

Reflection of Environmental Toxins on the Chemical Quality of the African Catfish

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ABSTRACT

This study was conducted on fifty fish samples of *Clarias gariepinus* randomly collected from Zagazig, Sharkia governorate, Egypt. The collected fish samples were evaluated for heavy metals, antibiotics and aflatoxin residues. Arsenic and Zinc residues were detected in 100% of fish samples with a mean value of 0.07 ± 0.007 and 5.13 ± 0.39 ppm respectively, while lead and mercury residues were detected in 90% of fish samples with a mean value of 0.28 ± 0.05 ppm and 1.4 ± 0.24 ppm respectively. On the other hand, cadmium residue was detected in 50% of fish samples with a mean value of 0.018 ± 0.002 ppm. Sulfadimidin residue was detected in all examined *Clarias gariepinus* samples with a mean value \pm SD (50.97 ± 21.55 μ g/kg). All examined fish samples were below maximum residue limit 100 μ g/kg while aflatoxin was detected only in one fish sample with a concentration 5.32 μ g/kg (ppb) which declared that there is no human health risk from consumption of *Clarias gariepinus* flesh.

INTRODUCTION

Fish are currently getting expanding consideration in human food as it attracts a great percentage of the world's people for its palatability, nutritious and healthy animal protein. Fish is extensively consumed by humans in several parts of the world due to its high digestible protein content, essential amino acids, phosphorus, calcium, iron, trace elements like copper and a fair proportion of the B-vitamins to maintain good health also fish contains low amount of saturated fat, two types of omega-3 polyunsaturated fatty acids: docosahexenoic acid (DHA) and eicosapentaenoic acid (EPA). Omega-3 (n-3) fatty acids are very essential for normal growth as they diminish cholesterol levels and the incidence of heart disease, stroke, and preterm delivery (Al bader, 2008).

Cat fish (*Clarias gariepinus*) belong to the family Claridae, the air-breathing catfish. They are a diverse group of ray-finned fish named for their noticeable barbells that look like a cats whiskers. By increasing human activities of various kinds, such as domestic, commercial, agricultural, industrial and navigation activities, different types of waste materials enter the aquatic ecosystems led to its contamination and decrease in fish production level. (Authman, 2011). Contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation, and biomagnifications in the food chain. Moreover, some aquatic organisms accumulate heavy metals to concentrations, which are higher than that present in water so they used as bio-indicators for water pollution (Mitra et al., 2012).

The main sources of heavy metals in the water and in the fish tissues are the surrounding atmosphere. The entrance of heavy metals to the fish tissues occur through two major pathways, directly through the digestive tract by the consumption of contaminated food or water and across permeable membranes such as gills (Ukoha *et al.*, 2014). The human is mainly exposed for such toxic metals through ingestion of contaminated food and water, which accounted for more than 90 % compared to inhalation and other exposure routes (Loutfy *et al.*, 2006). Antibiotics are commonly used as food supplements for disease prevention and treatment and as growth promoters (Reda *et al.*, 2013; Pham *et al.*, 2015). However, such use of antibiotics without veterinary control leads inevitably to the presence of antibiotic residues in the animal-derived products and by-products (Mensah *et al.*, 2014). The utilization of antibiotic products in aquaculture is prejudicial to the aquatic environment and aqua life on one hand, and on the other hand, to the fish products consumers due to the toxicity risk of antibiotic residues (Dhaouadi *et al.*, 2015).

This mycotoxin group mainly occurs in subtropical and tropical areas, contaminating mainly feedstuffs with high starch and lipid content, such as cottonseed, corn, peanut, wheat, and soya bean (Gonçalves *et al.*, 2016; Ostrowski-Meissner, 1984). AFB1 is one of the most potent carcinogens known, classified as a group I carcinogen by International Agency for Research on Cancer (IARC, 1993) and highly hepatocarcinogenic (Busby and Wogan, 1984; Sharma and Salunkhe, 1991). Therefore this study was carried out to determine the residual concentration of some heavy metals (As, Pb, Cd, Hg, and Zn), antibiotics and aflatoxin in muscle of *Clarias gariepinus* and to estimate the dietary intake of determined heavy metals, as well as to assess the potential health risks associated with the consumption of *Clarias gariepinus*.

MATERIALS AND METHODS

1-Collection of samples:

Fifty samples of *Clarias gariepinus* were collected from Zagazig, Sharkia governorate, Egypt. The collected samples were kept in an icebox and directly transferred to the central Laboratory, Faculty of Veterinary Medicine, Zagazig University for heavy metal analysis. Additionally portion of samples examined for antibiotic and aflatoxin residues in animal health research institute Dokkia, Giza, Egypt

2- Determination of heavy metals:

Digestion of tissue samples were carried out according to Zantopoulos *et al.*, (1996) then introduced for quantitative determination of heavy metals "Buck scientific 2/0 VGP Atomic Absorption Spectrometer" at the faculty of Veterinary Medicine, Zagazig University. The analysis was conducted by air/ Acetylene flow (5.5/1.11/m) flame A.A.S (Buck Scientific Model 210 VGP).

3- Human risk analysis of heavy metal exposure:

Estimated daily intake (EDI) ($\mu\text{g}/\text{kg}/\text{day}$) for arsenic, cadmium, lead, aluminum and mercury was obtained using the following equation described by the Human Health Evaluation Manual (US Environmental Protection Agency, EPA, 2010): EDI ($\mu\text{g}/\text{kg}/\text{day}$) was obtained using the following equation: $\text{EDI} = (\text{Cm} \times \text{FIR})/\text{BW}$, where Cm is the concentration of the heavy metal in the sample (mg/kg wet weight); FIR is the food (fish) ingestion rate in Egypt, which was estimated at 48.57 g/day (FAO, 2010); BW is the body weight of Egyptian adults, which was estimated at 70 kg then compared to Acceptable daily intakes (ADIs).

Target Hazard Quotient (THQ) this is the ratio between the exposure and the reference doses (RfD). Rfd represents reference oral dose that is an estimation of the daily exposure of a contaminant to which the human population may be continually exposed over a lifetime without an appreciable risk of harmful effects. (Akoto *et al.*, 2014).

RfD value for Cd, Pb, Hg, As, Zn and Cu is 0.001, 0.004, 0.0003, 0.0003, 0.3 and 0.04 (mg/kg bw/day) respectively (USEPA, 2006). The population will pose no risk if the ratio is less than 1 and if the ratio is equal or greater than 1 then population will experience health risk. The following equation is used (Chien *et al.*, 2002):

$$THQ = \frac{EF \times ED \times FIR \times C}{RFD \times BW \times AT} \times 10^{-3}$$

Where THQ is the target hazard quotient; EF is exposure frequency (365 days/year); ED is the exposure duration (70 years, average lifetime); FIR is the food ingestion rate (g/day); C is the heavy metal concentration in fish sample ($\mu\text{g/g}$); RfD is the oral reference dose (mg/kg/day); BW is the average adult body weight (70 kg); and AT is the averaging exposure time (365 days/year \times number of exposure years, assuming 70 years).

The hazard index is the sum of the hazard quotients as described in the following equation: $HI = \sum HQ = HQ_{As} + HQ_{Cd} + HQ_{Pb} + HQ_{Al} + HQ_{Hg}$ Where $\sum HQ$ is the summation of hazard quotients of metals and HQ_{As} ; HQ_{Cd} ; HQ_{Pb} ; HQ_{Al} and HQ_{Hg} are the hazard quotients for arsenic, cadmium, lead, aluminum and mercury, respectively. It is assumed that magnitude of adverse effect will be proportional to the sum of multiple metal Exposures. When the hazard index exceeds 1.0, there is a concern for potential health hazard effect (Huang *et al.*, 2008).

4. Quantitative analysis of sulfadimidine residues:

Chromatographic analysis was performed using high performance liquid chromatography system (HPLC, Agilent 1100 Series) and separation was achieved using a reversed phase C18 column (4.6 \times 150 mm, 5 μm , Agilent, ZORBAX SB-C18). Sulfadimidine was extracted from fish tissue using an extraction procedure based on less organic solvent consuming methodology (Lehotay *et al.*, 2005).

5. Quantitative analysis of total aflatoxins residues:

Total aflatoxins standards were obtained from Sigma (St. Louis, MO, USA) of 99% purity. Working solution was prepared in acetonitrile and stored at -20 $^{\circ}\text{C}$ in amber glass vials over a period of 12 months. Determination of aflatoxine residue is done by using Liquid chromatography: (HPLC) Agilent Series 1200 using fluorescence (FLD) detector with which was excitation at 360nm and emission at 440 nm. The chromatographic separation was performed with a reversed-phase column (Extend-C18, Zorbax column, 4.6 mm i.d., 250 mm, 5 μm , Agilent Co.). Extraction of aflatoxins Extraction of total aflatoxins residues from fish tissues was done according to Abd El Monem *et al.*, (2015). SPE (Solid Phase Extraction) and derivatization step was done according to Kalantari *et al.*, (1999), twenty μl were injected into HPLC (Anklam *et al.*, 2002).

6. Data analysis:

Statistical analysis of data was carried out using Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS, Inc., Chicago, IL, USA, 2007).

RESULTS AND DISCUSSION

I. Heavy metal residues:

The higher the contamination of heavy metals in the water body, the higher bioaccumulation of heavy metals contained in the network aquatic organisms and this represents a serious threat to humans (Tapia *et al.*, 2012 and Shafei, 2015).

I.1. Arsenic residues (As):

The content of arsenic in fish tissues is generally due to the arsenic naturally existed in fish feeding such as macroalgae that contain high level of arsenic (Maher and Clarke, 1984). Arsenic is present in food as different species, which vary in toxicity with inorganic forms being the most toxic. Most of the arsenic in the diet is present as the less toxic organic species (Edmonds and Francesconi, 1993; Buchet *et al.*, 1994 and Schoof *et al.*, 1999). The data in table (1) showed that the arsenic residue detected in 100% of examined *Clarias gariepinus* muscle with a concentration ranged from 0.0425 to 0.1075 with a mean value of 0.07 ± 0.007 ppm. Nearly similar level of arsenic (0.013 ± 0.002 ppm) was obtained in Assiut governorate by Salem (2003). Higher levels of arsenic were obtained by Rattanachongkiat *et al.*, (2004); Mukherjee and Bhupander (2011); Budiati (2010) and El Bayomi *et al.*, (2019) where they detected 5.8 ppm in fish samples collected from Thailand; 0.66 ± 0.09 ppm from fish samples collected from India; 2.14 ± 33 , 1.7 ± 0.24 and 1.05 ± 0.88 ppm in three types of fish collected from Malaysia and 0.69 ± 0.04 ppm in *Clarias gariepinus* collected from Egypt respectively. No existing legislated regulations on permissible limits for the total arsenic in seafood in Egypt but according to ISIRI No. 6952 (2014) all examined fish samples within the established limit 0.2 ppm for fish and fish products as shown in table (1).

Table (1) Occurrence, concentration and comparison with maximum permissible limits of heavy metal residues (ppm) in examined *Clarias gariepinus* muscle (n= 50)

	Arsenic	Lead	Cadmium	Mercury	Zinc
Occurrence	100%	90%	50%	90%	100%
Minimum	0.0425	0.08	0.01	0.08	3.36
Maximum	0.1075	0.48	0.03	2.2	7.44
Mean \pm SE	0.07 ± 0.007	0.28 ± 0.05	0.018 ± 0.002	1.4 ± 0.24	5.13 ± 0.39
MPL	0.2 ^b	0.1 ^a	0.05 ^a	0.2 ^a	40 ^c
Within MPL	50 (100%)	15 (30%)	50 (100%)	5 (10%)	50 (100%)
Exceed MPL	0	35 (70%)	0	45(90%)	0

SE = standard error

MPL= Maximum permissible limits

^a according to Egyptian standard (ES 7136, 2010).

^b according to ISIRI No. 6952

^c according to Egyptian standard (ES 1-889/ 2005).

Risk assessment of exposure to arsenic due to *Clarias gariepinus* consumption

Foods having toxic substances could present a toxic threat for the consumer which is dependent on the heavy metal concentration in diet and amount of food used (Hajeb *et al.*, 2009). Risk consists of detecting the toxicological characters related to a specific substance (Kuhnlein and Chan, 2000). The 'tolerable intake' is important to describe 'safe' levels of intake; and can be stated on a daily basis tolerable daily intake (TDI) regulated by (JECFA,

2004). PTWI is the maximum amount of a pollutant to which individual can be exposed per week over a life span without an unacceptable risk of health problems.

Estimated daily intake of arsenic (EDIAs)

In this study, dietary exposure assessment for As through *Clarias gariepinus* consumption was carried out by detecting the estimated daily intake in comparison to the tolerable daily intake. The average quantity of fish consumed per adult person (assuming a 70 kg person) every week was 12 ounces (approximately 340 grams weekly 48.57 g/ daily (FAO, 2010). The presented data in (table 2) revealed that estimated daily intake (EDI) of arsenic from *Clarias gariepinus* muscle 0.05µg/ kg body weight/day and not exceeded the tolerable daily intake (2.1µg/ kg body weight/day) established by (JECFA, 2004). The EDI of arsenic through consumption of fish and seafood worldwide was 2.9, 0.18, 1.37 and 0.550 µg/ kg body weight/day in Spain (Llobet *et al.*, 2003), in India (Mukherjee and Bhupander, 2011), in Malaysia (Budiati, 2010) and in Egypt (Morshdy *et al.*, 2015), respectively.

Target hazard quotient of arsenic (THQAs)

The THQ exceeding one indicates that there is potential risk to human safety, and THQ ≤ 1 indicated no adverse health effects. The showed data in table (2) and declared that As THQ value was 0.167(lower than 1). A higher THQ obtained 1.21 from consumption of fish caught in Portuguese waters (Vieira *et al.*, 2011).

Table (2). Estimated daily intake (EDI) µg/ kg body weight/day in comparison to the Tolerable daily intake (TDIs) µg/ kg body weight, target hazard quotient (THQ) and Hazard index (HI) of different metals.

Heavy metal	EDI	TDI ^a	THQ	Hazard index
As	0.05	2.1	0.167	3.49
Pb	0.20	3.57	0.049	
Cd	0.012	1	0.012	
Hg	0.97	0.228	3.25	
Zn	3.55	300-1000	0.012	

^aJECFA Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2004.

II.2. Lead residues (Pb):

Lead is non-essential heavy metal that have no biological role but present in all organisms. Extensive research shows that lead may have adverse health effects. Moreover, lead can mimic and construct the building blocks of DNA (nucleotides) and alter the activity of zinc in the synthesis of hemoglobin (Kirberger *et al.*, 2013).

The data in table (1) showed that lead detected in 90% of examined samples. The lead residue level ranged from 0.08 to 0.48 with a mean value of 0.28±0.05. The results were in line with the finding of Saei-Dehkordi and Fallah, (2011) and El Bayomi *et al.*, (2019) who detected 0.534 ppm of lead residue in fish samples collected from Iran and 0.42 ± 0.04 ppm in *Clarias gariepinus* collected from Egypt respectively while lower lead residue values were obtained by Jorhem and Sundström, (1993) and Suppin *et al.*, (2005) who detected 0.008 and 0.018 ppm of lead residue in fish samples respectively.

The data in Table (1) declared that the number of accepted samples according to lead residues was 15 (30%) and the exceeded samples was 35 (70%) according to the

Regulation No. **7136/2010 of EOS, (2010)**, the maximum permitted limit (MPL) has been reported at 0.1ppm wet weight for fish. The higher percentage of fish samples exceeding the permissible limits may be due to the increased lead sources in the environment such as; lead paint, lead from combustion of fuel containing tetraethyl lead, plumbing leachates from pipes or solder and lead from leaded chips, and batteries (**Committee of Environmental Health, 2005**).

Risk assessment of exposure to lead due to *Clarias gariepinus* consumption

Estimated daily intake of lead (EDI Pb)

The presented data in table (2) revealed that estimated daily intake (EDI) of lead from *Clarias gariepinus* sample was 0.2 µg/ kg body weight/day. The EDI of lead from consumption of *Clariasgariepinus* was lower than the tolerable daily intake (3.57-µg/ kg body weight/day) established by **JECFA (2004)** which clearly, indicates that Pb intake for the general population from *Clarias gariepinus* were below the guidelines. The obtained results of the EDI of lead in this study was nearly similar to those obtained worldwide through consumption of fish where they were 0.078, 0.09 and 0.372µg/ kg body weight/day in Spain (**Llobet *et al.*, 2003**), Portugal (**Vieira *et al.*, 2011**) and Egypt (**Morshdy *et al.*, 2015**), respectively.

Target hazard quotient of lead (THQ Pb)

The calculated data in table (2) declared that THQ of lead was 0.049 lower than 1. The THQ of lead was 0.021 from consumption of fish caught in Portuguese waters (**Vieira *et al.*, 2011**), 0.11, 0.06 and 0.12 from consumption of Brush tooth Lizard fish, Mackerel and Horse Mackerel (**Ali, 2018**) and 0.06 from consumption of *Clarias gariepinus* in Egypt (**El Bayomi *et al.*, 2019**)

I.3. Cadmium residues (Cd)

Cadmium accumulates in human's body from food chain magnification and has the ability to induce kidney dysfunction, skeletal damage, and also affect reproductive system (**WHO, 2003**). The results in table (1) showed that cadmium residue detected in 50% of examined samples where its concentration was ranged from 0.01 to 0.03 ppm with a mean value of 0.018 ± 0.002 ppm as shown in table (2). A higher Cd level was obtained by **El Bayomi *et al.*, (2019)** they detected (0.19 ± 0.02 ppm) in *Clarias gariepinus* collected from Egypt. Considering the existing legislated regulations on permissible limits of cadmium (**ES: 7136/2010**), the maximum permitted limit (MPL) has been reported at 0.05 ppm wet weight for fish which mean that all examined samples of *Clarias gariepinus* were accepted.

Risk assessment of exposure to cadmium due to *Clariasgariepinus* consumption

Estimated daily intake of cadmium (EDI Cd)

The presented data in table(2) revealed that estimated daily intake (EDI) of cadmium was 0.012 µg/ kg body weight/day which is lower than the tolerable daily intake (1µg/ kg body weight/day) established by **JECFA (2004)**. That's clearly indicates that Cd intake for the general population from *Clarias gariepinus* samples is below the guidelines. The EDI of cadmium through consumption of fish and seafood worldwide was 0.055, 0.13 and 0.007µg/ kg body weight/day in Spain (**Llobet *et al.*, 2003**), India (**Mukherjee and Bhupander, 2011**) and Egypt (**Morshdy *et al.*, 2015**), respectively.

Target hazard quotient of cadmium (THQ Cd)

The THQ exceeding 1 indicates that there is potential risk to human health, and $THQ \leq 1$ indicated no adverse health effects. The showed data in table (2) declared that THQ of cadmium 0.012 lower than one. The THQ of cadmium was 0.002 to 0.19 from consumption of fish caught in Portuguese waters (**Vieira *et al.*, 2011**). And 0.02, 0.024 and

0.03 from consumption of Brush tooth Lizard fish, Mackerel and Horse Mackerel in Egypt (Ali, 2018).

I.3. Mercury residues (Hg):

Mercury compounds are highly toxic, because they are fat-soluble, easily absorbed, and accumulated in erythrocytes and CNS (Castoldi *et al.*, 2003). The results in table (1) showed that the mercury residue detected in 90% of examined *Clarias gariepinus* samples where its level ranged from 0.08 to 2.2 ppm with a mean value of 1.4 ± 0.24 ppm. Nearly similar value for mercury level was obtained by El Bayomi *et al.*, (2019) detected (1.02 ± 0.08 ppm) of mercury residue in *Clarias gariepinus* collected from Egypt while lower values was obtained by Mukherjee and Bhupander (2011) who detected 0.62 ± 0.05 ppm of mercury residue in fish samples collected from India and Morshdy *et al.*, (2007) who reported 0.54 ± 0.03 and 0.56 ± 0.01 ppm in Brush tooth Lizard fish and Mackerel fish samples collected from Egypt respectively.

According to ES (7136/2010) the maximum permitted limit (MPL) is 0.2 ppm wet weight for fish. The results in table (1) declared that the numbers of accepted samples according to mercury residue were 5(10%) and the exceeded samples resemble 45 (90%) according to EOS (2010) permissible limits.

Risk assessment of exposure to mercury due to *Clarias gariepinus* consumption Estimated daily intake of mercury (EDI Hg)

The presented data in table (2) revealed that estimated daily intake (EDI) of mercury was $0.97 \mu\text{g}/\text{kg}$ body weight/day which is higher than the tolerable daily intake ($0.228\text{-}\mu\text{g}/\text{kg}$ body weight/day) established by JECFA (2004). That's clearly, indicates the Hg intake for the general population from *Clarias gariepinus* samples may lead to public health hazard and mercury toxicity. The EDI of mercury through consumption of fish and seafood worldwide was 0.14, (0.17 in summer and 0.086 in winter), 0.17, 0.021 and $0.56 \mu\text{g}/\text{kg}$ body weight/day in Spain (Llobet *et al.*, 2003), Iran (Maghtouie *et al.*, 2011), India (Mukherjee and Bhupander, 2011), Egypt (Morshdy *et al.*, 2015) and (El Bayomi *et al.*, 2019), respectively.

Target hazard quotient of mercury (THQ Hg)

The calculated data in table (2) declared that THQ of mercury 3.25 was more than one while lower results were obtained by Vieira *et al.*, (2011), Ali (2018) and El Bayomi *et al.*, (2019) who reported that THQ of mercury was ranged from 0.025 to 1.30 from consumption of fish caught in Portuguese waters, 0.025, 0.19 and 0.15 from consumption of Brush tooth Lizard fish, Mackerel and Horse Mackerel in Egypt and 0.35 from consumption of *Clarias gariepinus* collected from Egypt respectively.

I.4. Zinc residues (Zn):

Zinc is an essential nutrient for almost all plants. For this reason, algae growing in streams and lakes can absorb a large part of the zinc dissolved in water. In addition to its nutritive effect, zinc is also toxic to most forms of plants when present in amounts exceeding certain limits (Krishna *et al.*, 2014). The data in table (1) showed that Zn was detected in 100% of examined *Clarias gariepinus* muscle. The tissue concentrations of zinc ranged from 3.36 to 7.44 ppm with mean values of 5.13 ± 0.39 ppm. Nearly similar values was obtained by El-Sayed *et al.*, (2011) who detected (3.84 to 5.67 ppm) of Zinc concentrations in *Clarias gariepinus* while higher zinc content has been reported in the range 55.8-60 mg/kg in fish species from Lake Qarun by Mohamed and Gad (2008), 13-39 mg/kg in fish species from Rosetta Branch of River Nile by Gad and Yacoub (2009) and 0.1- 70 mg/kg in fish species from Nile delta Egypt by Zyadah (2005). The data in

table (1) declared that all examined *Clarias gariepinus* samples were located within the established limit 40 ppm for fish according to Egyptian slandered (EOS, 2010).

Risk assessment of exposure to zinc due to fish consumption

Estimated daily intake of zinc (EDI Zn)

The presented data in table (2) revealed that the estimated daily intake (EDI) of zinc from *Clarias gariepinus* samples was 3.68 µg/ kg body weight/day and not exceeded the tolerable daily intake (300 µg/ kg body weight/day) established by JECFA (2004). Higher value of EDI (5.37 µg/ kg body weight/day) of zinc through consumption of fish in India was obtained by Krishna *et al.* (2014) while lower results (1.703 µg/ kg body weight/day) was obtained by Morshdy *et al.* (2015) through consumption of fish in Egypt.

Target hazard quotient of zinc (THQ Zn)

The data shown in table (2) declared that Zn THQ value was 0.012 which is lower than one. On the other hand higher value of Zn THQ (17.9) was obtained by Krishna *et al.*, (2014) from consumption of fish caught from Machilipatnam coast, Andhra Pradesh, India. Hazard index (HI) defined as Σ THQ can be estimate the risk of mix pollutants (USEPA, 1989). HI value of *Clarias gariepinus* was higher than 1 (3.49) as shown in table (2) for adult, indicating that there is health risk for the consumer by ingesting multiple metals contained in *Clarias gariepinus*.

II. Antibiotic residues in *Clarias gariepinus*:

One of the most important concerns to consumer is the consumption of food containing chemical additives particularly animal drug residues, which produce adverse effects in humans (Donoghue, 2003).

The sulfonamide group includes a large number of compounds which have been used for a very long time, including sulfadiazine, sulfamethizole, sulfamethoxazole, sulfasalazine, sulfisoxazole, and various high-strength combinations of three sulfonamides. At present, sulfonamides and other drugs (chlortetracycline, penicillin, and several ionophores) are the most common contaminating antimicrobials in animal feed, generating potentially serious problems in human health (Wang *et al.*, 2006).

Table (3) Occurrence of sulfadimidine and aflatoxin residues in examined *Clarias gariepinus* muscle (n=5)

Sample	Sulfadimidine (µg/kg)	Aflatoxin (µg/kg)
1	50.83	ND
2	87.5	ND
3	37.5	ND
4	33.2	5.32
5	45.83	ND
Mean ± SD	50.97±21.55	-

SD = Standard deviation

ND = Not detected

The EU has set Maximum residue limit (MRL) for total sulfonamide concentration in fish at 100-µg kg⁻¹. The data in table (3) declared that sulfadimidin residue was detected in all examined *Clarias gariepinus* samples with minimum 33.2, maximum 87.5, and mean value ± SD (50.97±21.55 µg/kg). All examined samples were below Maximum residue limit 100 µg/ kg. Sulfonamide previously detected within different concentration in sea bass

271 µg/kg, and in rainbow trout sample with at concentration of 56 µg/kg (Yipel *et al.*, 2017).

III. Aflatoxin residues in *Clarias gariepinus*:

The aflatoxine occurrence in those fish samples could be attributed to the presence of mycotoxin in fresh fish tissues once the contaminated feed is ingested by farmed fish (Sun *et al.*, 2015). The results in table (3) declared that aflatoxin was detected only in one sample from five samples with a concentration 5.32 µg/kg (ppb) where nearly similar results was obtained by Sun *et al.*, (2015) who detected aflatoxin only in one sample from examined carp purchased from local supermarkets in Shanghai city, China with a concentration of 1.2 µg/kg. Meanwhile, aflatoxin was not detected in raw fish samples from Spain (Carballo *et al.*, 2018), Smoked fish and sushi from Japan (Tolosa *et al.*, 2019).

There is no Egyptian regulation for aflatoxin in fish flesh but because of the potential health hazards of mycotoxin for humans, threshold levels of aflatoxin in commodities have been established worldwide. A maximum residue limit of 20 µg/kg (FDA, 2000) and 4 µg/kg (FAO, 2004) for total aflatoxin in foods. Comparing the obtained results declared that there is no possibility aflatoxin exposure and human health risk from consumption of *Calais gariepinus* flesh.

CONCLUSION

The obtained results declared that *Clarias gariepinus* flesh was contaminated with various concentrations of heavy metals where the level of arsenic, cadmium, and zinc was within the permissible limit while the level of lead and mercury exceeded the permissible limit in most of examined samples. The estimated daily intake of arsenic, lead, cadmium and zinc below the tolerable daily intake. Meanwhile, mercury exceed the tolerable daily intake so, possible risk may occur in population consumed *Clariasgariepinus* flesh. Antibiotic residue (sulfadimidin) detected in all examined *Clariasgariepinus* flesh samples but within the permissible limit while Aflatoxin residue was detected only in one sample of *Clariasgariepinus* flesh and located below the permissible limit.

REFERENCES

- Abd El Monem, M.; Ragab, M.; Maher, M.A.; Ali, S.H.H.; Salah, N.; Hussein, H.M.A and Amr, F.H. (2015): Detection of aflatoxins in meat by modified HPLC method. Egypt. J. Chem. Environ. Health. 1, 945-954.
- Akoto, O.; Bismark Eshun, F.; Darko, G. and Adei, E. (2014): Concentrations and health risk assessments of heavy metals in fish from the Fosu Lagoon. International Journal of Environmental Research. 8(2): 403-410.
- Al bader, N. (2008): heavy metal levels in most common available fish species in Saudi market. J. of food technology. 6 (4): p. 173- 177.
- Ali, E.T. (2018): Chemical safety of imported frozen fish. M.V.Sci. Thesis. (Meat Hygiene). Fac. Vet. Med. Zag. Univ. Egypt.
- Anklam, E.; Stroka, J. and Boenke, A. (2002): Implementation of EU legislation with afocus on mycotoxins. Food Control, 13: 173- 183, 16th ed. Association of Analytical Communities (AOAC) (1995): Method 990.33, Official methods of analysis.
- Authman, M. M. (2011): Environmental and experimental studies of aluminium toxicity on the liver of *Oreochromis niloticus* (Linnaeus, 1758) fish. Life Science Journal, 8(4): 764-776.
- Buchet, J. P.; Pauwels, J. and Lauwerys, R. (1994): Assessment of exposure to inorganic arsenic following ingestion of marine organisms by volunteers. Environ Research. 66:44-51.
- Budiati, T. (2010): The presence of arsenic as heavy metal contaminant on salmon: a risk assessment. International Journal of Basic and Applied Sciences.10(5): 6-12.

- Busby, W. F. and Wogan, G. N. (1984):** Aflatoxins. In: Searle CEE (ed) Chemical carcinogenesis. American Chemical Society, Washington, DC: 945–1136.
- Carballo, D.; Font, G.; Ferrer, E. and Berrada, H. (2018):** Evaluation of mycotoxin residues on ready-to-eat food by chromatographic methods coupled to mass spectrometry in tandem Toxins, 10(6): 243.
- Castoldi, A. F.; Coccini, T. and Manzo, L. (2003):** Neurotoxic and molecular effects of methyl mercury in humans. Reviews on Environmental Health. 18:19- 31.
- Chien, L. C.; Hung, T. C.; Choang, K. Y.; Yeh, C. Y.; Meng, P. J.; Shieh, M. J. and Han, B. C. (2002):** Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. Science of the total environment. 285(1-3): 177-185.
- Committee on Environmental Health. (2005):** Lead exposure in children: prevention, detection, and management. Pediatrics. 116(4): 1036-1046.
- Dhaouadi, R.; Tarhouni, D.E. and Louati, A. (2015):** Utilisation des antibiotiques en aquaculture. [http://docplayer.fr/22592327-Utilisation-des antibiotiques en aquaculture. Html](http://docplayer.fr/22592327-Utilisation-des-antibiotiques-en-aquaculture.html). Accessed 10.3. 2018.
- Donoghue, D. (2003):** Antibiotic residues in poultry tissues and eggs, human health concerns. Poultry Science. 82: 618–621.
- Edmonds, J.S. and Francesconi, K.A. (1993):** Arsenic in seafood's: human health aspects and regulations. Marine Pollution. 26:665-674.
- Egyptian Organization for Standardization and Quality control (EOS.NO.7136) (2010):** Maximum level for certain contaminants in food stuffs. ES NO.7136/2010. Cairo, Egypt.
- El Bayomi, R. M.; Darwish, W. S. and Elawady, E. F. (2019):** Risk Assessment of some Heavy Metals from *Claris gariepinus* (African catfish) Consumed in Sharkia Governorate, Egypt. Zagazig Veterinary Journal, 47(2): 193-202.
- El-Sayed, E. A.; El-Ayyat, M. S.; Nasr, E. and Khater, Z. Z. K. (2011):** Assessment of heavy metals in water, sediment and fish tissues, from Sharkia province, Egypt. Egypt. J. Aquat. Biol. & Fish, 15(2): 125-144.
- FDA (2000):** Guidance for industry: Action levels for poisonous or deleterious substances in human food and animal feed <<http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm077969.htm#afla>> Last updated 11.09.11
- FAO “Food and Agriculture Organization” (2004):** Worldwide regulations for mycotoxins in food and feed in 2003. *Food and Nutrition Paper*, FAO, Rome. Paper 81.
- FAO (Food Agriculture Organization) (2010):** Nutrition Country Profiles – Egypt. FAO, Rome, Italy.
- Gad, N.S. and Yacoub A. M. (2009):** Antioxidant defense agents and physiological responses of fish to pollution of Rosetta Branch of River Nil Egypt, *Egyptian Journal of Aquatic Biology and Fisheries* 13(4): 109-128.
- Gonçalves, R.A.; Naehrer, K. and Santos, G.A. (2016):** Occurrence of mycotoxins in commercial aquafeeds in Asia and Europe: a real risk to aquaculture? Reviews in aquaculture, n/a-n/a.
- Hajeb, P.; Jinap, S.; Ismail, A.; Fatimah, A. B.; Jamilah, B. and Rahim, M. A. (2009):** Assessment of mercury level in commonly consumed marine fishes in Malaysia. Food Control. 20(1): 79-84.
- Huang, M.; Zhou, S.; Sun, B. and Zhao, Q. (2008):** Heavy metals in wheat grain: assessment of potential health risk for inhabitants in Kunshan, China. Science of the Total Environment. 405(1-3): 54-61.
- International Agency for Research on Cancer (IARC) (1993):** Some naturally occurring substances: food items and constituents, heterocyclic aromatic amine and mycotoxins, In Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC, Lyon.
- ISIRI No. 6952 (2014):** Institute of Standards and Industrial Research of Iran. Fish and fish products. <http://www.anytest.co.kr/isiri-7/?ckattempt=1>.
- JECFA Joint FAO/WHO Expert Committee on Food Additives (JECFA), (2004):** Safety evaluation of certain food additives and contaminants. WHO Food Additives Series No. 52. World Health Organization, Geneva
- Jorhem, L. and Sundström, B. (1993):** Levels of lead, cadmium, zinc, copper, nickel, chromium, manganese, and cobalt in foods on the Swedish market, 1983–1990. Journal of Food Composition and Analysis. 6(3): 223-241.

- Kalantari, H.; Zandmoqadam, H. and Abdolahilorestani, S. (1999):** Determination of aflatoxins B1 and M1 in liver by HPLC. *Journal of Ahwaz University Agriculture*, 22 (1): 10.
- Kirberger, M.; Wong, H. C.; Jiang, J. and Yang, J. J. (2013):** Metal toxicity and opportunistic binding of Pb²⁺ in proteins. *Journal of inorganic biochemistry*. 125: 40-49.
- Krishna, P. V.; Jyothirmayi, V. and Rao, K. M. (2014):** Human health risk assessment of heavy metal accumulation through fish consumption, from Machilipatnam Coast, Andhra Pradesh, India. *Journal Issues ISSN*, 2360, 8803.
- Kuhnlein, H. V. and Chan, H. M. (2000):** Environment and contaminants in traditional food systems of northern indigenous peoples. *Annual Review of Nutrition*. 20(1): 595-626.
- Lehotay, S. J.; Mastovska, K. and Yun, S. J. (2005):** Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. *J. AOAC Int.* 88: 630-638.
- Llobet, J. M.; Falco, G.; Casas, C.; Teixido, A. and Domingo, J. L. (2003):** Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *Journal of Agricultural and Food Chemistry*. 51(3): 838-842.
- Loutfy, N.; Fuerhacker, M.; Tundo, P.; Raccanelli, S.; El Dien AG and Ahmed MT (2006):** Dietary intake of dioxins and dioxin-like PCBs, due to the consumption of dairy products, fish/seafood and meat from Ismailia city, Egypt. *Sci Total Environ* 370:1-8.
- Maghtouie, A. H.; Neissi, G.; Nasser, S.; Gholaminezhad, E.; Shalamzari, N. and Nikpour, Y. (2011):** Determination of mercury in mullet fish (*Liza abu*) from Arvand River, Iran. *World Journal of Fish and Marine Sciences*. 3(6): 514-517.
- Maher, W. A. and Clarke, S. M. (1984):** The occurrence of arsenic in selected marine macroalgae from two coastal areas of South Australia. *Marine Pollution Bulletin*. 15(3): 111-112.
- Mensah, S.E.P.; Koudandé, O.D.; Sanders, P.; Laurentie, M.; Mensah, G.A. and Abiola, F.A. (2014):** Résidus d'antibiotiques et denrées d'origine animale en Afrique: risques de santé publique, *Rev. Sci. Tech. Off. Int. Epiz.* 33 (3): 1-27.
- Mitra, A.; Barua, P.; Zaman, S. and Banerjee, K. (2012):** Analysis of trace metals in commercially important crustaceans collected from UNESCO protected world heritage site of Indian Sundarbans. *Turkish Journal of Fisheries and Aquatic Sciences*, 12(1): 53-66.
- Mohamed, F.A.S. and Gad, N.S.: (2009):** Bioaccumulation, some blood biochemical changes and histological alteration in selected tissues of *Oreochromis niloticus* exposed to Zinc and/ or Cadmium. *American Eurasian Journal of Agricultural and Environment Science*, 5 (3): 441-455.
- Morshdy, A.; Darwaish, S.; Hussein, M. and El Ebidy O. (2015):** Heavy Metal Residues in Marketed Fish at Manzalla Dakahllyia. 2nd Conference of Food Safety, Suez Canal University, Faculty of Veterinary Medicine. Volume I August: 111-118.
- Morshdy, A.; Eldosky, K.E. and El-Sebaie, S. (2007):** Some heavy metal residues in mackerel and saurus fish. *Zag. Vet. J.* 35(4):114-120.
- Mukherjee, D. P. and Bhupander, K. (2011):** Assessment of Arsenic, Cadmium, and Mercury Level In Commonly Consumed Coastal Fishes from Bay of Bengal, India. *Food Science and Quality Management*. www. iiste. org. Diakses. 16.
- Ostrowski-Meissner, H.T. (1984):** Effect of contamination of foods by *Aspergillus flavus* on the nutritive value of protein. *J Sci Food Agric* 35:47-58.
- Pham, D.K.; Chu, J.; Do, N.T.; Brose, F.; Degand, G.; Dela- haut, P.; De Pauw, E.; Douny, C.; Van Nguyen, K.; Dinh Vu, T.; Scippo, M.-L. and Wertheim, H.F.L. (2015):** Monitoring antibiotic use and residue in freshwater aquaculture for domestic use in Vietnam. *Eco Health*, 12 (3): 480-489.
- Rattanachongkiat, S.; Millward, G. E. and Foulkes, M. E. (2004):** Determination of arsenic species in fish, crustacean and sediment samples from Thailand using high performance liquid chromatography (HPLC) coupled with inductively coupled plasma mass spectrometry (ICP-MS). *Journal of Environmental Monitoring*. 6 (4): 254-261.
- Reda, R. M.; Ibrahim, R. E.; Ahmed, E. N. G. and El-Bouhy, Z. M. (2013):** Effect of oxytetracycline and florfenicol as growth promoters on the health status of cultured *Oreochromis niloticus* Egypt. *J. Aquat. Res.*, 39(4): 241-248.
- Saei-Dehkordi, S. S. and Fallah, A. A. (2011):** Determination of copper, lead, cadmium and zinc content in commercially valuable fish species from the Persian Gulf using derivative potentiometric stripping analysis. *Microchemical Journal*. 98(1): 156-162.

- Salem, D.A. (2003):** Survey of some environmental pollutants in freshwater fishes in Assiut governorate, Egypt. *Ass. Univ. Bull. Environ. Res.* 6 (2): 15-38.
- Schoof, R.A.; Yost, L. J.; Eickhoff, E. A.; Crecelius, E.A.; Cragin, D. W.; Meacher, D. M. and Menzel, D. B. (1999):** A market basket survey of inorganic arsenic food. *Food and Chemical Toxicology.* 37:839-846.
- Shafei, H. M. E. L. (2015):** Some heavy metals concentration in water, muscles and gills of *Tilapia Niloticus* as biological indicator of Manzala Lake Pollution. *J. of Aquaculture Research and Development.* 6: 358.
- Sharma, R.P. and Salunkhe, D.K. (1991):** *Mycotoxins and phytotoxins*, Press, C., ed. (Boca Raton, FL).
- SPSS, 16 (2007):** Statistical Package for Social Science ver. 16 for Windows. SPSS (Inc., Chicago, IL.).
- Sun, W.; Han, Z.; Aerts, J.; Nie, D.; Jin, M.; Shi, W., & Wu, A. (2015):** A reliable liquid chromatography–tandem mass spectrometry method for simultaneous determination of multiple mycotoxins in fresh fish and dried seafoods. *Journal of Chromatography A*, 1387: 42-48.
- Suppin, D.; Zahlbruckner, R.; Krapfenbauer-Cermak, C. H.; Hassan-Hauser, C. H. and Smulders, F. J. M. (2005):** Mercury, lead and cadmium content of fresh and canned fish collected from Austrian retail operations. *Arbeitstagung Des Arbeitsgebietes Lebensmittelhygiene.* 46: 633.
- Tapia, J.; Chacoff, L. V.; Bertrán, C.; Cortés, F. P.; Hauenstein, E.; Schlatter, R.; Jiménez, C. and Tapia, C. (2012):** Heavy metals in the liver and muscle of *Micropogonias manni* fish from Budi Lake, Araucania Region, Chile: potential risk for humans. *Environ. Monitoring and Assessment.* 184:3141–3151.
- Tolosa, J.; Barba, F. J.; Font, G. and Ferrer, E. (2019):** Mycotoxin Incidence in Some Fish Products: QuEChERS Methodology and Liquid Chromatography Linear Ion Trap Tandem Mass Spectrometry Approach. *Molecules*, 24(3): 527.
- Ukoha, P. O.; Ekere, N. R.; Udeogu, U. V. & Agbazue, V. E. (2014):** Potential health risk assessment of heavy metals [Cd, Cu and Fe] concentrations in some imported frozen fish species consumed in Nigeria. *Int. J. Chem. Sci.* 12(2): 366-374.
- US Environmental Protection Agency, EPA (2010):** Integrated Risk Information System (IRIS). Cadmium (CASRN-7440-43-9) <http://www.epa.gov/iris/subst/0141.htm> (Accessed 18.04.2013).
- USEPA (2006):** United States, Environmental Protection Agency, Integrated Risk Information System, [<http://www.epa.gov/iris/substS>].
- USEPA, (United States Environment Protection Agency) (1989):** Risk Assessment Guidance for Superfund, Vol 1. EPA/540/1-89/002. Office of Emergency and Remedial Response, USEPA, Washington, DC.
- Vieira, C.; Morais, S.; Ramos, S.; Delerue-Matos, C. and Oliveira, M. B. P. P. (2011):** Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra-and inter-specific variability and human health risks for consumption. *Food and Chemical Toxicology*, 49(4): 923-932.
- Wang, S.; Zhang, H.; Wang, L.; Duan, Z. J. and Kennedy, I. (2006):** *Food Add Contam* 23: 362–384.
- WHO (2003):** Cadmium in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality Geneva, World Health Organization (WHO/ SDE/ WSH/ 03 04/80).
- Yipel, M.; Kürekeci, C.; Tekeli, İ. O.; Metli, M. and Sakin, F. (2017):** Determination of selected antibiotics in farmed fish species using LC- MS/MS. *Aquaculture research.* 48(7): 3829-3836.
- Zantopoulos, N.; Antoniou, V.; Petsaga, V. and Zdragas, A. (1996):** Copper concentrations in sheep liver and kidney in Greece. *Veterinary and human toxicology.* 38(3): 184-185.
- Zyadah, M.A.: (2005):** Monitoring of heavy metals in water and fish at northern region of Nile Delta Egypt. *Journal of the Egyptian German Society of Zoology* 46 (D): 141-157.