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RESEARCH ARTICLE

ANALYTICAL ENVIRONMENTAL STUDY ON THE POLLUTION EFFECT OF HEAVY METALS IN SOME TYPES OF THE FISH IN YEMEN

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ABSTRACT:

The fish samples were collected from the three different Cities of Yemeni coasts. Aden, Al-Hodeidah and AL-Mukalla were chosen for the sample collection. Lethrinusmahsena, Thunnustonggol, Sphyraenajello and Epinephelusareolatus fish samples were considered for the study as they are more common eatable fish among the population. The study was carried out in the all three seasons of winter 2011, summer 2012 and winter 2013 in order to check seasonal variation of heavy metal pollution. Total Fish (108 samples of each muscles, liver and gills) were analyzed. The four heavy metals lead, Cadmium, Mercury and arsenic which are considered highly toxic were detected in the samples in the year 2010, 2012 and 2013.

The mean concentration of Pb, Cd, Hg and as in muscle was 0.101 ± 0.012 , 0.046 ± 0.010 , 0.058 ± 0.002 and $0.089 \pm 0.002 \mu\text{g.g}^{-1}$ dry wt. respectively; whereas in liver was 0.196 ± 0.033 , 0.132 ± 0.020 , 0.102 ± 0.007 and $0.115 \pm 0.0005 \mu\text{g.g}^{-1}$ dry wt. respectively; whereas in gill was 0.294 ± 0.042 , 0.196 ± 0.063 , 0.016 ± 0.0006 and $0.034 \pm 0.0005 \mu\text{g.g}^{-1}$ dry wt. respectively.

The results showed that, the heavy metals concentrations were high in stations AL- Hodaydah and AL- Mukalla and low in station of Aden. Also the heavy metals concentrations were high in Seasons summer and low in Seasons winter.

From the heavy metal concentrations mentioned above we can see that somewhere the concentration is crossing the limits as permissible by the World Health Organization. It suggests a high risk to the health of human being on the consumption of contaminated fish. 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.

Keywords: Fish samples, heavy metals, Yemeni coasts, seawater.

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INTRODUCTION

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 environments¹. Fishes have been found to be good indicators of the heavy metal contamination levels in the aquatic systems because they occupy different trophic levels². According to² there are two main routes of heavy metals exposure:

1. 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.

2. The secondary route is through the intestine by food or sediment particles with subsequent transport across the gut.

The food may also be important source for heavy metal accumulation in fish³. In aquatic ecosystem, metals are transferred to the fish through food chain that could ultimately affect the health of people consuming this fish. 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 fish⁴. 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 reduceability to growth and reproduce⁵.

Bioaccumulation is the incorporation and retention of metals by organisms from their surrounding environment⁶.⁷ states that aquatic organisms bioaccumulate trace elements in considerable amounts which may stay in the organism over a long period of time⁷. According to⁸ metals can have the following effects on fishes: (i) act as mutagenic or genotoxic compounds; (ii) increased metal concentrations can change xenobiotic metabolic pathways and (iii) can affect various metabolic activities such as glycolysis, amino acid- and carbohydrate metabolism⁸.

The ability of aquatic organisms to digest heavy metals in the system determines the rate at which heavy metals bioaccumulate in aquatic organisms. Furthermore, the rate of bioaccumulation of heavy metals in aquatic organisms is determined by the concentration of metals in the aquatic system⁷, the feeding habits of the organism and the mode of exposure to heavy metals, which affects the amount of bioaccumulation in different tissues of organisms⁹. Gills and the liver are normally the prime sites for bioaccumulation of heavy metals⁹. Fishes are both situated at the top of the aquatic food chain. Crabs are typically benthic organisms and will give a better indication of the contamination of surface sediment¹⁰. Various studies have indicated a correlation of heavy metals in tissue of organisms and the size of the organism. Bigger organisms display higher bioaccumulation rates of heavy metals¹¹.

MATERIALS AND METHODS

2.1: Description of the study area

The Republic of Yemen lies on the southwestern tip (part) of the Arabian Peninsula bordering Saudi Arabia to the north and Sultanate of Oman to the east. It occupies an area of nearly 555000 square kilometers - excluding Al Ruba Al Khali Desert and has a sizeable coast line both to the Red Sea and to the Gulf of Aden/Arabian Sea (2500 km). Yemen has a large population (approximately 25.956 million in 2013, a high growth rate of approximately 3.7% per annum¹². Fish consumption is apparently increasing at the present time because of the large increases in the price of other alternative protein sources, such as beef, goats and chickens. Yemen's marine environment is characterized by a high level of productivity and fisheries are the second most important source of export revenues in Yemen after petroleum and play an important role in reducing poverty. Aden (on the northwestern side of the Gulf of Aden) AL-Hudaydah (on the southeastern side of the Red Sea) and AL-Mukalla (on the northeastern side of the Gulf of Aden) are the main coastal cities (Figure 1) with areas larger than others in Yemen.

For these problems and others, we chose this thesis to study the concentrations of heavy metals in the environment of Yemen coast (Aden, Al Hodeidah and Al Mukalla) sites, which is considered as the most

important indicator of the extent of the pollution. The study also included estimate of the heavy metals in selected tissues (muscles, liver and gills) of four commercially important fish species, *Lethrinusmahsena*, *Thunnustonggol*, *Sphyraenajello* and *Epinephelusareolatus*.

2.2: Sampling Stations

The trial were undertaken for the period of seasons: Winter 2011, Summer 2012 and Winter 2013, during which a total of 108 Sample of Muscles Fish, 108 Sample of Liver Fish and 108 Sample of Gills Fish were collected and analyzed.

Three stations were selected for sampling of large main coastal cities. Samples were collected from three stations. Aden city, overlooking the Gulf of Aden and Al Hodeidah, overlooking the Red Sea and Al Mukalla city, overlooking the Arabian Sea (Table 1).

2.3: Fish Species

To assess the health of the aquatic environment, aquatic organisms are usually used as biological monitors because they tend to accumulate pollutants from their environment and reflect the combined effects over a period of time¹³.

Study species are often chosen depending on the monitoring purpose and the information available on environmental contamination. If the information on pollutants is available, certain indicators could be chosen for a optimum use or cost.

In this study, the risk of trace metal exposure to humans consuming fish from the Yemen seas was an important question therefore fish were chosen for the study. In order to meet the requirements of monitoring species¹⁴. Table 2 shows the fish species used in the study.

This study examined trace metals in the muscle and the different fish tissues (liver and gill) of fish. Muscle was been chosen because it is the edible part and the result from measuring metals in muscle is used to assess the risk of trace metal exposure to humans consuming fish. liver and Gill, however, was chosen because the metal bioaccumulation in liver is relative higher than any other tissues in biota, so it can be a good environmental indicator of trace metal contamination¹⁵.

All collected organisms were weighed (wet wt.) and measured (total weight for fish and length) in Table (3) upon return to the laboratory. Characteristics (number of individuals, length and weight) of each of the 4 species collected are given in Table 3. This table also indicates the trophic level (i.e. grazer/ scavenger, predator of invertebrates, predator of invertebrates and small fish, predator of small fish) and the water-column distribution (benthic, nectobenthic and neritic) for every species.

While making a selection for the fish species to be taken for the present study, following criteria's were taken into consideration:

1. Edible status
2. Presence at all the selected sites
3. Popularity with the people of Yemen

Based on these criterias, four widely consumed fish species were selected viz. *Lethrinusmahsena*, *Thunnustonggol*, *Sphyraenajello* and

Epinephelusareolatus. Their common names in Yemen are Gahash, Zainoop, Kud and Khulkhul respectively.

2.4: Fish Sampling and Analysis

1. Fish Sampling

A total of 324 specimens of four commercially important fish species, *Lethrinusmahsena*, *Thunnustonggol*, *Sphyraenajello* and *Epinephelusareola us* 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 respectively¹⁴. After measurements, fish samples were washed with deionized water, sealed in polyethylene bags and kept in a freezer at -20°C until chemical analysis¹⁴.

2. 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, liver or gills) was accurately weighed and digested with 7ml of concentrated nitric acid (HNO₃ 65%) and 1ml of hydrogen peroxide (H₂O₂ 30%). Milestone Start Microwave Digestion Lab station with internal Temperature sensor and 260 terminal teach screen With HPR1000/10S High Pressure Segmented rotor (Application Note HPR-FO-07) and AOAC Official Method¹⁶ 999.10 and 974.14.

Microwave Program 2 Steps (1)15.00 Min (temperature 200) (2)15.00 Min (temperature 200).

After Finish left vessels 20 min until reach the room temperature, then the digested portion was diluted to a final volume of 50 ml using deionized water, before proceeding US EPA Method¹⁷ 3052.

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 ForPb, Cd, Hg and As Content.

i. 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 Method¹⁶ 999.10.

ii. Mercury and Arsenic analysis in fish tissues by Hydride analyzer

Cold Vapor Hg Analyzer (Buck Model 410), U.S.A Made, were used for analysis of Hg in fish tissue samples, Perfect for AOAC Official Method¹⁶ 974.14.

Arsenic Hydride Analyzer (Buck Model 411), U.S.A Made, were used for analysis of As in fish tissue samples, Perfect for EPA method¹⁸ 206.3.

Recovery studies were performed in order to establish the accuracy of the method. Recovery of the metals was determined by spiking one sample with increasing amounts of metal standard solution. The spiked samples were then taken through the same digestion procedure (as all other samples) and analyzed for

heavy metal concentrations. To assess the precision of the overall procedure, the samples were divided in batch of eight and for each batch; three replicate analyses of one of the samples were conducted. Analyses of all sample digests were performed in duplicate by the instrument. Certified Reference Material (CRM), DogFish (DORM-2) from the National Research Council, Canada was also included in quintuplicate.

STATISTICAL ANALYSIS

All heavy metals data (lead, cadmium and mercury) were analyzed and tested for differences between group means of stations and seasons for significance ($P \leq 0.05$) 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

3.1: Heavy Metals in different organs

3.1.1: Lead

The average concentration of Lead throughout all the three Sizes in Muscles Fish was 0.091 µg/g dry wt (At Small) to 0.184 µg/g dry wt (At large) for *L. mahsena* and from 0.080 µg/g dry wt (At Small) to 0.116 µg/g dry wt (At large) for *T. tonggol*. and from 0.029 µg/g dry wt (At Small) to 0.046 µg/g dry wt (At large) for *S. jello* and from 0.125 µg/g dry wt (At Small) to 0.152 µg/g dry wt (At large) for *E. areolatus*.

The average concentration of Lead throughout all the three Sizes in Liver Fish was 0.394 µg/g dry wt (At Small) to 0.456 µg/g dry wt (At large) for *L. mahsena* and from 0.115 µg/g dry wt (At Small) to 0.158 µg/g dry wt (At large) for *T. tonggol*. and from 0.043 µg/g dry wt (At Small) to 0.081 µg/g dry wt (At large) for *S. jello* and from 0.144 µg/g dry wt (At Small) to 0.174 µg/g dry wt (At large) for *E. areolatus*.

The average concentration of Lead throughout all the three Sizes in Gill Fish was 0.835 µg/g dry wt (At Small) to 0.853 µg/g dry wt (At large) for *L. mahsena* and from 0.120 µg/g dry wt (At Small) to 0.148 µg/g dry wt (At large) for *T. tonggol*. and from 0.052 µg/g dry wt (At Small) to 0.084 µg/g dry wt (At large) for *S. jello* and from 0.121 µg/g dry wt (At Small) to 0.144 µg/g dry wt (At large) for *E. areolatus*.

The high concentration of Pb (0.845± 0.009 µg/g dry wt.) was found in the Gill tissue of *L. mahsena* (Table 4), while in the lowest concentration of lead level (0.037±0.009 µg/g dry wt.) was detected in the muscle tissue of *S. jello* (Table 4). The mean concentration of Pb in the muscles, livers and gills of the four studied fish species varied from a minimum of 0.037± 0.009, 0.062±0.019 and 0.067±0.016 in *S. jello*, to a maximum value of 0.137±0.014; 0.428±0.031 and 0.845±0.009 in *E. areolatus*, *L. mahsena* (Table 4), (Fig. 2).

Table (5) shows the covariance analysis between mean concentration of Pb among in some types of fishes in

Yemeni seas and different variables. These different variables include; tissue, type and area of the fish.

After included the weight and length factors to show the related impact in the change of concentration of Pb to the three periods of time, the covariance analysis was not statistically significant in which the ($p < 0.01$). The mean concentration of Pb to the species of fish, among the tissues of the fish and among three different areas of fishing were also statistically significant with the p value are similar ($p < 0.01$), where the impact of these differences in concentration ratio of Pb were (37%, 21% and 6%) respectively.

Table (6) shows the mean concentration of Pb among the areas, type and the tissues of the fish. The concentration of Pb were concentrated at Between Al Mukalla and Aden, Al Hudaydah in which the mean concentration of Pb among fishes were (0.048 and 0.075) Towards Aden, Al-Hudaydah respectively, these results were statistically significant ($p < 0.01$).

The differences in the mean concentration of Pb among the different types of the fishes were found between *L. smahsen* and three other types and the mean concentration was in *T. tonggol* (0.446), *S. jello* (0.484) and *E. areolatus* (0.342), The direction of the type of fish *L. mahsena*, these difference was statistically significant ($p < 0.01$). Also found between *E. areolatus* and *S. jello* (0.143), The direction of the type of fish *E. areolatus*, these difference was statistically significant ($p < 0.05$).

According the concentration of Pb among the tissues of the fish was found among the muscles and liver (0.094), muscles and gill (0.193) The direction of the average lead concentration in the liver and Gill respectively, and liver and gill, (0.099) The direction of the average lead concentration in the Gill and these results were statistically significant ($p < 0.01$).

3.1.2: Cadmium

The average concentration of Cadmium throughout all the three Sizes in Muscles Fish was 0.091 $\mu\text{g/g}$ dry wt (At Small) to 0.184 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.029 $\mu\text{g/g}$ dry wt (At Small) to 0.046 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.020 $\mu\text{g/g}$ dry wt (At Small) to 0.040 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.047 $\mu\text{g/g}$ dry wt (At Small) to 0.088 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*. The average concentration of Cadmium throughout all the three Sizes in Liver Fish was 0.079 $\mu\text{g/g}$ dry wt (At Small) to 0.092 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.065 $\mu\text{g/g}$ dry wt (At Small) to 0.126 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.047 $\mu\text{g/g}$ dry wt (At Medium) to 0.073 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.277 $\mu\text{g/g}$ dry wt (At Medium) to 0.312 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*.

The average concentration of Cadmium throughout all the three Sizes in Liver Fish was 0.079 $\mu\text{g/g}$ dry wt (At Small) to 0.092 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.065 $\mu\text{g/g}$ dry wt (At Small) to 0.126 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.047 $\mu\text{g/g}$ dry wt (At Medium) to 0.073 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.277 $\mu\text{g/g}$ dry wt (At Medium) to 0.312 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*. The average concentration of Cadmium

throughout all the three Sizes in Gill Fish was 0.078 $\mu\text{g/g}$ dry wt (At Small) to 0.096 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.118 $\mu\text{g/g}$ dry wt (At Small) to 0.401 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.068 $\mu\text{g/g}$ dry wt (At Medium) to 0.083 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.333 $\mu\text{g/g}$ dry wt (At Medium) to 0.397 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*.

The high concentration of Cd (0.365 ± 0.032 $\mu\text{g/g}$ dry wt.) was found in the Gill tissue of *E. areolatus* (Table 4), while in the lowest concentration of Cadmium level (0.029 ± 0.010 $\mu\text{g/g}$ dry wt.) was detected in the muscle tissue of *S. jello* (Table 4). The mean concentration of Cd in the muscles, livers and gills of the four studied fish species varied from a minimum of 0.029 ± 0.010 , 0.061 ± 0.013 and 0.077 ± 0.008 in *S. jello*, to a maximum value of 0.069 ± 0.021 ; 0.289 ± 0.020 and 0.365 ± 0.032 in *E. areolatus* Table (4).

Table (7) shows the covariance analysis between mean concentration of Cd among in some types of fishes in Yemeni seas and different variables. These different variables include; time, tissue, type and area of the fish. After included the weight and length factors to show the related impact in the change of concentration of Cd to the three periods of time, the covariance analysis was statistically significant in which the ($p < 0.01$). The mean concentration of Cd to the species of fish, among the tissues of the fish and among three different areas of fishing were also statistically significant with the p value are similar ($p < 0.01$), where the impact of these differences in concentration ratio of Cd were (21%, 27% and 6%) respectively.

Table (8) shows the mean concentration of Cd among the areas, type and the tissues of the fish. The concentration of Cd were concentrated at Between Al Mukalla and Aden, Al Hudaydah in which the mean concentration of Cd among fishes were (0.048 and 0.075) Towards Aden, Al Hudaydah respectively, these results were statistically significant ($p < 0.05$).

The differences in the mean concentration of Cd among the different types of the fishes were found between *E. areolatus* and three other types and the mean concentration was in *L. mahsena* (0.173), *T. tonggol* (0.246) and *S. jello* (0.182), The direction of the type of fish *E. areolatus*, these difference was statistically significant ($p < 0.01$).

According the concentration of Cd among the tissues of the fish was found among the muscles and liver (0.086), muscles and gill (0.149) The direction of the average lead concentration in the liver and Gill respectively, and liver and gill (0.064) The direction of the average lead concentration in the Gill and these results were statistically significant ($p < 0.01$).

The focus of statistical significance in the ratio of the concentration of cadmium between time stages in 2011 differences than between 2012 and 2013 on the one hand and the differences towards the point in time in 2012 a difference in average (0.052) and stage time in 2013 a difference of (0.052) and at the level of statistical significance ($p < 0.01$).

3.1.3: Mercury

The average concentration of Mercury throughout all the three Sizes in Muscles Fish was 0.062 $\mu\text{g/g}$ dry wt

(At Small) to 0.063 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.023 $\mu\text{g/g}$ dry wt (At Small) to 0.036 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.056 $\mu\text{g/g}$ dry wt (At Small) to 0.078 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.058 $\mu\text{g/g}$ dry wt (At Small) to 0.083 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*. The average concentration of Mercury throughout all the three Sizes in Liver Fish was 0.093 $\mu\text{g/g}$ dry wt (At Small) to 0.063 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.098 $\mu\text{g/g}$ dry wt (At Small) to 0.149 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.043 $\mu\text{g/g}$ dry wt (At Small) to 0.074 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.106 $\mu\text{g/g}$ dry wt (At Small) to 0.141 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*. The average concentration of Mercury throughout all the three Sizes in Gill Fish was 0.011 $\mu\text{g/g}$ dry wt (At Small) to 0.016 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.015 $\mu\text{g/g}$ dry wt (At Small) to 0.025 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.016 $\mu\text{g/g}$ dry wt (At Small) to 0.022 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.009 $\mu\text{g/g}$ dry wt (At Small) to 0.011 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*. The high concentration of Hg (0.127 \pm 0.026 $\mu\text{g/g}$ dry wt.) was found in the livers tissue of *T. tonggol* (Table 4), while in the lowest concentration of Mercury level (0.010 \pm 0.001 $\mu\text{g/g}$ dry wt.) was detected in the gills tissue of *E. areolatus* (Table 4). The mean concentration of Hg in the muscles, livers and gills of the four studied fish species varied from a minimum of 0.030 \pm 0.006, 0.060 \pm 0.016 and 0.010 \pm 0.001 in *T. tonggol*, *S. jello* and *E. areolatus*, to a maximum value of 0.071 \pm 0.012; 0.127 \pm 0.026 and 0.020 \pm 0.005 in *E. areolatus*, *T. tonggol* (Table 4).

Table (9) shows the covariance analysis between mean concentration of Hg among in some types of fishes in Yemeni seas and different variables. These different variables include; tissue and type of the fish. After included the weight and length factors to show the related impact in the change of concentration of Hg to the three periods of time and area, the covariance analysis was not statistically significant in which the ($p>0.05$).

The mean concentration of Hg to the species of fish, among the tissues of the fish were also statistically significant with the p value are similar ($p<0.01$), where the impact of these differences in concentration ratio of Hg were (72%, 13%) respectively, and not statistically significant in the among three different areas of fishing. Table (10) shows the differences in the mean concentration of Hg among the different types of the fishes were found between *L. mahsena* and *T. tonggol*, *T. tonggol* and *E. areolatus*, *S. jello* and *E. areolatus* (0.030), (0.040) and (0.030) respectively, The direction of the type of fish *L. mahsena* and *E. areolatus*, these difference was statistically significant ($p<0.01$).

According the concentration of Hg among the tissues of the fish was found among the muscles and liver (0.043), muscles and gill (0.042) The direction of the average lead concentration in the liver and muscles respectively, and liver and gill (0.086) The direction of the average lead concentration in the liver and these results were statistically significant ($p < 0.01$).

3.1.4: Arsenic

The average concentration of Arsenic throughout all the three Sizes in Muscles Fish was 0.100 $\mu\text{g/g}$ dry wt (At Small) to 0.114 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.045 $\mu\text{g/g}$ dry wt (At Small) to 0.100 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.077 $\mu\text{g/g}$ dry wt (At Small) to 0.103 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.078 $\mu\text{g/g}$ dry wt (At Small) to 0.099 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*. The average concentration of Arsenic throughout all the three Sizes in Liver Fish was 0.114 $\mu\text{g/g}$ dry wt (At Small) to 0.128 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.121 $\mu\text{g/g}$ dry wt (At Small) to 0.184 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.053 $\mu\text{g/g}$ dry wt (At Small) to 0.087 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.114 $\mu\text{g/g}$ dry wt (At Small) to 0.134 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*. The average concentration of Arsenic throughout all the three Sizes in Gill Fish was 0.020 $\mu\text{g/g}$ dry wt (At Small) to 0.032 $\mu\text{g/g}$ dry wt (At large) for *L. mahsena* and from 0.027 $\mu\text{g/g}$ dry wt (At Small) to 0.060 $\mu\text{g/g}$ dry wt (At large) for *T. tonggol*. and from 0.020 $\mu\text{g/g}$ dry wt (At Small) to 0.029 $\mu\text{g/g}$ dry wt (At large) for *S. jello* and from 0.042 $\mu\text{g/g}$ dry wt (At Small) to 0.048 $\mu\text{g/g}$ dry wt (At large) for *E. areolatus*. The high concentration of As (0.147 \pm 0.032 $\mu\text{g/g}$ dry wt.) was found in the livers tissue of *T. tonggol* (Table 4), while in the lowest concentration of Arsenic level (0.025 \pm 0.005 $\mu\text{g/g}$ (dry wt.) was detected in the gills tissue of *S. jello* (Table 4). The mean concentration of As in the muscles, livers and gills of the four studied fish species varied from a minimum of 0.071 \pm 0.028, 0.070 \pm 0.017 and 0.025 \pm 0.005 $\mu\text{g/g}$ (dry wt.) in *T. tonggol*, *S. jello* and *S. jello*, to a maximum value of 0.132 \pm 0.048; 0.147 \pm 0.032 and 0.045 \pm 0.003 $\mu\text{g/g}$ (dry wt.) in *E. areolatus*, *T. tonggol* and *E. areolatus* (Table 4).

Table (11) shows the covariance analysis between mean concentration of As among in some types of fishes in Yemeni seas and different variables. These different variables include; tissue, type and area of the fish. After included the weight and length factors to show the related impact in the change of concentration of As to the three periods of time, the covariance analysis was not statistically significant in which the ($p>0.05$). The mean concentration of As to the species of fish, among the tissues of the fish and among three different areas of fishing were also statistically significant with the p value are similar ($p<0.01$), where the impact of these differences in concentration ratio of As were (68%, 15% and 5%) respectively. Table (12) shows the mean concentration of As among the areas, type and the tissues of the fish. The concentration of As were concentrated at Between Aden and Al Mukalla in which the mean concentration of As among fishes were (0.013) Towards Al Mukalla, these results were statistically significant ($p<0.01$).

The differences in the mean concentration of As among the different types of the fishes were found between *L. mahsena* and two other types and the mean concentration was in *T. tonggol* (0.049) and *S. jello* (0.033), The direction of the type of fish *L. mahsena*, these difference was statistically significant

($p < 0.05$). and the differences in the mean concentration of Hg among the different types of the fishes were found between *T. tonggol* and *E. areolatus*, *S. jello* and *E. areolatus* (0.049), (0.033) respectively, The direction of the type of fish *E. areolatus*, these difference was statistically significant ($p < 0.05$).

According the concentration of As among the tissues of the fish was found among the muscles and liver (0.027), muscles and gill (0.055) The direction of the average lead concentration in the liver and muscles respectively, and liver and gill (0.081) The direction of the average lead concentration in the liver and these results were statistically significant ($p < 0.01$).

3.2: Statistically Evaluation

The details of analysis of each statics ANOVA

3.2.1: Correlation analyses between metals in Muscles

The relationship between metals level in Muscles tissue are represented by correlation coefficient (r) in Table 13. Notes from the table 13 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.

3.2.2: Correlation Analyses between Metals in Liver

The relationship between metals level in Liver tissue are represented by correlation coefficient (r) in Table 14. Notes from the table 14 above for the Aden site and the presence of significant positive correlations at the level of ($p < 0.01$) between the Pb in the tissue of fish liver with As in the same Liver tissue, also has been associated with Cd significant positive correlations morally at a level ($p < 0.01$) with Hg and As in the same Liver tissue, and has been associated with Hg significant positive correlations morally at a level ($p < 0.01$) with As in the same Liver tissue.

In Al-Hudaydah site, show a significant positive correlations at a level ($p < 0.05$) between the Cd into the tissue of fish liver with As in the same Liver tissue, also has been associated with Hg significant positive correlations morally at a level ($p < 0.01$) into the fabric of fish liver As with metal arsenic in the same Liver tissue.

In AL-Mukallala site, show a significant positive correlations at the level of ($p < 0.05$) between the Pb in the tissue of fish liver with Hg and As in the same Liver tissue, and has been associated with Cd into the tissue of fish liver significant positive correlations morally at a level ($p < 0.01$) with Hg and As in the

same Liver tissue, also has been associated with Hg in fish liver tissue was significant positive correlations at the level of ($p < 0.01$) with As in the same Liver tissue.

3.2.3: Correlation analyses between metals in Gill

The relationship between metals level in Gill tissue are represented by correlation coefficient (r) in Table 15. Notes from the table 15 for the Aden site and the presence of significant negative correlations at the level of ($p < 0.01$) between the Pb in the tissue of the gills of fish with Hg in the same gill, while linked to Hg in the tissue of the gills of fish significant positive correlations morally at a level ($p < 0.01$) with As in the same gill. In Al-Hudaydah site, showed a significant negative correlations at the level of ($p < 0.05$) between the Pb in the tissue of the gills of fish with Cd in the same gill, while correlation to Hg in the tissue of the gills of fish significant positive correlations morally at a level ($p < 0.01$) with As in the same gill.

In AL-Mukallala site, showed a significant negative correlations at the level of ($p < 0.05$) between the Pb in the tissue of the gills of fish with Hg and as in the same gill, and has been associated with Hg is significant negative correlations at the level of ($p < 0.01$) with As in the same gill, while the Cd was associated in the gills of fish tissue significant positive correlations morally closely at the level of ($p < 0.05$) with Hg in the same gill.

DISCUSSION

4.1: Heavy Metals in different Organs

During the present study, Pb levels have been seen to be maximum in gill from *L. mahsena* and *S. jello*, and in liver from *T. tonggol* and *E. areolatus*, minimum in muscle in all the fish species. Cd levels, too, are found to be maximum in gill, minimum in muscle and intermediate in liver in all the fish species.

Hg and As levels, are found to be maximum in liver in all the fish species, except *S. jello* It was higher in muscle, minimum in gill in all the fish species and intermediate in muscle in all the fish species except *S. jello*. In all the investigated fish species, *L. mahsena*, *T. tonggol*, *S. jello* and *E. areolatus*, gill accumulates the highest levels of Pb and Cd. Liver accumulates the highest levels of Hg and As.

The highest accumulation of metals in gill as compared to other organs has been recorded a similar trend has been observed in *E. fasciatus* by¹⁹ and in *E. areolatus* by²⁰. The highest accumulation of metals in liver as compared to other organs has been widely recorded in earlier studies conducted by^{21, 22, 23, 24, 25, 26, 27, 28, 29, 30}.

Liver is the major detoxification organ and many poisonous materials absorbed from the environment are detoxified in the liver. Studies carried out with different fish species have shown that heavy metals accumulate mainly in metabolically active liver that stores metals to detoxicate by producing metallothioneins³¹. Metallothioneins (MTs) are cysteine rich low molecular weight proteins having capacity to bind to physiological as well as xenobiotic heavy metals through a thiol group of cysteine. The higher levels of trace elements in liver relative to other tissues are, therefore, attributed to the affinity of MT proteins with these heavy elements³².

During the present study, muscle of all the fish species has been found to accumulate lesser metals as compared to liver and gill. Muscle tissue is not considered to be an active site for metal accumulation^{33, 34}. Studies comparing the metal accumulation in muscle and liver of fish show lower metal concentration in the former. This trend has been recorded in marine fishes, in *L. mahsena*, by^{35,36,37}, in *T. albacores*, by³⁸, in *S.putnamae*, by³⁹ and in *Soleasolea*, and *Sparusaurata*, by²⁷. Cd and Pb, have no biological role and hence they are harmful to living organisms even at considerably low concentrations.

In this study, the overall mean concentrations of metals were found to accumulate in the order of Pb > Hg > As > Cd, Except in Fish species *S. jello* mean concentrations of metals were found to accumulate in the order of Hg > As > Pb > Cd. Although it is not always the rule, these results were in conformity with the observations of Al sulami, 2002 (Pb > Hg > Cd > As) and Burger et al., 2005 (Hg > As > Pb > Cd).

Analysis of heavy metals in sediments offers more convenient and more accurate means of detecting and assessing the degree of water pollution⁴⁰.

Although it is well known that fish muscle is not an active tissue in accumulating heavy metals⁴¹, the present study concerned with the heavy metal concentrations in the fish muscles because it is the most consumed portion by the Yemen people. Furthermore it was documented that some fish in polluted regions may accumulate substantial amounts of metals in their tissues which sometimes exceeded the maximum acceptable levels.

Lead accumulation in different organs showed the order *L. mahsena* > *E. areolatus* > *T. tonggol* > *S. jello*.

Overall ranking revealed from the results that among the four fish species the *L. mahsena* accumulated the highest concentration of all the heavy metals, which indicates that this species have more potential to accumulate these metals in each liver and gills. It may be due to the feeding habits of the fish, lipid content in the tissue and excretion percentage of these toxic metals from their body.

Cadmium, Mercury and Arsenic accumulation in different organs showed the order *E. areolatus* > *T. tonggol* > *L. mahsena* > *S. jello*.

The arrangement order of Pb and Cd content in tissues of the polluted fish was gill > liver > muscle, whereas in case of Hg and As the order was liver > gill > muscle (Table 4).

The great amount of Pb and Cd in gills of fish may be the result of a water contamination caused by environmental pollution.

4.2: Heavy Metal Concentrations vs. International dietary Standards and Guidelines.

4.2.1: Lead

The FAO/WHO⁴² and Yemen Standardization⁴³ guidelines for a prescribed maximum permissible limit of Lead in Fish are 1.50 µg/g (dry wt.) and 1.00 µg/g (dry wt.).

The main highest concentration of Pb in the muscles, livers and gills of the four studied fish species was 0.137± 0.014 µg/g (dry wt.) in large *E. areolatus* and

0.428± 0.031; 0.845± 0.009 µg/g (dry wt.) in *L. mahsena*. At site AL- Hudaydah.

The values obtained for Pb in the muscles, livers and gills were below the Pb prescribed standard safe limits of 1.00 - 1.50 µg/g dry wt.) for food fish (FAO/WHO⁴² and Standard Specification for Yemen⁴³). Based on this information, Yemen coast in the present study is low polluted when it is compared with other locations.

4.2.2: Cadmium

The FAO/WHO⁴² and Yemen Standardization⁴³ guidelines for a prescribed maximum permissible limit of Cadmium in Fish are 1.00 µg/g (dry wt.) and 0.2 µg/g (dry wt.).

In summer, the main highest concentration of Cadmium in the muscles and livers of the four studied fish species were 0.069±0.021 and 0.289±0.020 µg/g (dry wt.) in large *E. areolatus* in Year 2012 at Site AL-Hudaydah whereas in gills was having 0.365±0.032 µg/g (dry wt.) in *E. areolatus* in Year 2012 at Site Aden. The values obtained for Cd in the muscles, livers and gills were below the Cd prescribed standard safe limits of 1.0 µg/g (dry wt.) for food fish FAO/WHO⁴².

But, the Yemen Standardization⁴³ guidelines for maximum permissible limit of Cadmium in Fish are given as 0.2 µg/g (dry wt.). As the range of Cadmium detected was higher than the permissible limit in livers and gills *E. areolatus* and gills *T. tonggol* fish.

4.2.3: Mercury

The FAO/WHO⁴² and Yemen Standardization⁴³ guidelines for prescribed maximum permissible limit of Mercury in Fish are 0.50 µg/g (dry wt.).

The main highest concentration of Hg in the muscles, livers and gills of the four studied fish species was 0.071±0.012 µg/g (dry wt.) in *E. areolatus* (at large); 0.127± 0.026 and 0.020± 0.005 µg/g (dry wt.) in large *T. tonggol* at Site AL-Mukalla. The FAO/WHO⁴² and Yemen Standardization⁴³ guidelines for prescribed maximum permissible limit of Mercury in Muscles Fish are 0.50 µg/g (dry wt.).

As the detected Mercury remained below the FAO/WHO⁴² and Yemen Standardization⁴³ permissible limits.

4.2.4: Arsenic

The FAO/WHO⁴² and Yemen Standardization⁴³ guidelines for a prescribed maximum permissible limit of Arsenic in Fish are 0.10 - 5.00 µg/g (dry wt.) and 1.0 µg/g (dry wt.). The main highest concentration of As in the muscles, livers and gills of the four studied fish species was 0.106±0.007 µg/g (dry wt.) in *L. mahsena* (at large); 0.147±0.032 µg/g (dry wt.) in *T. tonggol* (at large) and 0.045±0.003 µg/g (dry wt.) in *Epinephelusareolatus* at Site AL-Mukalla.

As the detected Arsenic remained below the FAO/WHO⁴² and Yemen Standardization⁴³ permissible limits.

CONCLUSIONS

The following can be concluded from this study results:

The Fish samples were collected from the Three different Cities of Yemeni coasts. Aden, Al-Hodeidah

and AL-Mukalla were chosen for the sample collection.

Lethrinusmahsena, Thunnustonggol, Sphyrænaajello and Epinephelusareolatus fish samples were considered for the study as they are more common eatable fish among the population. The study was carried out in the all three seasons of winter 2011, summer 2012 and winter 2013 in order to check seasonal variation of heavy metal pollution.

Total Fish (108 samples of each muscles, liver and gills) were analyzed. The four heavy metals lead, Cadmium, Mercury and arsenic which are considered highly toxic were detected in the samples in the year 2010, 2012 and 2013. Four species of fish Lethrinusmahsena, Thunnustonggol, Sphyrænaajello and Epinephelusareolatus was examined and Lead, Cadmium, Mercury and Arsenic concentration .

The highest mean concentration of Pb in the muscles, livers and gills of the four studied fish species was $0.137 \pm 0.014 \mu\text{g/g}$ dry wt in large Epinephelusareolatus and 0.428 ± 0.031 ; $0.845 \pm 0.009 \mu\text{g/g}$ dry wt in Lethrinusmahsena. At Site AL- Hudaydah.

The values obtained for Pb in the muscles, livers and gills were below the Pb prescribed standard safe limits of 1.00 - 1.50 $\mu\text{g/g}$ dry wt. (for food fish (FAO/WHO⁴² and Standard Specification for Yemen⁴³).

In Summer, the highest mean concentration of Cadmium in the muscles and livers of the four studied fish species were 0.069 ± 0.021 and $0.289 \pm 0.020 \mu\text{g/g}$ dry wt in large Epinephelusareolatus in Year 2012 at Site AL- Hudaydah whereas in gills was having $0.365 \pm 0.032 \mu\text{g/g}$ dry wt in Epinephelusareolatus in Year 2012 at Site Aden.

The values obtained for Cd in the muscles, livers and gills were below the Cd prescribed standard safe limits of 1.0 $\mu\text{g/g}$ (dry wt) for food fish.

But, the Standard Specification for Yemen⁴³ guidelines for maximum permissible limit of Cadmium in Fish are given as 0.2 $\mu\text{g/g}$ dry wt. As the range of Cadmium detected was higher than the permissible limit in livers and gills Epinephelusareolatus and gills Thunnustonggol fish. The highest mean concentration of Hg in the muscles, livers and gills of the four studied fish species was $0.071 \pm 0.012 \mu\text{g/g}$ (dry wt) in Epinephelusareolatus (at large) ; 0.127 ± 0.026 and $0.020 \pm 0.005 \mu\text{g/g}$ (dry wt) in large Thunnustonggol at Site AL-Mukalla .

The WHO and Standard Specification for Yemen⁴³ guidelines for prescribed maximum permissible limit of Mercury in Muscles Fish are 0.50 $\mu\text{g/g}$ dry wt. As the detected Mercury remained below the WHO and Standard Specification for Yemen⁴³ permissible limits. The highest mean concentration of As in the muscles, livers and gills of the four studied fish species was 0.106 ± 0.007 in Lethrinusmahsena (at large) ; 0.147 ± 0.032 in Thunnustonggol (at large) and $0.045 \pm 0.003 \mu\text{g/g}$ (dry wt.) in Epinephelusareolatus at Site AL-Mukalla. As the detected Arsenic remained below the FAO/WHO⁴² and Yemen Standardization (2006) permissible limits. From the heavy metal concentrations mentioned above we can see that somewhere the concentration is crossing the limits as

permissible by the World Health Organization. It suggests a high risk to the health of human being on the consumption of contaminated fish. 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.

RECOMMENDATIONS

From the study results outcome the following can be recommended: The following recommendations might be of particular interests

- Establishment of Risk analysis system in Yemen would enable authorities working in the field of marine protection to achieve safety of fish and shellfish used for human consumption.
- Devoting more efforts for carrying out further studies on assessment of contamination in other marine species with other pollutant would help in drawing a complete picture with regards of pollution status in regional sea catchments area of Yemen.

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Table 1: Sampling stations details

Station	Longitude (E)	Latitude (N)	Description
Aden city	45°04'88"E	12°77'53"N	overlooking the Gulf of Aden
AL-Hudaydahcity	42°94'05"E	14°91'35"N	overlooking the Red Sea
AL-Mukalla city	49°10'67"E	14°52'87"N	overlooking the Arabian Sea

Table 2: Target Species for fish from three sites

No	Family name	LETHRINIDAE
1	Common name	Gahash (emperor)
	FAO name	Ee-Mahsenaemeror
	Scientific name	<i>Lethrinusmahsena</i>
	Habitat	Demersal, Life style and Feeding , mportant benthic feeding fish found on coral reefs
	Trophic level	3.4±0.42
		
2	Family name	SCOMBRIDAE
	Common name	Zainoop (Longtail tuna)
	FAO name	En-Longtail tuna
	Scientific name	<i>Thunnustonggol</i>
	Habitat	Pelagic – naritic, Life style and Feeding , Important commercial and angling species Carnivore on small fish and crastease
Trophic level	4.5 ±0.6	
		
3	Family name	SPHYRAENIDAE
	Common name	Kud, Kunat (Pickhandle Barracuda)
	FAO name	En-Barracuda
	Scientific name	<i>Sphyraenajello</i>
	Habitat	Pelagic – naritic, Life style and Feeding , Carnivorous fish (feeding on other
Trophic level	4.5 ±0.6	
		
4	Family name	SERRANIDAE
	Common name	Khulkhul (Areolate grouper)
	FAO name	En-Areolate grouper
	Scientific name	<i>Epinephelusareolatus</i>
	Habitat	Demersal, Life style and Feeding , Important benthic feeding fish found on coral reefs Carnivore on small fish and crustaceans
Trophic level	3.7±0.3	
		

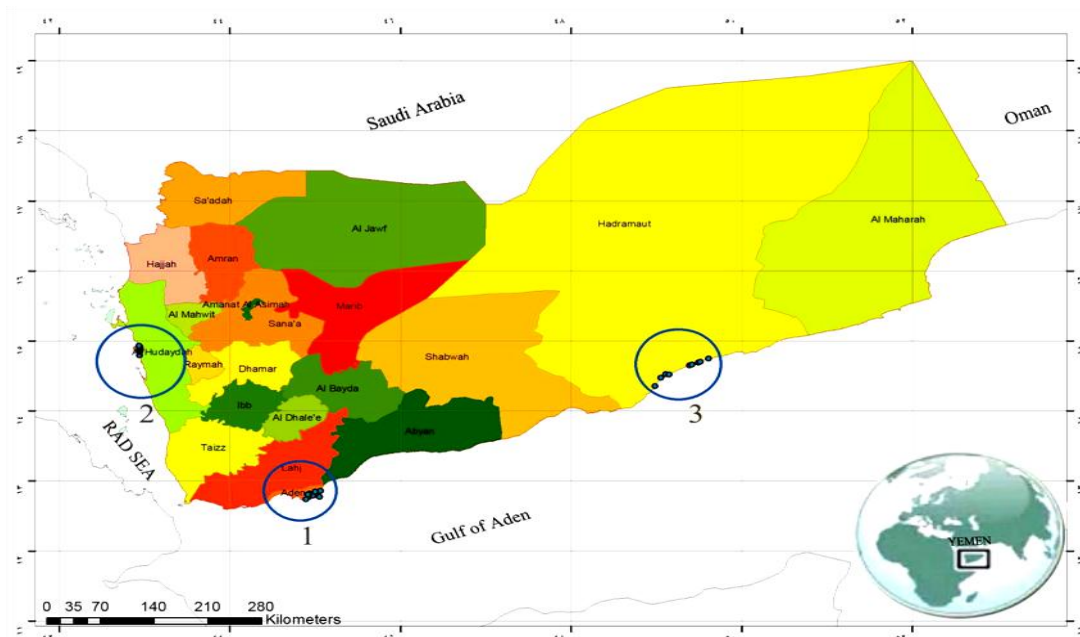


Figure 1: Sampling locations along the Coast of Yemen.

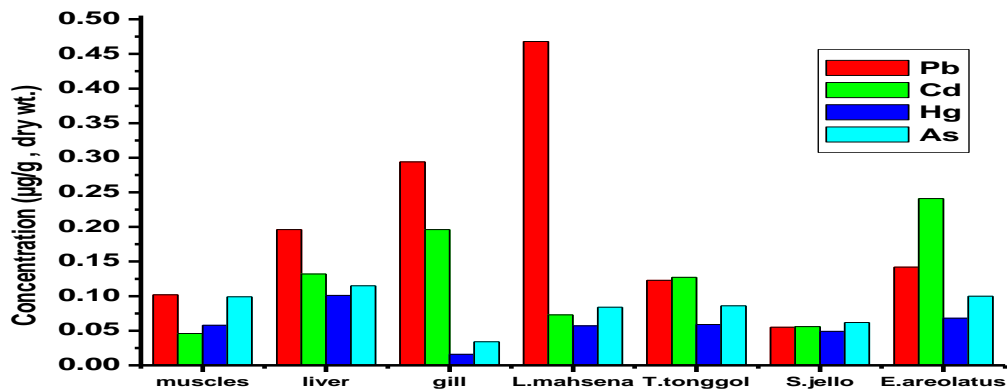


Figure 2: The mean of Concentration µg/g (dry wt.) for lead, cadmium, mercury and Arsenic in different organs muscles, livers and gills of the four studied fish species collected from Aden, AL-Hudaydah and AL-Mukalla station.

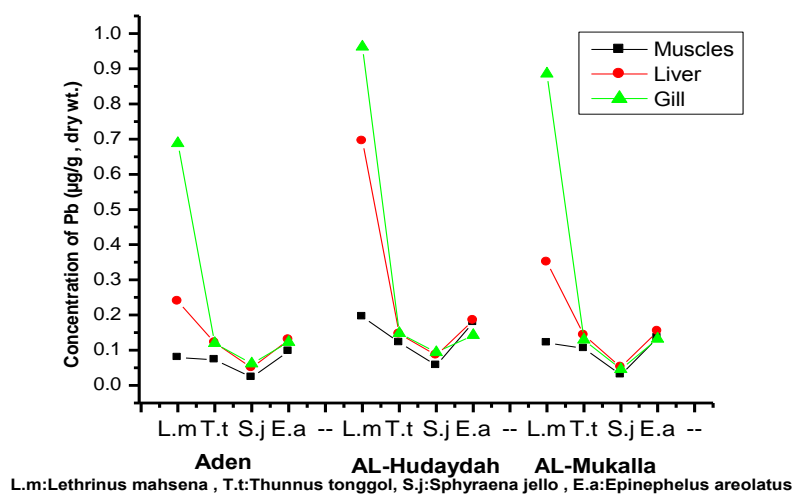


Figure 3: The mean of Concentration µg/g (dry wt.) for Lead in different organs muscles, livers and gills of the four studied fish species collected from Aden, AL-Hudaydah and AL-Mukalla station.

Table 3: The Mean weight and length of fish collected during the seasons from Aden , AL-Hudaydah and AL-Mukalla Stations, Yemen coast

Site	Species	Size	Weight (g)	Length (cm)
			Mean±Std	Mean±Std.
Aden	<i>Lethrinusmahsena</i>	Large	445.89±14.31	29.32±1.09
		Medium	358.83±54.32	26.24±1.61
		Small	252.52±24.21	24.25±1.15
	<i>Thunnustonggol</i>	Large	3513.05±411.4	73.33±2.04
		Medium	2652.85±214.79	60.4±1.65
		Small	1860.49±78.34	52.93±1.93
	<i>Sphyraenajello</i>	Large	890.56±160.38	52.73±1.6
		Medium	741.84±30.8	48.37±0.7
		Small	587.24±30.31	44.5±0.79
	<i>Epinephelusareolatus</i>	Large	798.95±49.43	40.4±0.91
		Medium	444.11±17.44	31.53±2.06
		Small	242.24±47.95	23.93±1.47
Al Hudaydah	<i>Lethrinusmahsena</i>	Large	1085.77±75.04	37.07±0.82
		Medium	746.49±139.89	32.63±2.08
		Small	238.13±60.67	23.27±1.78
	<i>Thunnustonggol</i>	Large	3416.47±421.55	64.47±2.84
		Medium	2338.1±97.98	56.5±0.85
		Small	1878.68±94.29	52.2±0.96
	<i>Sphyraenajello</i>	Large	1156.43±78.07	57.17±1.21
		Medium	694.31±79.85	47.5±1.42
		Small	484.48±24.65	42.37±1.28
	<i>Epinephelusareolatus</i>	Large	911.37±66.14	39.13±0.7
		Medium	678.33±51.94	35.5±0.79
		Small	374±32.24	29.17±0.98
Al Mukalla	<i>Lethrinusmahsena</i>	Large	746.8±50.18	34.1±1.53
		Medium	544.82±51.53	30.77±0.97
		Small	359.78±37.32	26.43±1.09
	<i>Thunnustonggol</i>	Large	2893.21±147.23	62.37±1.05
		Medium	2268.93±218.43	57.57±1.82
		Small	1614.56±107.72	51.53±1.13
	<i>Sphyraenajello</i>	Large	1037.37±175.26	55.37±3.19
		Medium	625.67±93.35	46.57±2.34
		Small	454.82±34.13	41.8±1.06
	<i>Epinephelusareolatus</i>	Large	857.36±51.75	41.37±1.18
		Medium	353.8±81.85	35.33±1.92
		Small	187.48±53.95	24.13±2.4

Table 4: The mean of Concentration $\mu\text{g/g}$ (dry wt.) for lead, cadmium, mercury and Arsenic in different organs muscles, livers and gills of the four studied fish species collected from Aden, AL-Hudaydah and AL-Mukalla station.

species	Metal ion	Organ			Total mean \pm SD
		Muscles	Liver	Gill	
<i>Lethrinusmahsena</i>	Pb	0.132 \pm 0.048	0.428 \pm 0.031	0.845 \pm 0.009	0.468 \pm 0.358
	Cd	0.049 \pm 0.016	0.084 \pm 0.007	0.087 \pm 0.009	0.073 \pm 0.021
	Hg	0.062 \pm 0.0005	0.094 \pm 0.001	0.014 \pm 0.002	0.057 \pm 0.040
	As	0.106 \pm 0.007	0.121 \pm 0.007	0.026 \pm 0.006	0.084 \pm 0.051
<i>Thunnustonggol</i>	Pb	0.100 \pm 0.018	0.137 \pm 0.022	0.133 \pm 0.014	0.123 \pm 0.020
	Cd	0.037 \pm 0.008	0.092 \pm 0.031	0.253 \pm 0.142	0.127 \pm 0.112
	Hg	0.030 \pm 0.006	0.127 \pm 0.026	0.020 \pm 0.005	0.059 \pm 0.059
	As	0.071 \pm 0.028	0.147 \pm 0.032	0.041 \pm 0.017	0.086 \pm 0.055
<i>Sphyraenajello</i>	Pb	0.037 \pm 0.009	0.062 \pm 0.019	0.067 \pm 0.016	0.055 \pm 0.016
	Cd	0.029 \pm 0.010	0.061 \pm 0.013	0.077 \pm 0.008	0.056 \pm 0.024
	Hg	0.069 \pm 0.011	0.060 \pm 0.016	0.019 \pm 0.003	0.049 \pm 0.027
	As	0.090 \pm 0.013	0.070 \pm 0.017	0.025 \pm 0.005	0.062 \pm 0.033
<i>Epinephelusareolatus</i>	Pb	0.137 \pm 0.014	0.156 \pm 0.015	0.133 \pm 0.011	0.142 \pm 0.012
	Cd	0.069 \pm 0.021	0.289 \pm 0.020	0.365 \pm 0.032	0.241 \pm 0.154
	Hg	0.071 \pm 0.012	0.124 \pm 0.017	0.010 \pm 0.001	0.068 \pm 0.057
	As	0.132 \pm 0.048	0.123 \pm 0.010	0.045 \pm 0.003	0.100 \pm 0.048
Total mean \pm SD	Pb	0.102 \pm 0.046	0.196 \pm 0.160	0.294 \pm 0.368	0.197 \pm 0.096
	Cd	0.046 \pm 0.017	0.132 \pm 0.106	0.196 \pm 0.139	0.124 \pm 0.075
	Hg	0.058 \pm 0.019	0.101 \pm 0.031	0.016 \pm 0.005	0.058 \pm 0.043
	As	0.099 \pm 0.026	0.115 \pm 0.032	0.034 \pm 0.010	0.083 \pm 0.043

Table 5: The covariance analysis between mean concentration of Pb ($\mu\text{g/g}$) among in some types of fishes in Yemeni seas and different variables.

Source	Type III Sum of Squares	df	Mean Square	F	p	Partial Eta Squared(R^2)
Corrected Model	11.090a	11	1.008	42.000	0.000	0.597
Intercept	0.005	1	0.005	0.202	0.653	0.001
Seasons	0.121	2	0.060	2.513	0.083	0.016
Tissue	2.013	2	1.007	41.940	0.000	0.212
Species	4.315	3	1.438	59.921	0.000	0.366
Site	0.515	2	0.257	10.727	0.000	0.064
Weight (Kg)	0.000	1	0.000	0.016	0.899	0.000
Length (M)	0.035	1	0.035	1.474	0.226	0.005
Error	7.489	312	0.024			
Total	31.180	324				
Corrected Total	18.579	323				

a. R Squared =0.597 (Adjusted R Squared =0.583)

Table 6: The mean concentration of Pb ($\mu\text{g/g}$) among the areas, type and the tissues of the fish.

(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	P ^b
Al Mukalla	Aden	-0.048*	0.017	0.012
	Al Hudaydah	-0.075*	0.017	0.008
(I) Species <i>Lethrinusmahsena</i>	(J) Species <i>Thunnustonggol</i>	0.446*	0.051	0.000
	<i>Sphyraenajello</i>	0.484*	0.057	0.000
	<i>E. areolatus</i>	0.342*	0.027	0.000
	<i>Sphyraenajello</i>	0.143*	0.046	0.013
(I) Tissue Muscles	(J) Tissue Liver	-0.094*	0.021	0.000
	Gill	-0.193*	0.021	0.000
Liver	Gill	-0.099*	0.021	0.000

Based on estimated marginal means

*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Table 7: The covariance analysis between mean concentration of Cd ($\mu\text{g/g}$) among in some types of fishes in Yemeni seas and different variables.

Source	Type III Sum of Squares	df	Mean Square	F	P	Partial Eta Squared
Corrected Model	3.789 ^a	11	0.344	23.659	0.000	0.455
Intercept	0.022	1	0.022	1.530	0.217	0.005
Seasons	0.192	2	0.096	6.599	0.002	0.041
Tissue	1.212	2	0.606	41.640	0.000	0.211
Species	1.705	3	0.568	39.048	0.000	0.273
Site	0.289	2	0.144	9.922	0.000	0.060
Wight (Kg)	0.084	1	0.084	5.745	0.017	0.018
Length (M)	0.004	1	0.004	0.301	0.584	0.001
Error	4.542	312	0.015			
Total	13.350	324				
Corrected Total	8.331	323				

a. R Squared =0.455 (Adjusted R Squared =0.436)

Table 8: The mean concentration of Cd (µg/g) among the areas, type and the tissues of the fish.

(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	P b
Al Mukalla	Aden	-0.048*	0.017	0.012
	Al Hudaydah	-0.075*	0.017	0.000
(I) Species	(J) Species			
<i>E. areolatus</i>	<i>Lethrinusmahsena</i>	0.173*	0.021	0.000
	<i>Thunnustonggol</i>	0.246*	0.038	0.000
	<i>Sphyraenajello</i>	0.182*	0.036	0.000
(I) Tissue	(J) Tissue			
Muscles	Liver	-0.086*	0.016	0.000
	Gill	-0.149*	0.016	0.000
Liver	Gill	-0.064*	0.016	0.000
(I) Time	(J) Time			
Phase 2011	Phase 2012	-0.052*	0.016	0.006
	Phase 2013	-0.052*	0.017	0.005

Based on estimated marginal means *. The mean difference is significant at the .05 level. b. Adjustment for multiple comparisons: Bonferroni

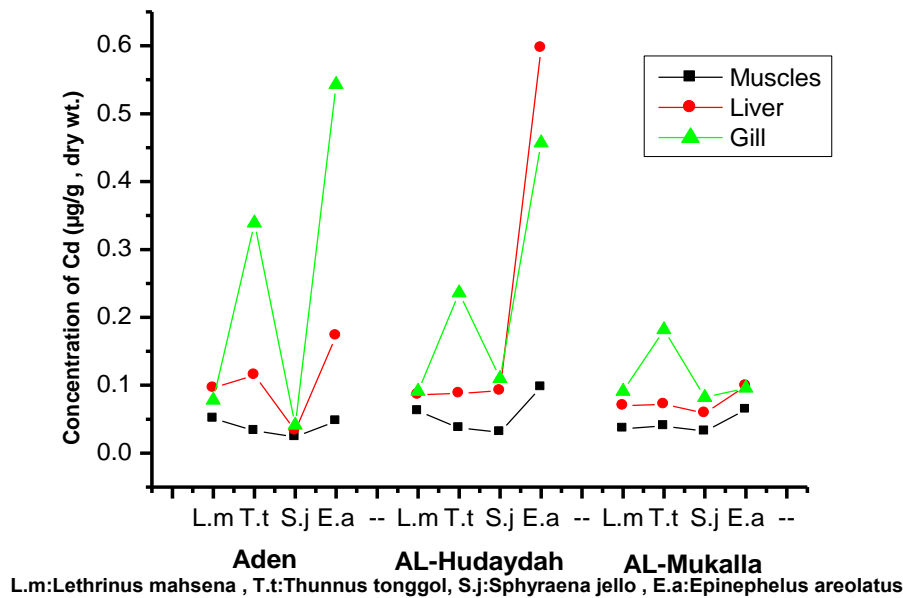


Figure 4: The mean of Concentration µg/g (dry wt.) for Cadmium in different organs muscles, livers and gills of the four studied fish species collected from Aden, AL-Hudaydah and AL-Mukalla station.

Table 9: The covariance analysis between mean concentration of Hg (µg/g) among in some types of fishes in Yemeni seas and different variables.

Source	Type III Sum of Squares	df	Mean Square	F	P	Partial Eta Squared
Corrected Model	0.428 ^a	11	0.039	79.945	0.000	0.738
Intercept	0.002	1	0.002	4.235	0.040	0.013
Seasons	0.001	2	0.000	0.970	0.380	0.006
Tissue	0.397	2	0.198	407.259	0.000	0.723
Species	0.024	3	0.008	16.126	0.000	0.134
Site	0.001	2	0.000	0.551	0.577	0.004
Wight	0.001	1	0.001	1.611	0.205	0.005
Length	0.001	1	0.001	1.367	0.243	0.004
Error	0.152	312	0.000			
Total	1.683	324				
Corrected Total	0.580	323				

a. R Squared = 0.738 (Adjusted R Squared = 0.729)

Table 10: The mean concentration of Hg (µg/g) among the areas, type and the tissues of the fish.

(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	P ^b
<i>Lethrinusmahsena</i>	<i>Thunnustonggol</i>	0.030*	0.007	0.000
<i>Thunnustonggol</i>	<i>E. areolatus</i>	-0.040*	0.007	0.000
<i>Sphyraenajello</i>	<i>E. areolatus</i>	-0.030*	0.007	0.000
(I) Tissue	(J) Tissue			
Muscles	Liver	-0.043*	0.003	0.000
	Gill	0.042*	0.003	0.000
Liver	Gill	0.086*	0.003	0.000

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

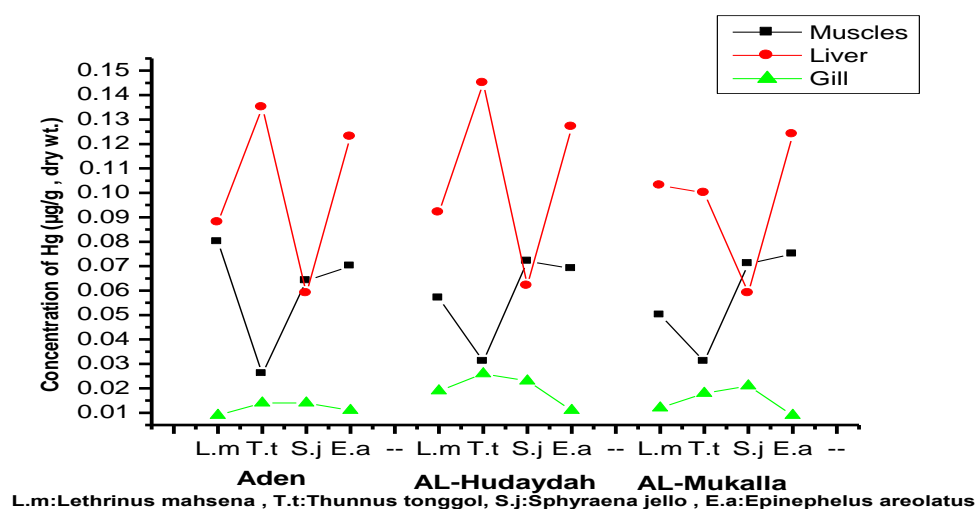


Figure 5: The mean of Concentration µg/g (dry wt.) for Mercury in different organs muscles, livers and gills of the four studied fish species collected from Aden, AL-Hudaydah and AL-Mukalla station.

Table 11: The covariance analysis between mean concentration of As (µg/g) among in some types of fishes in Yemeni seas and different variables.

Source	Type III Sum of Squares	df	Mean Square	F	P	Partial Eta Squared
Corrected Model	0.446 ^a	11	0.041	72.024	0.000	0.717
Intercept	0.005	1	0.005	9.118	0.003	0.028
Seasons	0.000	2	0.000	0.023	0.977	0.000
Tissue	0.370	2	0.185	328.712	0.000	0.678
Species	0.030	3	0.010	17.989	0.000	0.147
Site	0.009	2	0.004	7.855	0.000	0.048
Wight (Kg)	0.005	1	0.005	9.093	0.003	0.028
Length (M)	0.000	1	0.000	0.452	0.502	0.001
Error	0.176	312	0.001			
Total	2.666	324				
Corrected Total	0.622	323				

a. R Squared = 0.717 (Adjusted R Squared = 0.707)

Table 12: The mean concentration of As ($\mu\text{g/g}$) among the areas, type and the tissues of the fish.

(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	P ^b
<i>Lethrinus mahsena</i>	<i>Thunnustonggol</i>	0.049*	0.007	0.000
	<i>Sphyraenajello</i>	0.033*	0.007	0.010
<i>Thunnustonggol</i>	<i>E. areolatus</i>	-0.049*	0.006	0.000
<i>Sphyraenajello</i>	<i>E. areolatus</i>	-0.033*	0.006	0.010
(I) Tissue	(J) Tissue			
Muscles	Liver	-0.027*	0.003	0.000
	Gill	0.055*	0.003	0.000
Liver	Gill	0.081*	0.003	0.000
(I) Site	(J) Site			
Aden	Al Mukalla	-0.013*	0.003	0.000

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

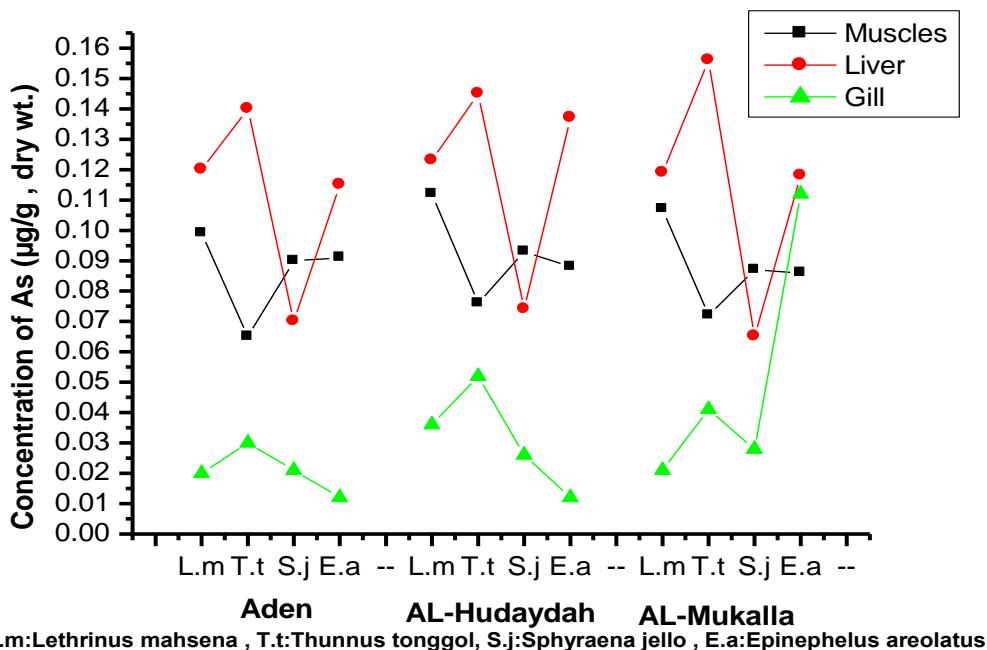


Figure 6: The mean of Concentration $\mu\text{g/g}$ (dry wt.) for Arsenic in different organs muscles, livers and gills of the four studied fish species collected from Aden, AL-Hudaydah and AL-Mukalla station.

Table 13: 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 ($P < 0.01$)

Table 14: Correlation analyses between metals in liver tissue

Site		Pb	Cd	Hg	As
Aden	Pb	1	-	-	-
	Cd	0.266	1	-	-
	Hg	0.194	0.474**	1	-
	As	0.510**	0.481**	0.805**	1
AL- Hudaydah	Pb	1	-	-	-
	Cd	-0.202	1	-	-
	Hg	-0.176	0.325	1	-
	As	0.093	0.331*	0.887**	1
AL- Mukalla	Pb	1	-	-	-
	Cd	0.123	1	-	-
	Hg	0.375*	0.584**	1	-
	As	0.367*	0.493**	0.560**	1

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

Table 15: Correlation analyses between metals in gill tissue

Site		Pb	Cd	Hg	As
Aden	Pb	1	-	-	-
	Cd	-0.294	1	-	-
	Hg	-	0.183	1	-
	As	0.006	0.083	0.652**	1
AL- Hudaydah	Pb	1	-	-	-
	Cd	-0.357*	1	-	-
	Hg	-0.065	-0.298	1	-
	As	0.173	-0.200	0.822**	1
AL- Mukalla	Pb	1	-	-	-
	Cd	-0.134	1	-	-
	Hg	-0.393*	0.363*	1	-
	As	-0.382*	0.090	-0.499**	1

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