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Bioaccumulation of Heavy Metals in Nile Tilapia *Oreochromis Niloticus*, Collected from Karachi, Pakistan

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Abstract

This study was conducted to evaluate heavy metals in Nile tilpia, muscle sample gathered from Nile Tilapia *Oreochromis niloticus* collected from Fish Market, Musa Colony, Karachi, Pakistan were analyzed to determine heavy metal concentrations (Aluminum [Al], Lead [Pb], Zinc [Zn], copper [Cu], and cadmium [Cd]). All samples were divided for three groups according their sizes given as follows: Group A: 15.0-20.0 cm; Group B: 20.1-25.0 cm; Group C: 25.1-30.0 cm and were analyzed using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). It was observed that the average measured level of metals in muscle (Al 9.47±0.13 µg/g, Pb 0.40±0.01 µg/g, Zn 37.64±2.35 µg/g, Cu 12.42±1.42 µg/g, Cd 0.22±0.02 µg/g), Skin (Al 8.00±0.13 µg/g, Pb 0.48±0.02 µg/g, Zn 42.66±2.52 µg/g, Cu 13.30±1.27 µg/g, Cd 0.28±0.01 µg/g), Liver (Al 11.92±1.21 µg/g, Pb 0.70±0.22 µg/g, Zn 64.00±4.31 µg/g, Cu 15.79±3.25 µg/g, Cd 0.30±0.01 µg/g) and Gills (Al 8.80±2.17 µg/g, Pb 0.70±0.11 µg/g, Zn 14.83±2.98 µg/g, Cu 10.78±1.42 µg/g, Cd 0.28±0.08 µg/g) respectively. The correlation between size groups and metal accumulation in organs were investigated for all the groups. As a result of the analysis, Al, Pb, Zn, Cu and Cd accumulations in muscle tissues of Nile tilapia *Oreochromis niloticus* collected from Musa Colony Pakistan did not exceed limit values.

Keywords: Heavy Metal Accumulation, Karachi Fish Market, *Oreochromis Niloticus*, Pakistan

Introduction

Tilapia (*Oreochromis niloticus*) have become one of the most important fish species for aquaculture and play an increasing role in the international aquatic food trade [1, 2, 3, 4]. Heavy metals are essential components of the freshwater and marine environment, usually found in really low concentrations. The Majority part of the heavy metals released into the earth discover their way into the aquatic environment as input of direct air deposition, climatic, and disintegration due to rainwater. The level of trace metals is too high, in the area where mechanical, cultivating and mining activities are across the natural areas [5, 6, 7].

Heavy metals accumulated in the aquatic organisms by direct consumption of water and food through the digestive system or indirectly across the permeable membranes such as gills and skin. Concentration levels of heavy metals in fish organs indicate their levels in water and sediments. The level of accumulation of heavy metals in fish organs can exceed the environmental levels. The toxic effect usually occurs when the rate of uptake is exceeding the mechanism of metabolism, storage, and detoxification [8].

Contamination of the aquatic environment by heavy metals has been reported by many authors such as [8, 9, 10, 11] have discovered that lead, mercury, zinc, copper, arsenic, chromium, and cadmium are the important heavy metals which contaminate the water and harmful to aquatic organisms. Many aquatic organisms (fish, shrimp, and crab) at the highest point of the natural feeding ground items and aggregates high quantities of elements from the water [12, 13]. The basic dangerous contaminants make undesirable consequences for the fish and their effects move to the human body after using the polluted aquatic organisms and deteriorate the status of human health [14, 15].

The levels of dangerous contaminants in aquatic living things are a critical factor in view of their potential effects on the organisms themselves and the life status that utilize them which include individuals. Health institutions, for example, Food and Drug Administration (FDA) have started late raised stresses over the security of fisheries obtained from business sources [16].

Heavy metals pollution in water also leads to changes in the chemical components of the aquatic environment, usually influences the behavioural, physiological and bloodstream patterns, cell structures ionic balance [17], carbohydrate metabolism and liver function [17, 18] of fishes. Earlier reports, also showed that industrial and domestic effluent constitute largest sources of heavy metal which contribute to the steadily increasing metallic contaminant in aquatic and terrestrials environment in most part of the world [19].

In general, metals can be categorized as biologically essential and nonessential. The nonessential metals (e.g., saluminum (Al), cadmium (Cd), and lead (Pb)) have no proven biological function (also called xenobiotics or foreignele- ments), and their toxicity rises with in creasing concentration. Essential metals (e.g.,copper (Cu) and zinc (Zn) on the other hand, have a known biological role, and toxicity occurs either at metabolic deficiencies or at high concentrations [20]. The deficiency of an essential metal can therefore cause an adverse health effect, whereas it's high concentration can also result in negative impacts which are equivalent to or worse than those caused by non- essential metals [20, 21]. The most commonly found heavy metalsin fish organisms are cadmium, lead, mercury, zinc, copper, nickel, cobalt, molybdenum, chromium and tin. Amongst them, the most frequently studied, with respect to fish deformities, include cadmium, copper, lead, zinc, mercury and chromium.

Research Methods

Sampling for heavy metals determination

Nile tilapia (*Oreochromis niloticus*) were collected from Fish Market, Musa Colony Karachi, Pakistan. These fish were packed in clean zipped polythene bags and transported to the research facility of International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, in an ice filled polystyrene protection box. Samples were cleaned from sticky materials and put in the Industerial Analytical Center (IAC) laboratory freezer at −20°C to decrease organic decay before and during the investigation. The speciments were sorted with respect to their sizes given as follows: Group A: 15.0-20.0 cm; Group B: 20.1-25.0 cm; Group C: 25.1-30.0 cm. Samples were dealt with and flushed with distilled water before dissection for the isolation of the muscle tissues, liver, skin and gills of Nile tilapia (*Oreochromis niloticus*). The speciments were dissected out into little pieces with stainless-steel scissors, forceps and honed blades. The speciments were washed with Milli-Q water and dried at 80°C for 12 h and ground to fine powder with mortar and pestle. The dried tissue powder was measured precisely to the closest (1 g) and exchanged to a glass container. 10 mL of concentrated acid (60% HNO_3: 70% $HClO_4$) were added and left at room temperature for 12 h. The processed specimens were heated gradually to 180°C till the sample volume was diminished to 2–3mL. Every sample was filtered and made up to 25 mL with Milli-Q water. The heavy metals were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES AvioTM 500 PerkinElmer USA).

Calibration

Calibration of a particular element's concentration at ICP-OES was done by using a set of standards and blanks. In ICP-OES, a matrix matched solution of 1% nitric acid was used as the calibration solution and calibration concentrations ranged from 0 to 5 ppm for all analyzed elements. Standards used in preparing the calibration curve include 0.01, 0.05, 0.1, 0.5 and 2 ppm as described by [22, 23]. Standards to prepare the calibration equation had 0, 10, 25, 50, 75, and 100 ppb concentrations. Internal standards were spiked in calibration solution, blanks, and samples to serve as ionization buffers and monitor effects on analytes during calibration. For ICP-OES, Y and Cs were the internal standards [24].

Instrumentation

(ICP-OES AvioTM 500 PerkinElmer USA) were used for this analysis. The main parts of the instruments are shown in Figure 1. ICP-OES (Fig. 1) has a carrier gas tube and a torch made of a quartz tube and connected to a radio-frequency (RF) generator. Argon is introduced to the torch and RF is applied to create a magnetic field and produce ions and electrons. The resultant current flow heats the gas so that once sample introduction is done via the nebulizer, it is converted to aerosol and directed to the torch [22, 24]. Light emitted by atoms of metals from samples in plasma is converted to quantifiable electrical signals.

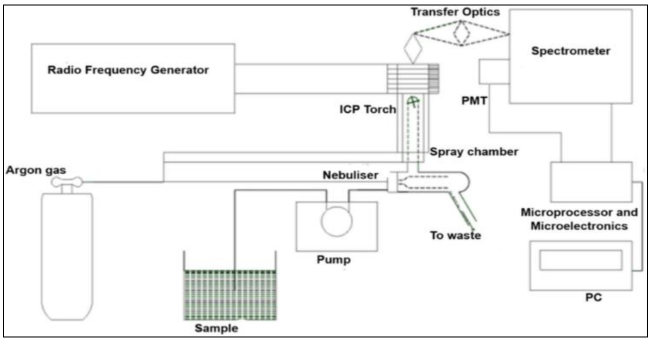

Fig 1: Schematic of a typical ICP-OES AvioTM 500 PerkinElmer USA) system. ICP-OES Inductively Coupled Plasma Optical Emission Spectrometry; PMT, photo-multiplier tube; PC, personal computer [22, 24, 25]

Digested samples were filtered, diluted, and assayed using the ICP-OES instruments. After analysis, the final concentrations of each element were determined in ppm (mg/kg):

Metal concentration (mg/kg)=

$$\frac{Concentration\ X\ volume\ of\ sample\ x\ dilution\ factor}{Weight\ of\ digested\ sample} x\ 1000$$

Statistical Analysis

All heavy concentrations of the metals in Nile tilapia (*Oreochromis niloticus*) within muscle tissues among the groups were determined by carrying out analyses of variance (ANOVA) using Tukey's HDS post-hoc comparison method. The results were assessed on the basic of homogenous groups with a significant level of ($p < 0.05$). The elements which were common in the muscle tissue of Nile tilapia (*Oreochromis niloticus*) were assessed by means of Pearson's correlation coefficients. Then, the correlation between weight groups and metal accumulation were investigated. Finally, the data collection and statistical calculations were performed using (IBM SPSS software version 24).

Results and Discussion

Results

The mean measurements based on (µg/g dry weight) of heavy metals (Al, Pb, Zn, Cu and Cd) of Nile tilapia (*Oreochromis niloticus*) has shown in Table (1-2) and figure 2. It was observed that the amount of the metals in muscle tissues of (*Oreochromis niloticus*) decreases in the group A (weight= <5g) compared to group B (weight= 5g >). The amount of Al, Pb, Zn, Cu and Cd concentrations in different organs of (Oreochromis niloticus) ranged from (8.00-11.92 µg/g), (0.40 – 0.70 µg/g), (14.83-64.00 µg/g), (10.78 – 15.79 µg/g) and (0.22 – 0.30 µg/g) respectively.

Table 1: Table 1. Mean±SD, length (cm) and weight (g) of Nile tilapia (*Oreochromis niloticus*)

	Length (cm)	**Weight (g)**
Group A	17.6±0.43	61.6±1.9
Group B	23.3±1.4	102.5±1.3
Group C	27.8±1.6	510.5±3.4

Table 2: Average concentrations of heay metals (µg/g ± SD) in different organs of Nile tilapia (*Oreochromis niloticus*)

		Al	**Pb**	**Zn**	**Cu**	**Cd**
	Muscle	5.77±0.24^{c}	0.33±0.13^{d}	12.21±0.13^{d}	6.28±0.53^{d}	0.13±0.03^{d}
	Skin	4.69±0.47^{c}	0.32±0.11^{d}	15.93±0.24^{d}	7.59±0.32^{d}	0.12±0.02^{d}
A	Liver	10.30±0.33^{b}	0.63±0.14^{b}	31.12±0.22^{c}	8.55±0.42^{d}	0.20±0.01^{c}
	Gills	2.12±0.50^{d}	0.62±0.19^{b}	10.17±0.25^{d}	5.62±0.42^{e}	0.25±0.03^{b}
	Muscle	12.52±0.60^{a}	0.42±0.20^{c}	33.31±0.19^{c}	13.42±0.13^{c}	0.28±0.03^{b}
	Skin	9.73±0.78^{b}	0.36±0.06^{d}	48.18±0.37^{bc}	11.17±0.29^{c}	0.37±0.11^{a}
B	Liver	12.70±0.04^{a}	0.49±0.19^{c}	76.55±0.28^{a}	18.49±0.62^{ab}	0.34±0.01^{a}
	Gills	11.72±0.76^{ab}	0.56±0.04^{bc}	13.55±0.96^{d}	12.51±0.19^{c}	0.26±0.01^{b}
	Muscle	10.12±0.70^{b}	0.46±0.11^{c}	67.41±1.14^{b}	17.55±0.22^{b}	0.26±0.02^{b}
	Skin	9.58±0.12^{b}	0.76±0.05^{b}	63.88±1.28^{b}	21.13±0.26^{a}	0.35±0.14^{a}
C	Liver	12.76±0.25^{a}	0.99±0.02^{a}	84.34±1.22^{a}	20.33±0.58^{a}	0.36±0.01^{a}
	Gills	12.56±0.59^{a}	0.91±0.08^{a}	20.78±1.05^{cd}	14.22±0.52^{bc}	0.33±0.02^{a}

Data on the same column with different superscripts are significantly difference ($P < 0.05$)

Table 3: Comparison of the metal concentrations (µg/g) in Nile tilapia (*Oreochromis niloticus*) with international standards

	Metal concentration (µg/g)					
	Al	Pb	Zn	Cu	Cd	References
Group A	5.72	0.48	17.36	7.01	0.18	Present study
Group B	11.67	0.46	42.90	13.90	0.31	Present study
Group C	11.26	0.78	59.10	18.31	0.33	Present study
International Limits						
WHO	-	2	100	30	0.5	[26]
FAO	-	0.5-6	30-100	10-100	1	[27]
IAEA-407	13.8	0.12	67.1	3.28	0.189	[28]
European Community	-	0.2	-	-	0.05	[29]
England	-	2	50	20	0.2	[30]
USA	-	1	75	6	1	[31]
EU limits	-	0.1	-	10	0.1	[32]
Korean	-	4-53	70-316	5-7	<1-2	[33]

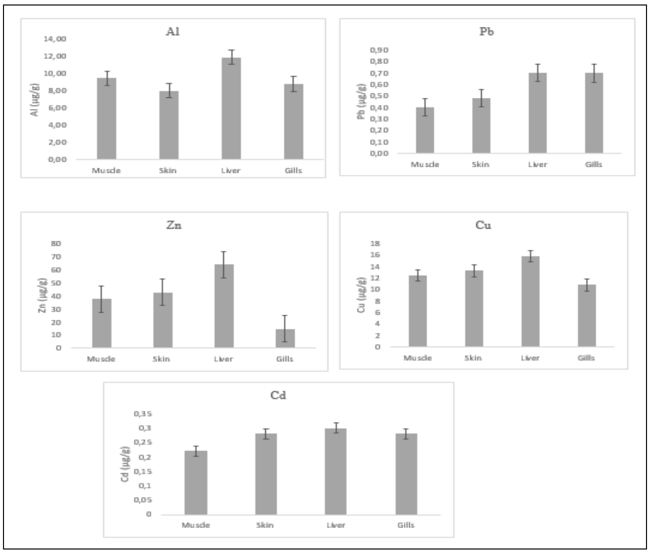

Fig 2: Average concentrations of heay metals (µg/g ± SD) in different organs of Nile tilapia (*Oreochromis niloticus*)

Discussion

Heavy metals accumulated in the aquatic organisms by direct consumption of water and food through the digestive system or indirectly across the permeable membranes such as gills and skin. Concentration levels of heavy metals in fish organs indicate their levels in water bodies, the level of accumulation of heavy metals in fish organs can exceed the environmental levels. The toxic effect usually occurs when the rate of uptake is exceeding the mechanism of metabolism, storage, and detoxification [8].

Heavy metals like Aluminum (Al), Cadmium (Cd), Lead (Pb), Copper (Cu) and Zinc (Zn) have high level of toxicity, and persistence capacity possessing potential for biomagnification, bioaccumulation and incorporation into the food chain after reaching a certain limit in the aquatic environment [34, 35, 36, 37, 38]. Bioaccumulation and biomagnification denote the processes and pathways of heavy metal pollutants from one trophic level to others in the foodweb [39, 38]. In view of this, a number of aquatic organisms have been used as bioindicators as Nile tilapia *Oreochromis niloticus* [40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55] Red Tiapia [56]. Contamination of the aquatic environment by heavy metals has been reported by many authors such as [9, 57, 8, 58] have discovered that lead, mercury, zinc, copper, arsenic, chromium, and cadmium are the important heavy metals which contaminate the water and harmful to aquatic organisms. Many aquatic organisms (fish, shrimp, and crab) at the highest point of the natural feeding ground items and aggregates high quantities of elements from the water [12, 13].

In Table 3. the levels of the heavy metal concentrations in different organs of Nile tilapia *Oreochromis niloticus* are compared with the international standards for metals compiled by the [26, 27, 28, 29, 30, 31, 32]. The maen concentrations of the Pb in group A,B and C above than EU, Cd in group B and C above the EC, EU, IAEA-407 and MAFF England standards. Heavy metals pollution in water also leads to changes in the chemical components of the aquatic environment, usually influences the behavioural, physiological and bloodstream patterns, cell structures ionic balance [17], carbohydrate metabolism and liver function [17, 18] of fishes. Earlier reports, also showed that industrial and domestic effluent constitute largest sources of heavy metal which contribute to the steadily increasing metallic contaminant in aquatic and terrestrials environment in most part of the world [19, 59]. The accumulation of Al, Pb, Zn, Cu and Cd in in different organs of Nile tilapia *Oreochromis niloticus* was found to be significantly different between the three different groups ($p < 0.05$). Table 4. sumarizes the correlation coefficients among the metal levels in different organs in Nile tilapia, significant correlations ($p < 0.05$) and ($p < 0.01$) are in asterisks (*) and (**) respectively. Clearly seen from the table, the most noticeble correlations were found between the pairs (Cu-Pb in muscle 0.998*), (Zn-Pb skin-muscle 1.000*), (Zn-Zn gills-muscle 0.997*), (Zn-Cu gills –muscle 0.999*), (Cd-Cd skin-muscle 0.999*), (Cd-Al skin –muscle 0.999*), (Al-Al skin-liver 0.999*), (Cd-Pb gill-skin 0.999*), (Zn-Cu gill-skin 0.998*), (Al-Al gill-liver 0.999*), (Cu-Zn liver-liver 1.000**), (Cd-Zn liver –liver 1.000*), (Al-Zn gill-liver 0.998*), (Cu-Zn gill-liver 0.999*), (Cd-Cu liver-liver 1.000*), (Al-Cu gill-liver 0.997*), (Cu-Cu gill-liver 0.999*), (Al-Cd gill-liver 0.999*) and (Cu-Cd gill-liver 0.997*).

Conclusion

The results of this study show that Al, Pb, Zn, Cu and Cd accumulations of Nile tilapia collected from Karachi fish market were below the international limits. The present study shows that precautions are needed to be taken in order to obviate metal pollution in the future. It is thought that intake values may trigger some health problems in case of excessive consumption because these pollutants can be detrimental for the health of fish population and human consuming them.

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