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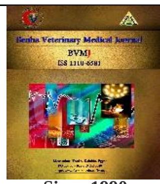

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Original Paper

Detection of cadmium and copper in fresh and marine fishes

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ABSTRACT

Cadmium and copper are very toxic heavy metals found in the environment and have public health hazard. Therefore, the current study was performed on 100 samples of freshwater fish (*Clarias lazera* and *Oreochromis niloticus*) and marine fish (*Sardina pilchardus* and *Pagrus pagrus*) collected at different times from various fish markets in Kafr El-sheikh governorate, Egypt. The collected samples were analyzed for heavy metal residues (cadmium and copper) by Atomic Absorption spectrophotometer (AAS). The obtained results showed that the mean values of cadmium concentrations (mg/kg) were 0.25 ± 0.01, 0.17 ± 0.01, 0.09 ± 0.01 and 0.08 ± 0.01 in *C. lazera, O. niloticus, S. pilchardus* and *P. pagrus,* respectively. While, the average concentrations of copper were 3.05 ± 0.27, 2.62 ± 0.19, 2.10 ± 0.13 and 1.73 ± 0.08 mg/kg in such examined samples. The sources of contamination of freshwater and marine fishes with heavy metals and their public health significance as well as some recommendations to control such serious pollutants were discussed

1. INTRODUCTION

Fish is very important source for high quality protein, minerals, vitamins and other nutrients as well as omega-3 that can decrease the danger of cardiac diseases and neurodevelopment in infants (Olmedo et al., 2013). From the nutritional point of view, consumption of fish regulates the blood glucose level (Patterson and Rashjitha, 2009).

Fish can accumulate heavy metals more than water because they their nutrients are organic matter from aquatic system, algae and other small organisms (Olaifa et al., 2004). The accumulation of heavy metals differs according to the age, sex, size and species of the fish. Furthermore, the highly active organs as gills, kidneys and liver are the target organs (Shivakumar et al., 2014).

The sources of heavy metals are earth crusts, weathering, fertilizers, and pesticides, leaching from harbor activities and sailing as well as from industries and water wastes (Elhussien and Adwork, 2018).

Cadmium causes elevation in AST, ALP and ALT activities of liver; also, it increases urea and cholesterol levels. On contrary, it reduces the levels of albumin and protein that will lead to severe damage in many organs as spleen, testis and kidneys (Rhaman et al., 2011). However, cadmium compounds as cadmium oxide, cadmium chloride and cadmium sulphate are carcinogenic. In addition, cadmium chloride can cause impairment in fertility and can harm the unborn fetus (Court et al., 2011).

Copper has hepatotoxic effect in mice causing severe variation in the levels of ascorbic acid and glutathione. Further, copper can cause changes in the levels of glutathione and malonaldehyde concentrations due to the impairment of cell lining. Copper sulphate can cause liver damage as it results in granular damage, necrosis and dysfunction in hepatic cells. Also, copper has severe toxic effect to all living organisms (Oguz et al., 2010). However, copper gluconate is added to the food of infant as supplement. In addition, chromium copper arsenate (CCA) can be used as protecting for wood building substance and can cause kidney damage, and tubular dysfunction and necrosis (Rita et al., 2009).

Therefore, the aim of this study is to determine the concentrations of cadmium and copper in some freshwater fish and marine fish in Kafr El-sheikh governorate. Also, the hazardous toxic effects of these heavy metals and public health importance were studied.

2. MATERIAL AND METHODS

Collection of samples:

A random 100 samples of fresh and marine fishes represented by *C. lazera, O. niloticus, S. pilchardus and P. pagrus* (25 of each) were purchased from fish markets in Kafr El-shiekh governorate, Egypt.

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All collected samples were examined for their cadmium and copper concentrations based on wet weight method using Atomic Absorption Spectrophotometer (AAS).

Washing procedures

In general, all equipment and instruments were perfectly rinsed to avoid their contamination with heavy metals according to Lars (2003).

Digestion technique:

The technique recommended by Staniskiene et al. (2006) was applied one gram of each sample was digested by 10ml of digestion mixture (60ml of 65% HNO_3 and 40ml of 70% HCL) in screw capped tube for estimation of their cadmium and copper levels.

The tubes were shaken and left overnight at room temperature. Further, the tubes were put in water bath at temperature starting from 60ºC to 110ºC for 4 hours to ensure complete digestion of the samples. Thereafter, the tubes were left to cool at room temperature and diluted with 1ml deionized water (30%). However, such tubes were diluted with deionized water till reach 25 ml. All the digests were filtered.

Analysis

All the digests (standard, digested and blanks) were absorbed by AAS (VARIAN, Australia, model AA240 FS) and examined for cadmium and copper residues.

Condition	Cadmium	Copper
Lamp wavelength (nm)	228.8	324.8
Lamp current (m/amp)	4	15
Fuel flow rate	1.2	1.00
Used gas	Argon	Argon
Measurement time (seconds)	4	4
Detection limit (ppb)	2-8	8-40

The concentration of cadmium, and copper was estimated according to the following equation:

$C = R \times (D/W)$

Where, C=concentration of cadmium and copper (mg/kg) wet weight. R=reading of digital scale of AAS. D= Dilution of prepared sample. W= Weight of the sample.

N.B. The concentration of each heavy metal in the blank solution was also calculated and subtracted from each analyzed sample.

3. RESULTS

The results recorded in table (1) and fig (1) revealed that the cadmium was detected in *C. lazera, O. niloticus, S. pilchardus* and *P. pagrus* with percentages of 52%, 36%, 32% and 20%, respectively.

Table (2) and fig (2) showed that the concentrations of cadmium (mg/Kg) in *C. lazera, O. niloticus, S. pilchardus* and *P. pagrus* ranged from 0.05 to 0.44, 0.03 to 0.28, 0.01 to 0.19 and 0.01 to 0.16 with an average of 0.25±0.01, 0.17±0.01, 0.09±0.01 and 0.08±0.01, respectively.

Table 1 Incidence of Nile and marine fishes contaminated with cadmium (n=25).

Fish species	.No	%
lazera Clarias	13	52
Oreochromis niloticus	9	36
Sardina pilchardus	8	32
Pagrus pagrus	5	20
Total	35	35

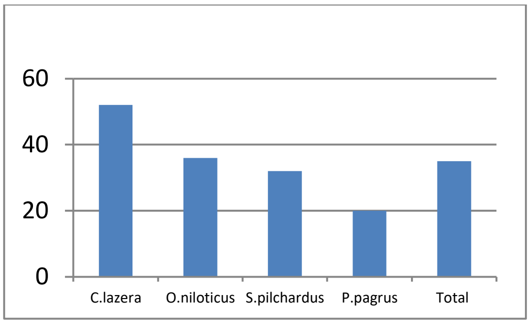

Fig. 1 Incidence of cadmium in the examined Nile and Marine fishes

Table 2: Statistical analytical results of cadmium residues (mg/Kg) in the examined samples of Nile and marine fishes (n=25)

Fish species	Min	Max	Mean ± S.E
Lzera Clarias	0.05	0.44	[a]0.01 ± 0.25
Oreochromis niloticus	0.03	0.28	[b]0.01 ± 0.17
Sardina pilchardus	0.01	0.19	[c]0.01 ± 0.09
Pagrus pagrus	0.01	0.16	[d]0.01 ± 0.08

The difference between different letters in the same column were significant High significant differences ($P<0.01$)

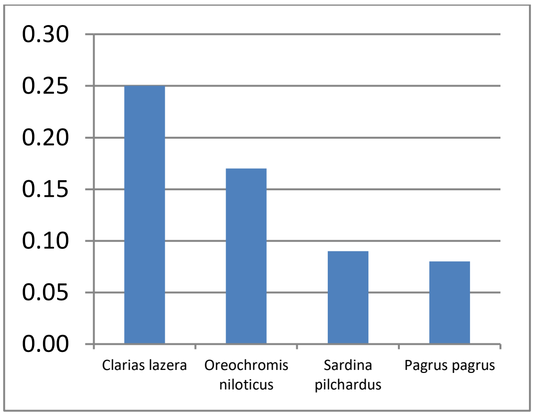

Fig. 2 Averages of cadmium residues (mg/Kg) in the examined Nile and marine fishes.

The results in table (3) and fig (3) indicted that the accepted samples of *C. lazera, O. niloticus, S. pilchardus* and *P. pagrus* according to their contents of cadmium were 52, 72, 76 and 84%, respectively.

Results given in table (4) and fig (4) showed that the copper residues in *C. lazera, O. niloticus, S. pilchardus* and *P. pagrus* were detected in all examined samples of fresh water and marine fishes.

Table 3: Validity of the examined fresh and marine fishes according to their cadmium residues (n=25).

Fish species	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
Fish species		No.	%	No.	%
C. lazera	0.05	13	52	12	48
O.niloticus	0.05	18	72	7	28
S.pilchardus	0.05	19	76	6	24
P.pagrus	0.05	21	84	4	16
Total (100)		71	71	29	29

* Maximum Residual Limit stipulated by Egyptian Organization for Standardization "EOS" (2010).

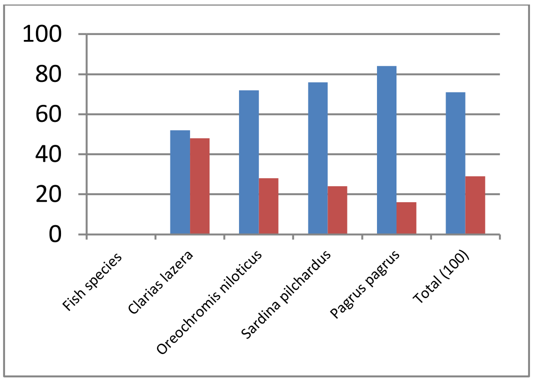

Fig 3 Incidence of accepted and unaccepted fish samples based on their levels of cadmium.

Table 4 Incidence of fresh and marine fishes contaminated with copper residues (n=25).

Fish species	No.	%
C. lazera	25	100
O.niloticus	25	100
S.pilchardus	25	100
P.pagrus	25	100
Total	100	100

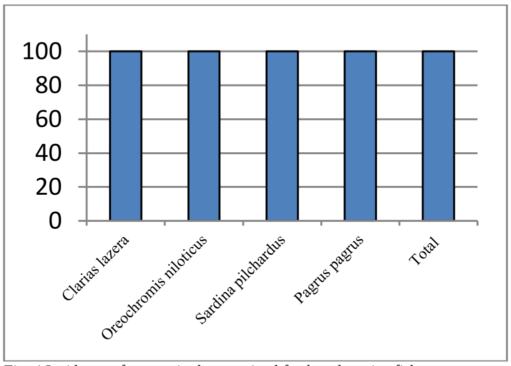

Fig. 4 Incidence of copper in the examined fresh and marine fishes.

Table (5) and fig (5) showed that the concentrations of copper residues (mg/Kg) in the examined samples of *C. lazera*, *O. niloticus*, *S. pilchardus* and *P. pagrus* ranged from 1.58 to 4.76, 1.30 to 4.12, 0.89 to 3.25 and 0.74 to 2.91with averages of 3.05±0.27, 2.62 ±0.19, 2.10± 0.13 and 1.73 ±0.08, respectively .

The results achieved in table (6) and fig (6) showed that all examined samples of fresh water and marine fishes were accepted based on their levels of copper.

4. DISCUSSION

The impacts of the heavy metals of primary concern are mainly cadmium and copper because of their known toxicity to human as well as their health hazard as lethal, sublethal, acute and chronic toxicity (Levensen and Barnard, 1988).

Table 5 Statistical analytical results of copper residues (mg/Kg) in the examined samples of fresh and marine fishes (n=25).

Fish species	Min	Max	Mean ± S.E
C.lazera	1.58	4.76	3.05 ± 0.27^{a}
O.niloticus	1.3	4.12	2.62 ± 0.19^{b}
S.pilchardus	0.89	3.35	2.10 ± 0.13^{c}
P.pagrus	0.74	2.91	1.73 ± 0.08^{d}

The difference between different letters in the same column were significant High significant differences (P<0.01)

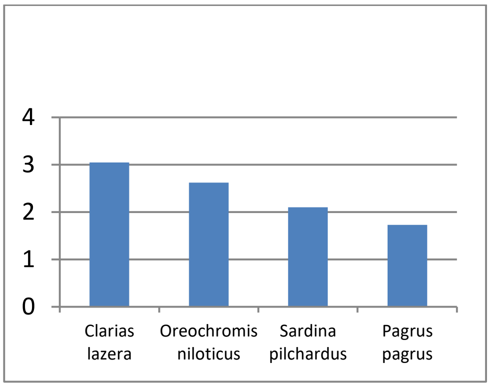

Fig. 5 Averages of copper residues (mg/Kg) in the examined Nile and marine fishes.

Table 6 Validity of the examined fresh and marine fishes according to their copper residues (n=25).

Fish species	MRL (mg/Kg)*	Accepted samples		Unaccepted samples	
Fish species		No.	%	No.	%
C. lazera	20	25	100	0	0
O.niloticus	20	25	100	0	0
S.pilchardus	20	25	100	0	0
P.pagrus	20	25	100	0	0
Total (100)		100	100	0	0

Maximum Residual Limit stipulated by Food Stuffs Cosmetics and Disinfectant Act (2007)

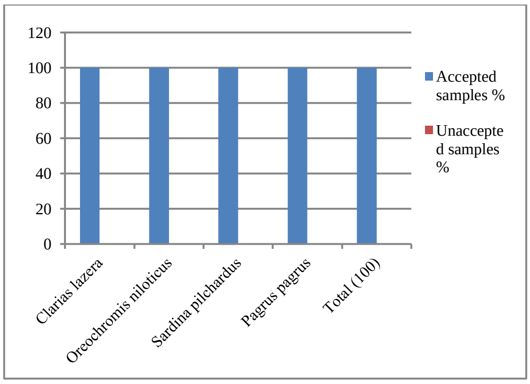

Fig. 6 Incidence of accepted and unaccepted fish samples based on their levels of copper residues

Cadmium was detected in C. lazera, O. niloticus, S. pilchardus and P. pagrus with percentages of 52%, 36%, 32% and 20%, respectively (Table 1 & Fig 1). Generally, the cadmium concentrations were recorded at highest levels in the examined samples of C. lazera followed by O. niloticus, S. pilchardus and P. pagrus (Table 2 & Fig 2).The difference between different letters in the same column were significant , high significant differences ($p<0.01$) as a result of type of product also high significant difference ($p<0.01$) appeared between such examined samples due to their origin .Moreover, non-significant differences appeared as a result of interaction between types of products and their origin .

Nearly similar results were recorded by Sohsah (2009) who recorded that the mean value of cadmium in C. lazera ranged from 0.05 to 0.37 mg/kg with an average of 0.20 ±0.03 mg/kg in large size while in T. nilotica ranged from 0.03 ±0.26 mg/kg and Abou-Donia (1990) who reported that such mean value was 0.208 mg/kg .

Lower results were recorded by El-Said (2016) who recorded that the mean value of cadmium T. nilotica and C. lazera were 0.051±0.011mg/kg and 0.049 ±0.012 mg/kg, respectively. Also, El-Nahas (2015) who recorded those values were 0.10±0.01mg/kg and 0.14±0.02 mg/kg and Gawish and Hosni (2017) who reported that such mean value was 0.049 mg/kg in sardine and 0.036 mg/kg in morgan.

Higher results were reported by Seddek et al. (1996) who reported that , the mean value of cadmium in T. nilotica ranged from 0.01 to 0.62 mg/kg with an average of 0.26 mg/kg , while much value in C. lazera ranged from 0.03 to 0.48 mg/kg with an average of 0.33 mg/kg, Latif (2018) who reported that the mean value of cadmium in sardine was 0.15±0.01mg /kg , Mehouel et al. (2019) who recorded that the mean value of cadmium in sardine was 0.55 ±0.14 mg/Kg and Hadeed et al. (2017) who recorded that such mean value in P. pagrus to be 0.14±0.02 mg/Kg.

Furthermore, table (3) and fig (3) revealed that the accepted samples of C. lazera, O. niloticus, S. pilchardus and P. pagrus were 52%, 36%, 32% and 20%, respectively according to EOS (2010) which stated that the maximum residual limit of cadmium should not exceed than 0.05 (mg/kg) in fresh and marine water fishes

The main sources of cadmium contamination are operations in industry and mining also the using in phosphate in fertilization process that can contaminate fish Kaoud and El-Dahshan (2010).

The toxicity of cadmium originated by its ability complete with other essential elements for binding sites, furthermore, its ability to interfere with sulfhydryl groups that are important for protein and enzymes function as cadmium can block these groups (Alan and Miller, 1998). Cadmium is naturally found in earth crust and in environment with minute can cause cancer at high level, the production of some metals as zinc, aluminium, steel and iron production are main sources of cadmium contamination as well as the using of phosphate as fertilizer (United States Environment Protection Agency, 2003).

Chronic toxicity with cadmium is manifested by bone disorders, fractures, impaired renal function with protein urea, phosphorus and calcium regulation is affected and (Itai –Itai) or (Ouch-Ouch) disease that include joints pain and bone disorders (Friberg and Elinder, 1985 and Kagamimori et al., 1986). Cadmium causes hypertension and renal damage (Nishiya –Ma et al., 1986), testicular atrophy, renal damage, cancer prostate and nausea are symptoms of cadmium toxicity (Kikuchi et al., 2002). Cadmium accumulates in brain causing damage in neurons cells, stroglial death through the deplete of glutathione (Imetal., 2006).

The copper residues in C. lazera, O. niloticus, S. pilchardus and P. pagrus were detected in all examined samples of fresh water and marine fishes as shown in table (4) and fig (4) by a percentage of contamination (100%) in all examined samples.

It is evident from the results recorded in table (5) and fig (5) that the copper residual level (mg/kg) in C. lazera, O. niloticus, S. pilchardus and P. pagrus ranged from 1.58 to 4.76 , 1.30 to 4.12, 0.89 to 3.35 and 0.74 to 2.91 with an average of 3.05 ±0.27, 2.62 ±0.19, 2.10 ± 0.13 and 1.73 ±0.08, respectively . The difference between different letters in the same column were significant, high significant differences ($p<0.01$) as a result of type of product also high significant difference ($p<0.01$) appeared between such examined samples due to their origin. Moreover, non-significant differences appeared as a result of interaction between types of products and their origin.

Nearly similar results was recorded by EL-said (2016) who found that the mean value of copper in O. niloticus and C. lazera were 2.49± 0.224 and 2.331 ±0.3257 mg/kg, Al-Weher (2008) who reported that such value in Tilapia was 2.90 ± 0.3 mg/kg and Kaoud and El-Dahshan (2010) who found that such value was 2.54 ± 0.05 mg/kg in O. niloticus.

Higher results were recorded by Sohsah (2009) who recorded that such mean value ranged from 2.04 to 5.91mg/kg and 1.76 to 5.13mg/kg with an average of 4.26 ±0.45 and 3.51 ± 0.37 mg/kg in C. lazera and T. nilotica, Olatunji and Osibanjo (2012) who recorded that such value was 3.30 ±0.79 mg/kg in Tilapia and Ilgar (2016), who recorded that such value was 4.17 mg/kg in sardine.

Lower results were recorded by Adeosun et al. (2015) who found that the mean value of copper in O. niloticus was 0.015 mg/kg, Badr (2014) who found that such value was 0.264 ±0.023 ppm in O. nilticus and Ayeloja et al. (2014) who recorded that such value was 0.23 ±0.11 and 0.108 ±0.03 mg/kg in T. nilotica and C. lazera, respectively.

The main sources of copper contamination are the using of air fallout and effluents as mining from industry, the using of copper sulphate in eradication killing of snails in water and the use of copper compounds such as pesticides, algicides and fungicides in soil treatment (Ali, 2010).

The chronic contamination with copper causes haemolytic anemia and even death, Jaundice, hypertension, diarrhoea and vomiting are symptoms of high dose with copper. Wilson`s diseases is genetic disorder caused by high copper dose and recognized by damage of neuron that leads to neurological disorders, cirrhosis, odema, ascitis and Fleischer ring is a golden ring on the cornea formed from copper (Gossel and Bricher, 1990) .

The results recorded in table (6) and fig (6) showed that all the examined samples of fresh and marine water fishes were accepted based on their levels of copper by a percentage of (100%) where, the don`t exceed the permissible limit of copper according to Food Stuffs Cosmetics and Disinfectant Act (2007) which stated that the maximum residual limit of copper should not exceed than 20 (mg/kg).

5. CONCULOSION

The current study proved that there are great variations in the levels of cadmium and copper in the examined samples of fish. In addition, the examined samples were significantly polluted with high levels of toxic metals. The continuous consumption of these contaminated fish may result in public health hazard through progressive irreversible accumulation of such toxic pollutants in the human body.

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