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# Accumulation of Some Heavy Metals in *Oreochromis niloticus* from the Nile in Egypt: Potential Hazards to Fish and Consumers

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## Abstract

**Exposure to heavy metals is an important environmental problem resulting from anthropogenic activities. The aim of this study was the evaluation of some heavy metals as cadmium (Cd), lead (Pb), chromium (Cr) and aluminum (Al), in muscle of fresh water fish *Oreochromis niloticus* from ten provinces all over Egypt to assess its possible hazards on fish and consumers. The analyzed metals could be detected in all examined samples and their order was Pb > Cr > Cd > Al. A positive correlation between Al concentration and the fish length was observed. The concentrations of Pb, Cd and Cr in fish samples were several times higher than their concentration in water and the bioaccumulation Factor (BAF) ranged from 8.22 - 122.6. The estimated weekly intake of Cd, Pb and Cr for a 70 kg person consuming fish in Egypt (7.94, 15.84 and 9.8 µg) is well below the Provisional Permissible Tolerable Weekly Intake (PTWI) recommended by FAO/WHO. Although heavy metal levels in Egypt exceed the maximum permissible limits recommended by Egypt and WHO in some fish samples, the consumption of Nile *O. niloticus* from Egypt is safe on human health.**

## Keywords

***Oreochromis niloticus*, Cadmium, Lead, Chromium, Aluminum, Nile River**

## 1. Introduction

The Nile in Egypt facing major environmental problems associated with the dispersal or disposal of agricultural, industrial and urban wastes generated by human activities. The contamination of freshwater with a wide range of pollutants has become a matter of great concern over the last few decades [1]. Discharge of heavy metals into

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river or any aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity, persistence and accumulative behavior [2]. Toxicity with heavy metals is due to disrupt the function of essential biological molecules such as protein, enzymes and DNA as metals lead to displacement of an essential metal cofactor of the enzyme and interaction of the metallic ions with DNA which proven to be carcinogenic to animals and humans [3]. In addition, digestive, renal, nervous, endocrine, reproductive and respiratory system defect were also, confirmed as a result of heavy metal exposure [4].

Fish consumption has increased in importance among health-conscious because it provides a healthy, low cholesterol source of protein and other nutrients including omega-3 (n-3) fatty acid that reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery [5]-[7]. In Egypt, Nile tilapia, *Oreochromis niloticus* is the main species of freshwater fishes that inhabit Nile River, and it is one of the most popular, cheapest, and available fish for all Egyptians. *O. niloticus* can survive in bad environmental conditions because their resistance to disease is physically powerful, and their respiratory demands are slight so that they can accept low oxygen and high ammonia levels [8]. Fish may concentrate large amounts of some metals from the water [9] and transfer throughout the web chain into humans. Metals of major interest in bioavailability studies, as listed by the U.S. Environmental Protection Agency are Al, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Sb [10]. These metals were selected because of their potential for human exposure and increased health risk [11].

Al is a harmful metal to the aquatic ecosystem, being responsible for events of toxicity with serious ecological consequences [12]. Different physiological alterations frequently observed in different fish species exposed to Al as cardiovascular, hematologic, respiratory, ionoregulatory, reproductive, metabolic, endocrine and gill damage [13]-[15]. Few studies evaluated Al concentration in edible tissues of aquatic organisms in Egypt. Authman 2011 [16], concluded that Al concentration represented threaten for the fish resources in Nile River and the accumulation factor (AF) in *O. niloticus* livers was ranged between 21.86 and 1475. Cd and Pb are of no biological function in human system and they are potentially toxic even at trace concentrations [17]. Moreover, many dissolved metals that enter rivers are adsorbed onto colloid particulates and at high alkalinity and pH, the metals, particularly lead and cadmium, precipitate by forming complexes, which dramatically influence further the metal toxicity [18]. Cr compounds are frequently encountered as environmental pollutants and have been known to produce toxic, mutagenic, and carcinogenic effects in biological systems, although Cr is an essential nutrient [19].

Publications on the concentrations of Cd and Al in fresh water fish and dietary intakes of toxic elements from fish in Egypt are limited. Al concentration in drinking water samples of Dakahlia Province was higher than the permissible limits (MPL) of the Egyptian Ministry of Health (EMH) and WHO [20]. Furthermore, Pb, Cd and Cr levels along most province lies in the Nile were higher than the MPL recommended by the same mentioned organizations [21]. In addition, the prevalence of renal failure and liver cirrhosis in human, the top food chain, was markedly increased in the last few years, which could be linked with heavy metal pollution in Egypt [22] [23]. According to the latest WHO data published in April 2011, the fourth and third cause of death in Egypt were due to kidney and liver disease which reached 5.19% and 7.34% of the total death, respectively [24]. Therefore, this study was conducted to investigate the tissue accumulation of Pb, Cd, Cr and Al in the edible part, of *O. niloticus* from 10 provinces along the Nile River in Egypt and to determine the relationship between the current aquatic contamination with heavy metals and the health hazards to fish consumers from higher trophic levels.

## 2. Material and Methods

### 2.1. Fish Sampling

Fish of Nile Tilapia (*Oreochromis niloticus*) were collected from 10 Provinces along the Nile River and its main branches (**Figure 1**), from fish men in the area. Four to six samples were collected from each location and transported in an ice box at 4˚C. Before dissection at the laboratory of Animal Hygiene Department, Assiut University, fish were weighed and their standard and total lengths were recorded; edible fillets (muscles) were removed and kept frozen at −20˚C until analysis.

### 2.2. Analysis of Heavy Metals and Quality Control

All laboratory equipments and containers were washed with 10% $HNO_3$ solution prior to each use. Each sample

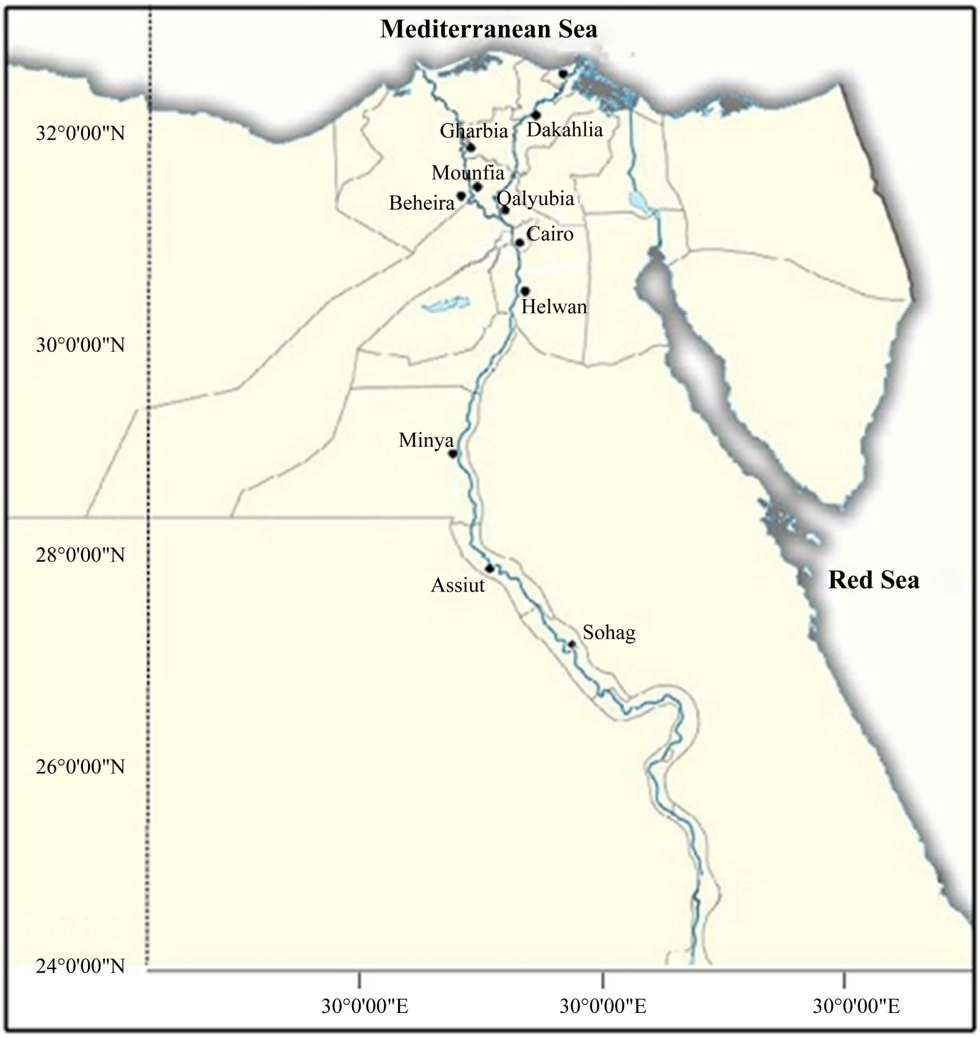

**Figure 1.** Fish sampling provinces along the Nile River in Egypt.

(about 6 - 8 gm) was cut into small pieces, placed in Petri-dish and dried in hot air oven at 80˚C until reaching a constant weight. Tissues were homogenized and grinded to a powder. One gram of each dry sample was weighed, transferred into screw caped glass bottled tube and 3 ml of nitric acid high grade (68%) was added to the sample. The mixture was left overnight at room temperature after tightly closing the tubes. Digestion were completed in a water bath at 90˚C for about 1 hour until all the tissues were dissolved and the solution become clear [1]. The digests were allowed to cool, filtered through Whatman (Ashless No. 42) filter paper, then transferred to 25 ml volumetric flasks and made up to mark with deionized water. Metal analysis (Pb, Cd, Cr and Al) was carried out in the Central Laboratory of the Faculty of Veterinary Medicine; Assiut University using a ZEEnit 700P Atomic Absorption Spectrophotometer with Graphite Furness Unite (AASG).

Aqueous standard stock solutions were prepared using appropriate salts of Cd, Pb, Cr and Al. Four working standards were prepared in triplicate for each metal by serial dilution of the stock solution. These standards and blank solution were aspirated into AASG as described by the manufacturers to obtain the absorbance of each of the samples and standard solutions for each of the metals. A calibration curve for the absorbance versus concentration of the standard metal concentrations was prepared for each metal from which calibration graph for each of the metals in the sample was determined as described by Nnaji *et al.*, 2007 [25]. The metal concentrations of the samples from the sites were determined from these calibration graphs.

The digestion method and the atomic AASG analysis were validated by preparation of a multi-element standard solution containing 1000 mg/l of each metal. Two grams of randomly selected muscle sample powder were spiked with four different concentrations 1, 5, 25 and 50 ppb of heavy metals, and each adjusted to run in triplicate. This was followed by the digestion of the spiked samples and determination of metal concentration using AASG. Unspiked samples consider our blank which was carried through the whole procedure described above. The amount of spiked metal recovered after the digestion of the spiked samples was used to calculate percentage recovery which was ranged from 90% - 120%. The Quality Control (QC) sample was run at a frequency of once in every 10 samples; to check for the calibration accuracy and the instrument drift. The results within 20% of the known QC values were deemed acceptable. Blank samples were run in duplicate in each analysis batch in a randomized order and were used to calculate the method detection limit (MDL). Heavy metal concentrations were determined and given as µg/gm dry weight (µg/g d wt). In the present study, concentrations are expressed on a dry weight basis. Moisture content of the muscle samples was 64.9% ± 11.2%. In order to compare our data to published values reported on a wet weight basis for WHO and Egyptian Organization for Standardization and Quality Control (EOSQC), we have converted our data to wet weight basis using a conversion factor 4.8.

### 2.3. Bioaccumulation Factor for Heavy Metals in Fish Muscle (BAF)

According to EPA guidelines, the BAF is defined as the ratio of chemical concentration in the organism to that in the surrounding water. Bioconcentration occurs through uptake and retention of a substance from water only, through gill membranes or other external body surfaces. BAF was calculated according to Kalfakakour and Akrida-Demertzi, 2000 [26] as follows:

BAF = M tissue/M water. Where; M tissue is the metal concentration in fish tissue mg/kg and M water, metal concentration in water mg/L. Metal concentration in water used for BAF calculation was obtained from previous study by [21].

### 2.4. Statistical Analysis

Statistical Analysis of data was carried out using IBM SPSS version 19 statistical package programs. A one-way analysis of variance (ANOVA) was performed. Differences in mean values were accepted as being statistically significant if $P < 0.05$. When the effect was significant ($P < 0.05$), means were separated using Tukey's test. The statistical significance of the correlation was reported at both $P \leq 0.01$ and $P \leq 0.05$ levels.

## 3. Results and Discussion

*O. niloticus* samples collected at total length (20.6 ± 4 cm) and standard length (17 ± 3.1 cm) with weight (181.6 ± 100 g) from ten Provinces along Egypt (**Figure 1**) were subjected to this study. We analyzed metal levels in muscle tissue (fillets) because they provide information on potential risk to the fish themselves and to the consumers of these fish. Pollution by heavy metals in aquatic ecosystem is growing at an alarming rate and has become an important worldwide problem [27]. Heavy metals (Cd, Pb, Al and Cr) could be detected in all examined *O. niloticus* muscle samples. **Figure 2** shows the mean values and the standard deviation (referred by error bars) of the tested metals in flesh fish samples from 10 Provinces along the Nile. Concentrations of heavy metal in tissues reflect past exposure via water and/or food and it can demonstrate the current situation of the animals before toxicity affects the ecological balance of populations in the aquatic environment [28].

### 3.1. Cadmium (Cd)

The highest level of Cd was observed in fish samples collected from Gharbia (0.31 ± 0.05 µg/g d wt), followed by those from Mounfia (0.299 µg/g d wt ± 0.004). However, the lowest level (0.197 µg/g d wt ± 0.06) was observed in fish samples collected from Minya (as shown in **Figure 2(a)**). In accordance, Cd concentration from Minya was significantly lower than that in Gharbia and Mounfia Provinces ($P < 0.05$). However, a non-significant difference was found between from Monufia and Gharbia Provinces for the same element. High incidence of Cd from Gharbia may be due to contamination of fish from super phosphate, Salt and Soda Company and pesticides factories at Kafr EI Zayat city or vast textile companies in El-Mahalla el-Kubra. Moreover, the use of phosphate and municipal sewage sludge to agricultural soils as a fertilizer and electrical appliances, ceramics, pigments and clothing industry could be considered as important sources of Cd pollution in Monufia Province. The use of Cd for several decades in many industrial fields and the application of fertilizer and sewage sludge to farm land [18] [29] have led to the increased Cd environmental levels. Cd concentration from *O. niloticus* muscle from different

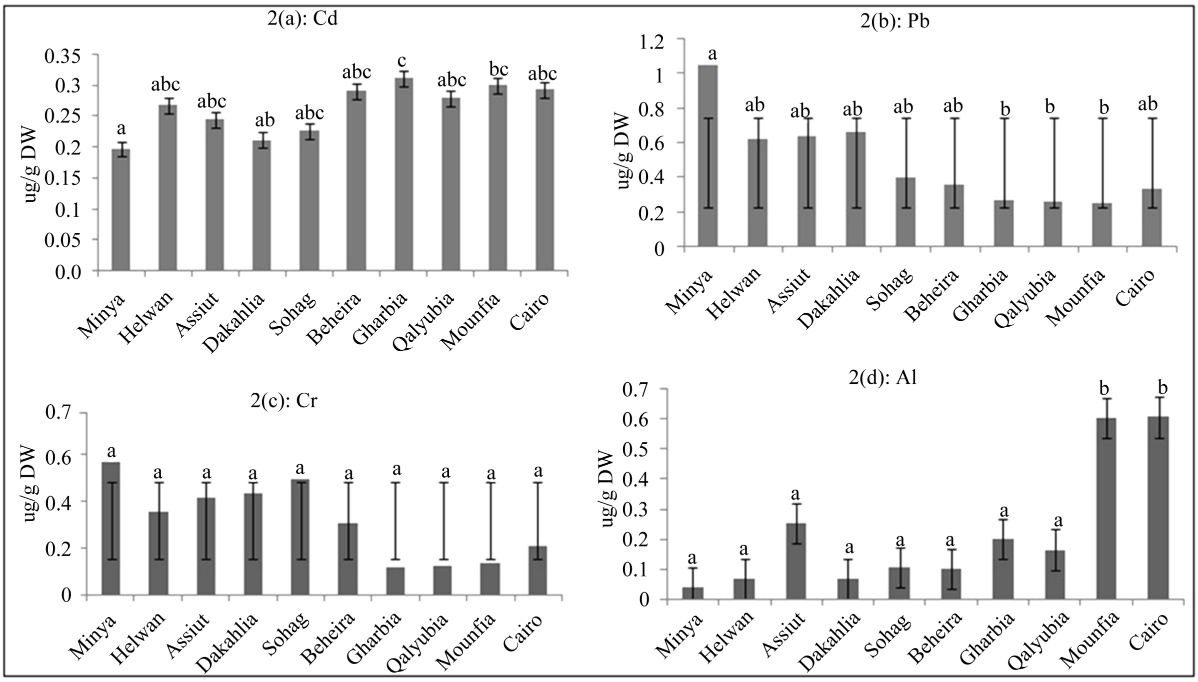

**Figure 2.** Mean concentration of the analyzed metals (Cd, Pb, Cr, and Al as shown in a, b, c and d, respectively) in fish muscle samples collected from 10 Provinces along the Nile. abc: means with different letters are significantly different ($P < 0.05$).

provinces compared to the permissible limits set by WHO 1993 and EOSQC 1993. None of the studied fish muscle was found to contain Cd concentration (0.008 - 0.19 mg/kg w wt) above the permissible limit recommended by EOSQC, 1993 [30]. However, 67% of the examined samples exceeded the permissible limits of WHO, 1993 [31] organization.

Cd is a serious environmental contaminant that is also transported atmospherically. In fish, it can cause anemia and vertebral fractures [32], osmoregulatory problems [33], decreased digestive efficiency [34], hematological and biochemical effects [35], growth deficits, erratic swimming, and mortality [36] [37]. Presumably, sublethal effects occur at lower levels. However, adverse effects from cadmium can occur with dietary levels of 0.1 μg/g [37]. The toxic effect of Cd is exacerbated by the fact that it has an extremely long biological half-life and is therefore retained for long periods of time in organisms after bioaccumulation [38]. Cd also may exert toxic effects to man including kidney damage and severe pain in bones which called itai-itai in Japan [39].

### 3.2. Lead (Pb)

The highest Pb concentration was found in fish samples from Minya (1.05 ± 0.7 μg/g d wt) followed by that from Dakahlia (0.67 ± 0.05 μg/g d wt). However, the lowest Pb concentrations were recorded in muscle fish samples from Mounfia (0.25 ± 0.03 μg/g d wt), Qalyubia (0.27 ± 0.05 μg/g d wt) and Gharbia (0.27 ± 0.06 μg/g d wt) as shown in **Figure 2(b)**. Moreover, Pb Concentration in muscle fish samples from Minya was significantly higher than that from Gharbia, Banha and Mounfia Provinces ($P < 0.05$). High levels of Pb may be attributed to water contamination from wide spread industrial production of perfumes, oils and fats, cement-making, quarrying (especially limestone), and brick-making, as well as, agricultural discharges, sewage effluents, high ways or motor boat traffic and from mine and smelting operations from Minya and Dakahlia Provinces. Release of industrial effluents into the water bodies without sufficient treatment increases Pb load to the environment [40]-[42].

The EOSQC, 1993 and WHO, 1993 [30] [31] established the maximum levels permitted for Pb in fish as 0.1 and 0.5 mg/kg w wt, respectively. Pb concentrations in our samples were within the permissible limit of WHO except for one sample from Minya, while 45% of the examined samples showed higher Pb concentration than the EQSQC recommended limits. Pb is a neurotoxin that causes persistent behavioral deficits in fish [43] and also causes decreases in survival, growth rates, development, learning, and metabolism, in addition to increased mucus formation in fish [44]. Exposure to lead is of concern mainly because of possible detrimental effects on intelligence. Studies on exposure to lead and children's intelligence have indicated an adverse effect of low-level lead exposure on neurophysiological development [45]. Food is one of the major sources of lead exposure; the others are air (mainly lead dust originating from petrol) and drinking water [46]. Although industrial and agricultural discharges are the primary source of lead poisoning of fish in Egypt [47], highways also pose a threat to fish because of lead contamination from automobile exhausts [48]. Chronic exposure to Pb has been linked to growth retardation causing potentially permanent learning and behavior disorders and death in children [49] [50]. Pb toxicity studies conducted on female mice revealed mostly miscarriages, premature delivery and infant mortality. Levels of Pb > 0.1 mg/l have been reported to be detrimental to foetuses and children, with possible development of neurological problems [51].

## 3.3. Chromium (Cr)

The lowest Cr value (0.12 ± 0.06 µg/g d wt) was found in fish samples from Gharbia, while the highest value (0.57 ± 0.39 µg/g d wt) was recorded in samples from Minya as shown in **Figure 2(c)**. However, non-significant differences between Cr values in fish samples from the 10 Provinces were observed. Cr is an essential trace element that is abundant in the earth's crust and occurs in several forms that differ widely in toxicity [52] [53]. High concentration of Cr may be attributed to anthropogenic sources include industrial, urban and agricultural runoff to the aquatic environment. Cr can cause serious problems in fish including swimming deficits, feeding disruption, fin ray erosion, ulcerations, and death [54]. Cr is of environmental and human health concern due to its carcinogenic and endocrine disrupting effects in humans [55]. Higher Cr levels increase incidences of post-implantation loss, decreased fetal body weight, reduced ossification, and decreased number of live fetuses [56]. The main health problems seen in animals following ingestion of chromium (VI) compounds are irritation and ulcers in the stomach and small intestine and anemia. Sperm damage and reproductive impairment (disruption of normal testicular physiology, have also been seen in laboratory animals exposed to chromium (VI) [57] [58]. The available permissible limit for Cr was found according to Brazilian regulatory limit of 0.1 mg/k w wt as reported by Tarley *et al*., 2001 [59]. It was found that 25% of fish samples in our study were higher than the above mentioned limits.

## 3.4. Aluminum (Al)

**Figure 2(d)** showed that fish samples collected from Cairo and Monufia Provinces represented the highest concentration of Al; 0.61 ± 0.16 and 0.6 ± 0.25 µg/g d wt, respectively. However, the lowest value was found in fish samples collected from Minya (0.04 ± 0.005 µg/g d wt). Al concentration in fish samples collected from Monufia and Cairo is highly significant ($P < 0.01$) comparing with other Provinces. Finally, the difference in Al concentrations in fish collected from Monufia and Cairo was non-significant. The sources of Al in fresh water include domestic wastes, manufacturing processes involving metals, and the dumped sewage sludge [60].

## 3.5. Heavy Metals and Health Hazards

The study showed that there was no correlation between Cd, Pb and Cr concentration and the fish size (weight and length) which may indicate that these metals do not increase in concentration with fish size because they are thought to be under haemostatic control [61]. However, there was a positive correlation between Al concentration and the fish length as the correlation coefficient was significantly ($P < 0.05$) positive. This positive correlation between Al concentration and fish length may be due to loss of haemostasis capacity in *Tilapia nilotica* under chronic metal exposure leading to bioaccumulation [62]. The concentrations of the analyzed metals (Pb, Cd and Cr) in the muscle of the sampled fish were several times higher than their concentration in water and the bioaccumulation Factor (BAF) ranged from 8.22 - 122.6. The order of the analyzed metals according to their BAF is Cr > Cd > Pb. Heavy metal concentration in tissues reflects past exposure via water and/or food and it can demonstrate the current situation of the animals before toxicity affects the ecological balance of populations in the aquatic environment [28]. The level of heavy metal bioaccumulation in fish tissues is influenced by biotic and abiotic factors, such as fish biological habitat, chemical form of metal in the water, water temperature and pH value, dissolved oxygen concentration, water transparency, as well as by fish age, gender, body mass, and physiologic conditions [63]. It was observed that there were non-significant differences between the BAF of Pb, Cd and Cr of the fish samples collected from Lower Egypt Provinces (Monufia, Gharbia, Dakahlia, Beheira and Qalyubia). However, there were significant ($P < 0.05$) differences between BAF of Pb, Cd and Cr of the fish samples collected from Upper and Middle Egypt Provinces (Cairo, Helwan, Minya, Assiut and Sohag). Additionally, The BAF of Cr was significantly ($P < 0.05$) higher than that of Pb and Cd. These results indicated that the water and fish from 10 Provinces along the Nile River were polluted with heavy metals.

Comparing the mean concentration of Cd, Pb, Cr and Al in *O. niloticus* from our results with the previously reported metals data was shown in **Table 1**. This variation could be attributed to the biological habitat and feeding behavior of fish species. The provisional permissible tolerable weekly intake (PTWI) values for Cd, Pb and Cr are 7 and 25 and 15 µg/kg body weight/week according to FAO/WHO, 2004 [64]. Therefore, PTWI of Cd and Pb for a 70 kg person are 490, 1750 and 1050 µg/week, respectively (**Table 1**). We hypothesis the average daily fish consumption of fish in Egypt is 20 g per person, so this will be equivalent to 140 g/week. The estimated daily intake (EDI) of Cd, Pb and Cr were 1.13, 2.26 and 1.4, respectively. The estimated weekly intake of Cd, Pb and Cr for a 70 kg person consuming fish included in this study (7.94, 15.84 and 9.8 µg) was well below the PTWI values respectively (**Table 1**).

**Table 1.** Comparison of Heavy metal concentrations in fish flesh (mg/kg dry wt) with values taken from the open literature and maximum permissible limits.

| Sample area | Fish type | Cd | Pb | Cr | Al | References |
|---|---|---|---|---|---|---|
| Ten governorates along Egypt | *Oreochromis niloticus* | 0.26 | 0.48 | 0.32 | 0.229 | **Our study** |
| Bayelsa state-Nigeria | *Tilapia nicolitica* | 0.95 | 3.88 | - | - | **Godwin *et al*., 2011** [65] |
| Bangi, Selangor, Malaysia | *Oreochromis niloticus* | 0.02 | 0.14 | 5.9 | 9 | **Taweel *et al*., 2011** [66] |
| Egypt | *Oreochromis niloticus* | 6.05 | 7.6 | - | - | **Kaoud and El-Dahshan, 2010** [67] |
| Malasya | *Oreochromis niloticus* | - | 0.09 | - | - | **Low *et al*., 2010** [68] |
| Madivala lakes of Bangalore | *Tilapia species* | 1.76 | 2.67 | 1.3 | 2 | **Begum *et al*. 2009** [69] |
| Lake Manzala Egypt | *Oreochromis niloticus* | 1.33 | 5.11 | - | - | **Abdel-Satar and Geneid Y, 2009** [70] |
| Kolleru Lake, India | *Different fish species* | - | - | 0.93 | - | **Adhikari *et al*., 2009** [71] |
| Manchar Lake, Pakistan | *O. mossambicus* | - | - | 0.46 | - | **Arain *et al*., 2008** [72] |
| Sabal canal, Monufia Gov, Egypt | *O. nilotica* | 3.4 | 31.95 | - | - | **Authman, 2008** [62] |
| Northern Jordan Valley, Jordan | *Oreochromis aureus* | 0.02 | - | - | - | **Al-Weher , 2008** [1] |
| Qena, Egypt | *Tilapia species* | 1.81 | 0.62 | - | - | **Labib *et al*., 2008** [73] |
| Iskenderun Bay, Turkey | *Saurida undosquamis, Sparus aurata and Mullus barbatus* | - | - | - | 2.71 | **Türkmen *et al*., 2005** [74] |
| Georgia and Alabama (USA) | *Mackerel, tuna, salmon, sardines and herrings* (canned) | - | - | 0.75 | - | **Ikem and Egiebor, 2005** [75] |
| Fayoum Governorate, Egypt | *Tilapia species* | - | - | 1.12 | - | **Mansour and Sidky, 2002** [9] |
| Selangor, Peminsular, Malysia | *O. nilotica* | 0.03 | - | 0.19 | - | **Taweel *et al*., 2012** [76] |
| Noethwest China | *Cyprinid fish species* | 0.11 | 0.17 | 0.01 | 6.30 | **Qin *et al*., 2015** [77] |
| * | | 0.1 | 0.1 | - | NA | **EQSQC, 1993** [30] |
| * | | 0.05 | 0.5 | - | NA | **WHO, 1993** [31] |
| * | | - | - | 0.1 | NA | **Brazilian standard, 2001** [59] |
| PTWI** | | 7 | 25 | - | - | **FAO/WHO, 2004** [64] |
| PTWI 70** | | 490 | 1750 | - | - | |
| PTDI** | | 70 | 250 | - | - | |
| EWI** | | 7.94 | 15.84 | - | - | |
| EDI** | | 1.13 | 2.26 | - | - | |

*mg/kg wet wt, **µg/kg wet wt, PTWI: Provisional permissible tolerable weekly intake, PTWI 70: PTWI for 70 kg person (µg/week), PTDI: Permissible tolerable daily intake for 70 kg person (µg /day), EWI: Estimated weekly intake (µg), and EDI: Estimated daily intake (µg).

## 4. Conclusion

It could be concluded that, *O. niloticus* is a good species for bio-monitoring pollution as it could withstand the adverse conditions in the ecosystem. The mean concentrations of Cd, Pb and Cr levels in muscle of *O. niloticus* were much lower than those obtained from the other studies in Egypt. However, this study was the first which could detect Al in *O. niloticus* in Egypt. The highest Pb and Cr concentrations were found in fish samples from Minya. The estimated daily and weekly intake for Cd, Pb and Cr were greatly lower than the established PTWI/ PTDI values; therefore, the consumption of the economic *O. niloticus* from the Nile in Egypt is not considered as a potential health hazard on human health.

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