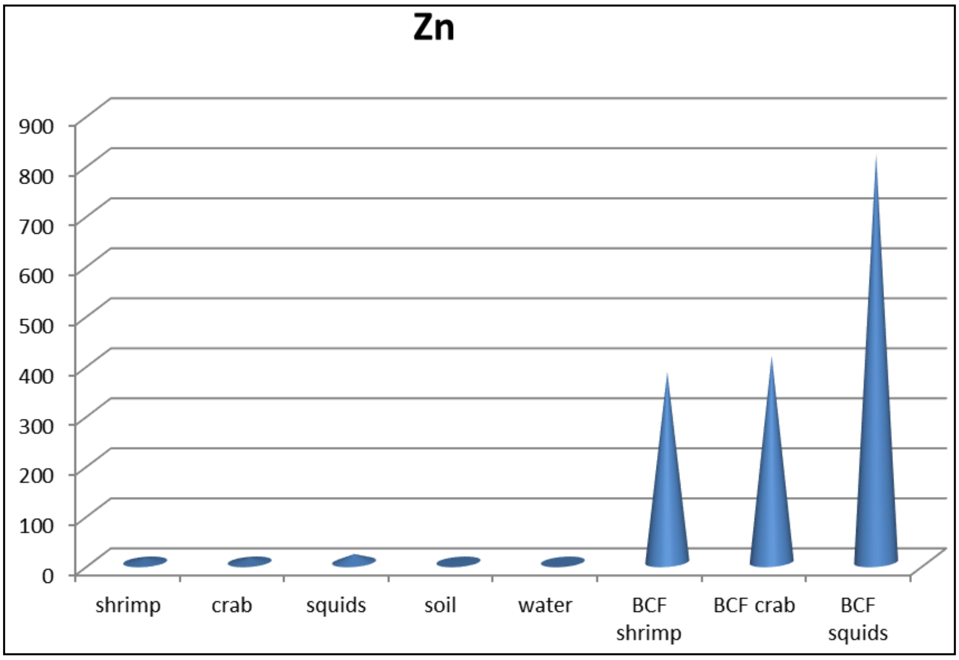
Table 3: Average means and standard error of heavy metals in Shrimp muscle, Shrimp exoskeleton, Shrimp whole body, Crabs whole body, Crabs muscle and Crabs Exoskeleton as ppm /dry weight.

	Shrimp muscle		Shrimp exoskeleton		Shrimp whole body		Crabs whole body		Crabs muscle		Crabs exoskeleton	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Hg	0.0552	0.0100	0.0472	0.0049	0.0512	0.0075	0.0580	0.0094	0.0495	0.0245	0.0650	0.0010
Zn	15.4101	1.0618	43.6318	17.8929	29.5209	9.4773	31.9835	5.5798	18.1196	1.1757	18.6079	0.6858
Pb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ni	0.0724	0.0293	0.3079	0.0840	0.1901	0.0567	0.1039	0.1039	0.1282	0.1282	0.0000	0.0000
Fe	0.0843	0.0334	0.0854	0.0222	0.0848	0.0278	0.0746	0.0118	0.1414	0.1134	0.0884	0.0070
Cu	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cr	0.9305	0.2094	0.9328	0.2578	0.9316	0.2336	0.5789	0.0844	0.9293	0.5782	0.3329	0.0071
Cd	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
As	30.7132	3.2228	20.3411	3.2835	25.5272	3.2532	20.6664	5.2864	23.4014	1.5043	26.5782	3.5370

Table 4: The average means of heavy metals in whole body for (shrimp, crabs, squids, ppm /dry weight and sediment and water) ppm and BCF for shrimp, crabs and squids

	Hg	Zn	Ni	Fe	Cr	As
shrimp	0.051167	29.52093	0.190145	0.084839	0.931625	25.52716
crabs	0.058	31.9835	0.103939	0.074622	0.578923	20.66636
squids	0.1065	63.39577	0.10632	0.068065	0.540511	30.06987
Sediment	0.0426	3.332751	1.56338	1.095032	5.985274	3.032048
water	0.008281	0.0194	0.000847	9.67E-05	0.005277	0.002768
BCF shrimp	6.178803	1521.698	224.5808	877.642	176.5555	9221.128
BCF crabs	7.003985	1648.634	122.7621	771.9561	109.7137	7465.272
BCF squids	12.86077	3267.823	125.5743	704.1189	102.4342	10862.08

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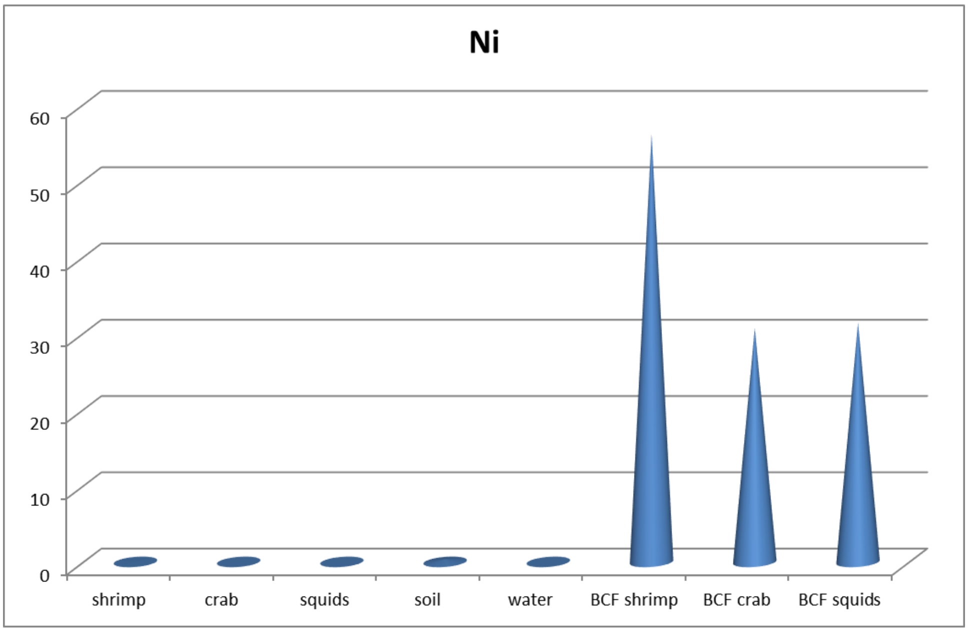
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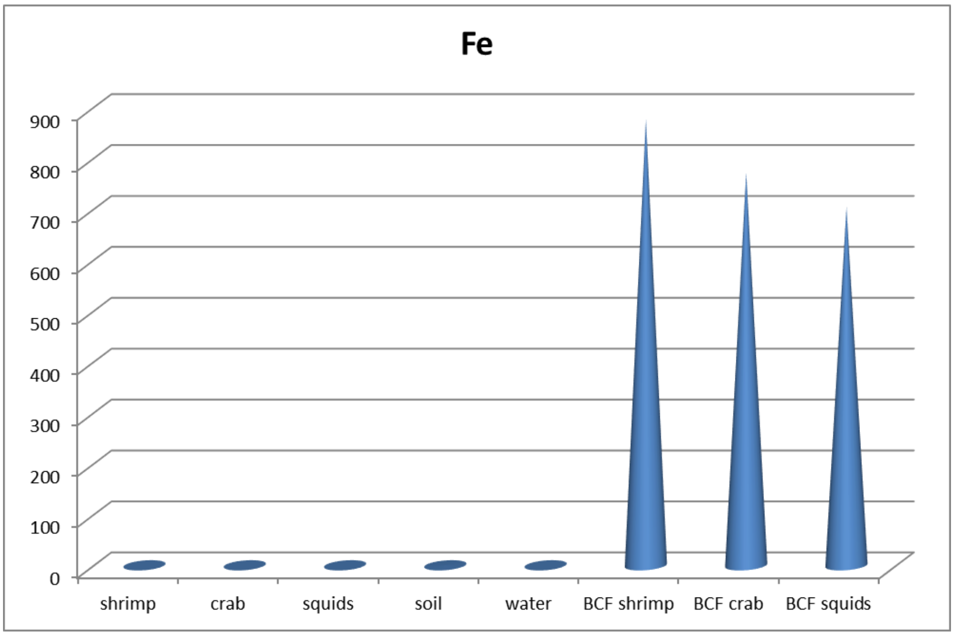
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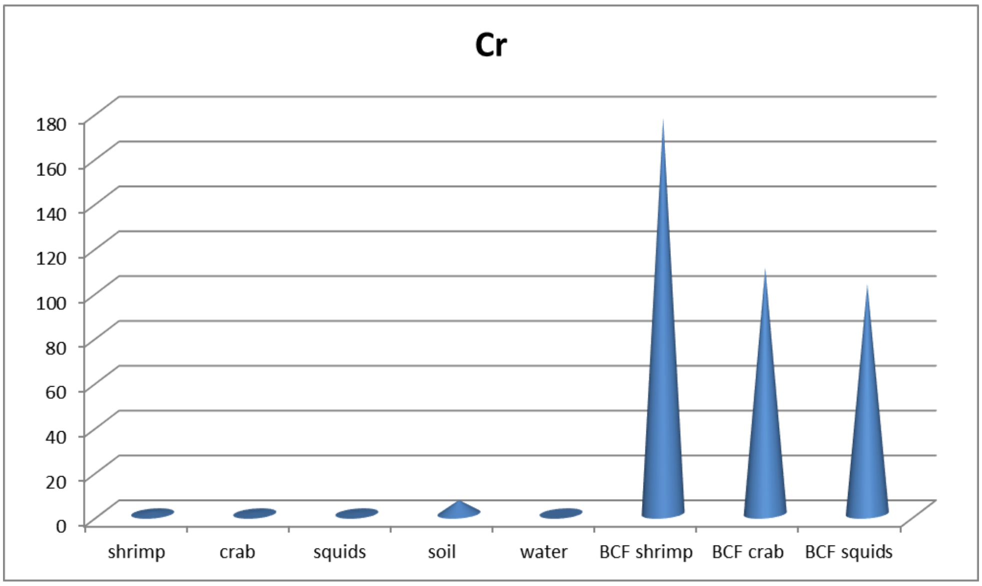


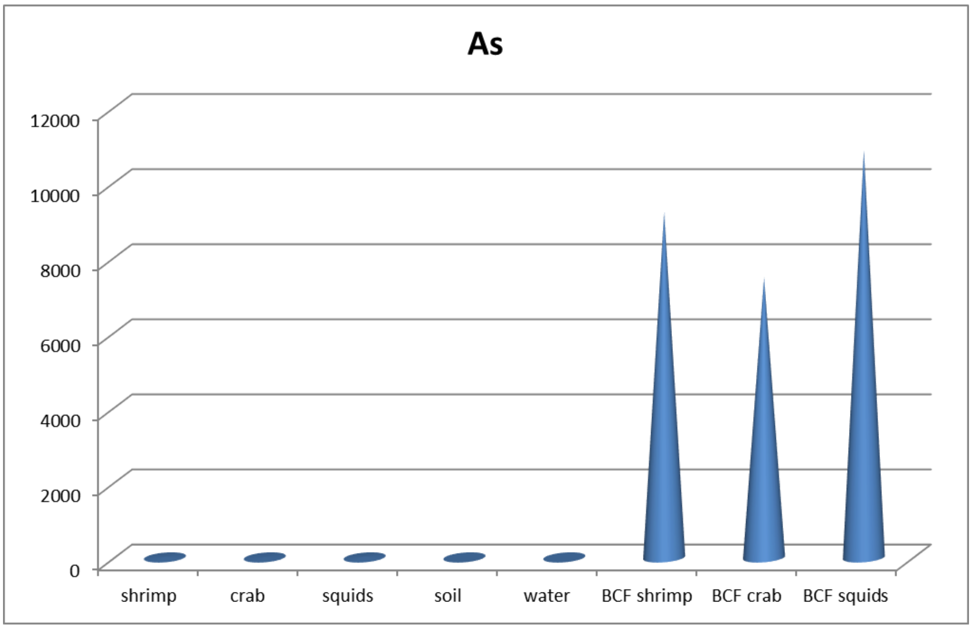
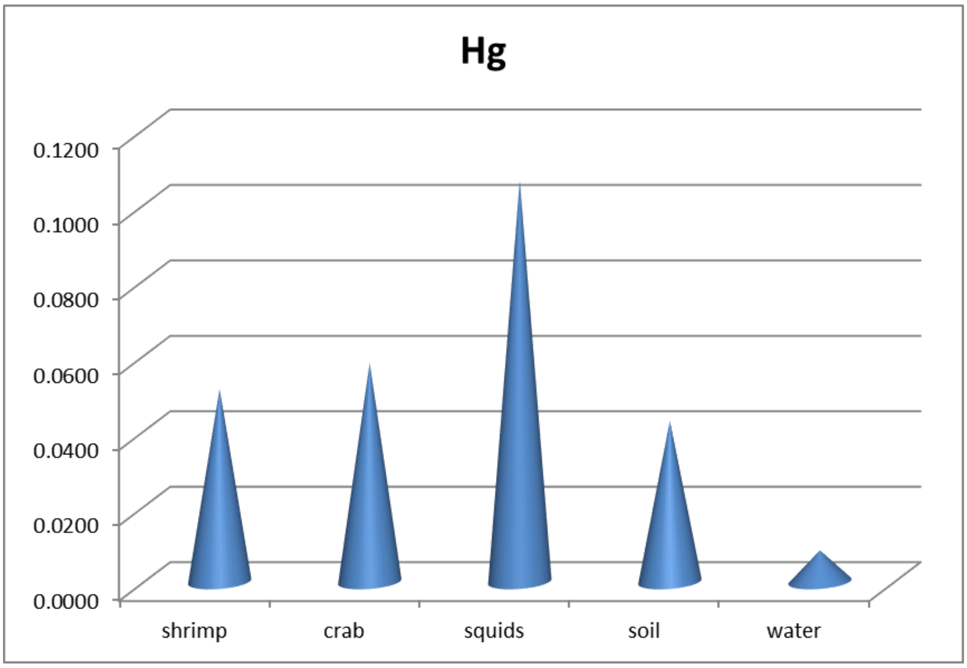
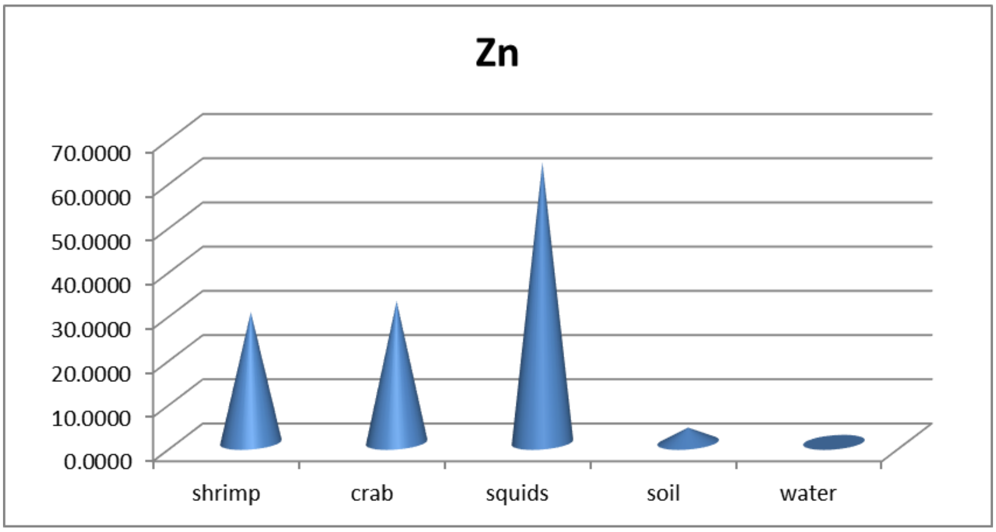


Fig 1: The heavy metals in shrimp, squids, Crabs, soil, water and BCF for shrimp, Squids and Crabs Wet wt.

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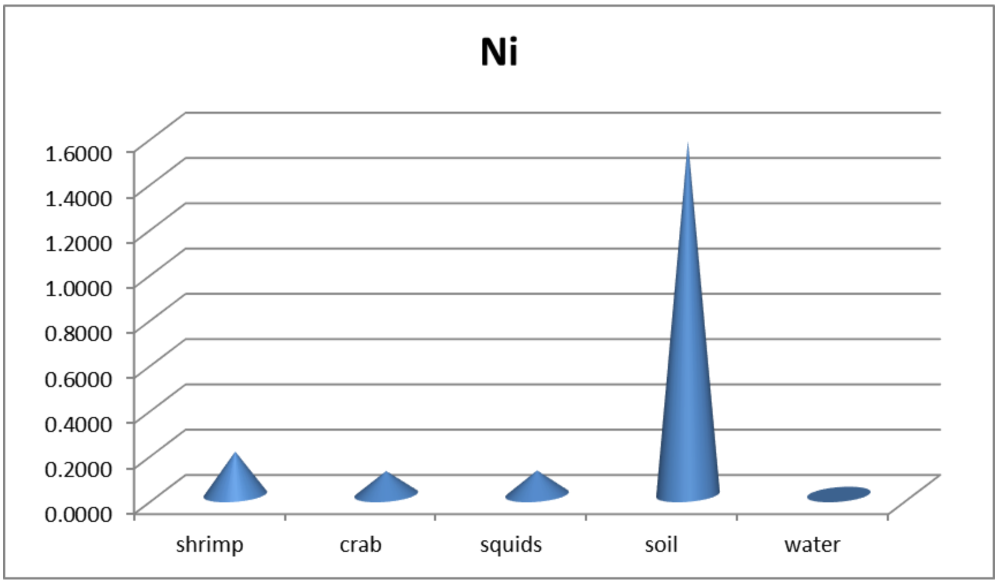
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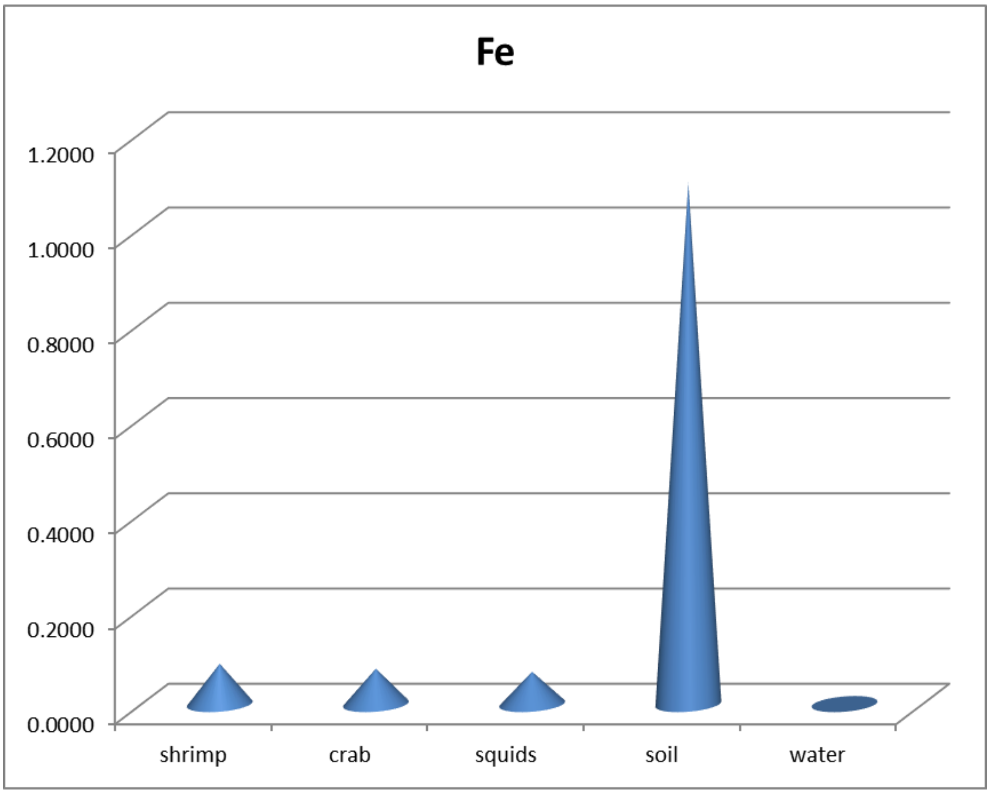
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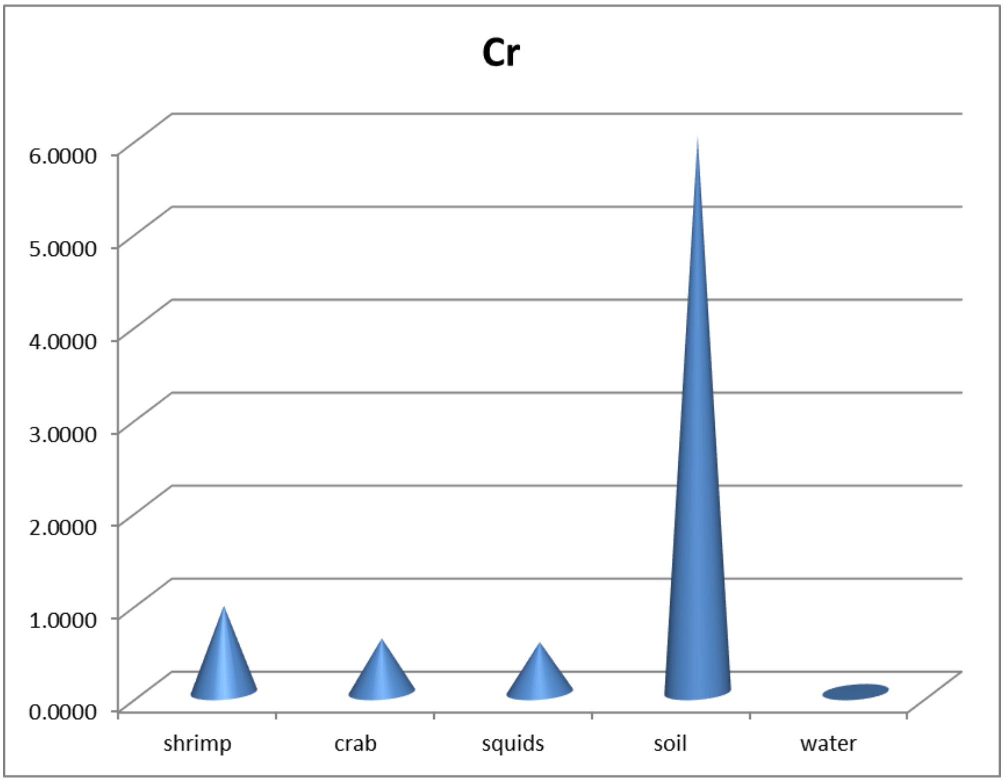

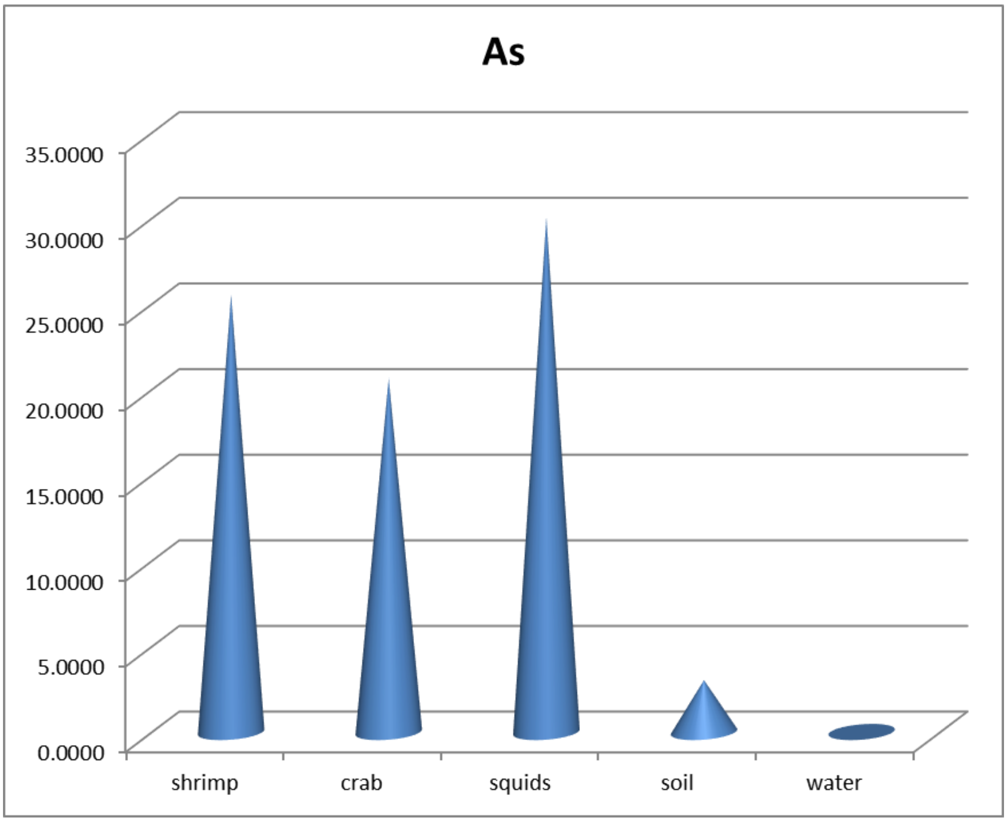

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