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**ARTICLES**

**Measurement of heavy metals accumulation in ctendia of Anadara ehrenbergi (Dunker, 1868) using energy-dispersive x-ray fluorescence (EDXRF)**

**1**

Saad, G. A., Bedeer, A. H. and Radwan, E. H.

Full Length Research Paper

## Measurement of heavy metals accumulation in ctendia of *Anadara ehrenbergi* (Dunker, 1868) using energy-dispersive x-ray fluorescence (EDXRF)

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Specimens were collected from three estuarine localities of the Arabian Gulf (Saudi Arabia) namely northern Khobar, southern Khobar and Ad-Dammam estuarine beaches during January and August 2013 and June 2014. Three collections in the form of twenty specimens were samples in each month from each locality. Immediately these samples were dissected and ctendia were isolated and frozen at -20°C. Using energy dispersive X Ray fluorescence (EDXRF), accumulations of Sodium, manganese, aluminium, phosphate, sulphur, chlorine, cadmium, calcium, iron, nickel, copper, zinc and lead were measured. This trail has been investigated in triplets. Concentration of the heavy metals studied was compared with United Kingdom Quality Standards, the United States Environmental Protection Agency (USEPA), Egyptian Organization for Standardization and Saudi Arabian Standards Organization. One way analysis of variance (ANOVA) with  $P < 0.05$  and Turkey's multiple analysis test were applied to all the data obtained from the software. This study concluded that clams live in southern Khobar estuarine beach contain percentage of heavy metals  $\geq$  Saudi Arabian Standards whereas the clams live the northern Khobar and Ad-dammam easuarine beaches contain percentage of heavy metals  $\approx$  Saudi Arabian Standards.

**Key words:** Biological markers, energy dispersive X-Ray fluorescence (EDXRF), *Anadara ehrengergi*.

### INTRODUCTION

Biological markers allow the direct determination of pollutant impact in marine ecosystems. Most studies on marine pollution have focused on animals nearer to the lower end of the food web. Heavy metals such as mercury, lead, arsenic, cadmium, tin, chromium, zinc,

and copper are among the most dangerous pollutants in the marine environment (Al-Mahroos and Al-Saleh, 2000; Al-Jedah and Robinson, 2001; Readman et al., 2002; De Forest et al., 2007). Pollution with organic and inorganic chemicals plays an important role in threatening marine

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organisms (Zyadah, 2010). Arabian Gulf is set as extremely arid region of a shallow semi-closed water basin, it receives a huge amount of waste water and other pollutants from global oil transportation, drilling oil, industrial and human activities, it leads to disturbance to the coastal environment (Sadiq and Alam, 1989; Khan and Al-Homaid, 2008; Zyadah, 2011). Therefore, the heavy metal contamination in water and marine organisms were monitored to determine the rate of accumulation in tissues of marine animals. The concentration of metals in the animal organs can be reasonable guide for public health standards and for the organisms condition (Zyadah, 2011). As a result high levels of heavy metals in aquatic ecosystems can be toxic and get incorporated into the food chain. Previous studies showed that heavy metals concentrations in marine organisms may exceed the allowable limits, where it may lead to a risk to ecosystems and humans consumption (El-Gendy, 2003; Zyadah and Al-Motairy, 2012). There are few published articles on the background of heavy metals in marine organisms in Arabian Gulf, Saudi Arabia (Sadiq and Alam, 1989; Sadiq et al., 1992; Zyadah and Almotairy, 2012). However, analysis of heavy metals bioaccumulation to identify mutations in the genes and molecular characterization proteins of *Ciona intestinalis* collected from three localities from the Mediterranean Sea, Alexandria, Egypt have been investigated (Saad et al., 2008, 2011). In these studies it was concluded that heavy metal pollution affects nearly all tissues and molecular structure characterization of this fouling animal. Historically, pearl oysters represent a major marine resource in the Arabian Gulf and Mediterranean Sea countries. Several studies have been conducted to detect heavy metal contamination in the pearl oyster, *Pinctada radiata* in the Mediterranean Sea. Selected heavy metals were determined in the tissue of this pearl oyster (Buo-Olayan and Subrahmanyam, 1997; Hedge et al., 2009; Radwan et al., 2009). These studies noted a significant increase in the levels of Cu, Pb and Zn in oysters. Molluscs have long been known to accumulate essential and non-essential elements in aquatic ecosystems (Al-Saleh and Doush, 2002; Al-Darwish et al., 2005). Mussels accumulate many chemicals due to their great filtration capacity, concentrate metals in their soft tissues, and hence serve as bioindicators of metal contamination (Naser, 2010).

The key objectives addressed in this present study is to understand the accumulation pattern of sodium, manganese, aluminium, phosphate, sulphur, chlorine, cadmium, calcium, iron, nickel, copper, zinc and lead in *ctendia* in *Anadara ehrengergi* under chronic exposure of metals. This study could determine the pollution capacity of each study locality. *A. ehrengergi* has an open circulatory system, and any change in their ambient environment will be reflected in the *ctendia* as soon as water bathes the body. A life in water requires that

*ctendia* and other body surfaces be designed for the efficient exchange of oxygen and other essential molecules.

## MATERIALS AND METHODS

Specimens of *A. ehrengergi* (Dunker, 1868) were collected from shallow water of three localities in Arabian Gulf, Saudi Arabia namely Ad-Dammam beach, northern Khobar at front of Merydien hotel and southern Khobar at the vicinity of Bahrain bridge during January and August 2013 and June 2014 (Figure 1). Three collections in the form of twenty samples were chosen in each collection from each locality. Identification of the clam was carried out according to Sahin et al. (2009), De Mora et al. (2010), Khade and Mane (2012) and Vedrana et al. (2012). 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 samples of *ctendia* were taken from each seasonally collections of each group from each study site and prepared for heavy metals analysis using energy dispersive X Ray fluorescence (EDXRF) without any chemical pre-treatment. These heavy metals are sodium, manganese, aluminium, phosphate, sulphur, chlorine, cadmium, calcium, iron, nickel, copper, zinc and lead. In EDXRF spectrometers, all of the elements in the sample are excited simultaneously, and an energy dispersive detector in combination with a multi-channel analyzer is used to simultaneously collect the fluorescence radiation emitted from the sample and then separate the different energies of the characteristic radiation from each of the different sample elements. Resolution of EDXRF systems is dependent upon the detector, and typically ranges from 150 to 600 eV. The principal advantages of EDXRF systems are their simplicity, fast operation, lack of moving parts, and high source efficiency.

Thin samples of *ctendia* were polished by abrasion on diamond discs and finally mirror polished with a nonaqueous 1 µm diamond suspension (ESCIL, PS-1MIC). The polished-thin samples were surrounded with a conductive silver paint to make contact on the surface, carbon-coated in a Balzers BAF-400 rotary evaporator, and then maintained in desiccators to prevent air-contact before analysis. Elemental energy-dispersive X-ray microanalysis were rapidly performed within two days of preparation in an environmental scanning electron microscope (FEI XL30 ESEM-FEG), operating at 15 to 20 kV and a working distance of 10 mm. A total of 9 polished thin slices for each season taken from 45 samples were imaged by back-scattered electrons (BSE) and analysed for the elemental composition of the minerals present. Elemental analyses have been carried out on the surface of 2µm size mineral concretions. EDX microanalyses with an acquisition time of 60 s have been obtained for the frozen samples in order to determine whether any mineral transformation took place through chemical reactions during sample preparation. The elemental quantitative analysis used an automatic background subtraction and a ZAF correction matrix to calculate the elemental composition in weight percent and atomic percent. For quantitative analysis of the mineral concretions, the contributions of the C-coating and the embedding resin containing C, O and trace of Cl were subtracted from the quantitative data of each spectrum. The contribution of C-coating was evaluated to 25 At % of C from a pure mineral sample (hydroapatite) C-coated in the same conditions. The remaining C was attributed to the resin and an amount of O (At %) was subtracted in the same proportion, deduced from reference spectra of pure resin (C/O ratio 8:1). More details about the techniques applied is given in Injuk et al. (2006), Corbari et al. (2008), Pete (2010) and Nicholas et al. (2012).

The XRF/AES software system has been applied for all calibrations. It consists of three procedures. The evaluation of the



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

spectra, the calculation of concentrations and the post-processing unit (Rindby, 1989; Perring and Blanc, 2008).

### Statistical analysis

Analysis of variance (ANOVA) is a broad group of techniques for

identifying and measuring different sources of variation within the data set. It consists of a set of procedures by which a variance of the random variable is broken down by certain sources of variation of its value. With the components of variance, depending on the sources, one can conclude if there is a significant difference between the values of dependent variable for different levels of the observed factor variables.

**Table 1.** Measurement of heavy metal contaminations in ctendia of *Anadara ehrenbergi*.

Label	Range (keV)	Gross	Net	% Total
NaKa1	0.947 - 1.128	2049	724	3.7
MgKa1	1.168 - 1.347	1610	90	0.5
AlKa	1.388 - 1.587	2782	362	1.8
PKa	1.908 - 2.128	14086	7354	37.3
SKa	2.207 - 2.408	8394	2493	12.6
ClKa	2.507 - 2.727	11867	4463	22.6
CdLb1	3.188 - 3.428	6021	1205	6.1
CaKa	3.568 - 3.807	7554	2309	11.7
CrKa	0	0	0	0
FeKa	0	0	0	0
NiKa	0	0	0	0
CuKa	7.887 - 8.208	3955	462	2.3
ZnKa	8.467 - 8.807	3104	143	0.7
HgLa1	0	0	0	0
PbLa1	9.807 - 10.167	1601	129	0.7

Samples were collected in January 2013 from Ad-Dammam estuarine beach.

**Table 2.** Measurement of heavy metal contaminations in ctendia of *Anadara ehrenbergi*.

Label	Range (keV)	Gross	Net	% Total
NaKa1,	0.947 - 1.128	1704	604	2.2
MgKa1	1.168 - 1.347	2039	389	1.4
AlKa	1.388 - 1.587	3104	486	1.8
PKa	1.908 - 2.128	18363	10221	37.5
SKa	2.207 - 2.408	8431	1930	7.1
ClKa	2.507 - 2.727	10280	3092	11.4
CdLb1	3.188 - 3.428	7174	1207	4.4
CaKa	3.568 - 3.807	20876	8721	32.0
CrKa	0	0	0	-v
FeKa	6.247 - 6.548	3320	120	0.4
CuKa	7.887 - 8.208	3970	383	1.4
ZnKa	8.467 - 8.807	3519	27	0.1
HgLa1	9.807 - 10.167	2015	20	0.1
PbLa1	10.368 - 10.727	1866	42	0.2

Samples were collected in August 2013 from Ad-Dammam estuarine beach.

In the present study, a one-way analysis of variance is used to compare the three groups of ctendia which have different levels of one variable. If the above-mentioned assumptions for ANOVA are not met, the Turkey's Multiple Comparison Test is used for determining whether three or more independent samples originate give a clear cut differences. When this test leads to significant results, at least one sample differs from the others. A principal component analysis is a standard tool in modern data analysis. It is a simple, nonparametric method for extracting relevant information out of confusing data sets. Principal component analysis is concerned with the interpretation of the variance and covariance structure of the original set of variables through a small number of their linear combinations. The general objectives of principal component analysis are data reduction and interpretation in order to

reduce the number of variables (Dijana et al., 2012).

## RESULTS

Heavy metals concentrations in ctendia of *A. ehengergi* in the three groups of collections ranged considerably depending upon the site of sampling. Using energy dispersive X Ray fluorescence (EDXRF), bioaccumulations of heavy metal are reported in Tables 1 to 9). The mean of percentages total of all heavy metal bioaccumulations over all estuarine beaches for all



**Table 3.** Measurement of heavy metal contaminations in ctendia of *A. ehrenbergi*.

Label	Range (keV)	Gross	Net	% Total
NaKa1,	0.947 - 1.128	2065	737	3.2
MgKa1	1.168 - 1.347	1624	80	0.7
AlKa	1.388 - 1.587	2765	373	2.6
PKa	1.908 - 2.128	14097	7363	35.1
SKa	2.207 - 2.408	8354	2476	11.2
ClKa	2.507 - 2.727	11832	4487	20.3
CdLb1	3.188 - 3.428	6064	1241	7.2
CaKa	3.568 - 3.807	7532	2317	12.3
CrKa	0	0	0	0
FeKa	6.247 - 6.548	4621	56	0.1
NiKa	7.327 - 7.648	3632	103	0.3
CuKa	7.887 - 8.208	3978	475	3.7
ZnKa	8.467 - 8.807	3134	151	0.8
HgLa1	0	0	0	0
PbLa1	9.807 - 10.167	1634	132	0.9

Samples were collected in June 2014 from Ad-Dammam estuarine beach.

**Table 4.** Measurement of heavy metal contaminations in ctendia of *A. ehrenbergi*.

Label	Range (keV)	Gross	Net	% Total
NaKa1,	0.947 - 1.128	4464	1791	8.2
MgKa1	1.168 - 1.347	3476	392	2.8
AlKa	1.388 - 1.587	6373	1497	7.9
PKa	1.908 - 2.128	14374	5868	36.9
SKa	2.207 - 2.408	11376	2997	30.4
ClKa	2.507 - 2.727	16585	6196	29.7
CdLb1	3.188 - 3.428	8084	1367	8.3
CaKa	3.568 - 3.807	8079	1387	8.1
CrKa	0	0	0	0
FeKa	6.247 - 6.548	4699	93	0.8
NiKa	7.327 - 7.648	3889	168	0.9
CuKa	7.887 - 8.208	5479	597	3.7
ZnKa	8.467 - 8.807	4886	291	2.5
HgLa1	9.807 - 10.167	2086	48	2.7
PbLa1	10.368 - 10.727	3074	314	3.7

Samples were collected in January 2013 from eastern Khobar estuarine beach.

collection sites are summarized in Table 10. The authors refer that concentrations of heavy elements studied were compared with the United Kingdom Quality Standards as Cd 5 lg l<sup>-1</sup>, Cu 5 lg l<sup>-1</sup>, Ni 30lg l<sup>-1</sup>, Pb 25lg l<sup>-1</sup>, Zn 40 lg l<sup>-1</sup>, etc. United States Environmental Protection Agency (USEPA) as Cd 8.8 lg l<sup>-1</sup>, Cu 3., Mn 50 lg l<sup>-1</sup>, Ni 610 lg l<sup>-1</sup>, Pb 8.1 lg l<sup>-1</sup>, Zn 81 lg l<sup>-1</sup>, etc., Egyptian Organization for Standardization (1993) and Saudi Arabian Standards Organization (SASO) (de Mora et al., 2004 , Tolosa et al., 2005; Juma and Al-Madany, 2008; Naser, 2010). SASO standards are cadmium, Cd 0.5 mg/Kg (ppm), mercury,

Hg 1.0 ditto (ppm), lead, Pb 2.0 ditto (ppm), Arsene, As 1.0 ditto (ppm), copper, Cu 20.0 ditto (ppm), Zinc, Zn 50.0 ditto (ppm) ([http://www.iso.org/iso/about/iso\\_members/](http://www.iso.org/iso/about/iso_members/)).

However, results of the different heavy metals analyzed in this study are shown in Tables 1 to 9 in original form from the software. However, concentration of all metals in ctendia will be summarized as follows:

(1) Sodium concentrations in mixed tissues of ctendia ranged from 2.1 to 3.6 mg/Kg dry weight in samples of

**Table 5.** Measurement of heavy metal contaminations in ctendia of *A. ehrenbergi*.

Label	Range (keV)	Gross	Net	% Total
NaKa1,	0.947 - 1.128	2333	943	3.3
MgKa1	1.168 - 1.347	2000	125	0.7
AlKa	1.388 - 1.587	3516	656	2.1
PKa	1.908 - 2.128	16079	8381	31.3
SKa	2.207 - 2.408	6573	1585	5.6
ClKa	2.507 - 2.727	9101	3119	11.3
CdLb1	3.188 - 3.428	5134	552	2.6
CaKa	3.568 - 3.807	19276	8876	32.3
CrKa	5.267 - 5.548	3184	49	0.5
FeKa	6.247 - 6.548	3057	25	0.6
NiKa	7.327 - 7.648	2683	125	0.4
CuKa	7.887 - 8.208	6290	1071	4.3
ZnKa	8.467 - 8.807	4584	669	2.6
HgLa1	0	0	0	0
PbLa1	10.368 to 10.727	1896	177	0.8

Samples were collected in August 2013 from eastern Khobar estuarine beach.

**Table 6.** Measurement of heavy metal contaminations in ctendia of *A. ehrenbergi*.

Label	Range (keV)	Gross	Net	% Total
NaKa1,	0.947 - 1.128	2333	943	3.6
MgKa1	1.168 - 1.347	2000	125	0.5
AlKa	1.388 - 1.587	3516	656	2.5
PKa	1.908 - 2.128	16079	8381	31.8
SKa	2.207 - 2.408	6573	1585	6.0
ClKa	2.507 - 2.727	9101	3119	11.8
CdLb1	3.188 - 3.428	5134	552	2.1
CaKa	3.568 - 3.807	19276	8876	33.7
CrKa	5.267 - 5.548	3184	49	0.2
FeKa	6.247 - 6.548	3057	25	0.1
NiKa	7.327 - 7.648	2683	125	0.5
CuKa	7.887 - 8.208	6290	1071	4.1
ZnKa	8.467 - 8.807	4584	669	2.5
HgLa1	0	0	0	0
PbLa1	10.368 to 10.727	1896	177	0.7

Samples were collected in June 2014 from eastern Khobar estuarine beach.

Ad-Dammam, 3.2 to 3.7 mg/Kg dry weight in samples of northern Khobar whereas 7.9 to 8.2 mg/Kg dry weight in samples of southern Khobar. Histogram (1) clarifies that sodium contamination in ctendia is Ad-Dammam < northern Khobar < southern Khobar (Tables 1 to 10) (Figure 2). The means significant difference ( $P < 0.05$ ) is obvious and Tukey's multiple comparison test with either  $P < 0.05$  or  $P > 0.05$  is denoted Table 11.

(2) Manganese concentrations in mixed tissues of ctendia ranged from 0.5 to 1.5 mg/Kg dry weight in samples of Ad-Dammam, 0.7 to 1.4 mg/Kg dry weight in samples of

northern Khobar whereas 1.5 to 2.8 mg/Kg dry weight in samples of southern Khobar. Histogram 2 clarifies that Manganese contamination in ctendia is Ad-Dammam  $\approx$  northern Khobar < southern Khobar (Tables 1 to 10). The means significant difference ( $P < 0.05$ ) is obvious and Tukey's multiple comparison test with either  $P < 0.05$  or  $P > 0.05$  is denoted in Table 12.

(3) Aluminium concentrations in mixed tissues of ctendia ranged from 2.1 to 2.5 mg/Kg dry weight in samples of Ad-Dammam, 1.8 to 2.6 mg/Kg dry weight in samples of northern Khobar whereas 2.7 to 7.9 mg/Kg dry weight in

**Table 7.** Measurement of heavy metal contaminations in ctendia of *A. ehrenbergi*.

Label	Range (keV)	Gross	Net	% Total
NaKa1,	0.947 - 1.128	2327	577	2.6
MgKa1	1.168 - 1.347	2347	347	1.5
AlKa	1.388 - 1.587	3286	322	1.4
PKa	1.908 - 2.128	16837	9001	40.1
SKa	2.207 - 2.408	6434	1281	5.7
ClKa	2.507 - 2.727	7211	1793	8.0
CdLb1	3.188 - 3.428	5670	938	4.2
CaKa	3.568 - 3.807	16111	7115	31.7
CrKa	0	0	0	0
FeKa	0	0	0	0
CuKa	7.887 - 8.208	3780	618	2.8
ZnKa	8.467 - 8.807	3218	383	1.7
HgLa1	0	0	0	0
PbLa1	10.368 - 10.727	1580	89	0.4

Samples were collected in January 2013 from western Khobar estuarine beach.

**Table 8.** Measurement of heavy metal contaminations in ctendia of *A. ehrenbergi*.

Label	Range (keV)	Gross	Net	% Total
NaKa1,	0.947 - 1.128	2127	911	2.1
MgKa1	1.168 - 1.347	2100	120	0.7
AlKa	1.388 - 1.587	3427	640	2.1
PKa	1.908 - 2.128	17057	8379	36.2
SKa	2.207 - 2.408	6546	1575	5.7
ClKa	2.507 - 2.727	9164	3103	10.6
CdLb1	3.188 - 3.428	5120	543	2.6
CaKa	3.568 - 3.807	19147	8889	31.6
CrKa	5.267 - 5.548	3176	45	0.4
FeKa	6.247 - 6.548	3046	21	0.2
NiKa	7.327 - 7.648	2649	129	0.4
CuKa	7.887 - 8.208	6300	1029	3.4
ZnKa	8.467 - 8.807	4568	667	2.1
HgLa1	0	0	0	0
PbLa1	10.368 - 10.727	1899	174	0.4

Samples were collected in August 2013 from western Khobar estuarine beach.

samples of southern Khobar. Histogram 3 clarifies that Aluminium contamination in ctendia is Ad-Dammam  $\approx$  northern Khobar < southern Khobar (Tables 1 to 10). The means significant difference ( $P < 0.05$ ) is obvious and Tukey's multiple comparison test with either  $P < 0.05$  or  $P > 0.05$  is denoted in Table 13.

(4) Phosphate concentrations in mixed tissues of ctendia ranged from 31.8 to 36.2 mg/Kg dry weight in samples of Ad-Dammam, 35.1 to 37.5 mg/Kg dry weight in samples of northern Khobar whereas 25.9 to 53.1 mg/Kg dry weight in samples of southern Khobar. Histogram 4 clarifies that phosphate contamination in ctendia is Ad-

Dammam  $\approx$  northern Khobar  $\approx$  southern Khobar (Tables 1 to 10). The means significant difference ( $P < 0.05$ ) is obvious and Tukey's multiple comparison test with either  $P < 0.05$  or  $P > 0.05$  is denoted in Table 14.

(5) Sulphur concentrations in mixed tissues of ctendia ranged from 5.7 to 6.0 mg/Kg dry weight in samples of Ad-Dammam, 7.1 to 12.6 mg/Kg dry weight in samples of northern Khobar whereas 4.6 to 30.4 mg/Kg dry weight in samples of southern Khobar. Histogram 5 clarifies that sulphur contamination in ctendia is Ad-Dammam < northern Khobar < southern Khobar (Tables 1 to 10). The means significant difference ( $P < 0.05$ ) is obvious and

**Table 9.** Measurement of heavy metal contaminations in ctendia of *A. ehrenbergi*.

Label	Range (keV)	Gross	Net	% Total
NaKa1,	0.947 - 1.128	2127	911	2.4
MgKa1	1.168 - 1.347	2100	120	0.6
AlKa	1.388 - 1.587	3427	640	2.3
PKa	1.908 - 2.128	17057	8379	35.2
SKa	2.207 - 2.408	6546	1575	5.8
ClKa	2.507 - 2.727	9164	3103	10.1
CdLb1	3.188 - 3.428	5120	543	2.7
CaKa	3.568 - 3.807	19147	8889	31.2
CrKa	5.267 - 5.548	3176	45	0.5
FeKa	6.247 - 6.548	3046	21	0.6
NiKa	7.327 - 7.648	2649	129	0.3
CuKa	7.887 - 8.208	6300	1029	3.5
ZnKa	8.467 - 8.807	4568	667	2.2
HgLa1	0	0	0	0
PbLa1	10.368 - 10.727	1899	174	0.3

Samples were collected in June 2014 from western Khobar estuarine beach.

**Table 10.** Measurement of percentage of heavy metal contaminations in ctendia of *A. ehrenbergi*.

Label	Ad Dammam (1)	Ad Dammam (2)	Ad Dammam (3)	North Khobar (1)	North Khobar (2)	North Khobar (3)	South Khobar (1)	SouthKh obar (2)	SouthKh obar (3)
NaKa1	3.6	2.6	2.1	3.7	2.2	3.2	7.9	4.2	8.2
MgKa1	0.5	1.5	0.7	0.5	1.4	0.7	1.5	2.7	2.8
AlKa	2.5	1.4	2.1	1.8	1.8	2.6	6.5	2.7	7.9
PKa	31.8	40.1	36.2	37.3	37.5	35.1	25.9	53.1	36.9
SKa	6.0	5.7	5.7	12.6	7.1	11.2	27.3	4.6	30.4
ClKa	11.8	8.0	10.6	22.6	11.4	20.3	27.3	3.1	29.7
CdLb1	2.1	4.2	2.6	6.1	4.4	7.2	5.8	2.9	8.3
CaKa	33.7	31.7	31.6	11.7	32.0	12.3	5.9	23.6	8.1
CrKa	0.2	0	0.4	0	-v	0	0	0	0
FeKa	0.1	0	0.2	0	0.4	0.1	0.3	0.1	0.8
NiKa	0.5	0	0.4	0	1.4	0.3	0.6	1.1	0.9
CuKa	4.1	2.8	3.4	2.3	0.1	3.7	2.6	0.9	3.7
ZnKa	2.5	1.7	2.1	0.7	0.1	0.8	1.0	1.1	2.5
HgLa1	0	0	0	0	0.2	0	0	0	2.7
PbLa1	0.7	0.4	0.4	0.7	0.2	0.9	1.3	0	3.7

Samples were collected in January and August 2013 and June 2014 from all study estuarine beaches.

Tukey's multiple comparison test with either  $P < 0.05$  or  $P > 0.05$  is denoted in Table 15.

(6) Chlorine concentrations in mixed tissues of ctendia ranged from 8.0 to 11.8 mg/Kg dry weight in samples of Ad-Dammam, 11.4 to 22.6 mg/Kg dry weight in samples of northern Khobar whereas 3.1 to 29.7 mg/Kg dry weight in samples of southern Khobar. Histogram 6 clarifies that chlorine contamination in ctendia is Ad-Dammam < northern Khobar < southern Khobar (Tables 1 to 10). The

means significant difference with ( $P < 0.05$ ) is obvious and Tukey's multiple comparison test with either  $P < 0.05$  or  $P > 0.05$  is denoted in Table 16.

(7) Cadmeium concentrations in mixed tissues of ctendia ranged from 2.1 to 4.2 mg/Kg dry weight in samples of Ad-Dammam, 4.4 to 7.2 mg/Kg dry weight in samples of northern Khobar whereas 2.9 to 8.3 mg/Kg dry weight in samples of southern Khobar. Histogram 7 clarifies that cadmium contamination in ctendia is Ad-Dammam <

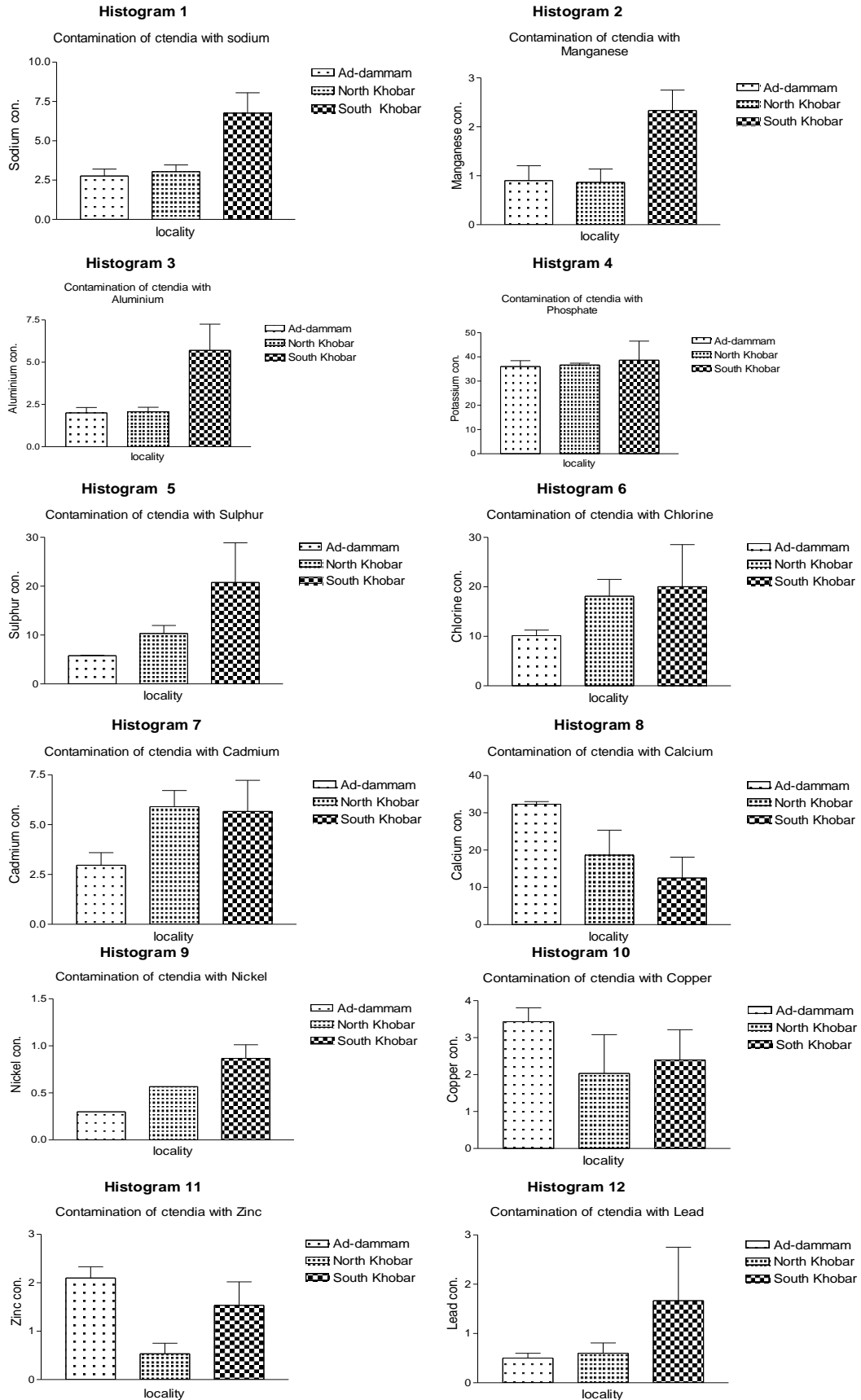


Figure 2. Histograms (1-12) showing contamination of ctendia with heavy metals.

**Table 11.** One-way analysis of variance (ANOVA) and Tukey's Multiple Comparison test analyses for sodium.

<b>Data table- analysis of sodium</b>				
<b>One-way analysis of variance</b>				
P value	0,0244			
P value summary	*			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	3			
F	7,343			
R squared	0,71			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	30,01	2	15	
Residual (within columns)	12,26	6	2,043	
Total	42,27	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	-0,2667	0,3231	P > 0.05	-3.848 to 3.314
Ad-dammam vs South Khobar	-4	4,847	P < 0.05	-7.581 to -0.4190
North Khobar vs South Khobar	-3,733	4,524	P < 0.05	-7.314 to -0.1524

**Table 12.** One-way analysis of variance (ANOVA) and Tukey's multiple comparison test analyses for manganese.

<b>Data table- analysis of manganese</b>				
<b>One-way analysis of variance</b>				
P value	0,0353			
P value summary	*			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	3			
F	6,146			
R squared	0,672			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	4,207	2	2,103	
Residual (within columns)	2,053	6	0,3422	
Total	6,26	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	0,03333	0,09869	P > 0.05	-1.432 to 1.499
Ad-dammam vs South Khobar	-1,433	4,244	P > 0.05	-2.899 to 0.03216
North Khobar vs South Khobar	-1,467	4,342	P < 0.05	-2.932 to -0.001176

northern Khobar  $\approx$  southern Khobar (Tables 1 to 10). The means significant difference (P < 0.05) is not obvious and tukey's multiple comparison test with either P < 0.05 or P > 0.05 is denoted in Table 17.

(8) Calcium concentrations in mixed tissues of ctendia ranged from 31.6 to 33.7 mg/Kg dry weight in samples of Ad-Dammam, 11.7 to 32.0 mg/Kg dry weight in samples of northern Khobar whereas 5.9 to 23.6 mg/Kg dry weight in samples of southern Khobar. Histogram 8 clarifies that

calcium contamination in ctendia is Ad-Dammam > northern Khobar > southern Khobar (Tables 1 to 10). The means significant difference (P < 0.05) is not obvious and Tukey's multiple comparison test with either P < 0.05 or P > 0.05 is denoted in Table 18.

(9) Chromium concentrations in mixed tissues of ctendia ranged from 0.2 to 0.4 mg/Kg dry weight in samples of Ad-Dammam, zero mg/Kg dry weight in samples of northern Khobar and zero mg/Kg dry weight in samples

**Table 13.** One-way analysis of variance (ANOVA) and Tukey's multiple comparison test analyses for sodium.

<b>Data table- analysis of aluminium.</b>				
<b>One-way analysis of variance</b>				
P value	0,049			
P value summary	*			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	3			
F	5,197			
R squared	0,634			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	26,9	2	13,45	
Residual (within columns)	15,53	6	2,588	
Total	42,42	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	-0,06667	0,07178	P > 0.05	-4.097 to 3.963
Ad-dammam vs South Khobar	-3,7	3,984	P > 0.05	-7.730 to 0.3299
North Khobar vs South Khobar	-3,633	3,912	P > 0.05	-7.663 to 0.3966

**Table 14.** One-way analysis of variance (ANOVA) and Tukey's multiple comparison test analyses for phosphate.

<b>Data table- analysis of phosphate</b>				
<b>One-way analysis of variance</b>				
P value	0,9233			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	0,08088			
R squared	0,02625			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	11,12	2	5,56	
Residual (within columns)	412,5	6	68,74	
Total	423,6	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	-0,6	0,1253	P > 0.05	-21.37 to 20.17
Ad-dammam vs South Khobar	-2,6	0,5431	P > 0.05	-23.37 to 18.17
North Khobar vs South Khobar	-2	0,4178	P > 0.05	-22.77 to 18.77

of southern Khobar. Chromium contamination in ctendia is Ad-Dammam  $\approx$  northern Khobar  $\approx$  southern Khobar (Tables 1 to 10). The means significant difference (P < 0.05) is not not obvious and Tukey's multiple comparison test with either P < 0.05 or P > 0.05 showed no difference.

(10) Iron concentrations in mixed tissues of ctendia ranged from 0.1 to 0.2 mg/Kg dry weight in samples of Ad-Dammam, 0.1 to 0.4 mg/Kg dry weight in samples of

northern Khobar whereas 0.1 to 0.8 mg/Kg dry weight in samples of southern Khobar. Iron contamination in ctendia is Ad-Dammam  $\approx$  northern Khobar  $\approx$  southern Khobar (Tables 1 to 10). The means significant difference (P < 0.05) is not not obvious and Tukey's multiple comparison test with either P < 0.05 or P > 0.05 showed no difference.

(11) Nickel concentrations in mixed tissues of ctendia ranged from 0.4 to 0.5 mg/Kg dry weight in samples of

**Table 15.** One-way analysis of variance (ANOVA) and Tukey's Multiple Comparison test analyses for sulphur.

<b>Data table- analysis of sulphur</b>				
<b>One-way analysis of variance</b>				
P value	0,1564			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	2,568			
R squared	0,4613			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	353,8	2	176,9	
Residual (within columns)	413,2	6	68,87	
Total	767	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	-4,5	0,9392	P > 0.05	-25.29 to 16.29
Ad-dammam vs South Khobar	-14,97	3,124	P > 0.05	-35.76 to 5.824
North Khobar vs South Khobar	-10,47	2,184	P > 0.05	-31.26 to 10.32

**Table 16.** One-way analysis of variance (ANOVA) and Tukey's multiple comparison test analyses for chlorine.

<b>Data table- analysis of chlorine</b>				
<b>One-way analysis of variance</b>				
P value	0,4312			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	0,9709			
R squared	0,2445			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	165,2	2	82,61	
Residual (within columns)	510,5	6	85,09	
Total	675,7	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	
Ad-dammam vs North Khobar	-7,967	1,496	P > 0.05	
Ad-dammam vs South Khobar	-9,9	1,859	P > 0.05	
North Khobar vs South Khobar	-1,933	0,363	P > 0.05	

Ad-Dammam, 0.3 to 1.4 mg/Kg dry weight in samples of northern Khobar whereas 1.5 to 2.8 mg/Kg dry weight in samples of southern Khobar. Histogram 9 clarifies that nickel contamination in ctendia is Ad-Dammam < northern Khobar < southern Khobar (Tables 1 to 10). The means significant difference (P < 0.05) is not obvious and Tukey's multiple comparison test with either P < 0.05 or P > 0.05 is denoted in Table 19.

(12) Copper concentrations in mixed tissues of ctendia ranged from 4.1 to 3.4 mg/Kg dry weight in samples of Ad-Dammam, 0.1 to 3.7 mg/Kg dry weight in samples of

northern Khobar whereas 0.9 to 3.7 mg/Kg dry weight in samples of southern Khobar. Histogram 10 clarifies that copper contamination in ctendia is Ad-Dammam > northern Khobar < southern Khobar (Tables 1 to 10). The means significant difference (P < 0.05) is not obvious and Tukey's means significant difference with either P < 0.05 or P > 0.05 is denoted in Table 20.

(13) Zinc concentrations in mixed tissues of ctendia ranged from 1.7 to 2.5 mg/Kg dry weight in samples of Ad-Dammam, 0.8 to 1.0 mg/Kg dry weight in samples of northern Khobar whereas 1.0 to 2.5 mg/Kg dry weight in



**Table 17.** One-way analysis of variance (ANOVA) and Tukey's multiple comparison test analyses for

<b>Data table- analysis of cadmium</b>				
<b>One-way analysis of variance</b>				
P value	0,1835			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	2,279			
R squared	0,4317			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	15,95	2	7,974	
Residual (within columns)	20,99	6	3,499	
Total	36,94	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	-2,933	2,716	P > 0.05	-7.619 to 1.753
Ad-dammam vs South Khobar	-2,7	2,5	P > 0.05	-7.386 to 1.986
North Khobar vs South Khobar	0,2333	0,2161	P > 0.05	-4.453 to 4.919

**Table 18.** One-way analysis of variance (ANOVA) and Tukey's Multiple Comparison test analyses for calcium.

<b>Data table- analysis of calcium</b>				
<b>One-way analysis of variance</b>				
P value	0,0768			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	4,057			
R squared	0,5749			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	616,4	2	308,2	
Residual (within columns)	455,8	6	75,96	
Total	1072	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	13,67	2,716	P > 0.05	-8.167 to 35.50
Ad-dammam vs South Khobar	19,8	3,935	P > 0.05	-2.034 to 41.63
North Khobar vs South Khobar	6,133	1,219	P > 0.05	-15.70 to 27.97

samples of southern Khobar. Histogram 11 clarifies that zinc contamination in ctendia is Ad-Dammam > northern Khobar < southern Khobar (Tables 1 to 10). The means significant difference (P < 0.05) is not obvious and Tukey's multiple comparison test with either P < 0.05 or P > 0.05 is denoted in Table 21.

(14) Mercury concentrations in mixed tissues of ctendia ranged from zero mg/Kg dry weight in samples of Ad-Dammam, zero to 0.2 mg/Kg dry weight in samples of

northern Khobar whereas zero to 2.7 mg/Kg dry weight in samples of southern Khobar. It can be concluded that mercury contamination in ctendia is Ad-Dammam ≈ northern Khobar < southern Khobar (Tables 1 to 10).

(15) Lead concentrations in mixed tissues of ctendia ranged from 0.4 to 0.7 mg/Kg dry weight in samples of Ad-Dammam, 0.2 to 9.0 mg/Kg dry weight in samples of northern Khobar whereas 1.3 to 3.7 mg/Kg dry weight in samples of southern Khobar. It can be concluded that

**Table 19.** One-way analysis of variance (ANOVA) and Tukey's multiple comparison test analyses for nickel.

<b>Data table- analysis of nickel</b>				
<b>One-way analysis of variance</b>				
P value	0,4008			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	1,069			
R squared	0,2627			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	0,4822	2	0,2411	
Residual (within columns)	1,353	6	0,2256	
Total	1,836	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	-0,2667	0,9725	P > 0.05	-1.456 to 0.9231
Ad-dammam vs South Khobar	-0,5667	2,067	P > 0.05	-1.756 to 0.6231
North Khobar vs South Khobar	-0,3	1,094	P > 0.05	-1.490 to 0.8898

**Table 20.** One-way analysis of variance (ANOVA) and Tukey's multiple comparison test analyses for copper.

<b>Data table- analysis of copper</b>				
<b>One-way analysis of variance</b>				
P value	0,4801			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	0,8312			
R squared	0,217			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	3,162	2	1,581	
Residual (within columns)	11,41	6	1,902	
Total	14,58	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	1,4	1,758	P > 0.05	-2.055 to 4.855
Ad-dammam vs Soth Khobar	1,033	1,298	P > 0.05	-2.422 to 4.488
North Khobar vs Soth Khobar	-0,3667	0,4605	P > 0.05	-3.822 to 3.088

sodium contamination in ctendia is Ad-Dammam  $\approx$  northern Khobar < southern Khobar (Tables 1 to 10). Histogram 12 clarifies that lead contamination in ctendia is Ad-Dammam < northern Khobar < southern Khobar (Tables 1 to 10). The means significant difference (P < 0.05) is not obvious and Tukey's multiple comparison test with either P < 0.05 or P > 0.05 is denoted in Table 22.

The most dramatic results came with the analysis of the samples of the southern Khobar estuarine beach in the three collections time. The data gathered showed very high concentration of total sodium, aluminium,

phosphate, sulphur, chlorine, cadmium, nickel, zinc, mercury and lead excluding iron that can cause significant adverse effects on bivalve species if concentrations continue to accumulate and bio-magnify in the food chain. Clams live in southern Khobar estuarine beach contain percentage of heavy metals  $\geq$  Saudi Arabian Standards whereas the clams live the northern Khobar and Ad-dammam easuarine beaches contain percentage of heavy metals  $\approx$  Saudi Arabian Standards. The accumulation of multiple metals and pollutants can have a synergistic effect that can increase the threat level

**Table 21.** One-way analysis of variance (ANOVA) and Tukey's multiple comparison test analyses for zinc.

<b>Data table- analysis of zinc</b>				
<b>One-way analysis of variance</b>				
P value	0,0421			
P value summary	*			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	3			
F	5,626			
R squared	0,6522			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	3,776	2	1,888	
Residual (within columns)	2,013	6	0,3356	
Total	5,789	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	1,567	4,684	P < 0.05	0.1155 to 3.018
Ad-dammam vs South Khobar	0,5667	1,694	P > 0.05	-0.8845 to 2.018
North Khobar vs South Khobar	-1	2,99	P > 0.05	-2.451 to 0.4511

**Table 22.** One-way analysis of variance (ANOVA) and Tukey's multiple comparison test analyses for lead.

<b>Data table- analysis of lead</b>				
<b>One-way analysis of variance</b>				
P value	0,4151			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	1,022			
R squared	0,2541			
<b>ANOVA table</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	
Treatment (between columns)	2,509	2	1,254	
Residual (within columns)	7,367	6	1,228	
Total	9,876	8		
<b>Tukey's multiple comparison test</b>	<b>Mean Diff.</b>	<b>q</b>	<b>P value</b>	<b>95% CI of diff</b>
Ad-dammam vs North Khobar	-0,1	0,1563	P > 0.05	-2.876 to 2.676
Ad-dammam vs South Khobar	-1,167	1,824	P > 0.05	-3.942 to 1.609
North Khobar vs South Khobar	-1,067	1,667	P > 0.05	-3.842 to 1.709

exponentially, but each individual metal can impact the organism alone. The northern Khobar estuarine beach contained moderate amount of heavy metals whereas Ad-Dammam estuarine beach lies within Saudi Arabian Standards. The presence of these identified heavy metals present in the tissues of the sampled bivalve could indicate serious problems within the aquatic biota of Arabian Gulf. Different bivalve and marine species will

react to metal toxicity in different manners, some will be more susceptible and others more resilient. The prevalence of the metals accumulating in all of samples is an indicator that more than likely these metals are accumulating in other aquatic species as well. If a metal is present in one species, especially in high concentrations, then one can assume that other species in the same ecosystem will have accumulated some level

of the same metal, resulting in widespread metal toxicity among an ecosystem's community. Aluminum has proven to be quite toxic especially at low pH levels associated with Alum ion concentrations. Mercury poses a large threat to the ecosystem once introduced due to its high potential and very efficient ability to bio-accumulate and bio-magnify through the trophic levels, because it is very persistent in the environment. Mercury has been proven to be a carcinogen and a mutagen and is under strict regulation. Nickel is known to be a carcinogen and a mutagen much like that of mercury. Ni is able to partition to dissolved and particulate matter, more often to carbon than other organic matter. The bioavailability of Ni can be in some correlated to the concentrations of calcium and magnesium. Research has shown that Ni is able to cause tissue damage, genotoxicity, growth inhibition in aquatic ecosystems. Phosphate is an essential nutrient for life, but when in excess it can cause devastating effects. P is found in numerous forms dependent on confounding factors present. Phosphates are high energy compounds and environmental removal is typically very slow. This study analysed a wide range of heavy elements simultaneously within the same sample. The method is non-destructive; hence samples can be stored for future reference or analysed by other laboratories for quality control purposes. This is important in regulatory pollution control work where analytical results can be vital. In addition, this study is sensitive to many elements over a broad span of concentrations and detection range is linear for a large number of elements. This alleviates the necessity for concentrating or diluting samples to within a range suitable for analysis, as required by some other techniques.

## DISCUSSION

The goal of this study was to determine the presence of heavy metals in ctendia of *Anadara ehrenbergi* collected from three estuarine beaches of Arabian Gulf (Saudi Arabia). A determination of metal concentrations in organisms should be a part of any assessment and monitoring program in the coastal zone. In the present study, Tables 1 to 10 showed that *A. ehrenbergi* concentrated heavy metals in its ctendia; however, little is known about the potential of the other species to do the same in the same study sites. It has been shown above that the samples collected from western estuarine beach in Khobar have anomalous heavy metal values. The most dangerous heavy metals measured in this study are aluminum, phosphate, calcium, nickel, potassium, mercury, iron, copper, chromium and zinc. metals that accumulate in ctendia of *A. ehrenbergi* pose a threat to the survivorship of individual species as well as the ecosystem. Several authors in the available literature interested to investigate the problem of heavy metals bioaccumulation in marine invertebrates and fishes (Khan

et al., 2001; Bu-Olayan and Thomas, 2001; Islam and Tanaka, 2004; Naithani et al., 2010; Freije, 2014; Radwan et al., 2014). The most interesting work concerning heavy metals accumulations in marine animals inhabiting the Arabian Gulf is Freije (2014). In this study, the Arabian Gulf environmental status was assessed based on studies conducted in Bahrain, Kuwait, Oman, Saudi Arabia, Qatar, and United Arab Emirates (UAE) during 1983 and 2011. The present study found many more or less parallel results with that work. Moreover, other authors interested to study the problem of heavy metals in marine organisms living in the Arabian Gulf. The present study accept the finding of Al-Sayed et al. (1994), Al-Madfa et al. (1998), De Mora et al. (2004), Al-Sayed and Dairi (2006) and Alyahya et al. (2011) and disagree with the results of (Al-Farraj et al., 2011). Levels of selected heavy metals in *P. radiata* collected from two oyster beds in Bahrain were determined by Al-Sayed et al. (1994). This study reported elevated levels of Pb and Cd that exceeded the recommended standards of the World Health Organization (WHO). Similarly, elevated levels of heavy metals were reported in *P. radiata* collected from areas that were subject to dredging and shipping activities along the Qatari coastline (Al-Madfa et al., 1998). This study reported high mean concentrations for V exceeded the international standards. De Mora et al. (2004) also found very high concentrations of Zn in pearl oysters near the oil refinery in Bahrain. Al-Sayed and Dairi (2006) measured selected heavy metals in the marine snail *Turbo coronatus* collected from nearshore sites around Bahrain. This study found that the levels of Cu and Pb were higher than the WHO acceptable limits for marine organisms. Selected heavy metals were measured in the edible clam *Meretrix meretrix* collected from stations along the coastline of Saudi Arabia (Alyahya et al., 2011). Elevated levels of Pb that exceeded the maximum permissible level recommended by the European Union standards. Nonetheless, this study concluded that the clam from the sampling region was within the safe limits for human consumption. Al-Farraj et al. (2011) determined the levels of selected heavy metals in the cuttlefish *Sepia pharaonis* collected from different fish markets at Al-Khober City in the Arabian Gulf. This study concluded that the levels of the investigated heavy metals in the cuttlefish were generally low and/or well within the maximum permitted concentrations imposed by different organizations and authorities, and consequently within the safe limits for human consumption. The study agrees with the finding of Al-Farraj et al. (2011), *Sepia pharaonis* is not a filter feeder as clams.

Heavy metal accumulation in the gill tissue of fishes from Arabian Gulf were reported (Islam and Tanaka, 2004). Mercury and other metals, such as lead and cadmium have been shown to accumulate in living organisms living in marine ecosystems (Al-Hashimi and Al-Zorba, 1991). Metal accumulation by the clam *Meretrix*

*meretrix* as lead, titanium, zinc, nickel, vanadium and copper were measured in Arabian Gulf (Sadiq, et al., 2002). In the present study, tissues of *ctendia* of *A. ehrenbergi* showed higher concentrations for most heavy elements than other body parts, except in the case of calcium. The concentrations of Cu, Zn, Fe and Pb are much more variable. Thus, it appears that a non-selective sampling strategy for different parts of the *ctendia* would be sufficient. The ratio of the metals Fe, Zn, Cu, and Pb remains approximately respectable of study site. This pattern may be changed in systems where predominantly one element is discharged. Further work is required to establish whether it is possible to use this ratio predictively. The EDXRF technique is well suited for multi-element determinations in environmental samples. Cadmium (Cd) is one of the most toxic heavy metals for humans; the main source of non-occupational exposure to Cd includes contaminated sea foods (Khan and Price, 2002). The World Health Organization (WHO, 1993) estimates that 80% of the world population presently uses herbal medicine. Several articles have reported of adverse effects of these herbal preparations due to the presence of high level of heavy metals such as Cd, lead, chromium, nickel, etc. (Naithani et al., 2010). The results revealed that the concentrations of some heavy metals, including Cd, were far greater than the permissible limits proposed by the International Regulatory Authorities for herbal drugs. Acute or chronic exposure of Cd causes respiratory distress, lung, breast and endometrial cancers, cardiovascular disorders and endocrine dysfunction (Nagata et al., 2005; Nadim, et al., 2008; Chang et al., 2009; Khamdan and Al Madany, 2009; Naithani et al., 2010; de Mora et al., 2010; Al Farraj, et al., 2011). In addition, Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources (Fowler et al., 2007). It can be accumulated in aquatic animals (crabs, shrimps, oysters, clams and mussels) after entering through different way such as respiratory tract, digestive tract, surface penetration etc. (Sokolova, 2004; Sokolova et al., 2004; USFDA, 2006; Juma and Al-Madany, 2008; Juma and Al-Madany, 2008; Khamdan and Al Madany, 2009; Naser, 2012). It is seriously harmful to the growth of aquatic life and survival, resulting in decline of their populations. At the same time, as aquatic food products, these animals exposed to Cd might threaten human health.

Several elemental, environmental, and organismal factors influence the absorption of these metals including: What the metal is bonded to chemically, if the ion is small, neutrally charged, and/or lipophilic, water hardness, pH, and temperature, concentration of compound, and route of exposure (gill, contaminated food, non-food particles, oral intake of water, and dermal absorption). After exposure it is transported by the blood, usually bonding to a protein at some point, and will then be distributed to and accumulates in a tissue which it has

a high affinity for. Over 80% of marine pollution comes from land-based activities (Al-Jamali et al., 2005; Al-Busaidi et al., 2011; Daoji and Dag, 2004; Sheavly and Register, 2007). From plastic bags to pesticides- most of the waste we produce on land eventually reaches the marine ecosystem, either through deliberate dumping or from run-off through drains and rivers. This includes oil, fertilizers, plastic garbage, sewage disposal and toxic chemicals. The prevalence of these metals accumulating in the *ctendia* of *A. ehrenbergi* might be an indicator of a disastrous future for the Arabian Gulf ecosystem.

A high prevalence of certain heavy metals and elements could cause what is known as a dead zone that will yield catastrophic effects to the ecosystem. A dead zone is an area on an aquatic ecosystem that is unable to support life due to lack of dissolved oxygen. When excess phosphate is in the watershed combined with excess nitrogen the populations of algae and phytoplankton drastically increase. When the large populations die they sink and begin to be broken down by bacteria. Bacteria that break down this large layer of biomass use dissolved oxygen and release carbon dioxide, which significantly depletes the aquatic environment of dissolved oxygen. The depletion of dissolved oxygen renders the area unable to support life dependent on aerobic respiration, thus getting the name dead zone (SASO, 1997).

The results of this study showed serious heavy metals accumulation problems in the Arabian Gulf ecosystem. Heavy metals are accumulating and bio-magnifying and have a very real potential to increase the toxicity present throughout the Arabian Gulf ecosystem.

This study illustrated the use of the EDXRF technique in rapid assessment of metal contamination in biological material. The distribution of heavy metals in different parts of *ctendia* of *A. ehrenbergi* has been analyzed, and the results obtained agree well with those of other authors on other species collected from Arabian Gulf, especially Zyadah and Al-Motairy (2012) and Freije (2014).

### Conflict of Interest

The authors have not declared any conflict of interest.

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