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Evaluation of Some Marine Animals as Bio-indicators of Metal Pollution in the Red Sea at Jeddah and Yanbu, Kingdom of Saudi Arabia

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The present study investigated the metal pollution bio-indicators in red sea marine organisms at six sites at Jeddah and Yanbu coastal area. Tow fish species (*Siganus canaliculatus* and *Epinephelis morio),* one crustacean species *(Panulirus homarus)* and one mollusca species *(Donax trunculus)* were used to monitor the bio-indicators of Zn, Cr, Cd, Pb and As pollution in the Red Sea water. The concentration of Zn, Cr, Cd, Pb and As were determined in water, sediment, fish tissues (skin, gills, liver and muscle) and muscular tissue of *Panulirus homarus* and *Donax trunculus.* The results indicated that, the concentrations of the studied metals were high in water, sediment and marine animal's tissues that were collected from Jeddah coastal areas. The highest concentration of metals was in the muscle tissue of mollusca, crustacean and finally fish species. The animal tissue metal levels were dependent on their water and sediment levels. The present study concluded that, significant spatial variations in metal contents among species and between tissues of each species were observed. These variations might be due to variation of species, location, and feeding habits. The study also shows that, molluscs are the best bio-indicators for metal pollution, then crustacean and finally fish species.

Key words: Metal pollution; Bio-indicators; Marine organisms.

Metal are the most important pollutants in aquatic ecosystems. Zn, Pb, Cu, Hg, Cd, Cr and Ni are considering the most water pollutant metals (Bhalchandra and Gajanan, 2012). Metal are accumulating in the food chain, producing disturbances in aquatic ecosystems and constitute a risk to the different flora and fauna species, including humans (Abdel- Salam and Hamdi, 2014) even at the minute level produce serious health problems, as growth and mental retardation, cancer, nervous system damage and may lead to death (Bhalchandra and Gajanan, 2012). Water and sediments metal analyses are not a representative indicator for metal pollution (Issam *et al.,* 2003). Metals are accumulated in the aquatic organisms lead to the magnitude of their concentration than that of the surrounding water (Casas *et al.*, 2008), so the investigation of metals in aquatic organisms is a reliable indicator for metal pollution than chemical analysis of sediment and water (Teodorovic *et al.,* 2000 ; Abdullah 2008). Many

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aquatic organisms were used as bio-indicators for metal pollution (Hung *et al.*, 2001). Fish, Molluscs and Crustacean are of the most important organisms that used as Biomonitoring for metal. Fish species are a good indicator for water pollution Biomonitoring. Fish have a special character as a prolonged life cycle , large body size, easy to raise. Fish species are at the top position in the aquatic food chain and directly affect the human health (Qunfang *et al.*, 2008). Fish bio-indicators such as metallothioneins (MTs), hematological, immunological, reproductive, endocrine, histology and morphology parameters were used to investigate the effects of environmental metal pollution on aquatic ecosystems (Van der Oost *et al.*, 2001). Cr, Cu, Mn, Fe, Pb and Zn were magnified in fish through the food chain (Zheng *et al.*, 2007). Molluscs have several biological characters making them the most popular aquatic organism for metal monitoring (Hung *et al.*, 2001). They are regionally abundant, long lived, filter feeder, have large tissue mass for analysis, accumulate metals from food and water, hence they possess good criteria as bio-indicators (Huang *et al.*, 2007). The enzymes that metabolizing the organic pollutants are of low activity in bivalves when compared with crustacean and fish. So contaminant levels in bivalve's tissues are an accurate bio - indicator for the aquatic ecosystem contamination (Phillips, 1990). Consequently, bivalves have been widely used in biological monitoring programs (Rutzke *et al.*, 2000). Over the last decades, the concern regarding the metal into the Red Sea environment was increased. This concern arises from the low available data about the metals concentration within the environment and marine organisms. Therefore, this study aimed to establish a monitoring network using four commercially important species of fish (*Siganus canaliculatus* and *Epinephelis morio*) crustaceans (crabs, *Panulirus homarus*) and mollusks (*Donax trunculus*) to assess trends of five metal (Zn, Cr, Cd, Pb and As) in the Read sea ecosystem at six sites at Jeddah and Yanbua.

MATERIALS AND METHODS

Ethical statement

The procedures of our experiment were approved by the Committee of the Faculty of

Table 1. Metal concentration µg L^{-1} dry weight in sediment collected from Jeddah and Yanbu

	Yanbu			Jeddah		
	I	II	III	IV	V	VI
Zn	0.12±0.003	0.26±0.02*	0.18±0.2	0.14±0.006	0.6±0.014**#	0.30±0.02*#
Cr	0.08±0.001	0.19±0.014*	0.13±0.3*	0.05±0.005	0.25±0.002**#	0.11±0.01*
Cd	0.05±0.003	0.12±0.009*	0.09±0.003	0.06±0.002	0.18±0.01**#	0.1±0.002
Pb	7.2±0.9	12.6±1.5*	9±1	9±1.3	20±1.1*#	10±1.4
As	0.06±0.004	0.13±0.04*	0.09±0.008	0.09±0.007	0.21±0.05**#	0.18±0.07*#

Within the same row means carrying star are significantly different *P < 0.05, **P < 0.01 comparing with the reference sites, # P < 0.05 comparing Jeddah sites with the corresponding Yanbu sites.

Table 2. Metal concentration µg L^{-1} dry weight in water collected from Jeddah and Yanbu

	Yanbu			Jeddah		
	I	II	III	IV	V	VI
Zn	0.10±0.003	0.13±0.02	0.11±0.2	0.10±0.006	0.136±0.02	0.12±0.02
Cr	0.03±0.001	0.12±0.012**	0.09±0.3*	0.02±0.005	0.3±0.02**#	0.15±0.001*#
Cd	0.01±0.003	0.07±0.002*	0.04±0.003	0.03±0.002	0.09±0.001**	0.07±0.002*
Pb	0.3±0.9	0.4±0.09	0.35±0.001	0.04±0.002	0.054±0.001	0.045±0.002
As	0.07±0.004	0.14±0.001*	0.09±0.008	0.08±0.007	0.6±0.08**#	0.3±0.0*#

Within the same row means carrying star are significantly different *P < 0.05, **P < 0.01 comparing with the reference sites, # P < 0.05 comparing Jeddah sites with the corresponding Yanbu sites.

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Study areas

Six sampling areas were selected along the Red Sea coast at Jeddah and Yanbu provinces. Four contaminated sites and two reference areas were investigated. At Yanbu coast area, I reference site, II collected close to Yanbu industrial harbor and III behind the oil refineries and petrochemical factories. At Jeddah coast area IV was the reference site, V north of Jeddah Islamic seaport and VI, in front of petromine refinery (Afifi *et al.*, 2014).

Sampling and analytical procedures

Sediment, water and fish samples were collected from the studded sites during mid of March 2014. Polyvinyl Chloride (PVC) tubes were used for water sample collection, at half meter depth from the surface of the water. Superficial sediment samples were collected as described by Boyd and Tucker (1992). Two kg of each of two fish species; *Siganus canaliculatus* and *Epinephelis morio*, one crustacean species*; Panulirus homarus* and one mollusca species *Donax trunculus* were sampled all animals in each species are of the same weight. Liver, gills and muscle samples from *Siganus canaliculatus and Epinephelis morio* and muscle from all species were stored at -20°C prior to analysis. Known quantities of sediment and fish

Table 3. Metal concentration µg g $^{-1}$ dry weight in liver of *S. canaliculatus* and *E. morio* collected from Jeddah and Yanbu

Fish species		Yanbu			Jeddah		
		I	II	III	IV	V	VI
S. canaliculatus	Zn	10.8±1.2	17±1.7**	14±1.5*	12±1.4	25±3**#	16±1.7*#
	Cr	0.19±0.02	0.52±0.047**	0.35±0.02*	0.24±0.02	0.67±0.05**#	0.4±0.03*
	Cd	0.17±0.02	0.38±0.02**	0.24±0.03	0.2±0.01	0.45±0.04**	0.29±0.01*
	Pb	0.116±0.08	0.16±0.003**	0.123±0.02*	0.126±0.01	0.2±0.02**#	0.14±0.01*#
	As	0.16±0.01	0.5±0.1*	0.35±0.01	0.3±0.01	0.6±0.1*	0.45±0.04
E. morio	Zn	3±0.4	8.8±1.2**	7.3±1.4**	3.7±0.2	11±1**#	8.4±1.1*
	Cr	nd	nd	nd	0.17±0.03	0.47±0.04**	0.3±0.03*
	Cd	0.12±0.01	0.29±0.02**	0.22±0.01*	0.14±0.009	0.4±0.02**#	0.25±0.01*
	Pb	0.08±0.002	0.12±0.002**	0.08±0.001	0.09±0.003	0.19±0.002**#	0.11±0.001**#
	As	0.11±0.01	0.35±0.02**	0.23±0.01*	0.2±0.02	0.42±0.03**	0.31±0.02*

Within the same row means carrying star are significantly different *P < 0.05, **P < 0.01 comparing with the reference sits, #P < 0.05 comparing Jeddah sites with the corresponding Yanbu sites.

Table 4. Metal concentration µg g $^{-1}$ dry weight in gills of *S. canaliculatus* and *E. morio* collected from Jeddah and Yanbu

Fish species		Yanbu			Jeddah		
		I	II	III	IV	V	VI
S. canaliculatus	Zn	21±3	33±7**	27±5*	26±02	39±6**#	31±4*
	Cr	0.8±0.1	2.2±0.47**	1.68±0.03*	1±0.2	2.8±0.5**#	1.8±0.2*
	Cd	0.40±0.02	0.53±0.02*	0.50±0.03*	0.46±0.04	0.60±0.03*	0.50±0.08
	Pb	0.23±0.02	0.44±0.02**	0.33±0.02*	0.24±0.02	0.66±0.02**#	0.50±0.01*#
	As	0.34±0.03	0.67±0.02**	0.35±0.01	0.38±0.03	0.7±0.02**	0.50±0.04*#
E. morio	Zn	12±2	24±3**	18±3*	14±2	28±3**	20±2*
	Cr	0.36±0.02	1.2±0.2**	0.72±0.1*	0.42±0.03	1.3±0.4**	0.9±0.03*
	Cd	0.14±0.02	0.32±0.2**	0.23±0.1*	0.17±0.009	0.45±0.02**#	0.29±0.01*
	Pb	0.13±0.02	0.34±0.02**	0.23±0.06*	0.14±0.03	0.45±0.08**#	0.34±0.001**#
	As	0.2±0.01	0.45±0.02**	0.30±0.01*	0.23±0.02	0.55±0.03**#	0.40±0.02*#

Within the same row means carrying star are significantly different *P < 0.05, **P < 0.01 comparing with the reference sits, #P < 0.05 comparing Jeddah sites with the corresponding Yanbu sites.

samples were dried in the oven at 105 °C for 24 h. The dried samples were ûnely ground and homogenized. One gram of the dried powdered samples was digested with 10 ml of 4:1 (v/v) Nitric and Perchloric acid mixture. The digested samples were used for analyzing of metals by the atomic absorption spectrometer (Perkin-Elmer, AA 700) . The metal concentrations were expressed as µg g^{-1} dry weight (Kingston and Jassie, 1988). Metal in water samples were analyzed according to the Standard Method 3110 (APHA 1992).

Statistical analysis

SPSS version 20 statistical packages (IBM, New York, NY, USA) were used in the statistical analysis of the obtained data, that were presented as a mean ± SD, n=10. One-way analysis of variance (ANOVA) was used for determination of statistical differences between the groups. Duncan's test was used for testing the inter-grouping homogeneity. Statistical significance was set at p<0.05.

Table 5. Metal concentration µg g^{-1} dry weight in muscle of *S. canaliculatus*, *E. morio*, *P. homarus* and *D. trunculus* collected from Jeddah and Yanbu

Fish species		Yanbu			Jeddah		
		I	II	III	IV	V	VI
S. canaliculatus	Zn	0.4±0.05	1±0.07*	0.8±0.02	0.7±0.06	1.4±0.08*	0.9±0.06
	Cr	0.2±0.012	0.28±0.04	0.22±0.02	0.23±0.01	0.3.±0.05*	0.25±0.03
	Cd	0.06±0.004	0.092±0.002*	0.075±0.003	0.08±0.004	0.12±0.03*	0.09±0.008
	Pb	0.022±0.001	0.04±0.002*	0.031±0.001	0.023±0.002	0.043±0.002**	0.034±0.003
	As	0.013±0.001	0.028±0.002*	0.017±0.001	0.016±0.001	0.031±0.002*	0.019±0.003
E. morio	Zn	0.2±0.01	0.6±0.04*	0.3±0.01	0.3±0.002	0.8±0.04*	0.5±0.02
	Cr	nd	nd	nd	0.1±0.012	0.16±0.04	0.11±0.02
	Cd	0.04±0.004	0.061±0.002*	0.05±0.003	0.053±0.005	0.08±0.006*	0.06±0.007
	Pb	0.018±0.001	0.03±0.002*	0.026±0.002	0.025±0.001	0.036±0.003*	0.028±0.001
	As	0.009±0.001	0.02±0.0011*	0.012±0.001	0.011±0.001	0.022±0.0014*	0.014±0.001
P. homarus	Zn	14.4±3	28.8±2**	21.6±3*	16.8±3	33.6±5**	24±3.1*
	Cr	1.44±0.5	3.96±0.8**	3±0.2*	1.8±0.6	5.04±0.8**#	3.6±0.3*#
	Cd	0.64±0.1	1.056±0.2**	0.8±0.03*	0.74±0.05	1.2±0.3**#	0.96±0.07*#
	Pb	0.44±0.04	0.64±0.06*	0.5±0.04	0.46±0.041	0.66±0.05*	0.53±0.04
	As	0.44±0.02	0.67±0.07*	0.46±0.04	0.49±0.04	0.91±0.08**	0.53±0.05
D. trunculus	Zn	35.7±4.2	56.1±8.2**	45.9±5.2*	40.2±6	66.3±7**#	52.7±4*
	Cr	2.64±0.8	7.26±1**	5.5±0.9*	3.3±0.4	9.24±1.2**#	7±0.6*#
	Cd	1.2±0.2	1.98±0.26**	1.5±0.09*	1.38±0.23	2.25±0.4**#	1.8±0.3*#
	Pb	0.78±0.06	1.49±0.4**	1.1±0.3*	0.82±0.08	2.2±0.2**#	1.7±0.6*#
	As	1.1±0.1	2.1±0.2**	1.1±0.1	1.3±0.3	2.38±0.3**	1.7±0.4*#

Within the same row means carrying star are significantly different *P < 0.05, **P < 0.01 comparing with the reference sits, #P < 0.05 comparing Jeddah sites with the corresponding Yanbu sits. Within the column values with different letters are significantly different (P < 0.05).

DISCUSSION

In the present work, we tended to evaluate some fish species collected from different six areas at Yanbu and Jeddah coastal areas in KSA as bio indictors for metal pollution. It is very important to include the aquatic organisms in the assessment of aquatic environment as the traditional toxicity tests and chemical-specific sensors cannot provide comprehensive real-time information relating to toxic events in an aquatic system (van der Schalie *et al.*, 2001). Fish species are the most suitable organisms for monitoring the aquatic environment (Shedd *et al.*, 2001). Bio monitoring/bio indication often includes collecting the organisms from representative sites and referring their quality level based on proportions of crayfish species present (Reynolds and Souty-Grosset, 2012), as well as sampling the tissues from collected organisms for evaluation of respective pollutant bioaccumulation level (Alcorlo *et al.,* 2006). In the current study, we collected fishes from two references non contaminated areas and four petroleum contaminated areas at Yanbu and Jeddah; liver, gills and muscles were collected for determination of some heavy metals in these tissues. The data obtained showed that the water and sediments collected from contaminated areas showed the highest concentrations of Zn, Cr, Cd, Pb and As respectively, comparing to the reference areas at both sites (table 1 and 2). In the same line; the concentrations of these heavy metals were high in the liver, gills and muscles of fishes collected from contaminated areas. The highest concentrations were found in livers, gills and muscles of *S. canaliculatus* if compare with *E. morio* species (tables 3 and 4). *D. trunculus* showed the higher concentrations of these metals than *P. homarus* species (table 5).

There are many reports mentioned that heavy metals, are slow bioaccumulate and do not immediately appear in fishes (Styrishave and Depledge, 1996; Bamber and Depledge, 1997; Kouba *et al.,* 2010). While the obtained results of our study showed a great correlation between the concentrations of heavy metals in fish species, and their concretions in water and sediments collected from the contaminated areas. The bioaccumulation of the metals was clearer in *P. homarus* and *D. trunculus* than fish species (table 5). This indicates their availability to be used a bio indictors for heavy metal pollutions more than fish species. George *et al.* (2013) showed the correlation of heavy metal accumulations in aquatic creatures and their use as a bio-indicator of the aquatic environmental pollution, the obtained data agreed with our observations.

Other studies have similarly measured metal concentrations in the shell and muscle tissues of *V. cyprinoides* collected from the Cochin backwaters. Babukutty and Chacko (1992) noted that metals like Cu, Zn, and Cd were more abundant in the muscle while Pb, Mn, and Co were preferentially concentrated in the shells. Pb concentrations from the present study agree well with the Cu, Zn and Pb concentration respectively, reported by Lakshmanan and Nambisan (1983). Similarly, Cu, Zn and Pb concentrations obtained during the present study agree well with Cu, Zn and Pb concentration respectively, reported by Raveenderan and Sujatha (2011). Cd concentrations from the present study are lower than the Cd concentration reported by Lakshmanan and Nambisan (1983); Raveenderan and Sujatha (2011). A study of Schuwerack *et al.* (2001) for evaluating the potential use of *Potamonautes warreni*, as a bio-indicator for heavy-metal contamination showed an accumulation of Cd and Zn in gills with high sensitivity to Cd ; this came in agree with our results. In addition; MacFarlane *et al.* (2000) proved the positive relationships between the concentrations of Zn and Pb in sediment, water and animal tissues this came agreed with our results. Others have proven that Zn was regulated in crustacean tissues to a certain threshold level but was not accumulated (Rainbow and White, 1989). However, when Zn levels are exceeded their threshold in the environment, it may be accumulated in the animal's tissues. This was seen in the current study for Zn at sites with highest Zn sediment levels (areas II& V). On the other side; Studies point out that heavy metals usually accumulate in non-edible parts of fishes, like gills, liver, guts and kidney. These metals only exceed the safe limits for human consumption in muscles when they already did in aforementioned organs (Sunjog *et al.,* 2012; Taweel *et al.,* 2012). This is clear in our study that all studied metals were accumulated in both gills, and livers showed also high concentrations in muscles.

It is important to mention that fish viscera are usually uneaten by people. So, comparisons of metal concentration in these tissues are valuable to show that environment is already presenting high levels of them, and metals are getting into fishes bodies. Once they are present in fish viscera, it is very plausible to consider that they may accumulate in edible parts as well (de Jesus *et al.,* 2014). The highest concentrations of heavy metals in the current study were found in gills of the examined species; this may be due to the feeding habit of the fish (Fonge *et al.,* 2011). Metal concentration in the gills could also be due to accumulation of the elements with mucus remaining between the gill lamella, which is hard to remove completely from the gills before preparation of the tissues for analysis (Demirak *et al.,* 2006). From previously mentioned; there is a variation in the concentration of heavy metals between species and tissues in the same species this may be due to several factors that influence metal bioaccumulation, like the type of food hydrochemistry conditions, metal bioavailability, genetic differences, and physiological state (Wang *et al.*, 1999). These factors determine variations of metal concentration that can sometimes mask the organism responses to the temporal or spatial gradient of pollution. Furthermore, ecological needs, sex, size, seasonal changes and moult of marine animal's changes were also found to affect metal accumulation in their tissues, Moulting has often been considered as one of the main excretory mechanisms of crustaceans since large amounts of metals may be lost with the moulted carapace (Yilmaz, and Yilmaz 2007). Moreover, many laboratory and field studies reported that metal accumulation in tissues of organisms depends on metal concentration in the water and also the exposure period, Physio-chemical parameters such as the temperature, pH, salinity and hardness of the water play a crucial role in heavy metal accumulation (Y1lmaz *et al.*, 2012; Viswanathan *et al.*, 2013). Also bioaccumulation varies among organisms based on uptake, detoxification and outside environment (Bryan 1973). With regards to the objectives of this research and the results obtained, this current study has provided useful information and a baseline for future along with continuous studies on the heavy metal's concentrations in fishes, crustaceans and mollusks. This work is not only important for human health, but it represents a comparative tool for the studded metal's concentrations in the edible aquatic organisms that collected Yanbu and Jeddah coasts. The present study has shown that significant spatial variations in heavy-metal contents among species, and between tissues of each species were observed these variations in metal bioaccumulation in studied organisms might be due to variation of species, and location. The study also proved that the studied organisms are a good bio-indictors for heavy metal pollution.

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