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Metal Pollution and Hazard Indexes of Heavy Metal Contents for some Fish Tissues Collected from Some Libyan Coasts

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ABSTRACT
This study was basically designed to estimate the pollution and hazard indexes of fish samples included (Mullus, Mugil SP, and Marbled) collected from the Benghazi coast. Different types of heavy metals (Cr, Cu, Pb, Ni, Zn, and Cd) were selected, then determined in three tissues in gills, liver, and muscle tissues in each type of the studied fishes. The ion coupling plasma (ICP) was used in this study. The results showed that the heavy metals concentrations were fluctuated in the following ranges: In the gills, Cr(3.75-12.5), Cu(11.25 -31.35), pb (23.75-50), Zn (126.25-277.5), Ni(non-detected) and Cd (2.5 -7.5), whereas for the Liver samples the concentration of heavy metals were ranged as follows: Cr(1.25 -5), Cu(26.25 -48.75), Pb (21.75-41.25), Zn(126.25 -726.25), Ni (non -detected), Cd(0 – 3.41μg/g).On the other side the studied metals were in the muscle samples were fluctuated as follows: Zn(0-3.75), Cu(16.25 -17.50), pb(28.75 – 31.75), Zn(172.5 – 193.35), Ni(0 -22.5) and for Cd (0 -1.61 μg/g). Generally, the Mugli fish exhibited higher concentrations of the detected heavy metals compared to the other studied fishes in this study. there are high levels of some heavy metals recorded in this study, and this is mainly due to the pollution effects of the studied area, coming mainly from the outlets of the city. The final metal pollution index (MPI) of each site is a weighted mean value, as it was obtained taking into account the total weight of fish. The calculated sequence of MPI in tissues of Mullus was as follows: liver > gills > muscle. Agreement with tissues of Mugil SP that were ordered as liver> gills > muscle, while the sequence of MPI in tissues of Siganus was as follows: gills > liver > muscle.

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INTRODUCTION

Marine pollution is one of the most important parts of ecotoxicology. The term ‘ecotoxicology" was first introduced by the toxicologist Prof. Truhaut in the late 1960s, when it was considered a sub-discipline of medical toxicology [1]. Three sources of pollution may broadly be distinguished, namely coastal sources, including river influx, atmospheric deposition, and offshore inputs. Coastal sources are either point sources or diffuse sources. Point sources include direct outflow through pipes discharging contaminated water from coastal industry, sewage discharges, and development sites [2]. Heavy metals, often at very low concentrations, are naturally occurring elements in the terrestrial environment. While some heavy metals may be relatively inert at low levels in aquatic environments, human activities can concentrate heavy metals in aquatic systems at levels hundreds to thousands of times the natural background rates resulting in adverse effects to aquatic organisms including commercially, recreationally, culturally and ecologically important fishes (e.g., salmon, steelhead, sturgeon, lamprey [3]. Many heavy metals can adversely affect fish and other aquatic organisms when they enter waterways. Elements such as mercury and arsenic are more bioactive in the aquatic environment than lead and are therefore more likely to harm fish and other aquatic organisms. Heavy metals enter the bodies of fish and interfere with several essential processes such as respiration, fitness, migration, and reproduction. Measuring heavy metals in aquatic organisms may be a bioindicator of their impact on organisms and ecosystem health [4].
Environmental pollution and its hazards are the most important problems of societies and living creatures. On the other hand, increased population with the development of technology and production can cause a lack of attention to environmental safety [5]. Heavy metals into the marine environment, causing permanent disturbances in marine ecosystems, leading to environmental and ecological degradation and constitute a potential risk to a number of flora and fauna species, including humans, through food chains [6].

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Industrialization leads to the pollution of ecosystems. Therefore, recognition of pollutants and prevention of their environmental dispersion are one of necessities in this field. Therefore, we must determine the pollution sources, their marine environmental effects, and prevention methods; also, filtration of industrial wastewater and education of instructions for environmental protection are vital works to control and protect against pollutants [7]. Pollutants enter fish through several routes: via skin, gills, oral consumption of water, food, and non-food particles. Once absorbed, pollutants are transported in the bloodstream to either a storage point (i.e, bone) or to the liver for transformation and/or storage [8]. This study aims to determine the contents of some heavy metals and calculate pollution and hazard indexes in some tissues of fish collected from some Libyan coasts.

METHODS

Area of Study

The Benghazi Planning Region lies between 19°50' and 25° east longitude, and 28°00' and 32°55' north latitude (Figure 1) of Libya. It is situated in the north-east of Libya, being bounded to the east by the Arab Republic of Egypt, to the south and southwest by Al Khalij Sub-Region, and the north and west by the Mediterranean [9]. The city of Benghazi is located on the Mediterranean Coast, almost halfway between Tripoli in the west and Alexandria in the east. The hinterland of Benghazi varies and is a semi-arid flat plain known as the plain of Benghazi, varying in width from north to south according to the divergence of the interior escarpment and the coastline. The Mediterranean Sea is an almost closed marine basin between Europe, Asia, and Africa. It is connected with the Atlantic Ocean by the Straits of Gibraltar, which are fifteen kilometers wide and have an average depth of 290 m to a maximum of 950 m. In addition to this natural connection, it has been connected to the Red Sea by the Suez Canal since 1869. The Suez Canal is one hundred and twenty meters wide and twelve meters deep. The number of fish species recorded for the Northeast Atlantic and the Mediterranean Sea totals about 1,255 [10]. A total of 540 fish species were listed for the Mediterranean Sea, including 362 shore dwellers, 62 of them endemic. It is unreasonable to assume that the whole Mediterranean Sea has the same species composition, due to the evident regional speciation in this sea (Whitehead *et al.*, 1984-1986). Benghazi Coast is one of the most densely populated areas of Libya. According to the Libyan Ministry of Planning, Interest of Statistics and Census, the Distribution of Population (Libyan & Non-Libyan) of Benghazi in 2012 was about 541,669 inhabitants [11] (Figure 1).

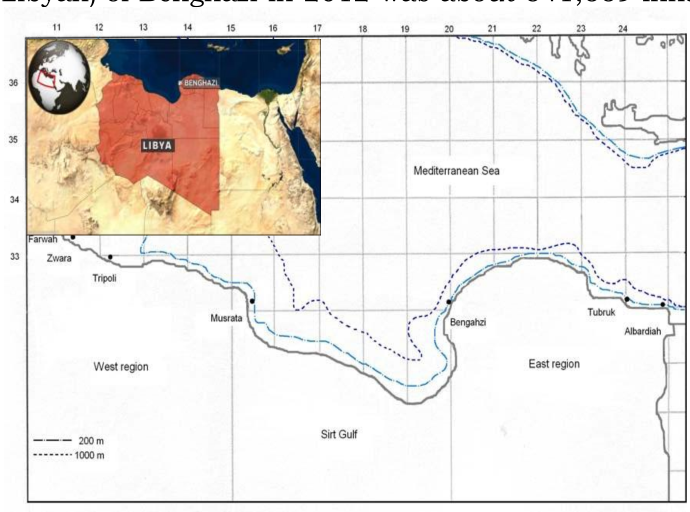

Figure 1. The map shows the study area of the Benghazi Coast

The fishing sector is still very little developed in Libya. Total catch for the Libyan coast is low, estimated in 1991 as 7,700 tons, whereas it was 90,710 tons in Tunisia and 40,192 ton in Egypt in 1991. In 1994 Libyan production was estimated at 33,469 tons. The total fleet in Libya during 1995 was 1911 and 1,866 during the year 2000; and trawlers were 105, however, the number of trawlers was only 65 boats during the year 2002. Marine Protected Areas along Libyan coast [12]. Due to the importance of the creek of its aesthetic value and important fishing route to the sea, Environmental Impact Assessment (EIA) was taken up to assess the impacts of anthropogenic climate changes on coastal waters of selected sites in Benghazi. The study was conducted in one of the lesser-known creeks near fish market; runs into the main land of city and was surrounded by houses. Major pollutants come from either land based or sea-based activities and both point sources, such as industrial discharge, oil spill incidents from boats, domestic sewage and nonpoint sources like agricultural runoff could add major pollutants to the creek. The depth of the creek varies from 1-3 feet at the border of the fish market and more than 6 feet in the center. Due to rapid urbanization, most of the creek is being closed and encroached by different kinds of construction had made the creek narrow and shallow. The creek is almost enclosed except at Northern part, where it is connected to sea for navigation for commercial fishing [13].

Samples Collection
A total of 90 of three fish species were collected from the Benghazi coast. Fish were first identified by the Marine Biology Research Center in Tripoli. Species of fish were *Mullus surmuletus, Mugil cephalus,* and *Siganus Rivulatus.*

Fish Handling and Preservation
After taking the measurements and identification, fish were washed with de-ionized water, sealed in polyethylene bags, and kept in a freezer at 20°C until chemical analysis.

Reagents
De-ionized water was used to prepare all aqueous solutions. All plastic and glassware used were rinsed and soaked in 10% (v/v) HNO_3, and were of the highest quality from Merck, Germany.

Digestion of Fish Samples
Sample Preparation and Digestion
Three different dry fish samples were brought directly from the local fish market and were kept sealed in resealable polypropylene bags. Digestion processes are made according to the U.S. Geological Survey National Water Quality. The samples were kept at room temperature, and before sampling were dried at 65˚C in a laboratory oven until they attained constant weight. The liver, muscle, and gills were isolated. For microwave digestion, about 0.2g of homogenized and dried samples of fish was accurately weighed directly into the PTFE-TF M digestion vessels. To each sample, 5.0 mL of concentrated nitric acid, 2.0 mL of hydrogen peroxide, and 1.0 mL of water were added. The analytical reagent blanks were also prepared, and these contained only the acids. The vessels were sealed and placed into the Rotor 8 for the microwave digestion. After the digestion process, the digestate liquids were transferred to the 25 auto-sampler polypropylene vials water was added to a final total volume of 25 mL [14-27].

Analytical Technique
Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)
ICP-OES conditions
Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) with a radial torch equipped with an argon saturation assembly was used for the determination of lead and cadmium. High-purity (99.99%) argon was used as plasma, auxiliary, and nebulizer gas. The gas flows were kept at 15.0 l/min for plasma, 1.50 l/min for auxiliary, and 0.56 l/min for nebulizer. The radio frequency (RF) power of the plasma generator was 1.35 KW. The vertical height of the plasma was fixed at 7 mm. Sample uptake time of 30.0 sec, delay time of 5 sec, rinse time of 10 sec, initial stabilization time of 10 sec, and time between replicate analyses of 5 sec were maintained throughout the studies for ICP-OES. All the observations of emission were recorded of the studied heavy metals, which correspond to the most sensitive emission wavelengths: Cd, 214.439 nm; Co, 238.892 nm; Cr, 267.716 nm; Cu, 327.395 nm; Ni, 231.604 nm; Pb,220.353 nm. The instrument was calibrated for various parameters before the studies.

Calibration of Instrument
Calibration requires the establishment of a relationship between signal response and a known set of standards. The standards in atomic absorption spectrometry refer to the production of a series of aqueous solutions of varying concentrations (working standards) of the analyte of interest. By measuring the signals for a series of working solutions of known concentrations, it is possible to construct a suitable graph. Then, by presenting a solution of unknown concentration to the instrument, a signal is obtained which can be interpreted from the graph, thereby determining the concentrations of the element in the unknown. The actual concentration of each metal was calculated using the formula: Actual concentration of metal = R (μg/g) × dilution factor.

Where:

R (μg/g) = AAS, Reading of digest

Dilution Factor = Volume of digest used / Weight of digested sample.

Data Analysis
Descriptive statistics such as average, range, standard deviation, and standard error values were calculated. Kruskal-Wallis nonparametric one-way analysis of variance was used to examine differences among fish types. One-way analysis of variance (ANOVA) was used after the logarithmic transformation was done on the data to improve normality, followed by the Duncan multiple range test to assess whether the means of metal concentrations were varied significantly among fish species. Possibilities less than 0.05 were considered statistically significant ($p < 0.05$). The nonparametric Kendall tau correlations were used to examine relationships among metals in the same fish species. The Kendall tau rank correlation coefficient (or simply the Kendall tau coefficient, Kendall's Ù or Tau test (s) is used to measure the strength of the relationship (or the degree of correlation) between two variables. It is feasible to carry out the hypothesis tests (assessing the

significance) with the help of Kendall's tau. Interpretation of the output of Kendall's tau (T). The interpretation of the strength of correlation of Kendall's tau was based on the following: 0.00 correlation indicates no correlation i.e., there is no relationship between the two variables 0.00 - .20 - Very Weak • 0.21 - .40 – Weak 0.41 - .60 – Moderate 0.61 - .80 – Strong 0.81 - 1.00 - Very Strong. All statistical calculations were performed with SPSS 11.0 for Windows. Microsoft Excel (2010) was used to calculate mean and standard deviation, also Microsoft Excel was used to plot graphs.

RESULTS

The analysis of the results shown in Tables (1-3) shows that the results obtained by the ICP technique. Heavy metal concentrations expressed as µg/g dry wt. The results showed that the contents of the detected heavy metals were fluctuated in the following ranges: In the gills, Cr(3.75-12.5), Cu(11.25 -31.35), pb (23.75-50), Zn (126.25-277.5), Ni(non-detected) and Cd (2.5 -7.5), whereas for the Liver samples the concentration of heavy metals were ranged as follows: Cr(1.25 -5), Cu(26.25 -48.75), Pb (21.75-41.25), Zn(126.25 -726.25), Ni (non -detected), Cd(0 – 3.41µg/g).On the other side the studied metals were in the muscle samples were fluctuated as follows: Zn(0-3.75), Cu(16.25 -17.50), pb(28.75 – 31.75), Zn(172.5 – 193.35), Ni(0 -22.5) and for Cd (0 -1.61 µg/g).

The results indicated that the higher concentration of the detected heavy metals was observed for Zinc (Zn), followed by Lead (Pb), Copper (Cu), and Chromium(Cr). On the other side, the lower contents were recorded for Nickel (Ni) and Cadmium (Cd). The results also showed that the gills contained high concentrations of Chromium (Cr) and lead(Pb). on the other side, the Liver samples showed bioaccumulation of Copper (Cu) and Zinc(Zn). There is an increase in Nickel content in muscle samples for some fish samples. Generally, the *Mugli* fishes exhibited higher concentrations of the detected heavy metals compared to the other studied fishes in this study.

Table 1. ICP results of Heavy metal concentrations (µg/g) in gill tissues

Fish Name	Tissue	Cr	Cu	Pb	Zn	Ni	Cd
Mullus	Gills	3.75	11.25	23.75	233.75	0	2.5
Mugil SP		3.75	13.75	31.25	126.25	0	3.41
Siganus		12.5	31.35	50	277.5	0	7.5

Table 2. ICP results of Heavy metal concentrations (µg/g) in liver tissues

Fish Name	Tissue	Cr	Cu	Pb	Zn	Ni	Cd
Mullus	Liver	1.25	48.75	21.75	726.25	0	1.25
Mugil SP		3.75	32.5	28.75	126.25	10	3.41
Siganus		5	26.25	41.25	243.75	5	0

Table 3. ICP results of Heavy metal concentrations (µg/g) in muscle tissues

Fish Name	Tissue	Cr	Cu	Pb	Zn	Ni	Cd
Mullus	Muscle	1.25	17.5	30	181.25	0	0
Mugil SP		0	16.25	28.75	172.5	22.5	1.61
Siganus		3.75	16.25	31.25	193.75	10.9	1.25

DISCUSSION

The concentration of chromium in the three organs under investigation for the selected species is illustrated in Tables 1-3 and Figures 2 -4. The concentration of chromium in the organs of *Mullus* was 3.376, 9.14, and 1.28 µg/g in the gills, liver, and muscles, respectively. While in *Mugil* sp fish sample, the chromium concentrations were 3, 2.68, and 2.43 µg/g dry wt in the gills, liver, and muscles, respectively. On the other side, the chromium concentrations in *Siganus* fishes were 11.7, 2.95, and 5.89 µg/g in the gills, liver, and muscles, respectively. Chromium was more accumulated in gills than in muscles and liver for most studied samples. comparing the present study results in *Mugil* sp with those reported in [14] which recorded in some fish's samples collected from Derna coast, we can say that, there is an agreement between the results of two studies for Cr concentrations measured in liver, but Cr concentrations in present study were less than those reported in, study were record 2.29 and 0.31 µg/g which found in gills and muscle respectively. When we compare the results of *Siganus* tissues in present study with there is an agreement between the two studies, in Cr concentrate measured in liver, but Cr concentration in gills is higher than those reported in study, the study record 1.29 µg/g, and less than in muscle, those reported in study that recorded 0.31 µg/g.

For *Mullus* tissues the result of the present study recorded for Cr concentration in gills and muscle are higher than that recorded in study, which is recorded (0.06 and 0.44) µg/g for gills and Muscle respectively, but it is an agreement with present study in liver tissues. The Cr bioaccumulation pattern in the tissues of the selected fish showed no clear pattern. Higher concentrations of Cr were mostly recorded in the gills followed by the liver. The concentrations of Cr found in the gills, liver, muscle and skin during this study can also be supported by various other studies. The accumulation of Cr in gill tissue is usually associated with structural damage to the gill epithelium as well as impaired respiratory and some regulatory function. These effects have often been cited as the acute mechanism of metal toxicity. The liver also showed high Cr concentrations, which was not unexpected because the liver is associated with storage and detoxification functions., in this study bio accumulated most Cr in the gill tissue, followed by the liver.

Copper was the third most abundant element for all studied samples, and the highest concentration of copper was found in liver of *Mullus* 43.69 μg/g while the lowest was found in gills and muscles of *Mullus* 10.11 and 13.58 μg/g respectively, the highest concentration of Cu reported in this study was higher than the ERL (34 μg/g), and less than the maximum acceptable limits by [15]100 μg/g.

Comparing Cu concentrations in the results for *Mugil* sp with those reported in we can say that, the result recorded in present study in muscle and gills tissues are higher than fishes collected from Derna coast that recorded (5,2.9) μg/g gills and muscle respectively, but Cu concentration in liver samples of present study are less than kemask study, which is recoded 60 μg/g. When we compare the results of *Siganus tissues* in present study with we found all tissues recorded results higher than Hamada study.

For *Mullus* tissues the results of present study recorded for Cu concentration in liver and muscle are higher than that recorded in [16] study, Mohammed recorded (18.45 and 6.55) μg/g for liver and Muscle respectively, but his study is agreement with present study in gills tissues at 10.11 μg/g. The concentration of Cu in different selected fish species were illustrated in Table 2 and figure (2) the result show that the liver is the organ which accumulated the highest amount of copper relative to other organs irrespective on the species studied. Similar results were reported by many investigators. Residues in the liver of ell, whiting and flounder from Medway Estuary (UK) were 5 - 60 times greater than those in muscle. The relatively high liver content recorded for *Mullus* Tables (1-3) can be related to their food habit

In all species, gills are the second organ which accumulate copper, it is observed that *Mullus* and *Mugil* SP which recorded the highest concentrations in liver and gills content relative to *Siganus.* This can be related to the species food habit. reported highest levels of Cu in the liver and gills of some fish's species. The higher levels of trace elements in liver relative to other tissues was attributed to the affinity or strong coordination of metallothionein protein with these elements were found that the concentration of distribution pattern of copper in organs under study is in the order of liver > gills > muscle. The low concentration of copper in muscle is investigated by many studied, Muscle of *Siganus* recorded the highest concentration of copper relative to other species. According to WHO guide lines the allowable concentration of copper for human consumption as 30 μg/g for the results it is appears that the concentration of copper in the all studied tissues are below the acceptable safety ranges except in liver of *Mullus*.

Lead concentrations in this study were ranged between 18.27 and 50.404 μg/g dry wt, which they are higher than the ERL (46.7 μg/g) in *Siganus* liver tissue, and lower than the ERL in other species tissues. Lead was the second abundant metal accumulating in tissues following the Zinc. The highest concentrations of lead were found in gills followed by liver in most studied samples. Comparing Pb concentration in present study for *Mugil* SP with those reported in recorded (8.9, 22.6 and 15) μg/g dry wt, for gills liver and muscle tissues respectively. Present study recorded results higher than fishes collected from Derna coast. But the Pb concentration in the results of *Siganus* tissues recorded in present study, also higher than Hamad study [14]. He recorded (0.96, 2.56, and 0.76) for gills, liver and muscle, respectively.

For *Mullus* tissues the result of the present study recorded for Pb concentration in gills and muscle are higher than that recorded in the study, which recorded (12.23 and 8.66) μg/g for gills and Muscle respectively, but it is less than present study in liver tissues, Mohammed recorded 30.11 μg/g. Gills are considered hard tissues, so it is recorded a high value of lead. the high content of lead in gills is approved by NRCC, attributing these findings to the possibility of particulate or organic lead adsorbed onto the gills of fish. Lower pH at the gill surface due to respired CO_2 may cause dissolved lead to a soluble form, which could diffuse into the gill tissue. In other words, lead is more accumulated in the hard tissues (as gills) than soft tissues. The highest accumulation of metals in the gills could be because gills serve as the respiratory organ in fishes through which metal ions are absorbed. The gills are in direct contact with the contaminated medium (water) and have the thinnest epithelium of all the organs, and metals can penetrate through the thin epithelial cells [17]. The concentration of metals in gills also reflects their concentration in water where the fish lives.

Additionally, absorption of toxic chemicals through gills is rapid, and therefore toxic response in gills is also rapid. The adsorption of metals onto the gill surface could also be an important influence on total metal levels of the gill. Gill surfaces are the first target of waterborne metals. The microenvironment of the gill surface consists of an epithelial membrane, which primarily contains phospholipids covered by a mucous layer. The gill surface is negatively charged and thus provides a potential site for gill-metal interaction for positively charged metal. The gill of Synodontis budgetti tends to accumulate the highest concentrations of all the metals, while Oreochromis niloticus shows the lowest concentrations. Laboratory experiments have indicated that in fishes that take up heavy metals from water, the gills generally show higher concentration than in the digestive tract. On the other hand, fish accumulating heavy metals from food show elevated metal levels in the digestive tract as compared to the gills. The gills of all the fish tend to accumulate significantly higher levels of heavy metals than other tissues.

Zinc is the First most abundant element for all studied samples, which accumulates with concentrations comparable to accumulation of lead, the concentration of zinc in liver ranged between 80 and 400 µg/g. zinc concentrations in the liver of *Siganus* were much higher than in liver of other fishes. The highest concentration of zinc was 260.2 µg/g reported in *Mugil* SP, and the lowest one was found in gills of *Mullus* (71.34 µg/g).
Comparing the Zn concentration in present study for *Mugil* SP with those reported in [14], we found the results recorded in present study (99.53, 260.2 and 149.73) for gills, liver and muscle tissues, these results are higher than fishes collected from Derna coast which recorded (94.28, 48.14, and 18.7) µg/g for gills, liver and muscle respectively. Also, comparing Zn concentration in the results for *Siganus* tissues in the present study with some studies found all tissues recorded higher than them [14]. He recorded (20.84, 48.4 and13,47) for gills, liver, and muscle, respectively. For *Mullus* tissues, the result of the present study recorded for Zn concentration in all tissues, gills, liver, and muscle are similar to those recorded in [16] study, he is record (32.54, 8.33and 15.87) µg/g for gills, liver, and Muscle, respectively. These results indicate that the liver appears to be one of the most important sites for Zn accumulation it also evident from some of the earlier findings of (Heath, 1987) and the high levels of Zn in liver can be ascribed to the bindings of Zn to metallothionein (MT) which was at highest concentration in liver. The Zn concentration in the liver (not in direct contact with Zn in water) which play a major role in detoxification as well as storage, would therefore differ from the concentration detected in the gill (in direct contact with the Zn in the water) which play a role in the uptake and excretion of the Zn [18].
The concentrations of zinc in three organs under investigation for the selected species are illustrated in Tables 1-3 and Figures 2-4. The concentration of zinc in the organs of *Mullus* was 71.34, 207.6, and 176.76µg/g in the gills, liver, and muscle, respectively. While in *Mugil* SP fish samples, the zinc concentration was 99.53, 260, and 149.73 µg/g in the gills, liver, and muscle. On the other side, the zinc concentration in *Siganus* fishes was 209, 239.2, and 194.19µg/g in the gills, liver, and muscle tissues, respectively. From the results, it is clear that the highest level of zinc was recorded in the *Mugil* SP relative to the other species under study. The zinc levels in soft tissues (gills, liver, and muscle) depend mainly on the food habit of the species rather than the other environmental parameters, while in the case gills, dependence on both food habit and environmental parameters. the highest concentration recorded in this study was lower than ERM (410 µg/g) and less than the maximum acceptable limits by [19] (750 µg/g).
Nickel concentrations ranged from non-detected in the organs of *Mullus* to 21.7 µg/g in muscles of *Mugil* SP, Aquatic environments generally have low concentrations of nickel. The concentration of nickel in the muscle tissue of the studied fishes ranged from not traceable to 8.97 µg/ g. Fish are known to accumulate Ni in different tissues, when they are exposed to elevated levels in their environment [20] The Ni concentration in present study for *Mugil* SP with which recorded for muscle and liver tissues are higher than recorded for fishes collected from Derna coast (5.4, 15.8) µg/g for muscle and liver tissues respectively.
For *Siganus* tissues the results of present study recorded for Ni concentration in liver and gills tissues are agreement with that recorded in [14] study, which recorded (4.62, 9.57) µg/g in the liver and gills tissues, respectively, some studies recorded 9.57 µg/g for muscle tissues, his results higher than results of present study. [14] Nickel is also extensively bio accumulated from the intake of contaminated food . In this study it was found that the selected fish species showed accumulation of Ni in all the tissues, but the data indicated that the gills contained the highest levels, followed by the liver, and then the muscle. Some researches indicate that Ni is taken up via the gills as a result of its close blood-water contact .Therefore, the gills are the main site for absorption of Ni from the surrounding medium. It should also be remembered that the gills play an important role in the secretion of metals, probably via the secretion of mucus.
The Ni concentrations in the gills of fish during this increased incidence of cancer of the lung and nasal cavity caused by high intake of Ni has been also been reported in workers in Ni smelters. The highest concentration of Ni (0.95 µg/g) was measured in the gill, while the lowest detectable level of 0.11 µg/g was detected in the flesh. The Estimated maximum guideline [21] For Ni is 70 - 80 µg/g. Thus The concentrations of Ni In all the samples were far below the stipulated limit.
Cadmium Concentrations in the studied fishes were ranged between 2.29 in gill, 1.57 in liver and 0.89 in muscle. for the organs of *Mullus,* while in *Mugil* SP fish samples were 2.90, 3.85 and 1.02µg/g in the gills, liver and Muscle respectively. on the other side the concentrations in *Siganus* fishes were 1.09, 3.85 and 7.86 µg/g for the gills, liver and Muscle tissues, respectively.
For *Mugil* SP tissues, the result of present study recorded for Cd concentrations in all tissues liver muscle and gills are similarly that recorded in [14]study he is record (1.24, 1.03, 2.07) µg/g for liver Muscle and gills, respectively. When we comparing Cd concentration in present study for *Siganus* with those reported in we found the results recorded in present study for gills, liver and muscle tissues are higher than fishes collected from Derna coast that recorded (0.13, 0.40 and 0.55) µg/g for gills, liver and muscle tissues respectively.For *Mullus* tissues the result of present study recorded for Cd concentration in liver and muscle tissues are agreement with that recorded in study, he is record (4.62, 9.57) µg/g in liver and muscle tissues respectively, but some study results [14] are less than results of present study, in gills tissues, he is recorded 0.86 µg/g.
From the results of all studied fish samples in the Table (1-3) and as it present in Figure (3). The liver seemed

to be the most organ which accumulate a higher value of cadmium. This is in agreement with the literature (which reported that Cd is stored in the body in various tissues, but the main site of accumulation in aquatic organisms is in the kidney and liver, beside other tissues, notably the gills, bone and exoskeleton. However, In this study, cadmium concentrations in tissues were higher than the maximum acceptable limits reported by [22] but less than that reported by both [24]. There is no great difference between the concentration of Cd in muscle in all collected fishes. Cadmium is not an essential element for any organisms, although for unknown reasons, it enhances phytoplankton photoplankton. Luoma, reported that although Cd in non - essential metal, it is accumulated by marine animals via the routes common to essentials metals. [25] Generally, the determined levels of the heavy metals in three species of fish samples collected from Benghazi coast are presented in the Tables (1-3). And presented in figures (2-4). The concentration metals followed the decreasing order of Zn > Pb > Cu > Cr > Cd > Ni in *Mullus* gill; and Zn > Cu > Pb > Cd > Cr > Ni in *Mullus* liver; and Zn > Pb > Cu > Cr > Cd > Ni in *Mullus* Muscle. The heavy metal levels in *Mugil* SP gills were: Zn > Pb > Cu > Ni > Cr > Cd ; in liver Zn > Cu > pb > Ni > Cr > Cd; and in Muscles Zn > Pb > Ni > Cu > Cr > Cd. The same heavy metal concentration in gills of *Siganus* were: Zn > Pb > Cu > Cr > Cd > Ni; in liver Zn > Pb > Cu > Ni > C r > Cd; and in muscles Zn > Pb > Cu > Ni Cr > Cd.

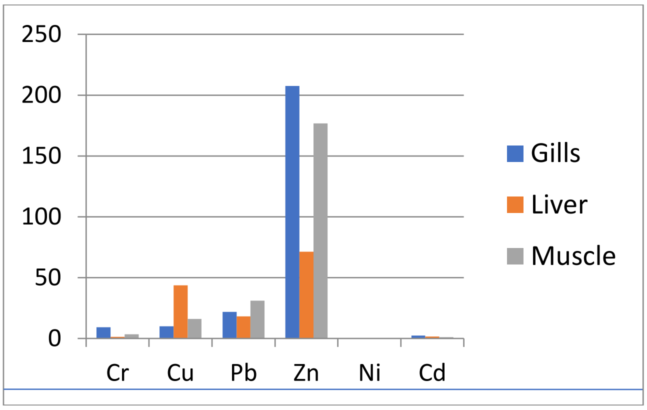

Figure 2. concentrations of metals in tissues of Mullus

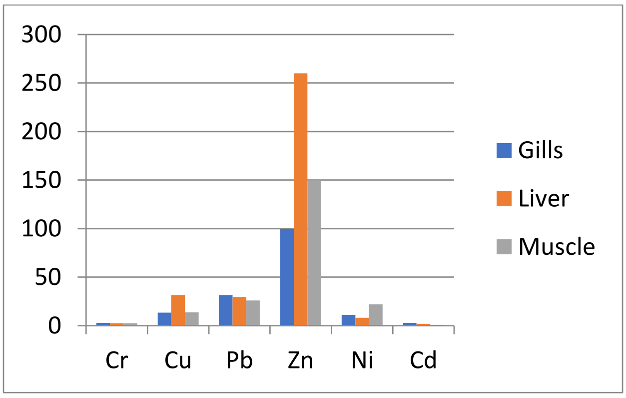

Figure 3. Concentrations of metals in tissues of Mugil SP

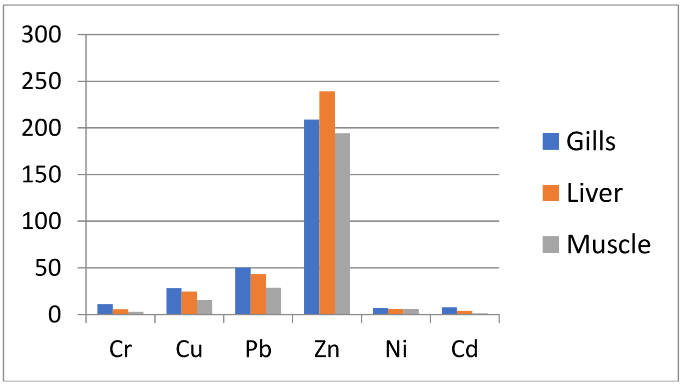

Figure 4. Concentrations of metals in tissues of Siganus

The final metal pollution index (MPI) of each site is a weight mean value, as it was obtained taking into account the total weight of fish. The calculated MPI was fluctuating between 10.27 to 23.49 µg/g with an average value of 14.37± 4.13, The highest MPI were recorded for liver tissues for *Mullus* and *Mugil* SP samples, while the lowest MPI was found in muscles tissues of all fish species studied. All results are given in Table 4 and figures (5-8). The sequence of MPI in tissues of *Mullus* was as following: liver > gills > muscle, Agreement with tissues of *Mugil* SP that ordered as liver> gills > muscle, while the sequence of MPI in tissues of *Siganus* was as following: gills > liver > muscle.

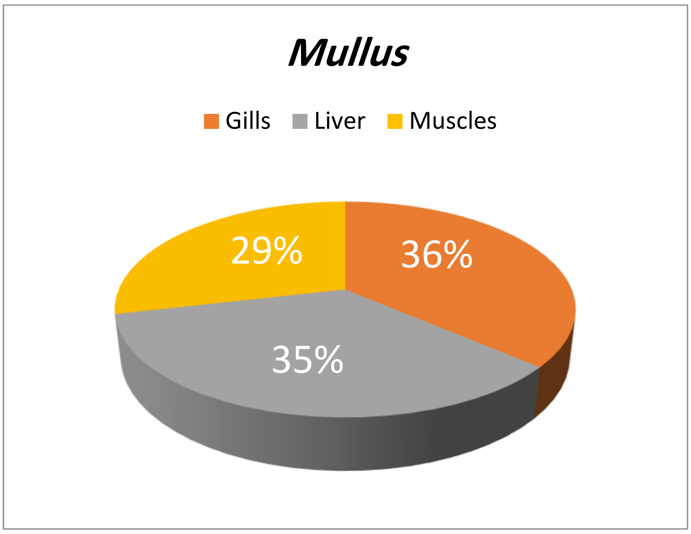

Figure 5. percentage of MPI in tissues of Mullus

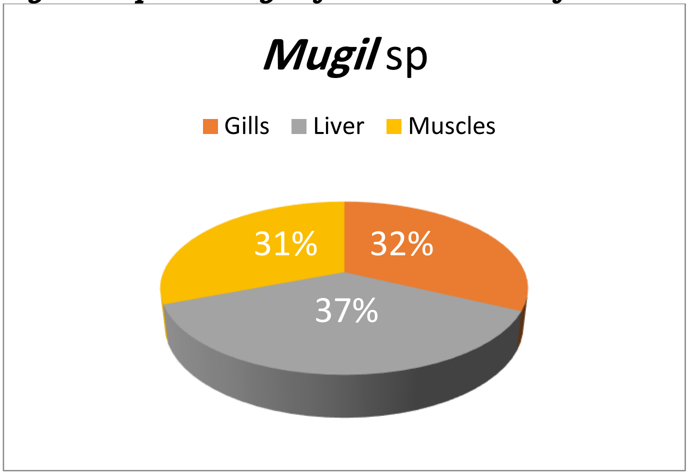

Figure 6. percentage of MPI in tissues of Mugil SP

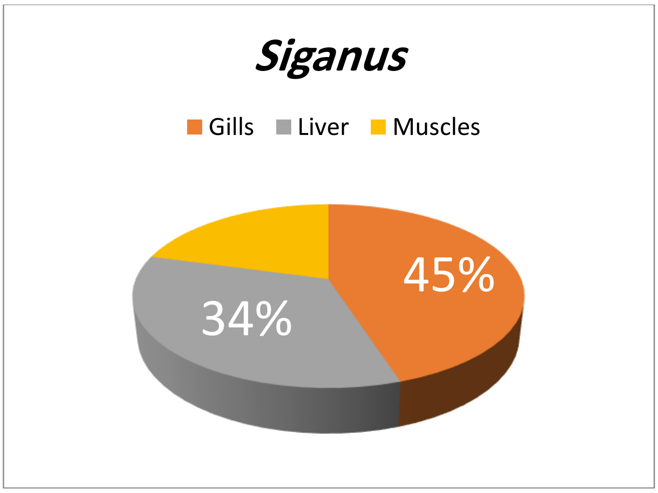

Figure 7. percentage of MPI in tissues of Siganus

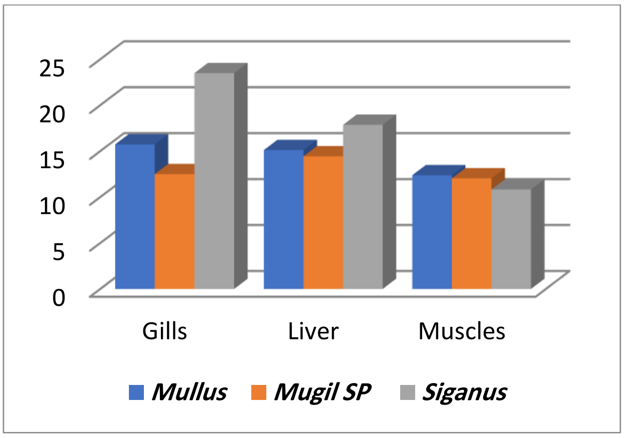

Figure 8. The MPI values of heavy metals in all fishes

The variability in heavy metal levels in deferent species depends on feeding habits, ecological needs, and metabolism, age, size, and length of the fish and fish habitat [26] Concentrations of trace metals detected in the muscle, gill, and liver samples indicate deferent bioaccumulation potentials. Muscles seem to be a transitory tissue in the pathway of metal uptake and in metal storage, whereas the liver appears to be the tissue, specialized in metal storage and detoxification. The gills comprise the chief exposure tissue and early uptake site of the soluble, waterborne metals in which metal concentrations are the highest in the early stages of exposure, before these metals are transported to other fish tissues [27]. The results of the present study suggest that, at some point, sources of heavy metal contaminations are present in the coast of Benghazi activities. In present study liver of the three species accumulated more concentrations of the metals when compared to other tissues. The gill tissue came second in terms of metals tissue accumulation after liver. The results of the current study showed presence different heavy accumulated in different tissues including Cr, Cu, Pb, Zn, Ni and Cd in gills liver and muscles of three fish species. The results indicated that some of the heavy metals recorded in this study were higher than the recommended limits of NHMRC guide lines for humaHasan n health. The study also showed differences between the studied heavy metals in the selected tissues of gills, liver and muscles. The study concluded that the contents of heavy metals in some tissues showed toxic influence for human health.
The calculation of fish ingestion, lifetime daily intake (e.g 70 years) and hazard index had been reported in table (10). The concentration of heavy metals recorded for fish tissues are still lower than acceptable daily intake. Results gave low hazard index values for all studied fish tissues. The hazard index was lesser than 1 for all studied samples, indicating no health risk on the consumers. Results gave low hazard index values for all studied fish tissues. The hazard index was lesser than 1 for all studied samples, indicating no health risk on the consumers. The study of heavy metals in different marine samples as water, sediments , fishes , alge in Libya was taken place in many studies through the near last year's [41-54].

Table 10: Metal pollution index calculated for heavy metals concentrations µg/g

Fish name	Tissue	Cr	Cu	Pb	Zn	Ni	Cd	Co	MPI
	Gills	9.14	10.11	21.97	207.67	ND	2.29	ND	15.73
Mullus	Liver	1.28	43.69	18.27	71.34	ND	1.57	ND	15.12
	Muscle	3.37	16.12	30.99	176.76	ND	0.98	ND	12.38
	Gills	3	13.54	31.5	99.53	11	2.9	ND	12.5
Mugil SP	Liver	2.43	31.61	29.73	260.2	8.144	2	ND	14.44
	Muscle	2.68	13.85	25.9	149.73	22	1.024	ND	12.04
	Gills	11.07	28.23	50.404	209	7	7.86	ND	23.49
Siganus	Liver	5.89	24.73	43.42	239.2	5.9	3.9	ND	17.86
	Muscle	2.954	15.84	28.69	194.19	6	1.09	ND	10.83
ERL	Tissue	81	34	46.7	150	20.9	1.2	NA	
EMR	Tissue	370	270	218	410	51.6	9.6	NA	

]

Table 11: calculation of ingestion of commercial fish studied for child and adult, and calculated lifetime daily intake (µg $g^{-1}day^{-1}$) (CLTDI).

Fish name	Ingestion for	Cr	Cu	Pb	Zn	Ni	Cd	Co
	Child	0.0045	0.008	0.015	0.085	0.00045	0.0075	ND
Mullus	Adult	0.0035	0.0063	0.012	0.069	0.0003	0.0059	ND
	CLTDI	0.032	0.058	0.11	0.63	0.0027	0.054	ND
	Hazard Index	0.4	0.01	0.52	0.03	0.009	0.9	
	Child	0.001	0.0065	0.02	0.01	0.011	0.006	ND
Mugil SP	Adult	0.001	0.0054	0.0101	0.058	0.017	0.0047	ND
	CLTDI	0.009	0.049	0.093	0.061	0.156	0.045	ND
	Hazard Index	0.11	0.016	0.44	0.0032	0.52	0.75	
	Child	0.001	0.005	0.014	0.095	0.003	0.05	ND
Siganus	Adult	0.0011	0.0062	0.011	0.076	0.0023	0.00043	ND
	CLTDI	0.0092	0.057	0.101	0.702	0.021	0.008	ND
	Hazard Index	0.115	0.019	0.48	0.036	0.07	0.135	

ND = not detected; NA = not available; MPI = metal pollution index calculated for dry weight; ERL = Effect Range - Low; EMR = Effect Range -Median = not detected. The hazard index can be calculated by dividing the CLTDI by the tolerable daily intake (TDI)

The tolerable daily intake approach in view of avoiding undesirable health hazards consequent of "excessive" intake of toxicants (including toxic metals), international and national scientific organisms such as FAO/WHO, FDA, European Union, etc, have used the safety factor approach for establishing acceptable or tolerable intakes of substances that exhibit threshold toxicity. The acceptable daily intake (ADI) or tolerable daily intake (TDI) are used to Describe "safe" levels of intake for several toxicants including toxic metals [28]. For chemicals that give rise to such Toxic effects, a tolerable daily intake (TDI), i.e. an estimate of the amount of a substance in food, expressed on a body weight basis (µg.g1 of body weight) that can be ingested over a lifetime without appreciable health risk. However, the estimated intakes of chromium, copper, lead, nickel, zinc and cadmium from daily consumption of fishes shows no risk since they are lower than the respective established permissible tolerable daily intakes for these elements.

CONCLUSION

From the results which recorded in this study, there are some heavy metals values higher than the WHO and NHMRC guide lines for human consumer. Also, the obtained data, may be give the following recommendations: A study other metals which not carried out in this study. Select other fishes to determine the other heavy metals. Selected organs of *Mullus, Mugil* sp and *Siganus*, were chosen for tissue Cr, Cu, Pb, Zn, Ni and Cd analysis. Heavy metals contents were measured and compared in fish species. Gills, liver and muscles were chosen for tissue heavy metals analysis. The mean concentrations of Cr, Cu, Pb, Zn, Ni and Cd in gills liver and muscles of three fish species. In liver of all fish species Cr(33.346, ppm), Cu (30.481 ppm) and Pb (190.25 ppm) concentrations were higher significantly different (p= 0.000) than gills and muscles. However, in gills Zn, Ni and Cd concentrations were significantly higher than concentrations in liver and muscles (P= 0.005). Values for all variables in the fish groups. The three groups differ in Cr, Cu, Pb, Zn, Ni and Cd contents in gills, liver and muscles. In fish gills, *Siganus* had significantly higher (p=0.000) concentration of Cr, Cu, Pb and Zn than concentrations in gills of *Mullus* and *Mugil* SP. However, *Mugli sp* fish had higher Ni content in gills than that found in gills of *Siganus* and *Mullus* (p=0.000). In liver, *Siganus* group had higher significantly concentrations of Pb and Cd than concentrations in liver of *Mullus* and *Mugil* SP. Nevertheless, liver of *Mugil* SP group had higher concentrations of Zn and Ni than other fish species (p= 0.005). In fish muscles high significant were found between fish species in Cr, Cu, Pb, Zn and Ni (P= 0.000). Although, non-significantly different in concentration of Cd between fish species (p= 0.559). In liver of all fish species Cr(33.346, ppm), Cu (30.481 ppm) and Pb (190.25 ppm) concentrations were higher significantly different (p= 0.000) than gills and muscles. However, in gills Zn, Ni and Cd concentrations were significantly higher than concentrations in liver and muscles (P= 0.005).

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