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RESEARCHARTICLE

MEASUREMENT OF HEAVY METALS CONTAMINATION AND THE SUBSEQUENT DYSFUNCTION OF HEPATOPANCREAS IN THE BRACHYURAN CRAB *PORTUNUS PELAGICUS* (LINNAEUS, 1758)

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ABSTRACT

Females of *Portunus pelagicus*were collected from shallow water of three localities in Arabian Gulf, Saudi Arabia namely Ad-Dammam, northern Khobar and southern estuarine beaches during October and November 2012 and June 2013. Three collections in the form of twenty females were chosen in each collection from each locality. Gill tissues and hepatopancreas were isolated and analysed for heavy metal accumulations. Each trail was made three times. The heavy metalsMn, Cd, Zn, Pb, Al, As, Ni, Cu, Fe, TBTwere determined using Graphite Furnace Atomic Absorption Spectroscopy (Perkin-Elmer model 2300) under the recommended condition limits (DL) in the manual for each metal. At each location water samples were collected using polyethylene bottles (2-litres capacity). The polyethylene bottles were previously cleaned with detergent rinsed several times with distilled water. Soaked in in HCL for several days and finally rinsed with redistilled water. Results are presented as means and standard errors. This study concluded that crabs live in southern Khobar estuarinebeach contain percentage of heavy metals ≥ Saudi Arabian Standards whereas the crabs live thenorthern Khobar and Ad-dammam easuarine beaches contain percentage of heavy metals ≈ SaudiArabian Standards.GSH,AST, SOD, ALP and LDH activities were measured in hepatopancreas and commented. Data obtained were subjected to analysis of variance (ANOVA) means signif. Different $P > 0.05$ or $P < 0.05$, column comparison test Df 2-10 and Tukey post hoc test. For all statistical tests, Bartlett's test for equal variances P value 0,5569 has been applied.

INTRODUCTION

Heavy metals have been defined as metals with a relative density to water of greater than five (**Walker *et al*., 2001**). The pollution status of the Arabian Gulf is generally attributed to the direct discharge of wastes (domestic and industrial etc.) due to the high level of urbanization and industrialization in the environs. Since the later part of the 19th century, the Arabian Gulf has served as the ultimate sink for disposal of untreated domestic sewage. The Arabian Gulf was the scene of three wars during the last three decades; the Iraq–Iran War in 1980–1988, the first and second Gulf War in 1991and 2003 respectively. As a result, the Arabian Gulf was subjected to a massive oil spill in 1991 in which 6–8 million barrels of Kuwait crude oil were released in the Arabian Gulf as well as various spills from normal oil operation and tanker-related spills (**Madany *et al*., 1987; Kureishy, 1993; Sheppard *et al*., 2010**). The oil spill associated with the 1991 Gulf War was considered the largest oil spill in the history.

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Therefore, large number of studies focused on the fate of this spill and provided evidence that the oil spill effect was limited to 400 km from the spillage point to Saudi Arabian coastline and that the main contaminants were rapidly degraded (**De Mora *et al*., 2010**). Both urban and industrial activities on the Arabian Gulf have resulted in elevated levels of metals in filter feeding marine crustacean and bivalvia (**Thrower and Eustace, 1973a, Rainbow, 2006, Doherty *et al*., 2010**). Of particular interest are the metals zinc, cadmium and copper which accumulate in concentrations up to 10% dry weight with no apparent effect on marine filter feeders (**Thrower and Eustace, 1973b**).

Some heavy metals such as Zn, Cu, Mn and Fe are essential for growth and vitality of Jiving organisms including man. However, they are likely to show toxic effects when organisms are exposed to levels higher than the normally required. Other elements such as Pb and Cd are not essential for metabolic activities and exhibit toxic properties. Lead, cadmium and mercury have no known biological function. Other elements of concern are aluminium, chromium, selenium, silver, arsenic, and antimony, which have contributed to serious problems in freshwater, estuarine, and coastal ecosystems.

At low concentrations, many heavy metals including Hg, Cd, Pb, As and Cu, inhibit photosynthesis and phytoplankton growth. Effects at higher trophic levels include delayed embryonic development, malformation and reduced growth of adult fishes, molluscs and crustaceans (**FAO, 1992**). The metals are classified into Class A (oxygen seeking), Class B (sulphur or nitrogen seeking) or Borderline elements. Only a few metals with proven hazardous nature are to be completely excluded in food for human consumption. Thus, only three metals, lead (Pb), cadmium (Cd) and mercury (Hg) have been included in the regulations of the European Union for hazardous metals (**EC 2005**), while the USFDA has included a further two metals, chromium (Cr) and nickel (Ni) in the list (**UN, 2010 a, b**). Therefore, a determination of metal concentrations in organisms should be part of any assessment and monitoring program in the coastal zone. Utilization of marine resources for human consumption has increased rapidly worldwide.

In 2004, about 75 % (105.6 million tons) of estimated world seafood production was used for direct human consumption (**FAO 2007**). Seafood is rich in protein, contains low cholesterol and a high percentage of n-3 polyunsaturated fatty acids (PUFA), as well as vitamins and essential minerals (**Zalloua *et al*., 2007; Chen *et al*., 2008**). Metal contamination of aquatic ecosystems is a matter of concern as many metals are persistent and potentially deleterious to aquatic life. All animals can accumulate metal dissolved in the ambient sea water and/or from the food. Environmental pollution by metals has become one of the most important problems in the world (**Chandran *et al*., 2005**). Most of the studies on marine environment, which have attempted to determine environmental poisoning by metals, have boosted up in the last decades due to concern on the growing use of metals in agricultural, chemical and industrial processes posing threats to lives of organisms.

It has been shown previously that elevated concentrations of heavy metals induce marked osmoregulatory and respiratory responses in crustaceans (**Kerkut and Munday, 1962; Thurberg *et al*., 1973; Vernberg *et al*., 1974; Bjerregaard and Vislie, 1986; Hunter, 1986; Spicer and Weber, 1991; Depledge, 1995**). As crustacean gills play a prominent role in osmoregnlation, ionic regulation and respiratory gas exchange (for a review see **Taylor and Taylor (1992)**. While there are many accounts of ultrastructural damage to crustacean gills resulting from heavy metal exposure (**Bubel, 1976; Couch, 1977; Ghate and Mulherkar, 1979; Papathanassiou and King, 1983; Papathanassiou, 1985; Anderson and Baatrup, 1988; Nonnotte *et al*., 1993**), information on the interactive effects of sublethal metal concentrations and environmental variables (such as salinity) on crustacean gill ultrastructure is relatively scarce (but see **Bubel (1976)**). Most metal toxicity studies are conducted at a single salinity and, for marine species, this is usually full strength seawater (**McLusky *et al*., 1986**). In marine crustaceans, a decrease in salinity has been associated with increased metal uptake rates (**O'Hara, 1973a,b; Hutcheson, 1974; Nugegoda and Rainbow, 1989a, b; Rainbow *et al*., 1993;Wright, 1995; Mohammed and Yassien, 2003, Jones, 2011**) and enhanced toxicity (**Bryan, 1976; McLusky *et al*., 1986; Chen, *et al*. 2005 and 2007**).

However, it has been difficult to extrapolate such findings to field situations, as many previous studies have employed heavy metals at concentrations which are not found in nature. A further problem is that many studies have employed static or recirculating seawater systems; yet it has been shown that metal uptake and physiological response differ between animals maintained in a static compared to a flow-through seawater system (**Vernberg and De Coursey 1977**). The species selected here as the bioassay organism, the crab *Portunus pelagicus* (**Linnaeus, 1758**), is found in Arabian Gulf where it is likely to be exposed to heavy metal contamination in combination with fluctuations in salinity. The present work tried to measure the concentration of Mn, Cd, Zn, Pb, Al, As, Ni, Cu, Fe, TB in the gills of the crab Portunus pelagicus in the three study localities namely Ad-dammam estuarine beach, Eastern Khobar estuarine beach and Western Khobar estuarine beach to comment on the possible dysfunction of hepatopancreas due to pollution stress.

MATERIALS AND METHODS

Animals

Specimens of *Portunus pelagicus* (**Linnaeus, 1758**) were collected from shallow water of three localities in Arabian Gulf, Saudi Arabia namely northern Khobar, southern Khobar at the vicinity of Bahrain bridge and Ad-Dammam beach during October and November 2012 and June 2013 (Fig.1). Three collections in the form of twenty female samples were chosen in each collection from each locality. Identification of the crab was carried out according to **Klinbungs *et al*. (2010)**. Immediately these samples were stored in an insulated box containing ice cubes and transferred to deep freeze (-20 °C) until the time for metal analysis. Three crabs having the same size (length and width) from the three collections were dissected. Three samples of gill tissues and other three samples of hepatopancreas were taken in each locality and analysed for heavy metal accumulations. Each trail was made three times. The trace metals Mn, Cd, Zn, Pb, Al, As, Ni, Cu, Fe, TBTwere determined using Graphite Furnace Atomic Absorption Spectroscopy (Perkin-Elmer model 2300) under the recommended condition limits (DL) in the manual for each metal.

Analysis of heavy metals in gills

The soft gill tissues from the dissected females of *Portunus pelagicus* were digested at 120 0C for 3hrs. in the nitric perchloric acids mixture (3:1). Ten metals; Mn, Cd, Zn, Pb, Al, As, Ni, Cu, Fe, TBTwere measured in the sea water and in the tissues following the method described by UNEP/FAO/IAEA/IOC (1984) and **El-Sikaily *et al*. (2004)**. To avoid contamination, all the glass wares were washed with double distilled water and soaked over- night in 20% nitric acid, analytical grade. All other chemicals were of highest purity. Sample preparation was undertaken in hoods to avoid any extraneous contamination. The water used in this study was double distilled in all glass apparatus. Briefly, the whole gill tissues of each site were homogenized in triplicate sample each 1 g and were digested using 4 ml of analar nitric acid in Teflon vessel, covered tightly and allowed predigesting at room temperature overnight.

Figure 1. Google earth map and land picture showing the three study sites. (1) Ad-dammamestuarine beach. (2) North Khobar estuarine beach. (3) South Khobar estuarine beach

The digestion block was placed on a preheated hot plat at 80 ºC for 3 h. The samples were cooled to room temperature and then were transferred to a 25 ml volumetric flask. Bi-distilled water was used for all preparations. The levels of different metals in the sea water (in the same sites) were also analyzed in triplicate. The reagents of analytical grade were utilized for the blanks and calibration curves; precision was checked against standard reference material provided by the National Research Council of Canada, and was within the range of certified values. Recovery of all metals studied was over 97%. The absorption wavelength and detection limits were as follows 228.8nm and 0.006 mg g¬-1 for Cd; 324.7 nm and 0.008 mg g-1 for Cu; 248.3 nm and 0.007 mg g-1 for Fe; 279.5 nm and 0.006 mg g-1 for Mn; 217.0 nm and 0.01 mg g-1 for Pb, respectively. All data are presented as concentrations per unit wet weight of the samples (as mg/kg).

Water Collection

Surface water samples were collected from the three locations (Ad-dammam, Eastern Khobar and Western Khobar. At each location water samples were collected using polyethylene bottles (2-litres capacity). The polyethylene bottles were previously cleaned with detergent rinsed several times with distilled water. Soaked in 1N HCL for several days and finally rinsed with redistilled water.

Reduced glutathione (GSH) assay

Levels of GSH were determined in the testeshomogenates (10%) according to the **Ellman (1959)**. One millilitreof supernatant was treated with 0.5 ml of Ellman's reagent(19.8mg of 5, 5`dithiobisnitro benzoic acid in 100 ml of 0.1%sodium citrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0).The absorbance was read at 412 nm in spectrophotometer. Toprevent the autoxidation of GSH, the samples were reduced withpotassium borohydride prior to analysis (**Kleinman and Richie, 2001**).

Asparatate aminotransferasse Assay

Dissolved enzyme at a concentration of one mg/ml in 0.1 M potassium phosphate pH 7.4. Immediately prior to use, dilute further in this buffer to a concentration of 0.05 - 0.25 u/ml.Adjust spectrophotometer to 340 nm and 25°C. Pipette 2.9 ml of the reagent mixture into cuvette and place in spectrophotometer. Incubate for 3 - 4 minutes to reach temperature equilibrium and establish blank rate, if any. At zero time add 0.1 ml of apropriately diluted enzyme and record the decrease in A340 for 4 - 5 minutes. Calculate ΔA340/minute from the initial linear portion of the curve.

Superoxide dismutase (SOD) Assay

Superoxide dismutase (SOD) activity was determined bythe method of Kakkar *et al*. (1984). Superoxide radicals react withnitroblue tetrazolium in the presence of NADH and produceformazan blue. SOD removes the superoxide radicals and inhibitsthe formation of formazan blue. The intensity of colour isinversely proportional to the activity of the enzyme.

Alkaline phosphatise Assay

Alkaline Phosphatase Assay Kit is designed to measure ALP activity directly inbiological samples without pretreatment. The improved method utilizes p-nitrophenyl phosphate that ishydrolyzed by ALP into a yellow coloured product (maximal absorbance at 405nm). The rate of the reaction isdirectly proportional to the enzyme activity.The kit is shipped at room temperature. Store pNPP Liquid at -20°C and other components at 4°C. Shelf life of12 months after receipt.ALP is stable for 48 hours at 4°C and 2 months at -20°C. EDTA, oxalate, fluoride, citrate are known inhibitorsof ALP and should be avoided in sample preparation. Serum, plasma (no EDTA/citrate, ideally unhemolyzed) and cell culture media can be assayed directly. To measure intracellular ALP, cell lysate can be prepared asfollows: 104 cells are washed with PBS and lysed in 0.5 mL 0.2% Triton X-100 in distilled water by shaking for20 min at room temperature.

Lactate dehydrogenase (LDH) Assay

Lactate dehydrogenase (LDH) activity was determinedby the method of **Wahlefeld (1983)** using sodium-lactate and NAD as the substrate. The reaction was initiated by the addition of thesubstrate and the increase in absorbance at 340 nm resulting fromthe formation of NADH was used for the calculation for LDHactivity.

Statistical analysis

Results are presented as means and standard errors. Data obtained were subjected to analysis of variance (ANOVA) means signif. Different $P > 0.05$ or $P < 0.05$, column comparisons test Df 2-10 and Tukey post hoc test. For all statistical tests, Bartlett's test for equal variances P value 0,5569.

RESULTS

Portunus pelagicus is one of the marine organisms affected by heavy metals. This specieswas used in this study as metal biological marker to measure toxicological effects inwhich it was substantiated with the highest sensitivity to toxic effect. This study tried to measure the enzymatic activity of hepatopanceas and whether this activity affected by heavy metals contamination. Samples were collected from three estuarine beaches of the Arabian Gulf, Saudi Arabia namely Ad-dammam, Eastern Khobar and Southern Khobar. Note all samples used in these measurements were females. Manganese concentration in the sea water of Ad-dammam estuarine beach ranged between 150-180 µg/g H_2O, in Eastern Khobarestuarine beach 360-390 µg/g H_2O and 440-590 µg/g H_2O in Western Khobarestuarine beach. It measured 340-390 $\mu g g^{-1}$gill in samples collected from Ad-dammam; 340-390 $\mu g g^{-1}$gillin samples collected from Eastern Khobar and 490-750 $\mu g g^{-1}$gillin samples collected from Western Khobar (see Histogram 1 and Tables 1-2). Cadmium concentration in the sea water of Ad-dammam estuarine beach ranged between 310-380 µg/g H_2O, in Eastern Khobarestuarine beach 411-480 µg/g H_2O and 630-690 µg/g H_2O in Western Khobarestuarine beach.

Table 1. Measurement of Manganese, Mn (µg/g H_2O, µgg^{-1}) in the sea water and gill of *Portunuspelagicus*

Ad-dammam water	gill tissue	Eastern Khobarwater	gill tissue	Western Khobarwater	gill tissue
180	340	390	660	440	490
150	390	340	620	470	680
179	350	360	610	590	750

Table 2. Two-way ANOVA of sea water and gills Mn contaminations

Table Analyzed	Data Table-Mn			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	90,25	P<0.0001		
Row Factor	1,75	0,3708		
Source of Variation	P value summary	Significant?		
Column Factor	***	Yes		
Row Factor	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	5	494900	98980	22,58
Row Factor	2	9619	4810	1,097
Residual	10	43830	4383	

Table 3.Measurement of Cadmium, Cd (µg/g H_2O, µgg^{-1}) in sea water and gill of *Portunus Pelagicus*

Ad-dammam water	gill tissue	Eastern hobarwater	gill tissue	Western Khobarwater	gill tissue
310	1020	440	630	660	910
360	970	480	710	690	880
380	1050	411	590	630	930

Table 4. Two-way ANOVA of sea water and gills Cd contaminations

Two-way ANOVA Cd				
Source of Variation	% of total variation	P value		
Column Factor	98,13	P<0.0001		
Row Factor	0,14	0,6852		
Source of Variation	P value summary	Significant?		
Column Factor	***	Yes		
Row Factor	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	5	985000	197000	113
Row Factor	2	1369	684,5	0,3927
Residual	10	17430	1743	

It measured 970-1020 µgg^{-1}in samples collected from Ad-dammam; 630-710 µgg^{-1}in samples collected from Eastern Khobar and 880-930 µgg^{-1}in samples collected from Western Khobar (see Histogram 2 and Tables 3-4).Zinc concentration in the sea water of Ad-dammam estuarine beach ranged between 1430-1550 µg/g H_2O, in Eastern Khobarestuarine beach 1640-1780 µg/g H_2O and 1410-1520 µg/g H_2O in Western Khobarestuarine beach. It measured 790-870 µgg^{-1}in samples collected from Ad-dammam; 1013-1220 µgg^{-1}in samples collected from Eastern Khobar and 1410-1520 µgg^{-1}in samples collected from Western Khobar (see Histogram 3 and Tables 5-6).

Lead concentration in the sea water of Ad-dammam estuarine beach ranged between 120-150 µg/g H_2O, in Eastern Khobarestuarine beach 340-390 µg/g H_2O and 650-690 µg/g H_2O in Western Khobarestuarine beach. It measured 240-290 µgg^{-1}in samples collected from Ad-dammam; 240-290 µgg^{-1}in samples collected from Eastern Khobar and 650-690 µgg^{-1}in samples collected from Western Khobar (see Histogram 4 and Tables 7-8). Aluminium concentration in the sea water of Ad-dammam estuarine beach ranged between 380-460 µg/g H_2O, in Eastern Khobarestuarine beach 430-490 µg/g H_2O and 830-880 µg/g H_2O in Western Khobarestuarine beach.

Table 5. Measurement of Zinc,Zn (µg/g H_2O , $µgg^{-1}$) in sea water and gills of *Portunus pelagicus*

Ad-dammam water	gill tissue	Eastern Khobarwater	gill tissue	Western Khobarwater	gill tissue
1430	870	1720	1013	1990	1410
1470	850	1640	1130	2040	1520
1550	790	1780	1220	2060	1470

Table 6. Two-way ANOVA of sea water and gills Zinc contaminations

Table Analyzed	Data Table-Zinc			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	98,13	P<0.0001		
Row Factor	0,58	0,1542		
Source of Variation	P value summary	Significant?		
Column Factor	***	Yes		
Row Factor	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	5	2673000	534700	152,3
Row Factor	2	15910	7957	2,267
Residual	10	35100	3510	

Table 7. Measurement of lead(µg/g H_2O , $µgg^{-1}$) in sea water and gills of *Portunus pelagicus*

Ad-dammam water	gill tissue	Eastern Khobar water	gill tissue	Western Khobarwater	gill tissue
130	290	340	240	560	690
120	240	370	290	520	680
150	260	390	250	510	650

Table 8. Two-way ANOVA of sea water and gills lead contaminations

Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	98,89	P<0.0001		
Row Factor	0,02	0,8963		
Source of Variation	P value summary	Significant?		
Column Factor	***	Yes		
Row Factor	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	5	591300	118300	181,3
Row Factor	2	144,4	72,22	0,1107
Residual	10	6522	652,2	

Table 9. Measurement of Aluminiumi(µg/g H_2O, $µgg^{-1}$)n sea water and gills of*Portunuspelagicus*

Ad-dammam water	gill tissue	Eastern Khobar water	gill tissue	Western Khobarwater	gill tissue
410	340	460	900	540	870
460	380	490	870	570	880
380	311	430	780	560	830

It measured 311-380 $µgg^{-1}$ in samples collected from Ad-dammam; 780-900 $µgg^{-1}$in samples collected fromEastern Khobar and 830-880 $µgg^{-1}$in samples collected fromWestern Khobar (see Histogram 5 and Tables 9-10). Arsenic concentration in the sea water of Ad-dammam estuarine beach ranged between 1370-1530 µg/g H_2O, in Eastern Khobarestuarine beach2000-2060 µg/g H_2O and 1010-1070 µg/g H_2O in Western Khobarestuarine beach. It measured 1740-1820 $µgg^{-1}$in samples collected from Ad-dammam; 750-790 $µgg^{-1}$in samples collected fromEastern Khobar and 1010-1070 $µgg^{-1}$ in samples collected fromWestern Khobar (see Histogram 6 and Tables 11-12).

Nickel concentration in the sea water of Ad-dammam estuarine beach ranged between 200-250 µg/g H_2O, in Eastern Khobarestuarine beach 400-450 µg/g H_2O and 510-590 µg/g H_2O in Western Khobarestuarine beach. It measured 320-395 $µgg^{-1}$ in samples collected from Ad-dammam; 370-420 $µgg^{-1}$in samples collected from Eastern Khobar and 510-590 $µgg^{-1}$in samples collected fromWestern Khobar (see Histogram 7 and Tables 13-14).Copper concentration in the sea water of Ad-dammam estuarine beach ranged between 30-50 µg/g H_2O, in Eastern Khobarestuarine beach 160-180 µg/g H_2O and 210-240 µg/g H_2O in Western Khobarestuarine beach.

Table 10. Two-way ANOVA of sea water and gills Al contaminations

Table Analyzed	Data Table-Al			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	97,76	P<0.0001		
Row Factor	1,44	0,0058		
Source of Variation	P value summary	Significant?		
Column Factor	***	Yes		
Row Factor	**	Yes		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	5	746300	149300	243,8
Row Factor	2	11010	5506	8,995
Residual	10	6122	612,2	

Table 11. Measurement of Arsenic(µg/g H_2O, µgg^{-1}) m in sea water and gills of *Portunus pelagicus*

Ad-dammam water	gill tissue	Eastern Khobar water	gill tissue	Western Khobarwater	gill tissue
1530	1820	2000	770	913	1010
1370	1740	2050	750	930	1020
1510	1790	2060	790	920	1070

Table 12. Two-way ANOVA of sea water and gills Arsenic contaminations

Table Analyzed	Data Table-Arsenic			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	99,4	P<0.0001		
Row Factor	0,17	0,1853		
Source of Variation	P value summary	Significant?		
Column Factor	***	Yes		
Row Factor	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	5	3880000	775900	461,7
Row Factor	2	6739	3369	2,005
Residual	10	16810	1681	

Table 13. Measurement of Nickel (µg/g H_2O, µgg^{-1}) in sea water and gills o f*Portunus pelagicus*

Ad-dammam water	gill tissue	Eastern Khobar water	gill tissue	Western Khobarwater	gill tissue
200	320	400	420	490	510
240	330	450	410	470	550
250	295	440	370	530	590

Table 14. Two-way ANOVA of sea water and gills Nickel contaminations

Table Analyzed	Data Table-Nickel			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	95,42	P<0.0001		
Row Factor	0,79	0,3859		
Source of Variation	P value summary	Significant?		
Column Factor	***	Yes		
Row Factor	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	5	206400	41270	50,35
Row Factor	2	1719	859,7	1,049
Residual	10	8197	819,7	

Table 15. Measurement of copper, Cu (µg/g H_2O, µgg^{-1}) in sea water and gills of *Portunus pelagicus*

Ad-dammam water	gill tissue	Eastern Khobar water	gill tissue	Western Khobarwater	gill tissue
50	320	180	410	240	680
30	270	160	480	200	595
35	290	170	390	210	570

Table 16. Column comparison of sea water and gills copper contaminations

Number of values (Cu)	6	6	6	1	6	6	6
Minimum	30	160	200	0	270	390	570
25% Percentile	32,5	164,5	205		273	395	580
Median	39	172,5	232,5	0	290	410	602,5
75% Percentile	47,5	182,5	241,5		320	450	647,5
Maximum	50	185	243	0	320	480	680
Mean	39,67	173,2	226,3	0	294,3	418,3	610
Std. Deviation	7,118	8,841	17,4	0	21,37	31,89	37,82
Std. Error	2,906	3,609	7,102	0	8,724	13,02	15,44
Lower 95% CI	32,2	163,9	208,1	0	271,9	384,9	570,3
Upper 95% CI	47,14	182,4	244,6	0	316,8	451,8	649,7

Table 17. Measurement of copper, Fe (µg/g H_2O, µgg^{-1}) in sea water and gills of *Portunus pelagicus*

Ad-dammam water	gill tissue	Eastern Khobarwater	gill tissue	Western Khobarwater	gill tissue
175	220	240	390	421	570
162	272	255	375	525	651
195	300	276	405	610	605

Table 18. Column comparison of sea water and gills iron contaminations

Number of values (Fe)	3	3	3	1	3	3	3
Minimum	162	240	421	0	220	375	570
25% Percentile							
Median	175	255	525	0	272	390	605
75% Percentile							
Maximum	195	276	610	0	300	405	651
Mean	177,3	257	518,7	0	264	390	608,7
Std. Deviation	16,62	18,08	94,66	0	40,6	15	40,62
Std. Error	9,597	10,44	54,65	0	23,44	8,66	23,45
Lower 95% CI	136	212,1	283,5	0	163,2	352,7	507,8
Upper 95% CI	218,6	301,9	753,8	0	364,8	427,3	709,6

Table 19. Measurement of Tributyl tin TBT (µgg^{-1}) in the tissues of *Portunus pelagicus*

Ad-dammam	Eastern Khobar	Western Khobar
300	351	500
250	320	450
290	290	390

It measured 270-320 µgg^{-1}in samples collected from Ad-dammam; 390-480 µgg^{-1}in samples collected fromEastern Khobar and 570-680 µgg^{-1}in samples collected fromWestern Khobar (see Histogram 8 and Tables 15-16). Iron concentration in the sea water of Ad-dammam estuarine beach ranged between 162-195µg/g H_2O, in Eastern Khobarestuarine beach 240-2760 µg/g H_2O and 570-651 µg/g H_2O in Western Khobarestuarine beach. It measured 220-300 µgg^{-1}in samples collected from Ad-dammam; 375-405 µgg^{-1}in samples collected fromEastern Khobar and 570-6510 µgg^{-1}in samples collected fromWestern Khobar (see Histogram 9 and Tables 17-18).

Tributyl tin concentration in gills of samples of Ad-dammam estuarine beach ranged between 250-300µg/g H_2O, in Eastern Khobarestuarine beach 290-351 µg/g H_2O and 390-500 µg/g H_2O in Western Khobarestuarine beach. (see Histogram 10 and Tables 19-20).GSH concentration in the sea water of Ad-dammam estuarine beach ranged between 48-51µg/g hepatopancreas , in Eastern Khobarestuarine beach 36-48 µg/g hepatopancreas and 37-42 µg/g hepatopancreas in Western Khobarestuarine beach. (see Histogram 11 and Tables 21-22).AST concentration in the sea water of Ad-dammam estuarine beach ranged between 257-310µg/g hepatopancreas, in Eastern

Table 20. Two-way ANOVA of gills TBT contaminations

Table Analyzed	Data Table-TBT			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	92,86	P<0.0001		
Row Factor	1,19	0,4028		
Source of Variation	P value summary	Significant?		
Column Factor	***	Yes		
Row Factor	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	5	267000	53400	31,2
Row Factor	2	3413	1707	0,9973
Residual	10	17110	1711	

Table 21. Measurement of GSH in hepatopancreas of *Portunus pelagicus*

Ad-dammam	Eastern Khobar	Western Khobar
48	36	37
49	44	39
51	48	42

Table 22. One-way analysis of variance and Tukey's Multiple Comparison Test of table 21

Table Analyzed (GSH)			
Data Table-12			
One-way analysis of variance			
P value	0,0007		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	3		
F	14,06		
R squared	0,7008		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	1,171		
P value	0,5569		
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	494,8	2	247,4
Residual (within columns)	211,2	12	17,6
Total	706	14	
Tukey's Multiple Comparison Test	Mean Diff.	q	P value
Ad-dammam vs Eastern Khobar	8,2	4,371	P < 0.05
Ad-dammam vs Western Khobar	14	7,462	P < 0.001
Eastern Khobar vs Western Khobar	5,8	3,091	P > 0.05

Table 23. Measurement of AST in hepatopancreas of *Portunus pelagicus*

Ad-dammam	Eastern Khobar	Western Khobar
263	330	260
257	270	269
310	274	258

Khobarestuarine beach 270-330 µg/g hepatopancreas and 258-269 µg/g hepatopancreas in Western Khobarestuarine beach. (see Histogram 12 and Tables 23-24).SOD concentration in the sea water of Ad-dammam estuarine beach ranged between 163-201µg/g hepatopancreas, in Eastern Khobarestuarine beach 139-189 µg/g hepatopancreas and 150-171 µg/g hepatopancreas in Western Khobarestuarine beach. (see Histogram 13 and Tables 25-26). ALP concentration in the sea water of Ad-dammam estuarine beach ranged between 210-260 µg/g hepatopancreas, in Eastern Khobarestuarine beach 286-300 µg/g hepatopancreas and 142-246 µg/g hepatopancreas in Western Khobarestuarine beach. (see Histogram 14 and Tables 27-28). LDH concentration in the sea water of Ad-dammam estuarine beach ranged between 63-71µg/g hepatopancreas , in Eastern Khobarestuarine beach 42-50 µg/g hepatopancreas and 29-37 µg/g hepatopancreas in Western Khobarestuarine beach. (see Histogram 15 and Tables 29-30).This study concluded that crabs live in southern Khobar estuarine beach contain percentage of heavy metals ≥ Saudi

Table 24. One-way analysis of variance and Tukey's Multiple Comparison Test of table 23

Table Analyzed (AST)			
Data Table-13			
One-way analysis of variance			
P value	0,0974		
P value summary	ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	3		
F	2,846		
R squared	0,3217		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	3,785		
P value	0,1507		
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	4042	2	2021
Residual (within columns)	8520	12	710
Total	12560	14	
Tukey's Multiple Comparison Test	Mean Diff.	q	P value
Ad-dammam vs Eastern Khobar	20,8	1,745	P > 0.05
Ad-dammam vs Western Khobar	40,2	3,374	P > 0.05
Eastern Khobar vs Western Khobar	19,4	1,628	P > 0.05

Table 25. Measurement of SODinhepatopancreas of *Portunuspelagicus*

Ad-dammam	Eastern Khobar	Western Khobar
163	151	150
170	139	168
201	189	171

Table 26. One-way analysis of variance and Tukey's Multiple Comparison Test of table 25

Table Analyzed (SOD)			
Data Table-16			
One-way analysis of variance			
P value	0,0031		
P value summary	**		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	3		
F	9,742		
R squared	0,6189		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	0,8076		
P value	0,6678		
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	96,69	2	48,34
Residual (within columns)	59,55	12	4,962
Total	156,2	14	
Tukey's Multiple Comparison Test	Mean Diff.	q	P value
Ad-dammam vs Eastern Khobar	3,82	3,834	P < 0.05
Ad-dammam vs Western Khobar	6,16	6,183	P < 0.01
Eastern Khobar vs Western Khobar	2,34	2,349	P > 0.05

Table 27. Measurement of ALPinhepatopancreas of *Portunuspelagicus*

Ad-dammam	Eastern Khobar	Western Khobar
260	300	142
250	290	243
210	286	246

Arabian Standards whereas the crabs live the northern Khobar and Ad-dammam easuarine beaches contain percentage of heavy metals ≈ Saudi Arabian Standards.

DISCUSSION

Crustaceans are frequently used as bioindicators in various aquatic systems. One reason is that they are a very successful group of animals, distributed in a number of different habitats including marine, terrestrial and freshwater environments. They are thus interesting candidates for comparative investigations. Some of the special features of crustaceans, particularly of reproduction strategies, may be highly important for the interpretation of data from bioindicator studies using these organisms, and for the development of ecotoxicological endpoints. A biomonitor refers to an animal species that can accumulate heavy metal in its tissues and may

Table 28. One-way analysis of variance and Tukey's Multiple Comparison Test of table 27

Table Analyzed (ALP)			
Data Table-14			
One-way analysis of variance			
P value	0,2146		
P value summary	ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	3		
F	1,755		
R squared	0,2263		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	0,1365		
P value	0,934		
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	15520	2	7760
Residual (within columns)	53070	12	4423
Total	68590	14	
Tukey's Multiple Comparison Test	Mean Diff.	q	P value
Ad-dammam vs Eastern Khobar	32,4	1,089	P > 0.05
Ad-dammam vs Western Khobar	78,4	2,636	P > 0.05
Eastern Khobar vs Western Khobar	46	1,547	P > 0.05

Table 29. Measurement of LDHinhepatopancreas of *Portunuspelagicus*

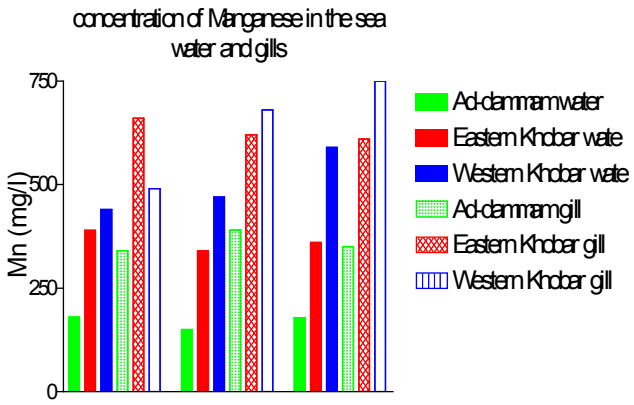
Ad-dammam	Eastern Khobar	Western Khobar
65	50	37
71	48	29
63	42	32

Table 30. One-way analysis of variance and Tukey's Multiple Comparison Test of table 29

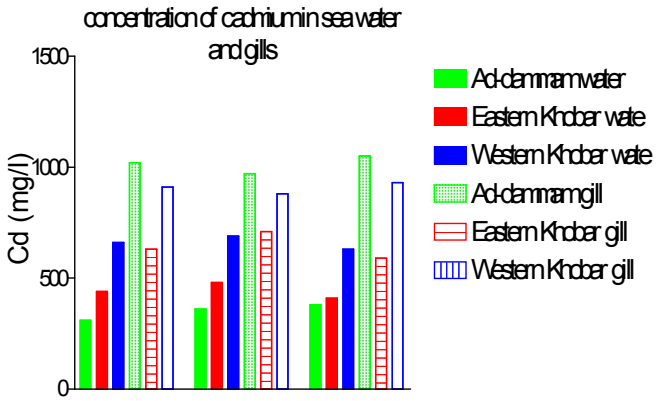
Table Analyzed (LDH)			
Data Table-15			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	3		
F	43,7		
R squared	0,8535		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	4,263		
P value	0,1186		
P value summary	ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	df	MS
Treatment (between columns)	4087	2	2044
Residual (within columns)	701,5	15	46,77
Total	4789	17	
Tukey's Multiple Comparison Test	Mean Diff.	q	P value
Ad-dammam vs Eastern Khobar	16,33	5,85	P < 0.01
Ad-dammam vs Western Khobar	36,83	13,19	P < 0.001
Eastern Khobar vs Western Khobar	20,5	7,343	P < 0.001

therefore be monitored as a measure of the bioavailability of the metals in the ambient habitat (**Rainbow, 1995**). Biomonitor species are widely used for monitoring coastal and estuarine environments for the bioavailability of metals (**Bryan and Langston, 1992; Rainbow, 1993**). An ideal biomonitor species must fulfill several criteria they must be easily identified, relatively abundant, cosmopolitan in geographical distribution, hardy enough to survive high concentrations of metals, long-lived and available for sampling throughout the year, of

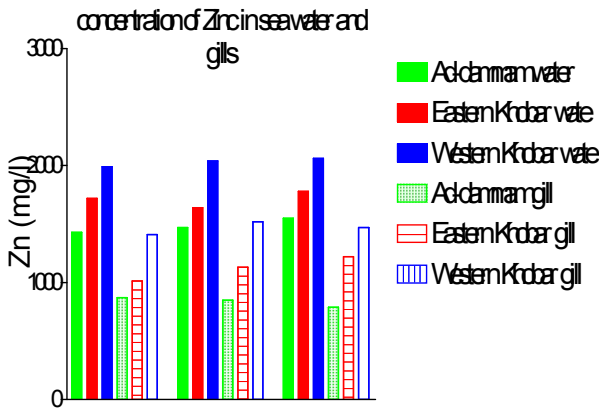
Histogram 1



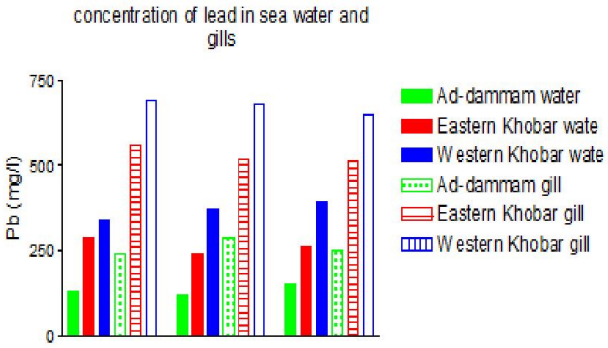
Histogram 2



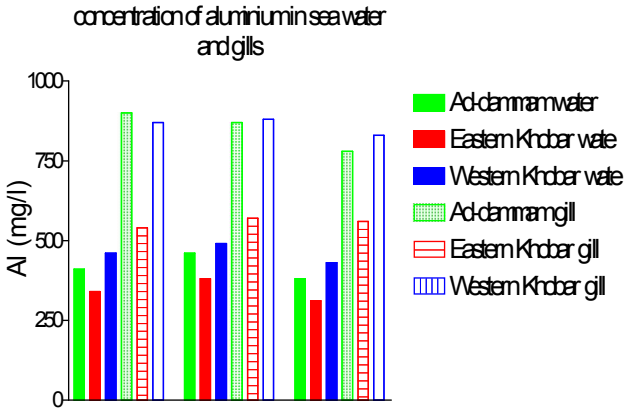
Histogram 3



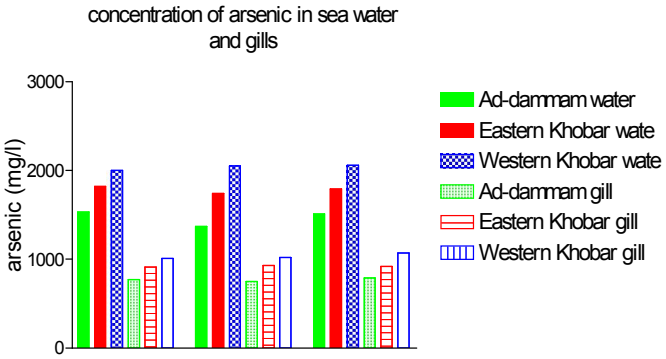
Histogram 4



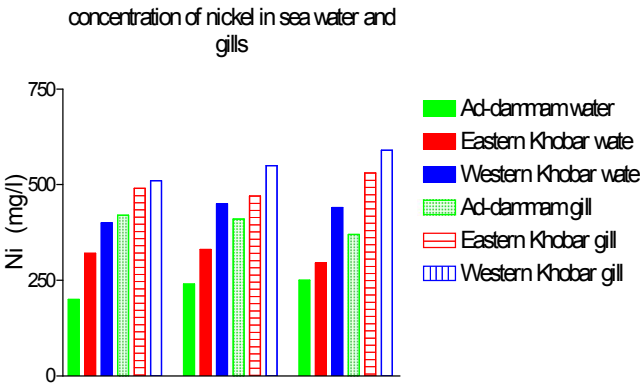
Histogram 5



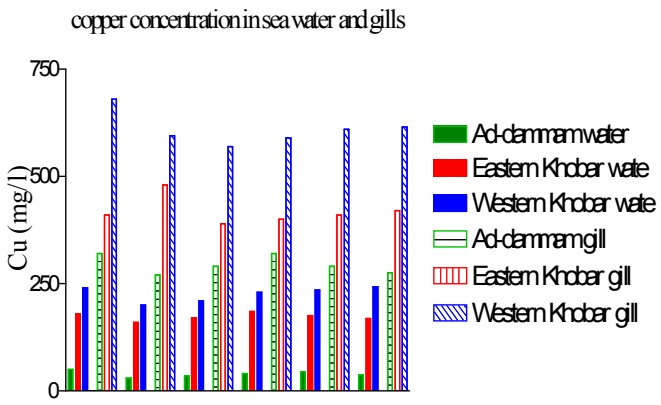
Histogram 6



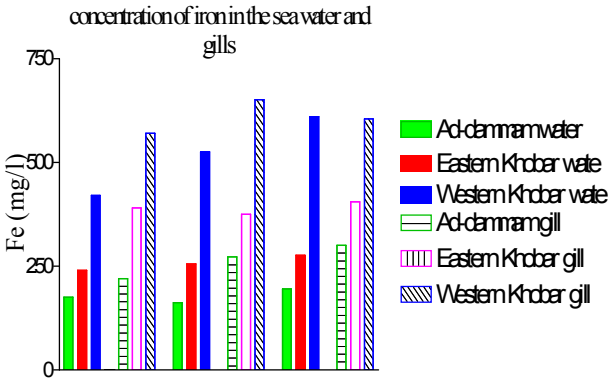
Histogram 7



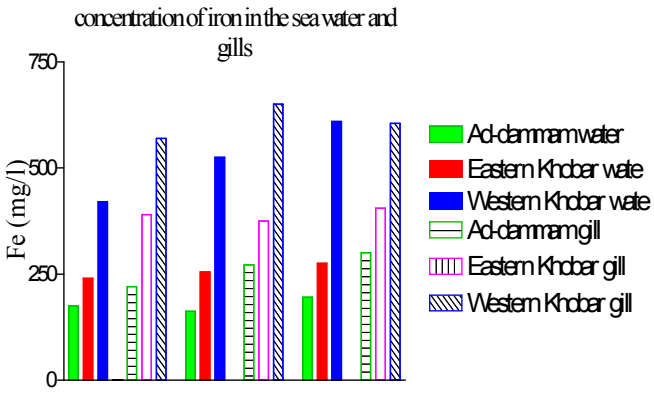
Histogram 8



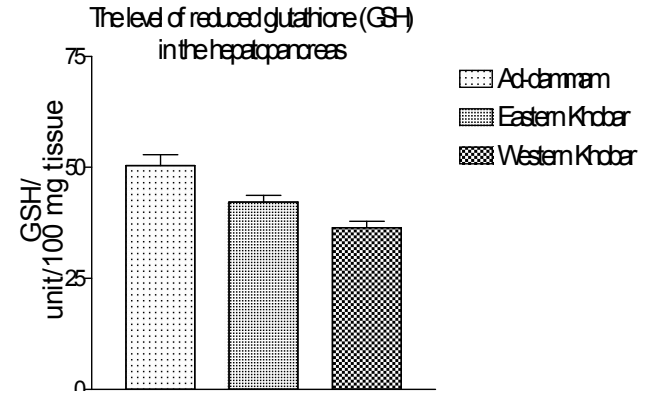
Histogram 9



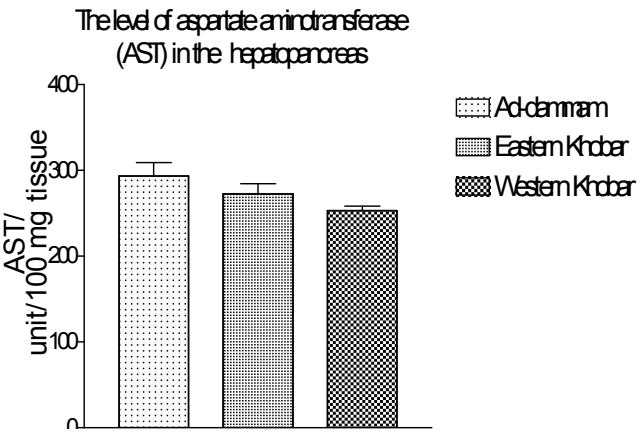
Histogram 10



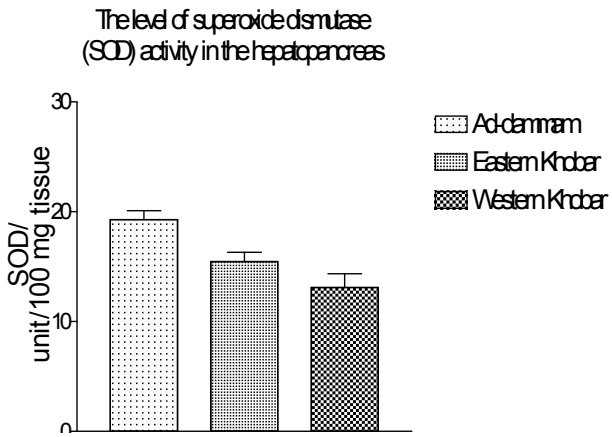
Histogram 11



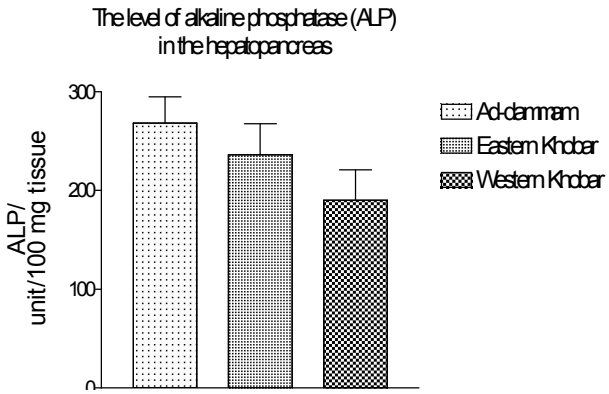
Histogram 12



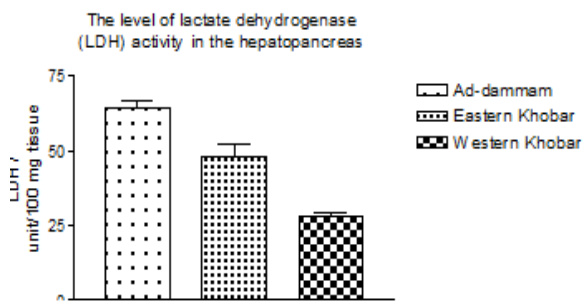
Histogram 13



Histogram 14



Histogram 15



sufficient size to allow the collection of enough samples for analysis and be net accumulators of the metal of interest (**Rainbow, 1995**). The trace metal content of a decapod crustacean can be divided into three components – metal passively adsorbed onto the cuticle, metal in the gut not assimilated into the body, and absorbed metal accessible to physiological processes. Passively adsorbed metal does not usually represent the major component, even of the cuticle component itself, since more cuticular metal is derived from internal sources and is in turn only a small fraction of the absorbed metal content incorporated via internal physiological processes (Rainbow, 1988). A vast number of invertebrates have been proposed as biomonitors of environmental pollutants. The biomonitoring concept arose from the realization that many contaminants could cause significant biological effects at low environmental concentrations. Research attention turned to effects monitoring rather than contaminant monitoring (**Lam and Gray, 2003**). Aquatic organisms may be exposed to heavy metals dissolved in the ambient water, either from natural sources or as pollutants released as a result of human activities such as mining or industrial processes. They may take up these metals, and have the potential to accumulate them to high concentrations (**Mortimer and Connell, 1993; Mortimer and Miller, 1994**). Human concern about metals has mainly focused on the highly toxic, rare and nonessential heavy metals like Pb, Hg and Cd.

In nature, Mn is one of the most abundant elements, particularly in the soft bottom sediments of oceans (**Hernroth *et al*., 2004**) and one of the important micronutrients in the aquatic environment (**Cover and Wilhm, 1982, Turoczy *et al.*, 2001**). Due to its high prevalence and possibly due to its status as an essential metal, the potential danger of Mn has been mostly neglected. It is an unforeseen toxic metal in the marine environment since it may occur in toxic concentrations in the bottom water after hypoxic conditions (**Baden and Eriksson, 2006**). At these high levels, it can pose a threat to aquatic organisms (**Vernberg, 1974 1977;Forestner and Prosi, 1979; Sanders *et al*., 1999**). In sea water, 58% of total Mn occurs as free hydrated ions (**Simkiss and Taylor, 1989**) which is believed to be readily available for uptake by exposed organisms. Environmental research efforts that commenced in the 1960s have revealed that many marine invertebrates accumulate metals in their gills from the environment (**Lam and Wu, 2003**). The metals might be taken up directly from the surrounding aquatic medium or they may be ingested via food particles or contaminated prey items. Therefore the relative proportion from each route varies with the invertebrate type and the relative availabilities of the metal in the water and diet (**Rainbow and Wang, 2001**).

Decapod crustaceans absorb trace metals from their food sources, and additionally in the case of aquatic species, via their permeable body surfaces such as the gills, without resort to active transport mechanisms (**Rainbow, 1988**).

The effects of contaminants on aquatic communities are used to indicate changes in environmental quality and conditions (**Turoczy *et al.*, 2001; Lam and Wu, 2003**). These responses might be behavioural, physiological, histopathological, biochemical or immunological (**Schuwerack *et al*., 2001**) or may affect other aspects of the biology of the organisms of choice. To date, numerous publications have reported the bioaccumulation of manganese in decapod crustaceans (the amount taken from water as well as ingestion via diet), and emphasis of these studies has been on the suitability of decapod crustaceans as bioindicator organisms for the marine ecosystem. Crabs in particular act as appropriate indicator organisms due to certain factors, including abundance in numbers and biomass, as well as relatively low mobility compared to other marine organisms such as fish (**Vernberg *et al.*, 1974, Vernberg and De Coursey, 1977; Mortimer, 2000; Monserrat *et al*., 2007**). Heavy metal content differs significantly among different organs in a freshwater crab, *Potamonautes perlatus* (**Peng *et al*., 2011**) and also in the marine Norway lobster, *Nephrops norvegicus*. Lobsters from an area with relatively high concentrations of manganese were found to accumulate a significantly higher concentration of the metal in the body, especially in the carapace, while muscle gills showed the lowest concentration (**McColl, 2004**).

The exoskeleton has been found to account for the major proportion of body burden of manganese (95%) in the shore crab, *Carcinus maenas* (**Bjerregaard and Depledge, 2002; Doherty *et al*., 2010**), which suggests that the carapace might act as a sink for deposition of the metal (**Steenkamp *et al*., 1994**). Different metals show different behaviours in bioaccumulation; for example they may be accumulated at different concentrations in different gills. In the shore crab *Carcinus maenas* exposed to metals for 32 days, cadmium bioaccumulates primarily in midgut gland and gills, copper in gill gills and zinc in muscle gills (**Martin-Diaz *et al*., 2007**). In the same experimental study, concentrations of cadmium in different gills seemed to reflect the exposure of the crabs to this metal, whereas concentrations of copper and zinc did not reflect the exposures.

When the mangrove crab *Ucides cordatus* was exposed to Mn in sea water, the metal accumulated in different gills in proportion to the exposure concentration, but to different absolute levels highest in the gills, followed by the hepatopancreas, and least in the muscle gills (**Correa Jr. *et al*., 2005**). Different species accumulate metals at different rates, depending on how they handle the metals (**Rainbow, 2006**). For marine crustaceans, metal accumulation processes vary not only between genera, but also between closely related species, some being net accumulators, while others are regulators (**Rainbow, 2002; Doherty, *et al*. 2010**). Net accumulation occurs when the rate of uptake into an organism exceeds the rate of excretion. Metal bioaccumulation can also be affected by the individual size of the organism. Smaller animals accumulate more metals in both freshwater crabs (**Steenkamp *et al*., 1994; Peng, *et al*., 2011**) and marine crabs (**Bjerregaard and Depledge, 2002**). However the statement does not apply to all taxa.

No significant relationship was found between the size of crustaceans, *Acanthephyra eximia, Aristeus antennatus* and *Polycheles typhlops* and total body manganese concentrations (**Kress *et al*., 1998**). Samples of a wide range of sizes are therefore essential to avoid over- or underestimation of the metal levels in a population under study. A sex-difference in

metal accumulation is not a general rule in decapods, but it has been reported in some species. **Mouneyrac *et al.* (2001)**, **Turoczy *et al.* (2001)** and **Al-Mohanna and Subrahmanyam (2001)** found no difference in metal accumulation (of Cd, Pb and Zn, and also Mn in the last study) between sexes in three different crab species. However, other studies reported that the accumulation does vary significantly between male and female animals (lobster *Nephrops norvegicus* – **Canli and Furness (1995), Canli *et al.* (1997)**; crab *Potamonautes warreni* – **Sanders *et al.* (1998)**; shrimp *Pleoticus muelleri* – **Jeckel *et al.* (1996)**.

The inconsistent relationship between sex and metal accumulation in biomonitor species suggests the importance of treating the data separately according to sexes for analyses in order to obtain more reliable outcomes of the impact of exposure.

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