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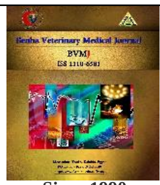

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Chemical Residues in Some Farmed Fish Species Marketed in Sharkia Governorate, Egypt.

Hania E. Abd El Maksod1, Saad M. Saad1, Gehan Fatahalla2, Maha M. Samir3

[1]*Department of Meat Hygiene, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2]*Fish Diseases Department, Animal Health Research Institute*

[3]*Food Control Department, Animal Health Research Institute, Zagazig Branch*

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ABSTRACT

This study was conducted to evaluate the chemical residues in some farmed fish species marketed in Sharkia Governorate and compare it with the Egyptian standards (ES) of chilled fish. A total of 150 random samples of farmed fish at Nile cages, concrete ponds, and earthen ponds (50 of each, including 25 of both *Tilapia nilotica* and *Mugil cephalus*), respectively were examined. The samples were collected during autumn 2021. The results of the chemical residues analysis revealed that the percentage of samples that exceeded the permissible limits according to ES (7136:2010) of mercury residues were 44% and 20% (Nile cages), 36% and 32% (concrete ponds), 14% and 12% (earthen ponds) for *Tilapia nilotica* and *Mugil cephalus*, respectively, for lead, such residues were 40% and 36% ; 20% and 28%, and 44% and 24%, respectively, while cadmium residues were 48% and 36% ; 20% and 16%, and 44% and 68%, respectively. Concerning, the hormonal residues (methyl testosterone and trenbotone acetate), all positive samples exceeded the permissible limits according to ES (3494:2005) which denotes that fish flesh should be free from hormonal residues. All examined samples were free from pesticide residues (Aldrin and Malathione). Therefore, to safeguard fish farms from pollution and lower environmental risk, a significant efforts and coordination among various authorities are required. This can be accomplished by treating sewage, industrial and agricultural wastes. It is also crucial to regularly check for toxic residues in farm water.

1. INTRODUCTION

An expanding worldwide population depends on the vital food that the aquaculture sector produces. It is crucial for the availability of affordable animal protein. Egypt is the top African nation and ranks ninth in the world for fish farming production. Despite being a significant sector, aquaculture is not permitted to use irrigation or Nile water. It is reliant on groundwater and water from agricultural drainage (Naziri, 2011). A decrease in fish output and a higher risk of disease outbreaks are both caused by poor water quality. Farmers now ask for freshwater because they reuse it on their crops. Due to the buildup of contaminants and probable fish contamination, this has a negative impact on the quality of farmed fish (FAO, 2014). Fish may concentrate huge amounts of some metals from the water in their tissues, which is a serious problem for heavy metal pollution of aquatic environments (Mansour and Sidky, 2002). The number of heavy metals in fish is a reliable indicator of pollution in the aquatic environment (Abdel-Mohsein and Mahmoud, 2015 ; Bayomy et al., 2015, and El-Shafei, 2015). When heavy metals build up in important human body organs including the kidneys, bones, and liver, they have neurotoxic and carcinogenic effects (Duruibe et al., 2007; Sapkota et al., 2008). It is also successful to add them to the water or treat fish diets with 17-Methyltestosterone and Trenbolone acetate to establish all fish male as tilapia populations (Ibrahim et al., 2015). The tissue residues of these drugs and their metabolites are the most dangerous side effects caused by these hormones. These residues have a higher impact on people since they can cause early puberty in both males and girls, liver tumors and increased embryo mortality (Ibrahim, 2009). Pesticides have been widely used in Egypt for the past 20 years, which has led to significant issues. Additionally, there are no ongoing programs in Egypt for identifying and quantifying various environmental contaminants. According to Yamashita et al. (2000), Egypt is the fourth-biggest importer of pesticides among developing nations and the largest market for pesticides in Arabian countries. Organochlorinated pollutants are mostly consumed through food, and fish and fisheries products appear to make up most of the dietary intake of these pollutants (Schnitzler et al., 2011). The lipid content of the fish and the quantity of seafood consumed determine the exposure levels. According to Zhang et al. (2014), these residues have the potential to disrupt the endocrine system, have neurotoxic and carcinogenic effects, and affect reproductive and developmental processes. Therefore, it is essential to continuously examined fish for these residues to determine any potential consequences on human health.

2. MATERIAL AND METHODS

Collection and preparation of the samples

A total of 150 random samples of farmed fish at Nile cages, concrete ponds, and earthen ponds (50 of each, including both 25 *T. nilotica* and 25 *M. cephalus* for each farming method), respectively, were examined. Samples were

* Corresponding author: Haniaosman2013@gmail.com

collected from Sharkia governorate during autumn ,2021. The samples were kept in a separate sterile plastic bag, labeled, and conserved in an ice box to transfer them to the laboratory at Faculty of Veterinary Medicine, Benha University.

Determination of heavy metals residues

Washing procedures (Lars, 2003)

Equipment cleaning is a crucial step in preventing contamination with the analyzed element. Deionized water was used to thoroughly clean all glassware and vessels. They were then submerged in hot, diluted HNO3 (10%) for 24 hours, risen many times, and dried to ensure that all equipment was free of metal. The digesting vessels were also immersed in water and soap for two hours before being repeatedly cleaned with tap water. Once with distilled water, once with a solution of 250 ml deionized water, 200 ml concentrated HCl (37% Merck), and 80 ml hydrogen peroxide (30% Marck), and once with 10% HNO3. Every container was carefully cleaned with deionized water and allowed to air dry in an incubator free of contamination or dust.

The digestion procedures (Staniskiene et al., 2006)

Concerning the purpose of determining the presence of lead and cadmium residues, each 1 g of sample was macerated with a sharp scalpel and digested with 10 ml of a digestion solution (60 ml of 65% nitric acid and 40 ml of 70% perchloric acid) in a screw-capped tube. To estimate mercury, 10 ml of a concentrated H2SO4/HNO3 solution (1:1) were added to 0.5 g of the macerated sample.

Preparation of the blank and standard solutions (Andreji et al., 2005)

Flame Atomic Absorption Spectrophotometer (VARIAN, model AA240 FS, Australia).

For quantitative determination of heavy metal residues

The AAS digital scale was used to immediately record the mercury absorption, and the following equation was used to determine the concentration of mercury:

$C_1= (A_1/A_2) \times C \times (D/W)$ mg/kg

While the concentration of lead and cadmium was estimated according to the following equation:

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

Determination of hormonal residues

The technique recommended by Nash et al. (2000) for determination of Methyl testosterone and trenbolone acetate in the examined samples of fish was applied by using Enzyme Linked Immunosorbant Assay (ELISA).

EIA methyl testosterone and EIA trenbolone acetate manual kits (CER Group/Health Department, Belgium) were used.

Determination of pesticides residues

Determination of organochlorine pesticides (Aldrin)

Estimation of aldrin as an organochlorine pesticide in the examined samples was applied according to Heck et al. (2007).

Determination of organophosphorus pesticides (Malathion)

The technique was carried out according to AOAC (2006). Determination was done in the faculty of Veterinary Medicine, Benha University.

3. RESULTS

The data shown in Table 1 revealed that the percentage of mercury residues that exceeded the legal limits for *T. nilotica* and *M. cephalus* in Nile cages, concrete ponds, and earthen ponds, respectively, was 44% and 20% ; 36% and 32% and 14% and 12%, according to ES (7136:2010).

Table 1 Mercury residues (ppm) in examined farm fish samples (25 for each).

	Nile cages		Concrete Ponds		Earthen Ponds	
	T.nilotica	*M.cephalus*	*T.nilotica*	*M.cephalus*	*T.nilotica*	*M.cephalus*
NO. (%) of +ve Samples	19(76%)	15(60%)	13(52%)	12(48%)	20(80%)	17(68)
Min	0.09	0.00	0.62	0.00	0.27	0.65
Max	1.26	1.13	1.43	1.07	1.48	1.15
Mean ±SE	0.57±0.08	0.53±0.08	0.72±0.08	0.71±0.08	0.76± 0.10	0.66±0.08
Samples exceed P.L.	11(44%)	5(20%)	9(36%)	8(32%)	14(56%)	12(48%)

Maximum permissible limits of Mercury 0.50 mg/kg according to ES (7136:2010).

Moreover, according to ES (7136:2010), the percentage of lead residues in Table 2 that exceeded the allowable limits for *T. nilotica* and *M. cephalus* in Nile cages, concrete ponds, and earthen ponds, respectively, was 40% and 36% ; 20% and 28% and 44% and 24%.

Table 2 Lead residues (ppm) in examined farm fish samples (25 for each).

	Nile cages		Concrete Ponds		Earthen Ponds	
	T.nilotica	*M.cephalus*	*T.nilotica*	*M.cephalus*	*T.nilotica*	*M.cephalus*
NO. (%) of +ve Samples	18(72%)	14(56%)	10(40%)	9(36%)	18(72%)	15(60%)
Min	0.00	0.02	0.14	0.07	0.11	0.02
Max	0.82	0.68	0.83	0.70	0.90	0.68
Mean ±SE	0.36±0.06	0.35±0.06	0.36±0.07	0.31±0.07	0.44±0.07	0.27±0.05
Samples exceed P.L.	10(40%)	9(36%)	5(20%)	4(28%)	11(44%)	6(24%)

Maximum permissible limits of Lead 0.30 mg/kg according to ES (7136:2010).

Concerning ES (7136:2010), the percentage of cadmium residues in Table 3 that exceeded allowed limits for *T. nilotica* and *M. cephalus* in Nile cages, concrete ponds, and earthen ponds, respectively, was 48% and 36%; 20% and 16% and 44% and 68%.

Table 3 Cadmium residues (ppm) in examined farm fish samples (25 for each).

	Nile cages		Concrete Ponds		Earthen Ponds	
	T.nilotica	*M.cephalus*	*T.nilotica*	*M.cephalus*	*T.nilotica*	*M.cephalus*
NO. (%) of +ve Samples	18(72%)	12(48%)	8(24%)	7(28%)	14(66%)	19(76%)
Min	0	0.00	0.04	0.00	0.00	1.30
Max	0.29	0.91	0.36	0.26	0.35	1.73
Mean ±SE	0.12±0.02	0.20±0.06	0.14±0.03	0.11±0.03	0.16±0.03	0.85±0.10
Samples exceed P.L.	12(48%)	9(36%)	5(20%)	4(16%)	11(44%)	17(68%)

Maximum permissible limits of Cadmium 0.05 mg/kg according to ES (7136:2010)

According to ES (3494:2005), acceptable fish flesh should be free of hormonal contaminants (methyl testosterone and trenbotone acetate), Table 4 demonstrated that all evaluated positive samples exceeded the allowable limits.

While organochlorine pesticides (aldrin) or organophosphorus pesticides (malathion) were not found in all samples in the current investigation.

Table 4 Hormonal residues in examined farm fish samples (25 for each).

		Nile cages		Concrete ponds		Earthen ponds	
		T.nilotica	*M.cephalus*	*T.nilotica*	*M.cephalus*	*T.nilotica*	*M.cephalus*
Testesterone (ppb)	Positive samples	25	24	25	18	25	25
	%	100%	96%	100%	72%	100%	100%
	level	1.1±0.12 (0.52-1.96)	0.81 ±0.08 (0.29-1.48)	0.92±0.10 (1.02-2.13)	0.77±0.09 (0-1.35)	1.15±0.15 (0.35-2.19)	0.85±0.10 (1.30-1.73)
T.acetate (ppb)	Positive samples	1	0	6	0	3	0
	%	4%	0	24%	0	12%	0
	level	0.28	----	0.17±0.0.2 (0-0.27)	---	0.22±0.03 (0-0.34)	----

Accepted fish meat should be free from hormonal residues according to ES (3494:2005).

4. DISCUSSION

Heavy metal environmental pollution is a severe issue everywhere, because of human activity, agricultural drainage water has higher concentrations of heavy metals, inorganic substances, and pesticide residues so, the environment is harmed (Ghannam et al., 2014). For the aquatic environment, heavy metals are toxic and damaging contaminants. It seriously endangers both the life and health of the person. According to Al-Kenawy and Aly (2015), the residues bioaccumulates in fish tissues and cannot be eliminated or broken down by biological processes. The Egyptian Organization for Standardization (ES: 7136: 2010) stated that the maximum allowed level of mercury in fish meat is 0.50 mg/Kg. Through the consumption of contaminated food, humans are exposed to heavy metals (Liang et al., 2016). The results were nearly in agreement with those recorded by Hassan and Salem (2003) who reported the mean concentration was 0.45±0.03 mg/g and relatively higher than that reported by Marzouk et al. (2016) who noted that the mean concentration was 0.105±0.005 ppm, and Eboh et al. (2006) and Hashim et al. (2008) who recorded that the mean concentration was 0.013±0.001 mg/kg. Meanwhile, Moustafa et al. (2011) noted high results reached 1.9 mg/kg as the mean concentration in tilapia. A mercury poisoning may affect immune system and developmental abnormalities as well as behavioral problems. During pregnancy, all types of mercury can cross the placenta to the embryo, where they may harm the growing central nervous system (Gochfeld, 2003).

Egyptian Organization for Standardization's (7136: 2010) stated that, the maximum allowed level of lead in fish meat is 0.10 mg/Kg. Almost the same outcomes as those reported by Kaoud and El Dahshan (2010); Authman et al. (2013). While Hashim et al. (2008) and Mousa et al. (2021) reported less favorable outcomes. Children that are exposed to lead may exhibit less intellectual acuity and delayed cognitive development. According to Bilandžić et al. (2011), lead toxicity in adults also results in elevated blood pressure, cardiovascular convulsions, coma, and renal failure illness. Fish raised in cages collect lead at levels that are higher than what is allowed, which poses a serious health risk to people who consume these fish as part of their meals (Nabil and Gamal, 2006).

The maximum allowable limit of cadmium residues was 0.05 mg/Kg in fish flesh was reported by Egyptian Organization for Standardization (7136: 2010). These results concur with those recorded by Ibrahim et al. (2008). However, Kaoud and El Dahshan (2010) ; Saeed and Shaker (2012) and Mousa et al. (2021) also produced outcomes that were less favorable. These outcomes surpass what recorded by Yosef and Gomaa Ghada (2011) and Neima et al. (2016). Both Hussien et al. (2011) and Lasheen et al. (2012) reported higher results. Meanwhile, Ismail and Rizk (2016) said that cadmium bioaccumulation results in renal failure and nephrotoxicity, due to its strong capacity to be excreted from the body or low tendency to bioaccumulate, cadmium may have a low concentration in the environment. Moreover, Emara et al. (2015) reveled that Nile tilapia tissues did not have heavy metal contents over the allowable levels.

Nabil and Gamal (2006) noticed a striking correlation between the levels of heavy metals in muscle tissue and the source of the water. Nath et al. (2016) cleared that the thoughtless and extensive use of organic fertilizers is regarded as a contaminant in aquatic ecosystems. While Martin et al. (2006) and Atafar et al. (2010) said that the application of liquid manure, soil manure, or inorganic fertilizers can lead to the buildup of heavy metals in the soil. So, Eneji et al. (2011) added that the bioavailability of heavy metals and the fish's capacity to both absorb and expel pollutants may be to blame for the various quantities of heavy metals that have built up in their tissues. The substantial fat content in muscle tissues, according to Uluturhan and Kucuksezgin (2007) explained why muscles have a decreased propensity to accumulate heavy metals. The largest concentrations of heavy metals were found in the organs of mullet species, while tilapia species had the lowest concentrations of various heavy metal components. This might be because mullet totally rely on filtering phytoplankton from the water column and debris left on the pond bottom, whereas tilapia only feeds partially on phytoplankton through the earthen pond's production cycle (Shaker et al., 2018).

All evaluated positive samples exceeded the allowable limits of hormonal contaminants (methyl testosterone and trenbotone acetate) according to ES (3494:2005), Lower results were reported by Marzouk et al. (2016) who showed that 25% of Tilapia fish samples contained 2 ppb of Methyl testosterone residue. El-Neklawey et al. (2009) noted 4.221 (ng/gm) in farm-raised Tilapia fish. The widespread usage of synthetic androgens such methyl testosterone in fish production in Egypt for its anabolic and androgenic action in fish may be the cause of the hormonal residues found in tilapia.

If used during the early phases of tilapia farming, particularly during the first month after the fry begin swimming freely, methyl testosterone therapy may be safe. To assure that no hormone residue is left in fish following the completion of the hormone treatment, wait at least five months.

The maximum levels of pesticide residues in fish flesh should be set at a rigorous level that is achieved by appropriate agricultural, fishing, and manufacturing practices while also considering the danger associated with food consumption (EOS, 2010). Organochlorine pesticides (aldrin) or organophosphorus pesticides (malathion) were not found in all samples in the current investigation. Additionally, none of the samples under examination revealed positive for aldrin contamination, as recorded by Marzouk et al. (2016) and Geng-Ruei Chang (2018) who suggested that no new pollution was washed into the aquaculture habitats from the land. Kalyoncu et al. (2009) were able to find aldrin residue in the majority of fish samples which collected from konya markets in Turkey. Malathione was not identified by Eman (2016) as an organophosphorus in some fish samples. The chemical family determined the toxicity of pesticide. According to Maroni et al. (2006), these residues cause acute poisoning, cancer, and detrimental effects on human and wildlife reproduction. Additionally, it may result in moderate skin irritation, endocrine disruption, tumors, genetic alterations, blood and nerve issues, and even coma or death. The lipid-rich tissues of aquatic creatures are where organophosphorus pesticides concentrate and are more resistant to microbial destruction. These pesticides have numerous uses in homes for pest management in addition to being utilized as agricultural insecticides (Akan et al., 2014). When agriculture drainage water doesn't contain greater quantities of both heavy metals and pesticide residues, the quality of cultured Nile tilapia is below allowable limits and suitable for human consumption (Soltan et al., 2016).

5. CONCLUSION

Farmed fish samples collected from Sharkia markets were found to contain chemical residues in high quantities that above the safe Egyptian permitted limits for human consumption. These residues included mercury, lead, cadmium, and hormones. Additionally, it was noted that *Nile Tilapia* followed by *Mugil cephalus* in the order of heavy metal bioaccumulation in fish muscles. Therefore, periodic monitoring studies for fish farms should be carried out to evaluate the contaminants and their concentrations that have negative effects on the water, other aquatic animals, and human health.

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