Assessment of Heavy Metals (Cd, Fe, Cu and Zn) levels in Oreochromis aureus and Cyprins Carpio fish species collected from Shat El-Arab River, Basra-Iraq, as possible indicator of heavy metals toxicity

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Abstract

Introduction: Environmental pollution caused by heavy metals has aroused widespread concern around the world. Fishes consider a connecting link for the transfer of toxic metals in human beings. Shatt AlArab is polluted by some different sources of pollutants including power stations, paper industry, oil refineries, and chemical fertilizer companies and overfishing. Accordingly the study was designed to measure Zn, Cu, Fe and Cd concentrations in the scale, bone, and muscle tissues of Bolti (Oreochromis aureus) and common carp(Cyprins Carpio) fish species in Basra, Iraq. Fishes were collected with the help of local fisherman; ten individuals of each species were stored in ice and transported to the laboratory immediately for heavy metals analysis.

Results: Zn concentration significantly high in Bolti compared to carpio, Cu level in bones and scales are significantly high in common carpio while muscles show an opposite result. Iron levels are comparable in in both fish species while Cd shows the highest concentration in bone of carpio species.

Conclusion: there were variation in heavy metals distribution in tested organs in both fish species, fortunately Zn, Cu, Cd and Fe concentrations in the edible part of the two fish species were below the limits proposed by various international standards and guidelines with significant increase in Cd concentration in fish's bones.

Key words: heavy metals, fish, pollution

Introduction

Globally environmental pollution occurred by heavy metals has aroused widespread concern around the world (1). Sea foods contain essential amino acids, fatty acids, protein, carbohydrates, vitamins and minerals commonly consumed and, hence, they are a connecting link for the transfer of heavy metals in human beings (2). According to FAO statistics, fish accounted for about 16% of the global human's intake of animal protein and 6% for all protein consumed (3). Since fish constitute a major constituent of human diet, it is not surprising that the quality and safety aspects are of particular interest. Over the past several decades, the concentrations of heavy metals in fish have been extensively studied in various places around the world because the diet is an important source of heavy metals for human being (4) and the major interest was in the edible commercial species. There fore, seas-onal investigation of heavy metals contents of fish species is important to analyze whether acceptable to human consumption at nutritional and toxic levels or it may be higher than the recommended legal limits. (5). Furthermore, concentrations of such elements in body fluids need to be tightly controlled, given their requirement in many metabolic reactions and their potential toxic effect at elevated levels (6). The rate of bioaccumulation of heavy metals in aquatic organisms depends on the ability of the organisms to accumulate the metals, the concentration of this metal in the river, concentration of the heavy metal in the surrounding soil sediments as well as the feeding habits of the organism, from this fishes have been recognized as a good accumulator of organic and inorganic pollutants[(7)].Furthermore fish have ability to collect these metals in concentrations higher than water and sediments due to feed on organic materials in aquatic environment also because they occupy different atrophic levels, all these factors make fish a good indicators and their chemical analysis can provide significant information on the assessment of heavy metals contamination in aquatic systems(8). Heavy metals are commonly found in natural waters and some are essential to living organisms, but they may become toxic when present in high concentrations (9). Heavy metals acquired through the food chain as a result of pollution are potential chemical hazards, threatening consumers, metals like (iron, copper and zinc) are essential metals since they play a crucial role in biological systems whether functional or structural, whereas metals (mercury, lead and cadmium) are non-essential metals and they are toxic even at low concentrations (10-13).

Copper and iron are required in trace amounts but is toxic in excess amounts due to free radical generating ability through fenton reaction which damages DNA and other macromolecules (14, 15). Cd concent-rates along the food chain and ultimately accumulate in the body of people eating such foods, once absorbed it irreversibly accumulates in the human body compartment, in particularly in kidneys and other vital organs such the lungs or the liver, Cd is also a highly toxic metal that can disrupt a number of biological systems, usually at doses that are much lower than most toxic metals (16). These metals also gain access into ecosystem through anthropogenic source and get distributed in the water body, suspended solids and sediments during their mobility (17). Therefore, it is important to monitor heavy metal in aquatic environments (water, sediment and biota). The Shatt al Arab River is formed after the confluence of the Euphrates and the Tigris Rivers near the city of Qurnah in southern Iraq (see Overview Map). The southern part of the river constitutes the border between Iran and Iraq until it discharges into the Gulf. With a total length of 192 km, the Shatt al Arab widens over its course, expanding from a width of 250-300m near the Euphrates-Tigris confluence to almost 700 m near the city of Basra and more than 800 m as it approaches the river mouth (18). Shatt Al-Arab River passes two major industrial agglomerations, the Iraqi port of Basrah and the Iranian port of Abadan before emptying into the Gulf. it is polluted by some different sources of pollutants including power stations, paper industry, oil refineries, petrochemical industry, chemical fertilizer companies and the sewage system and overfishing and the application of pesticides (19). Al-Higag assessed the concentration of Cd, Cu, Pb and Zn in Al Ashar and in Al-Khandak canals and has recorded high concentrations (20). Phillips et al measured trace elements conce-ntrations in a variety of seafood and reported elevated concentrations of arsenic and cadmium in fish (21). Since there is no formal control of effluent discharge from industries and homes into the river, it is important to monitor the levels of metals in shat al-alarab through measurement of heavy metals concentration in fish species. Accordingly, the objective of current study was the measurement of zinc, copper, iron and cadmium concentration in the scales, bones, and muscles tissues of Bolti (Oreochromis aureus) and common carp (Cyprins Carpio**)** fish species in order to assess the seafood consumption safety also it could establish a baseline for future studies of heavy metal pollution in Basra, Iraq.

Materials and methods

Chemicals

HNO_3, H_2O_2 (35%) and $HClO_4$ supplied from (Fluka-Garantie (Poch), Germany. All the plastic and glassware were cleaned by soaking in dilute HNO_3(1%) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 ppm of each element supplied from Buck Company, USA.

Sampling and sample preparation

In Aug 2013 Bolti (Oreochromis aureus) and common carpio (Cyprins Carpio) Fish species were collected with the help of local fisherman from shat El-arab River in Abu al-Khasib region. Ten individuals of each species were stored in ice and transported to the laboratory immediately, fish samples were washed with distilled water, dissected with clean stainless steel instruments on the same day. Fish samples were numbered and weighed and their lengths were measured on site. Fish having the same weight, length, and age were preferred (300±43-gram weight and 10.3±32 cm length). One gram of Muscle, bone, and scales of each individual fishes of both species were incised and stored in clean amber glass containers at -18 °C until further digestion and analysis of heavy metals.

Analyses of heavy metals Concentrations of Zn, Cu, Fe and Cd in muscles, bones and scales samples were first must be released from matrix by wet digestion method as mentioned below then determined by AAS using a Buck Model 211-VGP spectrophotometer according to operator's manual (February 2005 VER 3.94 C by Analyst: Gerald J. De Menna), with a detection limit of 0.005 ppm for (Zn, Cu and Cd), and 0.05 ppm for Fe (22). The flame conditions were fixed as recommended by the instrument manufacturer for Zn, Cu, Cd and Fe, (wavelengths 214, 324, 228 and 247 nm, respectively), the measuring time was 3 second. Standard solutions of those elements were first aspirated to calibrate the AAS before the aspiration of the samples (23). Six concentrations of standards for each element are prepared using 1000 ppm STD supplied by Buck company, and their absorbance by the instrument was constructed in preparation of the calibration curve was autoexcuted by the software of the instrument. From the prepared standard curves the concentration of each element was calculated using the following formula (9):

[Sample concentration= Read concentration × dilution factor/sample weight]

All metals concentrations were presented as (µg/g) on wet weight basis.

Wet digestion method One gram of each sample (muscle, bone, scales) was taken in a clean test tube and a mixture of 3.0 ml HNO_3:1.0 ml $HClO_4$ was added. The tube was plugged with cotton wool and left on the bench overnight to solubilize. On the second day, the digested sample was heated at 100°C on a hot plate for 20 min. Then it was allowed to cool, after a while 1.0 ml of hydrogen peroxide was added for each tube to prevent excessive foaming. The tubes were allowed to stand on the bench overnight again. On the third day, the samples were heated again at 100°C for 1.0 hr and allowed to cool at room temperature. Then the tube content was diluted with distilled water to a final volume of 10 ml, and stored in a 30 ml polyethylene bottle for later analysis by AAS (24-26).

Results

Figure (1) shows the concentration of zinc in two species of fish obtained from shat El-arab River in Abu al-Khasib region. Muscles, bones and scales in Bolti showed highly significant Zn concentrations when compared to common carpio. The bones showed the highest zinc concentration (Table1), regarding edible part (muscles) recorded high concentration of Zn in both species but this value acceptable for human consumption designated by standard guidelines on food safety by the WHO and FAO in 2011(table2). In figure2 the present study reported a significant increase in Cu concentration in muscle of Bolti fish species as compared to common carpio meanwhile their concentration acceptable for human consumption (table2), in bones and scales an opposite finding to that observed in muscles with highest level in common carpio than Bolti. Figure3 demonstrated that iron levels were comparable in muscle and bones in both fish species where no significant differences were found but there was a significant difference ($P< 0.05$) among species in scales with higher concentration in Bolti group, in general Fe concentration in both fish species acceptable for human consumption table (2). Cd **c**oncentration varied with no significant difference among fish species in muscle and scale except the bones that show the highest concentration in common carpio compared to Bolti (figure4), Cd concentration in the edible fish organ (muscle) was acceptable and below FAO/WHO 2011 maximum limit but their concentration passes designated limit where high Cd concentration was reported in bones and scales (Table 2). Different fish's organs showed different capacities for accumulating heavy metals and levels considerably varied among individuals of the same species. Table 1 summarized such variation, the highest metal concentrations were found in the bones and scales. However, the muscles tended to accumulate less metal and this could be favorable because muscle is the edible part of the fish. In *Oreochromis aureus*, the distribution pattern of Zn was differing >significantly in the following pattern: bones muscles. In *Cyprinus carpio* the >scales distribution pattern of Zn metal was different muscle=scales. In Cu the >as following bone >following distribution were observed bones muscles in Cyprinus carpio species >scales while in Bolti the distribution of Cu was comparable in all studied organs. Fe accumulations in both fish species were muscles. Regarding >comparable bones=scales Cd highest concentration observed in Cyprinus carpio species as following distribution muscles. While in Bolti the metals >bones>scales muscles.>accumulation as following scales=bones

Statistical analysis: Values were expressed as mean±S.D; the values were statistically evaluated using unpaired Student's ***t***-test and one way analysis of variance (ANOVA), supported by Bonferroni's*post hoc* analysis. Values with $P<0.05$ were considered significantly different. Analysis was performed using GraphPad Prism software for Windows (version 5.0, GraphPad Software, Inc., San Diego, CA).

Discussion

Metal toxicity may damage marine organisms at the cellular level and may affect the ecological balance and subsequent exposure for such polluted marine organisms as sea foods can cause health problems including neurological and reproductive problems (27). There were several studies on heavy metals concentrations in water, sediments, fishes and other aquatic organism conducted in rivers and lakes around the world but seasonal or regular evaluation of metals toxicity are required to assess and provide baseline information of pollution impact of sea foods particularly in Shat El-Arab river that constitute the main source of fishes in Basra city (Iraq). In the present study, two fish species were examined Bolti (Oreochromis aureus) and common carpio (Cyprins Carpio). The reasons behind the selection of such species are high human consumption and lack or scarcity of information available regarding heavy metals concentration in different organs of both species in Shat El-Arab River in Abu al-Khasib region. Meanwhile different feeding habits and habitats this gave variations in metal levels so different species provide more information about the main routes of heavy-metal uptake and toxicity (28). Zn concentration in muscles, bones and scales of Bolti fishes significantly high when compared to common carpio fishes with such pattern of muscles in Bolti >scales>distribution: bone while in common car pio muscles=scales. Generally, all the >bone studied organs showed high level of Zn in bone, scales and even the muscles particularly in Oreochromis aureus. This results were consistent with other previous reports in which Zn distribution occurs in similar manner (29). This value is higher than the acceptable value for Zn in edible fish 30 mg/ kg by FAO 1983 (30) but it is within acceptable limit of FAO/WHO 2011 (31). in general zinc deficiency caused by malnutrition, aging, certain diseases, or deregulated homeostasis is a far more common risk to human health than intoxication on the other hand zinc toxicity is rare, but at concentrations in water up to 40 mg/ l, may occur characterized by symptoms of irritability, muscular stiffness, loss of appetite and nausea (32). Furthermore, Zn appears to have a protective effect against the toxicities of cadmium (33). In the present study common carpio accumulated high concentrations of Cu in all tested organs except muscles while the highest concentration of Cu observed in Bolti fishes as compared to common carpio. Organs distribution as following bone =scales=muscles in Bolti while in common carpio muscles. Copper is present in high >scales>bone level in water this may be due to the extensive use of pesticides sprays containing copper compounds in agricultural purposes (34). Cu is required by human metabolism processes and inter in function or enzymes activity but at toxic level may lead to connective tissues and liver damage due to its potential toxicity and ability to generate free radical (35). The toxicity of copper depends upon the hardness and pH of the water; it is more toxic in soft water with low alkalinity this give an idea about variation in Cu concentrations in different rivers (36). The Cu concentrations found in the muscles of both tested fish species within normal limit and did not exceed the maximum legal limit designated by all comities. In the present study comparable Fe concentration were observed in both species except in scales high significant iron level observed in Bolti fishes. These iron values are within acceptable limit and lower than reported data from literatures (37,38). Iron is essential for normal physiology, as it is component of proteins involved in oxygen transports from the lungs to the tissues (39). In this study the mean±SD concentrations of iron 3.05±0.66 and 1.89±0.25 in Bolti and carpio respectively. These values are lower than WHO/FAO maximum permissible limit (31). Literature on Fe interactions in biological tissues has reported that excess amount of Fe causes rapid increase in pulse rate and coagulation of blood in blood vessels, hypertension and drowsiness (40).

Cadmium is a highly toxic non–essential heavy metal and it does not have a role in biological process in living organisms and could be harmful to living organisms (41). The mean±SD concentrations of Cd in muscles of both fishes lower than WHO/FAO maximum permissible limit of 0.5mg/kg, however it is greater than limit established by China's national standard [China National Standards Management Department, 2001] (42) and EU 2001(43). In general, the mean muscle concentrations were used to conduct health risk assessments for human consumption, the present study shows high Cd level in muscle of common carpio Cyprins Carpio) species as compared to Bolti species. Actually Cd concentration in bones and scales are significantly high and pass acceptable limit but fortunately this organ is not edible by human with least expected toxicity, however consumers might raise alarm for potential health risks of consuming contaminated food samples. Literature supports the view that, Cadmium which often accumulates in the human body via food negatively affects several organs: liver, kidney, lung, bones, placenta, brain and the central nervous system by competing with calcium at enzymatic locations (44). Moreover, accumulation of Cd in scales and bone indicated that the uptake of Cd could occur through hard tissues this in agreement with other study (45, 46) which reported that Cd is tored in kidney and liver, gills, bone and exoskeleton.

Conclusions

1- The present study provides a first estimate of the heavy metals concentrations in muscles bones and scales of Bolti (Oreochromis aureus) and common carpio (Cyprins Carpio) fish species in shat El-arab.

2- River in Abu al-Khasib region, Basra, Iraq. 2- There was no single type of fish that was consistently high for all metals.

3- The highest concentrations of Zn and Cu observed in bones in both fish species

4- Scales and bones showed higher concentration of iron compared to muscle in both examined fish species. And highest Cd level reported in bones and scales compared to muscles in each species.

5- Zn, Cu and Fe concentrations in all tested fish's organs were below the limits proposed for fish by various international standards and guidelines such as EU (2001), FAO/WHO (2011), MAFF (2000) Turkish guidelines and China National Standards 2001.

6- Cd concentration in the edible fish organ (muscle) was acceptable and below FAO/WHO 2011 maximum limit, but Cd accumulate in high concentration in bones and scales in both fish species exceeding acceptable limit.

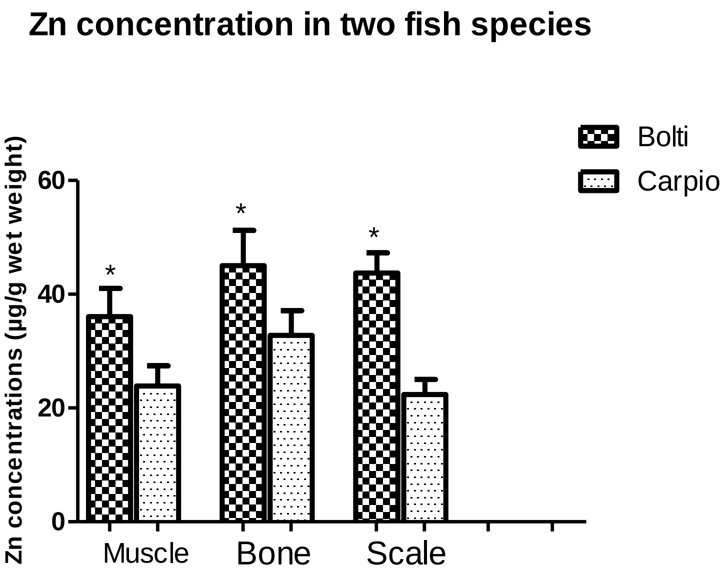


Figure (1) Zn concentrations in muscles, bones and scales in Bolti (Oreochromis aureus) and common carpio (Cyprins Carpio) Fish species. Values wereexpressed as mean±SD; number of fishs=10 in each species. * Significantly different among fish species in the same organ.

Figure (2) Cu concentrations in muscles, bones and scales in Bolti (Oreochromis aureus) and common carpio (Cyprins Carpio) Fish species. Values were expressed as mean±SD; number of fishs=10 in each species. * Significantly different among fish species in the same organ.

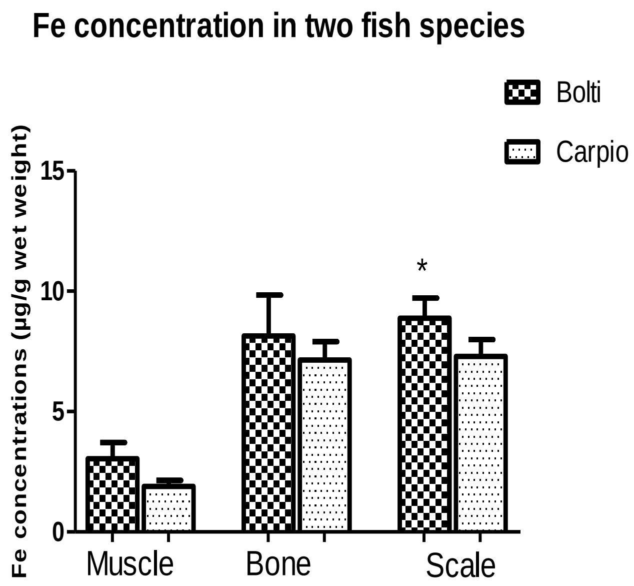
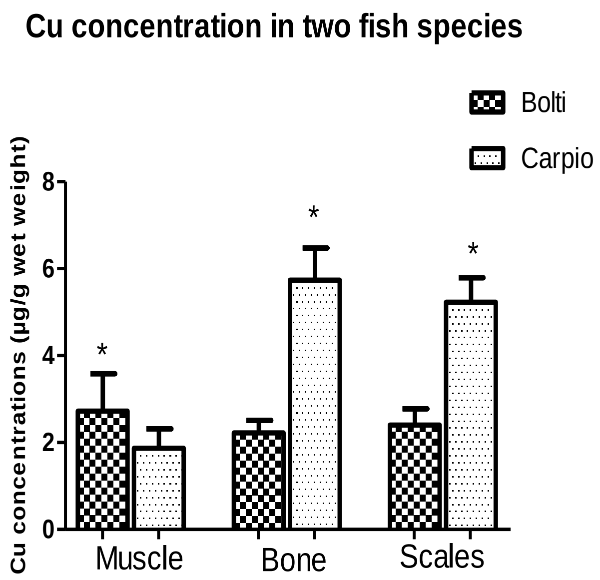


Figure (3) Fe concentrations in muscles, bones and scales in Bolti (Oreochromis aureus) and common carpio (Cyprins Carpio) Fish species. Values were expressed as mean±SD; number of fishs=10 in each species. * Significantly different among fish species in the same organ.



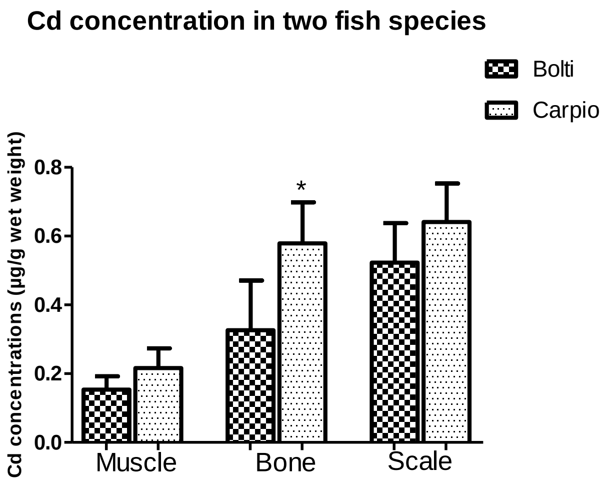


Figure (4) Cd concentrations in muscles, bones and scales in Bolti (Oreochromis aureus) and common carpio (Cyprins Carpio) fish species. Values were expressed as mean±SD; number of fishs=10 in each species. * Significantly different among fish species in the same organ.

Heavy metals	Fish species	organs			P-values
		muscle	bone	scale	
Zn	**Oreochromis aureus**	**36.1±4.9**	**45 ±6.23**	**43.7 ±3.5**	**0.05<**
	Cyprins Carpio	**23.9±3.5**	**32.8 ±4.3**	**22.3 ±2.6**	**0.05 except muscle Vs < scale**
Cu	**Oreochromis aureus**	**2.72±0.86**	**2.22 ±0.2**	**2.4±0.37**	**n.s.**
	Cyprins Carpio	**1.87 ±0.44**	**5.74±0.7**	**5.23±0.56**	**0.05<**
Fe	**Oreochromis aureus**	**3.05±0.66**	**8.15 ±1.6**	**8.88 ±0.8**	**0.05 except bone Vs < scale**
	Cyprins Carpio	**1.89 ±0.25**	**6.81±0.7**	**7.29±0.7**	**0.05 except bone Vs < scale**
Cd	**Oreochromis aureus**	**0.153 ±0.039**	**0.32±0.14**	**0.52±0.11**	**0.05<**
	Cyprins Carpio	**0.216 ±0.059**	**0.57±0.11**	**0.64 ±0.11**	**0.05 except bone Vs < scale**

Table (1). Concentrations of Zn, Cu, Fe and Cd, µg/ g wet wt. in muscles, bones and scales of two fish species collected from Shat El-Arab, southern Iraq during Aug, 2013.Values were expressed as mean±SD; number of fishs=10 in each species; (P<0.05): significant differences between organs within the same species. N.s.non significant differences

Table2. Maximum limit and standard levels in (µg/g wet weight) of metals in fish described in literature and concentrations found in edible parts(muscles) of Oreochromis aureus and Cyprins Carpio fish species collected from Shat El-arab river, southern Iraq(Basra). NA not available

Organization/country	Metals				References
	Cd	Cu	Zn	Fe	
European Community	0.05	–	–	–	EC (2005)(47)
FAO/WHO limits	0.5	30	40	42	FAO/WHO)٣١(2011)(
FAO (1983)	–	30	30		FAO)٣٠(1983)(
England	0.2	20	50		MAFF (2000)(48)
EU limits	0.1	10	NA		)٤٣EU (2001)(
					study
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Figure (5) Shat El-Arab Map

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تقييم مستويات المعادن الثقيلة (الكادميوم، الحديد، النحاس والزنك) في أسماك البلطي واسماك الكارب التي تم جمعها من نهر شط العرب في البصرة، العراق، كمؤشر لدرجة سمية العناصر الثقيلة

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الخلاصة

التلوث البيئي الناجم عن المعادن الثقيلة أثار قلقا واسعا في جميع أنحاء العالم. ان الأسماك هي همزة وصل لنقل المعادن السامة للبشر. هناك عدد من الملوثات التي تؤدي الى تلوث شط العرب بما في ذلك محطات توليد الطاقة وصناعة الورق ومصافي النفط وصناعة البتروكيماويات، وشركات الأسمدة والصيد الجائر. الهدف من الدراسة الحالية قياس تراكيز الزنك، النحاس، الحديد والكادميوم في القشور، العظام، والعضلات من سمك البلطي والكارب الشائع من أجل تقييم سلامة استهلاك المأكولات البحرية في البصرة، العراق.

جمعت اسماك البلطي والكارب بمساعدة الصيادين المحليين في نهر شط العرب حيث تم تخزين عشرة أفراد من كل نوع في الثلج ومن ثم تم نقلها إلى المختبر مباشرة لقياس المعادن الثقيلة.

النتائج: لوحظ ارتفاع تراكيز الزنك بشكل كبير في اسماك البلطي حيث أظهرت العظام أعلى مستوى ارتفاع. اما بخصوص مستوى النحاس في العظام والقشور فكانت التراكيز مرتفعة بشكل ملحوظ في أسماك الكارب بينما تظهر العضلات نتائج عكسية. لوحظ عدم وجود فروق ذات دلالة إحصائية في مستويات الحديد في كلا النوعين. فيما يخص الكادميوم كان اعلى تركيز في العظام لسمك الكارب.

الاستنتاج: أن هناك تفاوت في تراكيز وتوزيع المعادن الثقيلة في كل أنواع الأسماك، لحسن الحظ كانت تراكيز الزنك، النحاس، الكادميوم والحديد في الجزء الصالح للأكل تحت الحدود المقترحة وفقا للمعايير والمبادئ التوجيهية الدولية المختلفة مع ان هناك ارتفاع في تركيز الكادميوم في عظام الاسماك الخاضعة للدراسة.