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The Effects of Lead, Cadmium, Mercury and Arsenic on Fish and Seawater in Red Sea and the Gulf of Aden at Three Different Locations in Yemen

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Abstract

This study investigate concentrations of heavy metals (Pb, Cd, Hg and As) in water and muscle fish collected from three different sites (Aden, Al-Hodeidah and AL-Mukalla) of the Red Sea and the Gulf of Aden in Yemen's coastal. Fresh samples were purchased from near the sea public auction site locations. The four species of fish (emperor) *Lethrinus mahsena*, (Longtail tuna) *Thunnus tonggol*, (Pickhandle Barracuda) *Sphyraena jello* and (Areolate grouper) *Epinephelus areolatus* were collected and analyzed, 81 samples of Seawater and 108 samples of muscle Fish were digested using suitable procedures. The heavy metal concentrations in the samples were measured using the Atomic Absorption Spectrophotometer. The obtained results showed that the concentration (mg/l) of the heavy metal in seawater (Pb-0.061±0.005, Cd-0.007±0.001, Hg-0.007±0.0005 and As-0.008±0.0003) was lower than that of the concentration (µg/g) of heavy metal in muscle (Pb-0.101±0.012, Cd-0.046±0.010, Hg-0.058±0.002 and As-0.089±0.002). The results showed that, the heavy metals concentrations were high in stations AL- Hudaydah and AL- Mukalla and low in station of Aden. The highest mean concentration of Pb, Cd and As a in the muscles of the four studied fish species was 0.137± 0.014 and 0.069±0.021µg/g dry wt in large *Epinephelus areolatus* ; 0.106±0.007µg/g dry wt in large *Lethrinus mahsena* At Site AL- Hudaydah in Summer. The highest mean concentration of Hg a in the muscles of the four studied fish species was 0.071±0.012µg/g dry wt in large *Epinephelus areolatus* At Site Aden in summer. Positive relationships were found between metal levels and fish size. The results were analyzed and tested for differences between group means of stations and seasons for significance (P≤0.05) using the analysis of variance one way ANOVA and two ways ANOVA technique. The obtained results showed that the heavy metals concentrations were significantly higher, during the summer season for seawater and muscle fish samples in all stations during the study period. From these results, we conclude that the heavy metals (Pb, Cd, Hg and As) in the muscles of fish and seawater, at Yemen's coastal were still in range scale of international pollution standard However, the study recommends continuing the study of these pollutants and other contaminants and their impact on the environment and marine life.

Keywords: Fish; Heavy Metals; Water; Aden; Al-Hodeidah; AL-Mukalla; Red Sea; Gulf of Aden; Yemen

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Introduction

The pollution of aquatic systems has become a major concern worldwide [1]. There are a variety of sources that will pollute aquatic systems with heavy metals. These include animal matter, wet and dry fallouts of atmospheric particulate matter and human activities. The concentration, bioavailability and toxicity of heavy metals in aquatic systems can be affected by various factors, including pH and temperature [2]. Poor quality of surface water is caused in two ways. The pollution of surface water can either be due to point source (PS) or nonpoint source pollution (NPS). Point source pollution is mainly municipal sewage discharge and industrial wastewater loads. Municipal sewage discharge is from urban or highly residential areas, while industrial wastewater is from a variety of manufacturers [3]. When rainfall or irrigation water runs over land it will carry and deposit pollutants into rivers, lakes and coastal waters. This is seen as nonpoint source pollution [3]. Heavy metals will be distributed between the aqueous phase and bed sediments in aquatic systems [4]. Only a small percentage of the free metal ions stay dissolved in water. Fishes represent the peak

of consumers in the water system. Fishes have ability to collect these metals in concentrations higher than water and sediments because of feed on organic materials in aquatic environments [5]. Fishes have been found to be good indicators of the heavy metal contamination levels in the aquatic systems because they occupy different atrophic levels [6]. According to [6] there are two main routes of heavy metals exposure: The primary route of intake of these chemicals in fish species is via gill or transport of dissolved contaminants in water across biological membranes and ionic exchange. The secondary route is through the intestine by food or sediment particles with subsequent transport across the gut [6].

The food may also be important source for heavy metal accumulation in fish [7]. In aquatic ecosystem, metals are transferred to the fish through food chain that could ultimately affect the health of people consuming this fish [7].

Accumulation of these metals in the bodies of fish affected by different factors such as pH, water hardness and level of pollution in the surrounding water added to the age and physiological situation of fish [8]. Industrial and domestic waste containing heavy metals and hydrocarbon accumulate in aquatic food chains as possible to cause acute and chronic damages in fish communities and lead to reduce ability to growth and reproduce [9].

Determining the levels of such heavy metals and comparing the levels with guidelines will establish the potential health risk from the consumption of such fish species. Therefore, it is important to determine the concentrations of non-essential metals in fish in order to evaluate the possible risks of fish consumption. This can serve as an indicator for the extent of pollution in Yemen coastal waters.

Materials and Methods

Surface Seawater samples and fish samples were collected from winter 2011, summer 2012 and winter 2013, from 3 sampling sites (Figure 1-2). During which a total of 81 Sample of Surface Seawater, 108 Sample of Muscles Fish were collected and analyzed.

Surface seawater sampling and analysis

Surface seawater sampling: Seawater samples were collected seasons for analysis from one level; the surface Seawater of each station. In principle, collect seawater samples at high tide and avoid windy or rainy days). Before sampling, the bottles of samples were rinsed at least three times with water from the sampling station. The bottles were immersed to about 20-30 cm below the water surface to prevent contamination of heavy metals from air. For mercury analysis water samples kept in a sealable glass container that has been well washed before being transported [10]. For Arsenic analysis water samples were collected according to EPA Method 1632 [11].

A total of 81 of seawater samples were collected using cleaned plastic water sampler. Each sample was taken in 1 liter polyethylene bottles. All water samples were immediately brought to the laboratory where filtered through Whatman No.41 (0.45µm pore size) filter paper. The samples were acidified with 2ml nitric acid to prevent precipitation of metals, reduce adsorption of the analyses onto the walls of containers and to avoid microbial activity, and then stored at 4°C until the chemical analyses.

Surface seawater digestion and analysis

Surface seawater digestion for Pb and Cd analysis by GFAAS: Five milliliters of concentrated HCl was added to 250ml of each surface seawater sample placed in 600ml beaker and evaporated to 25ml volume. The concentrate was transferred to a 50ml volumetric flask and diluted to mark with deionized water. Prior analysis, the solutions were filtered through Whatman No.41 (0.45µm pore size) filter paper. Analyzed for Lead and Cadmium using Buck Model 210VGP, USA Made-Graphite furnace Atomic Absorption Spectrophotometer (GF AAS) in Seawater samples, before proceeding EPA method 200.13 [12].

Surface seawater digestion for Hg and as analysis by hydride analyzer: Forty five milliliter of surface seawater sample was measured. A volume of 5ml of concentrated nitric acid (HNO_3, 65%), 1ml of concentrated hydrochloric acid (HCl, 35%) was added to each sample. Vessels Sealed and placed in microwave system. Samples were heated according to time versus pressure profiles. Vessels allowed cooling to the room temperature and then each sample transferred to a final volume of 25ml using deionized water. The Sample may represent a safety hazard. Pre-digest sample in a hood, with vessel loosely capped to allow gases to escape, before proceeding EPA Method 3015A [13]. Cold Vapor Hg Analyzer (Buck Model 410), U.S.A Made, was used for analysis of Hg in Seawater samples, Perfect for EPA method 245.1 [14]. Arsenic Hydride Analyzer (Buck Model 411), U.S.A Made, was used for analysis of as in seawater samples, perfect for EPA method 206.3 [15].

Fish sampling and analysis

Fish sampling: A total of 108 specimens of four commercially important fish species, *Lethrinus mahsena, Thunnus tonggol , Sphyraena jello and Epinephelus areolatus* were collected seasons with the help of local fishermen from Aden, Al Hodeidah and Al Mukalla during the study period of seasons (winter 2011, summer 2012 and winter 2013). Samples were placed immediately in poly-ethylene bags, put into ice box, after that brought to the laboratory at the faculty of Environmental Sciences and Marine Biology, Hadramout University. The total length and the body wet weight of each specimen were measured to the nearest centimeter and gram respectively [16]. After measurements, fish samples were washed with deionized water, sealed in polyethylene bags and kept in a freezer at -20°C until chemical analysis [16].

Fish tissue digestion and analysis: Fish tissues were dried in oven at (80°C) until sample is at a constant weight. About 0.500g of dry tissue sample (muscles) was accurately weighed and digested with 7ml of concentrated nitric acid (HNO_3 65%) and 1ml of hydrogen peroxide (H_2O_2 30%). Milestone Stard D Microwave Digestion Lab station with internal Temperature sensor and 260 terminal teach screen With HPR1000/10S High Pressure Segmented Rotar (Application Note HPR-FO-07) and AOAC Official Method 999.10 [17]. And AOAC Official Method 974.14 [18]. Microwave Program 2 Steps (1)15.00Min (temperature 200) (2)15.00Min (temperature 200). After Finish left vessels 20min until reach the room temperature, then the digested portion was diluted to a final volume of 50ml using deionized water , before proceeding Method 3052 [19]. Pb Analyzed without Further Treatment, Cd diluted with Factor 2, Hg and As Diluted with Factor 100 . The Certified Reference Material DORM-2 Analyzed for For Pb, Cd, Hg and As Content.

Lead and Cadmium analysis in fish tissues by GFAAS: Graphite furnace atomic absorption spectrometry (Model 220 GF), U.S.A Made, were used for analysis of Cd and Pb in fish tissue samples, Perfect for AOAC Official Method 999.10 [17].

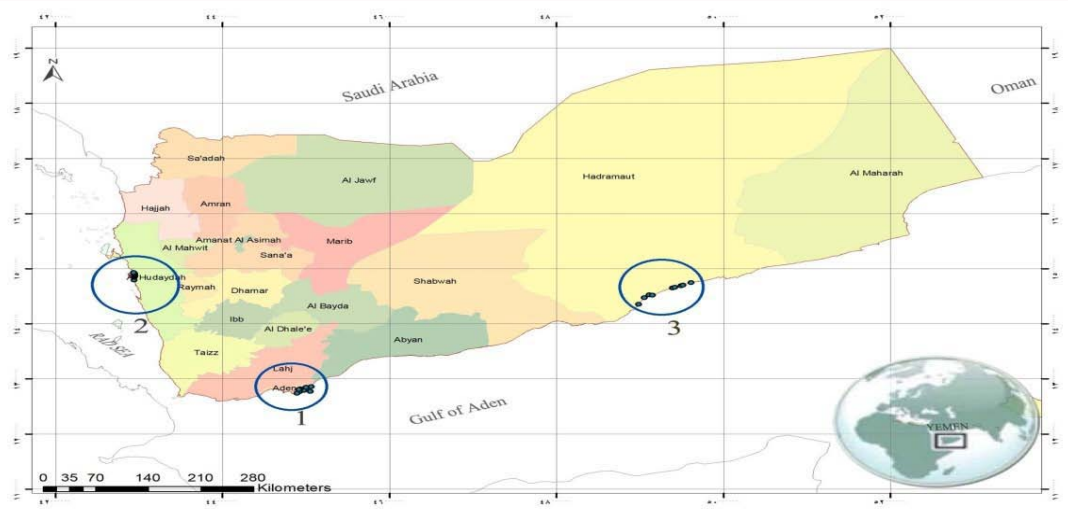

Figure 1: Sampling locations along the Coast of Yemen.

Table 1: The mean of concentration (mg/L) for lead, cadmium, mercury and Arsenic during the seasons in the filtered surface water of Aden , AL-Hudaydah and AL-Mukalla stations, Yemen coast.

Site	Metal ion	Seasons			Total mean ± SD
		Winter 2011	Summer 2012	Winter 2013	
Aden	Pb	0.055 ± 0.004	0.045 ± 0.007	0.051 ± 0.005	0.050± 0.005
	Cd	0.006±0.002	0.010±0.003	0.009±0.001	0.008±0.002
	Hg	0.005±0.000	0.003±0.000	0.007±0.002	0.005±0.002
	As	0.0057±0.000	0.0061±0.000	0.006±0.000	0.006±0.000
AL- Hudaydah	Pb	0.080±0.020	0.087±0.027	0.072±0.021	0.080± 0.008
	Cd	0.007±0.002	0.006±0.001	0.007±0.000	0.0070±0.000
	Hg	0.007±0.001	0.008±0.000	0.007±0.001	0.0073±0.000
	As	0.007±0.001	0.0082±0.000	0.0087±0.000	0.008±0.000
AL- Mukalla	Pb	0.033±0.002	0.064±0.026	0.064±0.018	0.054± 0.018
	Cd	0.006±0.000	0.0082±0.000	0.0083±0.000	0.0075±0.001
	Hg	0.009±0.000	0.0067±0.000	0.0069±0.000	0.0075±0.001
	As	0.010±0.002	0.011±0.002	0.010±0.000	0.010±0.000
Total mean ± SD	Pb	0.056± 0.023	0.065± 0.027	0.062± 0.018	0.061±0.005
	Cd	0.006±0.002	0.008±0.002	0.008±0.001	0.007±0.001
	Hg	0.007±0.002	0.006±0.002	0.007±0.001	0.007±0.0005
	As	0.0077±0.002	0.0083±0.002	0.0082±0.002	0.008±0.0003

Mercury and Arsenic analysis in fish tissues by hydride analyzer: Cold Vapor Hg Analyzer (Buck Model 410), U.S.A Made, was used for analysis of Hg in fish tissue samples, Perfect for AOAC Official Method 974.14 [18].

Arsenic Hydride Analyzer (Buck Model 411), U.S.A Made, was used for analysis of As in fish tissue samples, Perfect for EPA method 206.3 [15].

Statistics: All heavy metals data (Lead, Cadmium, Mercury and Arsenic) were analyzed and tested for differences between group means of stations and seasons for significance (P≤0.05) using the analysis of variance one way ANOVA and two ways ANOVA technique. Also, group means of environmental factors were analyzed by one way ANOVA technique. All statistical analysis was performed using the Origin 9 and SPSS software packages, version 17.0.

Results

Surface seawater

Concentrations of heavy metals in the filtered surface water collected in 3 seasons and 3 different stations at Yemen coast are given in Table 1 and the graphical representation in Figure 2. The maximum concentration of Mercury (0.007mg/l) is observed in winter season. The Maximum concentration Lead (0.065mg/l), Cadmium (0.008mg/l) and Arsenic (0.0083mg/l) are observed in summer season.

The highest concentration of Pb in filtered surface water of AL-Hudaydah was 0.080mg/L; however, the highest concentration of Cd in filtered surface water of Aden was 0.008mg/L. The concentration of Hg in filtered surface water had the same pattern of Pb and Cd; but, the highest concentration of Hg in the filtered surface water of AL-

Table 2: The mean of Concentration µg/g (dry wt.) for lead, cadmium, mercury and Arsenic during the seasons in the muscles samples for the study period.

Site	Metal ion	Seasons			Total mean ± SD
		Winter 2011	Summer 2012	Winter 2013	
Aden	Pb	0.059± 0.028	0.071± 0.032	0.075± 0.035	0.068± 0.031
	Cd	0.024±0.010	0.052±0.021	0.041±0.019	0.039±0.015
	Hg	0.059±0.022	0.061±0.025	0.059±0.023	0.060±0.023
	As	0.082±0.018	0.091±0.020	0.085±0.021	0.086±0.020
AL- Hudaydah	Pb	0.114±0.065	0.151±0.080	0.149±0.078	0.138± 0.073
	Cd	0.052±0.033	0.062±0.033	0.057±0.036	0.057±0.034
	Hg	0.060±0.017	0.058±0.016	0.053±0.021	0.057±0.018
	As	0.092±0.022	0.091±0.021	0.093±0.019	0.092±0.020
AL- Mukalla	Pb	0.091±0.038	0.098±0.052	0.104±0.049	0.098± 0.046
	Cd	0.028±0.010	0.050±0.026	0.051±0.024	0.043±0.016
	Hg	0.055±0.016	0.058±0.024	0.056±0.025	0.057±0.021
	As	0.088±0.020	0.091±0.018	0.085±0.019	0.088±0.019
Total mean ± SD	Pb	0.088± 0.051	0.107± 0.066	0.109± 0.064	0.101±0.012
	Cd	0.035±0.015	0.054±0.026	0.049±0.027	0.046±0.010
	Hg	0.058±0.018	0.059±0.022	0.056±0.023	0.058±0.002
	As	0.087±0.020	0.091±0.019	0.088±0.020	0.089±0.002

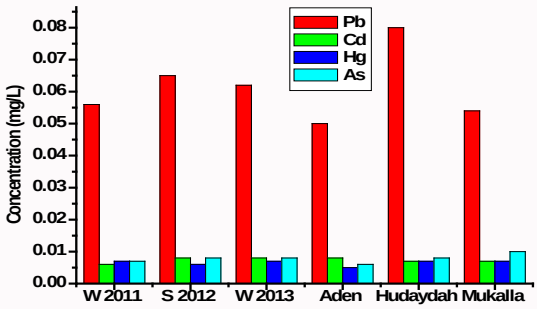

Figure 2: The mean of concentration (mg/L) for lead, cadmium, mercury and Arsenic during the seasons in the filtered surface water of Aden, AL-Hudaydah and AL-Mukalla stations, Yemen coast.

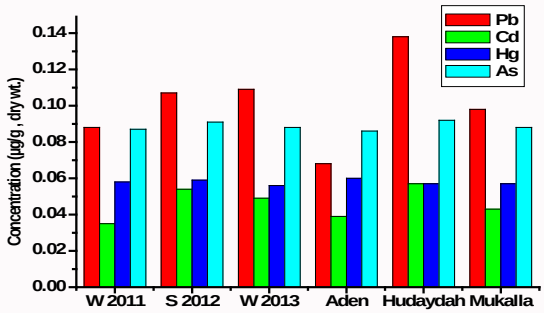

Figure 3: The mean of Concentration µg/g (dry wt.) for lead, cadmium, mercury and Arsenic during the seasons in the muscles samples for the study period.

Mukalla was 0.0075mg/L. The highest concentration of As in filtered surface water of AL-Mukalla was 0.010mg/L, as summarized in Table 1 and Figure 2.

Heavy metals in Muscles fish

The concentrations of heavy metals in spotted seer fish caught in 3 seasons and 3 different stations at Yemen coast are given in Table 2 and the graphical representation of the mean concentration in Figure 3. It is observed that the highest concentration of Lead (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (As), are in Winter 0.109µg/g (dry wt.), summer 0.054µg/g (dry wt.), summer 0.059µg/g (dry wt.) and summer 0.091µg/g(dry wt.) respectively.

The highest concentration of Pb in muscles of fish of AL-Hudaydah was 0.138µg/g (dry wt.); however, the highest concentration of Cd in muscles of fish of AL-Hudaydah was 0.057µg/g (dry wt.); but, the highest concentration of Hg in the muscles of fish of Aden was 0.060µg/g (dry wt.). The concentration of As in muscles of fish had the same pattern of Pb and Cd, and the highest concentration of As in muscles of fish of AL-Hudaydah was 0.092µg/g (dry wt.), as summarized in Table 2 and Figure 3.

The highest concentration of Pb in muscles of fish of *E. areolatus* was 0.137µg/g (dry wt.); however, the highest concentration of Cd in muscles of fish of *E. areolatus* was 0.069µg/g (dry wt.); however, the highest concentration of Hg in muscles of fish of *E. areolatus* was 0.071µg/g (dry wt.); but, the highest concentration of As in the muscles of fish of *L. mahsena* was 0.106µg/g (dry wt.).

The highest concentration of Pb , Cd, Hg and As in the muscles of fish was 0.124 , 0.060, 0.065 and 0.104µg/g (dry wt.) respectively on Large fish ,as summarized in Table 3 and Figure 4.

Correlation analyses between concentration of heavy metals in the seawater and the fish

The relationship between metals level in seawater and fish are represented by correlation coefficient (r) in Table 4.

Table 4 shows the comparison the concentration of heavy metals in the Filtered Surface water and the fish in the same water, there was significant negative correlations between the concentration of Hg in the *L. mahsena* and Filtered Surface water and *S. jello* and Filtered Surface water, the p value in both association were similar ($p<0.01$), and at a level ($p<0.05$) with the same metal in the fish type *T. tonggol*, also has been significant negative correlations with lead metal Pb in a sample of seawater closely morally backward at a level ($p<0.01$) with

Table 3: The mean of Concentration µg/g (dry wt.) for lead, cadmium, mercury and Arsenic during the fish size in the muscles samples for the different fish species study.

Species	Metal ion	Sizes of fish			Total mean ± SD
		Large	Medium	Small	
Lethrinus mahsena	Pb	0.184± 0.030	0.120± 0.014	0.091± 0.020	0.132± 0.048
	Cd	0.065±0.005	0.050±0.003	0.033±0.027	0.049±0.016
	Hg	0.063±0.002	0.062±0.006	0.062±0.010	0.062±0.0005
	As	0.114±0.005	0.105±0.009	0.100±0.008	0.106±0.007
Thunnus tonggol	Pb	0.116±0.018	0.104±0.019	0.080±0.018	0.100± 0.018
	Cd	0.046±0.017	0.036±0.012	0.029±0.010	0.037±0.008
	Hg	0.036±0.008	0.030±0.005	0.023±0.003	0.030±0.006
	As	0.100±0.009	0.067±0.007	0.045±0.002	0.071±0.028
Sphyraena jello	Pb	0.046±0.006	0.035±0.008	0.029±0.006	0.037± 0.009
	Cd	0.040±0.008	0.028±0.006	0.020±0.003	0.029±0.010
	Hg	0.078±0.008	0.072±0.006	0.056±0.010	0.069±0.011
	As	0.103±0.006	0.089±0.006	0.077±0.008	0.090±0.013
Epinephelus areolatus	Pb	0.152±0.008	0.134±0.013	0.125±0.012	0.137± 0.014
	Cd	0.088±0.027	0.073±0.025	0.047±0.020	0.069±0.021
	Hg	0.083±0.009	0.072±0.007	0.058±0.007	0.071±0.012
	As	0.099±0.008	0.088±0.004	0.078±0.004	0.088±0.010
Total mean ± SD	Pb	0.124± 0.059	0.098± 0.044	0.081± 0.040	0.101±0.046
	Cd	0.060±0.022	0.047±0.020	0.032±0.011	0.046±0.017
	Hg	0.065±0.021	0.059±0.020	0.050±0.018	0.058±0.019
	As	0.104±0.007	0.087±0.016	0.075±0.023	0.089±0.014

Table 4: Correlation analyses between concentration of heavy metals in the seawater and the fish in the same water.

Species		Pb Seawater	Cd Seawater	Hg Seawater	As Seawater
Lethrinus mahsena	R	-0.181	0.034	-0.371**	0.342**
Thunnus tonggol	R	-0.037	-0.016	-0.223*	0.305**
Sphyraena jello	R	-0.294**	0.049	-0.299**	0.499**
Epinephelus areolatus	R	-0.123	-0.028	-0.027	0.357**

*Significant correlation (*P<0.05*); **Significant correlation (*P<0.01*).

the same metal in one type of fish is *S. jello*, also, the concentration of As among four types of fishes (*L. mahsena*, *T. tonggol*, *S. jello* and *E. areolatus*) was also has significant positive correlations to the concentration of same metal in the Filtered Surface water Which are all ($p<0.01$).

Correlation analyses between metals in Muscles

The relationship between metals level in Muscles tissue are represented by correlation coefficient (r) in Table 5.

Notes from the Table 5 above for the province of Aden and the presence of significant positive correlations at the level of ($p<0.01$) between the Pb in fish muscle tissue with Cd in the same Muscle tissue, also has been associated with Cd morally significant positive correlations at the level of ($p<0.01$) with As in the same Muscle tissue, and has been associated with Hg significant positive correlations morally at a level ($p<0.01$) with As in the same Muscle tissue.

In Al-Hudaydah site, showed a significant positive correlations at a level ($p<0.01$) between the Pb in fish muscle tissue with Cd and at a level ($p<0.05$) with As in the same Muscle tissue, also it has been associated with Cd closely morally significant positive correlations at the level of ($p<0.05$) with Hg and As Hg in the same fabric, and has been associated with Hg significant positive correlations morally at a level ($p<0.01$) with As in the same Muscle tissue.

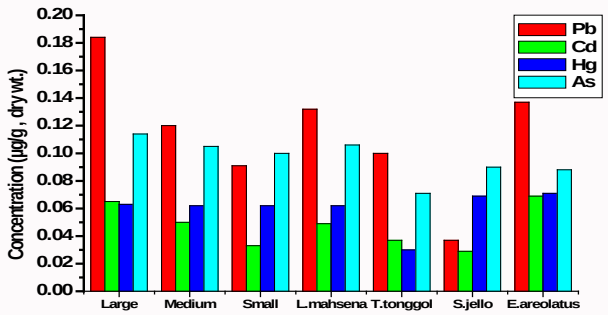

Figure 4: The mean of Concentration µg/g (dry wt.) for lead, cadmium, mercury and Arsenic during the fish size in the muscles samples for the different fish species study.

Discussion

Heavy metals in surface seawater

Overall, the results of the present study showed that they were significant differences (P<0.01), using one way ANOVA, regarding the concentration of Pb, Cd, Hg and As in the filtered surface water of Yemen coast (Aden, AL-Hudaydah and AL-Mukalla) sites, except As in Aden and AL Mukallala, and except Pb and Cd in AL-Hudaydah, for the period of seasons: winter 2011, summer 2012 and winter 2013 (Table 1).

On the other hand, thus results were analyzed by using two ways ANOVA, there were significant differences (P<0.01), regarding

Table 5: Correlation analyses between metals in Muscles tissue.

Site		Pb	Cd	Hg	As
Aden	Pb	1	-	-	-
	Cd	0.673**	1	-	-
	Hg	0.133	0.325	1	-
	As	0.208	0.454**	0.771**	1
AL- Hudaydah	Pb	1	-	-	-
	Cd	0.624**	1	-	-
	Hg	-0.046	0.390*	1	-
	As	0.399*	0.339*	0.472**	1
AL- Mukalla	Pb	1	-	-	-
	Cd	0.491**	1	-	-
	Hg	-0.072	0.494**	1	-
	As	0.226	0.132	0.383*	1

*Significant correlation (*P<0.05*); **Significant correlation *(P<0.01*).

the concentration of Cd and Hg, however, there was a significant difference (P<0.05) regarding the concentration of As in the filtered surface water of Yemen sites for the period of seasons: winter 2011, summer 2012 and winter 2013.

The highest concentration of Pb, Cd and As were (0.065± 0.027, 0.008±0.002 and 0.0083±0.002mg/L, respectively) in the filtered surface water of Yemen coast was obtained in the summer, whereas the highest concentration of Hg was 0.007±0.002mg/L in the filtered surface water of Yemen coast was achieved in winter (Table 1).

This result may be explained by the fact that amount of draining sewage on summer were higher compared with winter and also due to high water temperature on summer season.

The interpretation of these results is comparable to those reported by [20] reported that the concentrations of metals are increased during summer due to increase the water temperature; and [21] reported that the high heavy metal concentration during the summer may be attributed to increased water temperature during the summer that may result in increased metal toxicity. Also, Hg has a different character than Pb, Cd and As and has able to evaporate in the air; As supported also by [22] who pointed out that the distribution of Hg was different to the other heavy metals due to Hg is easy to vaporize and to move from one place to another.

Also the above results and the initial interpretation given are comparable to those reported in 2014 by [23] *et al*; showed that the highest concentration of Pb in summer, but the highest concentration of Hg in the filtered surface water was from Khawr-Mukalla, Hadhramout Coast, Yemen, in Autumn [23].

The results of the present study were analyzed by using two ways ANOVA in filtered surface water of Yemen sites during the seasons, it showed that there were significant differences (P<0.01) regarding the concentration of Pb, Hg and As, however, there was a significant difference (P<0.05) regarding the concentration of Cd in the filtered surface water of Aden, AL-Hudaydah and AL-Mukalla station.

The highest concentration of Pb was 0.080± 0.008 mg/L achieved in AL-Hudaydah, however, the highest concentration of Cd was 0.008±0.002 mg/L achieved in Aden, but the Hg was 0.0075±0.001mg/L and As was 0.010±0.000mg/L in the filtered surface water were found in AL-Mukalla.

The CSBTS [24], ANZECC and ARMCANZ [25] and ASEAN [26] guidelines for maximum permissible limit of Lead in Seawater is 0.001, 0.0044 and 0.0085mg/l. As the range of Lead detected was higher than the permissible limit.

There are several possible explanations for this result perhaps attributed to partially caused also by atmospheric input of local particulates from motor vehicle, mountainous regions which drain its water from Yemen highland to the Red sea through different vallies, precipitation, petroleum rich substrate of the area, influence of sewage discharge, agricultural and industrial effluents into this site, chemical distribution and partitioning between seawater and the sediment and the vigorous mixing of shallow coastal sediments increases the solubility of Pb in seawater as a result of oxygen saturated water [27-30].

The CSBTS [24] and ANZECC and ARMCANZ [25] guidelines for maximum permissible limit of Cadmium in Seawater is 0.001 and 0.0007mg/l. As the range of Cadmium detected was higher than the permissible limit. But ASEAN [26] guidelines for maximum permissible are limit of Cadmium in Seawater is 0.01mg/l. As the range of Cadmium detected is below than the permissible limit.

These high concentrations of Cd in Aden may be attributed to point source and non-point source pollution among which are PVC products, runoff from waste Ni-Cd batteries, paint, color pigments and solid waste [31].

These results further support the idea of Scrap-iron store at Labour Island in Aden site is the most likely source of Pb and Cd in the Seawater [32].

Significant positive correlations (Table 4) between the concentrations As in each type of fish and As in the surrounding sea water were obtained. The following correlations were determined from the content of As in *E. areolatus*, 0.357. This leads to the hypothesis of preferential as uptake from the water column.

The positive correlations presented in our data (table 4), recorded between the levels of As in the seawater with As contents in tissues of the fish may be explained by the high concentration of As detected in these tissues [30].

The CSBTS [24], ANZECC and ARMCANZ [25] and ASEAN [26] guidelines for maximum permissible limit of Mercury in Seawater is 0.00005, 0.0001 and 0.00016mg/l. As the range of Mercury detected was higher than the permissible limit.

The [24] guidelines for maximum permissible limit of Arsenic in Seawater is 0.020mg/l. As the range of Arsenic detected is below than the permissible limit.

The present high concentration of Hg and As in AL-Mukalla may be due to the petroleum rich substrate of the area, Oil pollution and atmospheric fallout could be responsible for the increased levels, also high values of As in the site may be attributed to agriculture.

Also the above results and the initial interpretation given are comparable to those reported by [30] pointed out that the concentration of Pb was 0.03±0.004mg/L and Cd was 0.02±0.004mg/L in summer from along the coast of Al-Shaykh Younes facing AL-Hudaydah city, Yemen [30].

The interpretation of these results is comparable to those reported by [23] pointed out that the concentration of Pb was 0.058-0.132mg/

L, Cd was 0.014-0.030mg/L, Hg was 0.005-0.008mg/L in Khawr-Mukalla, Hadhramout Coast, Yemen. This differs from the findings presented here may be attributed to drain sewage at first time into Khawr-Mukalla and non-coastal currents [23] and a good agreement with those are found by [33] pointed out that the concentration of Pb was 0.064-0.082mg/L, Cd was 0.002-0.005mg/L in Jeddah Coast, Saudi Arabia.

However, our results are in a good agreement with those found by [34] who reported that the concentration of Hg of filtered surface seawater was 0.002-0.005mg/L in the Langkawi Island, Malaysia [34]. Besides [35], pointed out that the concentration of Pb was 0.03-0.07mg/L, which is below the permissible limit of 0.1mg/L set for inland surface water, in the water samples collected from sea water in Międzyzdroje, Baltic coast, Poland.

However, our results were low concentration compared with other studies which mentioned by [34] who reported that the concentration of Pb of filtered surface seawater was 1.58-4.73mg/L and Cd was 0.01-0.02mg/L in the Langkawi Island, Malaysia [34]. Besides [36], pointed out that the concentration of Pb was 1.21mg/L, Cd was 0.04mg/L in Isalmic Port Coast, Red Sea, Jeddah, Saudi Arabia [36]. Besides [37], pointed out that the concentration of Pb was 0.065mg/L, Cd was 0.044mg/L in northern Delta Lakes, Egypt [37]. Besides [22], pointed out that the concentration of Pb was 0.61mg/L, Cd was 0.92mg/L and Hg was 0.030mg/L in Jinzhou bay, China [22]. Besides [35], pointed out that the concentration of Cd was 0.39-0.52mg/L, the values obtained were found to be below the permissible limit of 2.0mg/L set for inland surface water. Hg was 0.03-0.05mg/L which was very much above the maximum limit of 0.01mg/L set for inland surface water in the water samples collected from sea water in Międzyzdroje, Baltic coast, Poland.

Based on these information's, Yemen coast of the present study is low polluted compared with other locations.

Heavy metals in Muscles fish

Overall, the present study showed that there were no significant differences (P>0.05), using one way ANOVA regarding the concentration of Pb, Cd, Hg and As in muscles of four commercially important fish species, *Lethrinus mahsena*, *Thunnus tonggol, Sphyraena jello* and *Epinephelus areolatus* (Table 2) throughout the seasons: winter 2011, summer 2012 and winter 2013 at Yemen coast (Aden, Al-Hodeidah and AL-Mukalla) except Cd in Aden and Al-Mukalla sites there was significant difference (P<0.01).

On the other hand, these results of the present study were analyzed by using two ways ANOVA in muscles of fish of Yemen sites during the seasons, it showed that there were not significant differences (P>0.05) regarding the concentration of Pb, Hg and As, however, there was significant difference (P<0.01) regarding the concentration of Cd in the muscles of fish of Yemen sites for the period of seasons: winter 2011, summer 2012 and winter 2013.

The highest concentration of Cd was 0.054±0.026µg/g (dry wt.) in muscles of fish species studied was on summer.

The present high concentration of Cd in summer may be attributed to various factors as sewage, increase in the water temperature during summer increases heavy metals uptake in the fishes as compared to the winter season, higher metabolic rate in the fishes, migratory patterns of fishes, concentration, exposure time, route of metal uptake and feeding habits [20,23].

In addition, seasonal changes in feeding rate, in gaining or losing weight, or in synthesis of metal binding proteins, are all responsible for storage of the metals [38].

The interpretation of these results is comparable to those reported by [23] showed that the highest concentration of Cd in muscles of *M. seheli* was found on summer and the lowest concentration was obtained on winter who suggested that the amount of draining sewage on summer were higher compared with other seasons as a result of using water in this season by population of Mukalla city as well as seawater are more mixing by moving current (upwelling) in autumn that lead to dispersion the metals in seawater column. This suggestion also supported by environmental data for the high temperature in the summer at Khor al-Mukalla [23].

These results are in accord with recent studies indicating that heavy metal content of fish tissues indicate the influence of season as one source of variations or fluctuations on the metal concentration [39,40]. Seasonal fluctuations of metals might be resulted from different factors such as growth and reproductive cycles and changes in water temperature [39,41].

According to this study the muscles of fish usually accumulates higher levels of Cd was (0.054±0.026µg/g dry wt.) at summer. This result was nearly similar to other studies conducted in Turkey (0.060±0.000µg/g dry wt.) [42]. Also, in Turkey (0.01-0.08(0.03±0.01) µg/g dry wt.) [43].

On the other hand, our results illustrated high concentration compared with other studies which mentioned by [23] showed that the concentration of Cd was 0.01±0.002µg/g (dry wt.) in muscles of fish, Blue spot mullet, Moolgardaseheli from Khawr-Mukalla, Hadhramout Coast, Yemen, at summer [23].

These high differences between the results may reference to various factors such as temperature, migratory patterns of fishes, concentration exposure time, route of metal uptake and feeding habits.

It is assumed that the presence of the area near the strait of Bab Al-Mandab, where the water coming from both the Red Sea and the Indian Ocean mixes and consequently changes its nature, especially during the seasonal monsoons, is responsible mainly for elevated tissue Cd concentrations in the Muscles fish inhabiting the area. However, some sewage outflow can be additional anthropogenic sources of Cd in the Aden and Al- Hodiedah.

Overall, the results of the present study showed that they were significant differences (P<0.01), using one way ANOVA, regarding the concentration of Pb, Cd, Hg and As in the muscles of four commercially important fish species of Yemen coast (Aden, AL-Hudaydah and AL-Mukalla) sites, for the duration of the four types of fish studied: *Lethrinus mahsena*, *Thunnus tonggol, Sphyraena jello* and *Epinephelus areolatus*.

The highest concentration of Pb, Cd and Hg were 0.137±0.014, 0.069±0.021 and 0.071±0.012µg/g (dry wt.), respectively) in muscles of fish of *Epinephelus areolatus*; but, the highest concentration of As was 0.106±0.007µg/g (dry wt.) in the muscles of fish of *Lethrinus mahsena*, as summarized in Table 3.

The present high concentration of Pb, Cd and Hg in *E. areolatus* and high concentration of As in *L. mahsena* have been usually attributed to their habitat and feeding behavior. *E. areolatus* and *L.*

mahsena tend to be near the sediment region [444,45].

A possible explanation for this might be due to lipid content in the tissue and excretion percentage of these toxic metals from their body [46].

This result may confirm previous studies of several authors who reported that *E. areolatus* usually accumulates higher levels of heavy metals than other species [47-49].

In another study in Bahrain [50], found higher levels of Hg in muscles of *E. areolatus* than nine fish species from the manama city of Bahrain [50]. And [47] found higher levels of Hg in muscles of *E. areolatus* than other fish species from the Lihir Islands group is in Papua New Guinea's New Ireland [47].

These results are in agreement with [41] attributed the Trace element burden in aquatic inhabitants is associated with biological and ecological factors such as feeding habits and habitat, respectively [51-53]. Regarding to their habitat, demersal fish species had the highest muscular arsenic 0.481±0.307µg/g of wet weight concentrations.

There is strong correlation (Table 5) between Cd and Pb in Muscles (at Aden, r = 0.673; at Al-Hudaydah, r = 0.624; at Al-Mukalla, r = 0.491) . Associations between metals are important, as they determine the bioavailability and potential toxicity to fish in an aquatic system [54].

The interpretation of these results are comparable to those reported by [55] showed that the concentration of Pb was 0.10µg/g (dry wt.) in muscles of *Epinephelus tauvina* During summer in Red Sea, AL-Hudaydah, Yemen [55]. Also the above results and the initial interpretation given are comparable to those reported in 2010 by [56] showed that the concentration of Cd was 0.029µg/g (wet wt.) in muscles of *Epinephelus areolatus* from the Red Sea, Jeddah Coast, Saudi Arabia [56].

The obtained results for lead and Cadmium and their interpretation are comparable to those reported by [57] showed that the concentration of Pb was 0.127- 0.188µg/g (dry wt.) and Cd was 0.052µg/g (dry wt.) in muscles of *Epinephelus areolatus* from the Red Sea, Jeddah coast, Saudi Arabia [57].

However, our results are in a good agreement with those found by [58] showed that the concentration of Pb was 0.100±0.09µg/g (dry wt.) in muscles of *Epinephelus areolatus* from Tuticorin, India [58].

Besides [59], showed that the concentration of As was 0.25-0.9µg/g (dry wt.) and Hg was 0.08-0.2µg/g (dry wt.) in muscles of *L. mahsena* from Arabian Gulf Along the Eastern Coast, Saudi Arabia [59,60]. Pointed out that the concentration of Pb was 0.20 ± 0.02µg/g (dry wt.), As was 0.11±0.02µg/g (dry wt.), in *L. mahsena* from Red Sea, Egypt [60,61]. Pointed out that the concentration of Pb was 0.118-0.193µg/g (wet wt.), Cd was 0.013- 0.023µg/g (wet wt.), Hg was 0.012-0.184µg/g (wet wt.) in tuna fish Hadhramout Coast Yemen [61,62]. Pointed out that the concentration of Pb was 0.12±0.21µg/g (dry wt.), in *L. mahsena* from Red Sea of Al-Cornish Hodeidah Yemen [62,63]. Pointed out that the concentration of Cd was 0.09 ± 0.010µg/g (wet wt.), in *T. albacares* from Aden Yemen [63,64]. Pointed out that the concentration of Pb was 0.10 - 2.10µg/g (dry wt.), Cd was 0.06-1.06µg/g in *L. mahsena* from Jeddah Coast, Saudi Arabia [64].

However, our results were low concentration compared with other studies which mentioned by [62] showed that the concentration of Pb was 1.66±0.30-2.23±0.93µg/g (dry wt.) and Cd was 0.33±0.03-0.46±0.22µg/g (dry wt.) in muscles of *Epinephelus sexfasciatus* during winter in Red Sea, AL-Hudaydah, Yemen [62].

Besides [55], showed that the concentration of Cd was 0.50µg/g (dry wt.) in muscles of *Epinephelus tauvina* during summer in Red Sea, AL-Hudaydah, Yemen, who suggested that higher Cd concentrations might be either the fat content, or the diet consumed by this fish [55].

These high differences between the results may reference to their habitat and feeding behavior. *E. areolatus* and *L. mahsena* tend to be near the sediment region, as well as the metal content in food and the bioconcentration capacity of each species [38,65]. And location in relation to prey high in As from anthropogenic sources [66].

In general, cadmium residues in fish muscle cannot be related to concentrations in water [67]. And no such correlations (Table 3-4) were found in this study.

Significant positive correlations (Table 4) between the concentrations As in each type of fish and As in the surrounding sea water were obtained. The following correlations were determined from the content of As in *L. mahsena*, 0.342.

This leads to the hypothesis of preferential As uptake from the water column.

The results in the present study were analyzed by using two ways ANOVA in muscles of fish of Yemen sites during the seasons, it showed that there were significant differences ($P<0.01$) regarding the concentration of Pb and Cd in the muscles of fish of Aden, AL-Hudaydah and AL-Mukalla sites.

The highest concentration of Pb and Cd were 0.138±0.073 and 0.057±0.034µg/g (dry wt.), respectively achieved in AL-Hudaydah.

The present high concentration of Pb and Cd in AL-Hudaydah may be due to the geochemical nature of beach deposits and anthropogenic activities [68,69]. Feeding behavior of the fish species, fat content, the in their diet uptake [55,68].

The interpretation of these results are comparable to those reported by [62] showed that the concentration of Pb was 0.12±0.21-3.05±0.62µg/g (dry wt.) and Cd was 0.18±0.54-0.64±0.28µg/g (dry wt.) in muscles of five fish species collected from Red Sea, Hodeidah City, Yemen. Who suggested that higher Cd concentrations might be this confirms that Al-Mena (AL-Hudaydah) site is affected by industrial waste discharge taking place in that area, exposed to more pollutants and economic activities are very high at this site. Fishes and water quality within the area are influenced by sewage and oil spill from ships [62].

Also the above results and the initial interpretation given are comparable to those reported in 2014 by [55] showed that the concentration of Pb was 0.10 - 0.85µg/g (dry wt.) in muscles of greasy grouper (*Epinephelus tauvina*) and striped mackerel (*Rastrelliger kanagurta*) from Al-Hodeidah, Red Sea coast of Yemen [55].

The obtained results for lead and Cadmium and their interpretation are comparable to those reported in 2002 by [69] showed that the concentration of Pb was 0.03 ± 0.01- 0.25 ± 0.09µg/g (dry wt.) and Cd was 0.03±0.02 - 0.13±0.04µg/g (dry wt.) in muscles of different fish species in the Red Sea of Yemen [69].

However, our results are in a good agreement with those found by [65] pointed out that the concentration of Pb was 0.08±0.02µg/g (dry wt.) and Cd was 0.04±0.00µg/g (dry wt.) in *Lethrinus mahsena*

from Eastern Taiwan [65,70]. pointed out that the concentration of Pb was 0.25±0.07µg/g (dry wt.) and Cd was 0.05±0.01µg/g (dry wt.) in *Lethrinus sp*. from Red Sea, Egypt [70,71]. Pointed out that the concentration of Pb was 0.17±0.05µg/g (dry wt.) and Cd was 0.02±0.02µg/g (dry wt.) in Thunnus albacares from Abidjan [71,72]. Pointed out that the concentration of Pb was 0.07±0.03-0.13±0.08µg/g (dry wt.) and Cd was 0.06 ±0.01µg/g (dry wt.) in *Thunnus thynnus* from Korea [72,73]. Pointed out that the concentration of Pb was 0.113±0.035µg/g (dry wt.) and Cd was 0.049±0.024µg/g (dry wt.) in Sphyraena jello from Iran [73].

Some of these values are small compared with the obtained readings in the current study, however, not be surprising because the marine environment of the ocean is generally less polluting for the sea that are surrounded by industrialized countries, or that are over a lot of ships, such as the Gulf of Aden line.

However, our results showed low concentration compared with other studies which mentioned by [55] illustrated that the concentration of Cd was 0.30-0.50µg/g (dry wt.) in muscles of greasy grouper (*Epinephelus tauvina*) and striped mackerel (*Rastrelliger kanagurta*) from Al-Hodeidah, Red Sea coast of Yemen [55].

Besides [68], showed that the concentration of Cd was 0.60µg/g (dry wt.), in muscles of three Commercial Fish Species from Al Hudaydah, Red Sea Coast of Western Yemen [68].

Besides [74], showed that the concentration of Pb was 1.95-3.80µg/g (dry wt.) in muscles of different types of fish from Al Hudaydah, Yemen [74,75]. Showed that the concentration of Pb was 2.95- 3.13µg/g (dry wt.) and Cd was 0.14-2.56µg/g (dry wt.) in muscles of different types of fish from Al Hudaydah, Yemen [75,76]. Showed that the concentration of Pb was 1.60- 3.80µg/g (dry wt.) and Cd was 2.40-3.60µg/g (dry wt.) in muscles of different types of fish from Al Hudaydah, Yemen [76]. However, when comparing these results with previous studies in other areas, we found that our results were low concentration compared with other studies which mentioned by [36] pointed out that the concentration of Pb was 6.10µg/g (dry wt.) and Cd was 1.06µg/g (dry wt.) in *Lethrinus mahsena* from Red Sea at Jeddah Isalmic Port Coast, Saudi Arabia [36,77]. Pointed out that the concentration of Pb was 0.8±0.3µg/g (dry wt.) and Cd was 0.55 ± 0.2µg/g (dry wt.) in *L. mahsena* from Arabian Gulf Coast at the Eastern Province, Saudi Arabia [77,78]. Pointed out that the concentration of Pb was 0.53±0.08µg/g (dry wt.) and Cd was 0.16±0.11µg/g (dry wt.) in *T. tonggol* from Saudi Arabia [48,78] pointed out that the concentration of Pb was 5.0-5.6µg/g (dry wt.) and Cd was 0.9-1.0µg/g (dry wt.) in *Epinephelus fasciatus* from Jordan [48,79]. Pointed out that the concentration of Cd was 1.83µg/g (dry wt.) in *Sphyraena sphyraena* from Mediterranean Sea, Italy [79].

The high different between the results, may reference to various factors as the geochemical nature of beach deposits and anthropogenic activities, feeding behavior of the fish species, fat content, the in their diet uptake.

Based on this information, Yemen coast in the present study is low polluted when it is compared with other locations.

Also Overall, the results showed that there were significant differences ($P<0.01$), using two way ANOVA, regarding the concentration of Pb, Cd, Hg and As, in the muscles of fish in the muscles of Four types of fish studied of Yemen coast (Aden, AL-Hudaydah and AL-Mukalla) stations, for the period of size: Large, Medium and Small.

The highest concentration of Pb, Cd, Hg and As were 0.124±0.059, 0.060±0.022, 0.065±0.021 and 0.104±0.007µg/g (dry wt.), respectively in muscles of fish, were observed to be more concentrated in the larger sizes of fish, as shown in Table 3.

The present high concentration of Pb, Cd, Hg and As in the larger sizes of fish may be attributed to various factors as large fish that prey upon smaller fish can accumulate more of the chemical in their bodies. It is better to eat the smaller fish within the same species, the strong affinity of metallothionine protein with these elements. This is usually more pronounced in bigger fishes [80-84].

Many studies have recognized size as an influencing factor into the amount of concentration accumulated by fish [85-87].

Increase in body mercury level with fish size is probably related to the affinity of this metal to the muscle tissue [88,89].

The high concentrations of heavy metals in the surrounding water could result in continued metal accumulation in fish and increase of metal concentrations with fish size [90,91]. Metals like cadmium is low in juvenile stages but it are accumulated with age. The size and maturity stage of the fishes influence its accumulation levels. Cd concentration in the muscle tissue is found to increase with the size of the fishes [92].

The positive correlations presented in our data (Table 5) between specific heavy metal pairs in the Muscles indicated that these metals had the same distribution characteristics or may reflect similar levels of contamination and/or release from the same source of pollution [93,94].

Conclusions

Heavy metal concentration in Seawater samples shows that high concentration of Lead is found more At Site AL- Hudaydah (0.080± 0.008mg/l) in summer 2012, Site AL- Hudaydah is polluted highly. Lead content in all locations in all seasons was higher than the permissible limits according to international standards.

The Cadmium in Seawater in the Summer season shows that Site Aden is highly polluted in Summer 2012 with 0.008±0.002mg/l. As the range of Cadmium detected is below than the permissible limit.

The Arsenic concentration was found high at Site AL- Mukalla, 0.010±0.000mg/l in Year 2012 (summer season). As the range of Arsenic detected is below than the permissible limit.

The Mercury concentration was found high at Site AL- Mukalla, 0.0075±0.001mg/l in Year 2011 (winter season). Mercury content in all locations in all seasons was higher than the permissible limits according to international standards.

Four species of fish *Lethrinus mahsena*, *Thunnus tonggol, Sphyraena jello* and *Epinephelus areolatus* was examined and Lead, Cadmium, Mercury and Arsenic concentration.

The highest mean concentration of Pb in the muscles of the four studied fish species was 0.137± 0.014µg/g dry wt in large *Epinephelus areolatus* At Site AL- Hudaydah.

The WHO and Standard Specification for Yemen (2006) guidelines for prescribed maximum permissible limit of Lead in Muscles Fish are 0.2µg/g dry wt and 1.0µg/g dry wt. As the detected Lead remained below the WHO and Standard Specification for Yemen (2006) permissible limits.

In Summer, the highest mean concentration of Cadmium in the muscles of the four studied fish species were 0.069±0.021µg/g dry wt in large *Epinephelus areolatus* in Year 2012 at Site AL- Hudaydah.

The WHO and Standard Specification for Yemen (2006) guidelines for prescribed maximum permissible limit of Cadmium in Muscles Fish are 1.0µg/g dry wt and 0.2µg/g dry wt. As the detected Cadmium remained below the WHO and Standard Specification for Yemen (2006) permissible limits.

The highest mean concentration of Hg in the muscles of the four studied fish species was 0.071±0.012µg/g)dry wt(in *Epinephelus areolatus* (at large) at Site Aden.

The WHO and Standard Specification for Yemen (2006) guidelines for prescribed maximum permissible limit of Mercury in Muscles Fish are 0.50µg/g dry wt. As the detected Mercury remained below the WHO and Standard Specification for Yemen (2006) permissible limits.

The highest mean concentration of As in the muscles of the four studied fish species was 0.106±0.007 in *Lethrinus mahsena* (at large) at Site AL- Hudaydah.

The WHO and Standard Specification for Yemen (2006) guidelines for prescribed maximum permissible limit of Arsenic in Muscles Fish are 0.10µg/g dry wt and 1.0µg/g dry wt. As the detected Arsenic remained below the WHO and Standard Specification for Yemen (2006) permissible limits.

Therefore it is recommended that the practice of trace element detection should be continued in order to update whether the heavy metal concentration is above or below the permissible limits and if it is above the limit then precautions must be taken to avoid possible consumption of contaminated eatables. It is also recommended that awareness should be spread among the people regarding the hazards on consumption of polluted water and related eatables.

It is also essential that farmers should be educated to reduce such contamination and should be encouraged to use the controlled amount of pesticides, to avoid the leaching of waste water and cultivating in a field far away from industrial area as well as areas prone to contamination.

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