International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.12, pp 143-154, 2017

Determination of Some Contaminants in Silver Carp and Catfish Flesh from Wadi El-Rayan Lake and the Effect of Traditional Cooking Methods on Their Concentrations

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Abstract : In this study, the determination of some heavy metals, organochlorine pesticides residues (OCRs) concentrations and microbiological aspects were carried out of two fish species flesh; silver carp (*Hypophthalmichthys molitrix*) and catfish (*Clarias gariepinus*) from Wadi-El Rayan 1st Lakein El-Fayoum Governorate, Egypt during February, 2017. Also, the effect of traditional cooking methods (boiling, fryingand grilling)on these parameters levels were evaluated. According to the results, the determined heavy metals (lead, cadmium, manganese, copper and zinc) and detected organochlorine pesticide residues (p,p'-DDD, p,p'-DDE, p,p'-DDT, endosulfan-I, endosulfan-II, endosulfan-sulfate, heptachlore, endrin, endrin aldehyde, α-HCH, β-HCH, γ-HCH and δ-HCH) concentrations and total bacterial count (TBC) in raw flesh of two fish species were lower than the permissible limits set by International and Egyptian Standard Specifications and Legislations of food. By cooking methods; the thermal processes led to the decrease or increase of investigated heavy metals, boiling was the highest cooking method followed by grilling in the reduction of heavy metals (reduced all heavy metals) but, frying was increased most heavy metals concentrations. OCPs; most detected pesticide residues in raw flesh of two fish species were decreased, boiling was the highest in reduce OCRs concentrations followed by frying and grilling. Microbiologically; TBC was sharply reduced in all cooked fish samples, frying was the highest cooking method in reduction TBC followed by grilling and boiling. Also, neither *salmonella sp.* nor yeast and mould were detected in raw and cooked both silver carp and catfish flesh. Therefore, the fishes in Wadi-El Rayan 1st Lake especially investigated silver carp and catfish were considered very safe and validating for human consumption and traditional cooking methods (boiling, grilling and frying) were found appropriate for reducing the dangerous effect of heavy metals, organochlorine pesticides residues and bacterial counts.
Keywords : Wadi El-RayanLake; fish; pollutants; traditional cooking methods.

Introduction:

Wadi El-Rayan is a natural protected area in Egypt. Wadi El Rayan Lakes was declared a Protected Area, 1989. It is a great depression situated in the Western Desert, 60 km southwest of El-Fayoum city, 80 km west of the Nile River and about 200 km southwest of Cairo. The depression has been used as a reservoir for agricultural wastewater exceeding the capacity of Lake Qarun since 1973. Wadi El-Rayan depression was connected to the agricultural drainage system of El-Fayoum Governorate through El-Wadi Drain to decrease the accumulation of excess drainage water in Lake Qarun and to protect the nearby agricultural land from inundation[1].

Contaminants are usually present in natural environments as complex mixtures such as toxic heavy metals, bacterial contamination and pesticides that provide many vital indicators for a complete diagnosis of environmental degradation[2].

Heavy metal pollution is regarded as a severe problem because it injures the biological functions of the aquatic organisms and their accumulation in fish organs and flesh leading to serious healthy hazardous to the consumers[3]. Heavy metals such as cadmium, lead, copper and more specifically mercury are potentially harmful to most organisms even in very low concentrations and have been reported as hazardous environmental. Heavy metals are able to accumulate in the aquatic food chain with serious risks to animal and human health[4]. In the aquatic environment, heavy metals in dissolved form are easily taken up by aquatic organisms where they are strongly bound with sulfhydril groups of proteins and accumulate in their tissues[5]. Therefore, contamination of fish tissues by heavy metals is arisen mainly from the contamination of feed; water, air beside the accidental addition which can be associated with soils naturally high in these elements, environmental pollution from local industry, and feeding grain[6]. [7]recorded that heavy metals enters the fish bodies by three pathways: by gills, digestive track and body surface. The gills are considered as the significant site for direct uptake of metals from the water, though the body surface is normally estimated to take minor part in uptake of heavy metals in fish. The accumulation of heavy metal in tissues of organisms can result in chronic illness and cause potential damage to the population. Human exposures to heavy metals have become a major health risk [8].

Pesticides constitute a major group of potential environmental hazards to man and have been routinely used in most countries of the world to control harmful pests. Organochlorine (OCRs) pesticides residues are among the agrochemicals that have been used extensively for long periods. They have been used widely in agriculture, as well as, in mosquito, termite and tsetse fly control programs[9].OCRs are characterized by low polarization, low water solubility and high solubility in fats, and as a result have potential for bioaccumulation in the food chain, posing a major threat to human health and the global environment [10]. Also, [11] reported that pesticide residues have detrimental effect on human health like causing cancer, epilepsy, liver and kidney dysfunction, leukemia, decreased fertility and testicular cancer.

Fish is a group of great importance to humans as a major source of proteins and vitamins. On the other hand, it acts as a major route for the accumulation of toxic chemicals such as heavy metals and pesticides in human bodies. Regarding the importance of fish consumption, it is considered healthy food for most of the world's population, in particular developing countries. In contrast to the meat and poultry, it is a valuable source of high quality proteins, minerals and vitamins associated with health benefits and normal growth as well as high in unsaturated fatty acids contains omega-3 fatty acids that a decrease in the incidence of coronary heart diseases, depression, stroke, blood pressure, glycerin index, triglycerides, cancer and others[12,13].However, fish are able to accumulate a higher concentration of pesticide residues and heavy metals than the surrounding water[14]. [15] reported that pesticides and heavy metals can accumulate in the fish tissue through three primary ways; (i) dermally, direct absorption through the skin by swimming in pesticide contaminated water (ii) by breathing, direct uptake of pesticides through gills during respiration and (iii) orally, by drinking pesticide contaminated water or feeding on pesticide contaminated prey. Thus, the content of heavy toxic metals in fish can damage their beneficial effects[16]. The mineral content of fish can be affected by processing or cooking methods[17,18]andsome studies have reported a considerable reduction of the heavy metals in food after cooking[19,20].

Fish is usually cooked by different ways before consumption (boiling, baking, roasting, frying and grilling) to enhance their flavor and taste; increase shelf life[21]. Also, cooking processes are known to be reduce the risk of pollutants in fish, however, the mechanisms involved in the transfer and or degradation of pesticide residues contaminants during cooking process are not clear[22]. While,[18, 23]reported that the reduction in trace metals concentrations as affected by cooking methods may be due to the release of these metals with the loss of drip as free salts, possibly in association with soluble amino acids and un-coagulated proteins bounded with metals. Silver carp and catfish are major species of freshwater fish, which are found in large sizes in Wadi El Rayan 1st Lake. these two fish species can be prepared to fillets and consumed directly by using traditional cooking methods or using in producing high-quality fish products such as fish fingers, patties, kofta and chips that are favored by consumers [24]. Thus, fish safety just as food safety is an important public health issue because there are numerous diseases acquired by humans on the consumption of contaminated fish[25].

Therefore, the objective of this study is to determine some contaminants concentrations; heavy metals, organochlorine (OCPs) pesticide residues and microbial aspects in raw silver carp (*Hypophthalmichthys molitrix*) and catfish (*Clarias gariepinus*) flesh obtained from Wadi-El Rayan 1st Lake, where there is fresh water that is suitable for the survival of those Nile fish and compare these concentrations with the maximum permissible limit as recommended by the international and Egyptian regulations to assess potential risks from fish consumption on human health. Also, evaluate the effect of the traditional cooking methods used in this area of study; boiling, grilling and frying on concentrations of these contaminants.

Material and methods

Material

Study area

Wadi El-Rayan contains two main Lakes (Fig. 1), at different elevations, connected by waterfall. [26, 27] reported that the northern 1st Lake (upper lake) of Wadi El-Rayan covers an area of about 53 km^{2}at 10 m below the sea level and located between longitudes 30˚ 25\ 53.0\\& 30˚ 31\ 10.9\\ E and latitude 29˚ 11\ 30.0\\ & 29˚ 17\ 14.0\\ N. It has a circular shapeof 6.61 km length, 10.37 km maximum width and 23 m maximum depths, receives frequent effluent of agricultural drainage from El-Wadi Drain, about 200 million cubic meters of agricultural drainage water are transported annually. It is fully filled with water and excess water flows into the second through a shallow connecting channel (waterfall, Fig. 2). The southern 2nd Lake (lower lake) is located between longitudes 30˚ 21\ 8.6\\ & 30˚ 25\ 58.8\\ E and latitude 29˚ 05\ 10.3\\ & 29˚ 12\ 46.8\\ N. It has a conical shape of 15.72 km length, 4.49 km maximum width and 33 m maximum depths, with total surface area of 57.9 km². It is changing all the time, where newly flooded areas are continuously added at the southwestern side of the Lake. The 1st Lake is less saline (1.4-1.5 g/L) than the second one (4.5-6.1 g/L), where the salinity increases southward[28]. Nowadays, salinity degrees vary markedly between the two Lakes, averaging 1.95 g/L in the 1st Lake and an average of 21.97 g in the 2nd Lake[29].Wadi El Rayan Waterfall is considered to be the largest waterfall in Egypt.

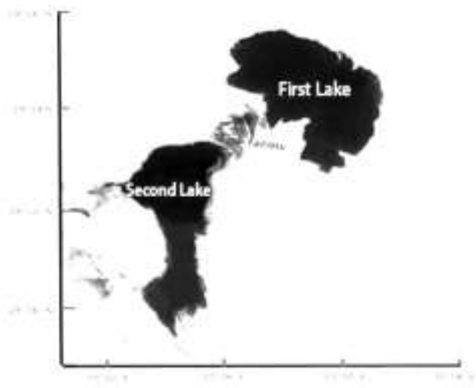

Fig. 1 Wadi El-Rayan Lakes **Fig. 2 Waterfall between upper and lower lakes inWadi El-Rayan, Egypt**

Fish samples

Silver carp (*Hypophthalmichthys molitrix*, 13 kg, 1600 ± 200g) and catfish (*Clarias gariepinus*, 12 kg, 2400 ± 300g) were obtained from Wadi-El Rayan 1st Lake during February, 2017. They were kept in iceboxs and cooled by quantities of ice then transported directly within about 1 hrs to the Laboratory of Fish Processing Technology in Shakshouk Fish Research Station, National Institute of Oceanography and Fisheries (NIOF), El-Fayoum Governorate, Egypt. The fish samples were beheaded, gutted, washed gently with tap water, then manually skinned and filleted.

Cooking methods

Fish fillets obtained from two fish species were divided into four groups to everyone, the first is raw fish fillets uncooked (control) and another three groups are cooked in the following methods: frying, grilling and boiling.

Frying: Fish samples were breaded with wheat flour and deep oil-fried at 160°C for 8 min. in sunflower oil using electrical fryer pan (Moulinex brand).

Grilling: Fish samples were rubbed with bran and grilled using electrical grill machine at 180°C for 10 min.

Boiling: Boiled fish fillets were prepared by cooking raw fish samples in water boiling for15 min.

Each type of control and cooked fish samples of both fish species were homogenized separately for determination the heavy metals, organochlorine (OCPs) pesticide residues and microbiological aspects.

Analytical methods

Heavy metals

lead (Pb), cadmium (Cd), manganese (Mn), copper (Cu), and zinc (Zn) were determined as described by[30]using Atomic Absorption Spectrophotometer in Chemistry Lab., Freshwater Division, Inland Water and Aquaculture Branch, El-Qanater El-Khiria Station for fish Research, National Institute of Oceanography and Fisheries (NIOF).

Organochlorine (OCPs) pesticides residues

OCPs were determined according to[31]by using TSQ 8000 GC/MS with HP5 column (25 m x 0.25 mm x 320 µm), which was used for identification and determination of pesticides and electron capture detector (ECD) for identification of pesticides in Central Lab., the Mediterranean Sea and Northern Lakes Branch in Alexandria, National Institute of Oceanography and Fisheries (NIOF). The results of both heavy metals and organochlorine pesticides residues were expressed as (ppm, wet weight).

Microbiological aspects

Total bacterial count (TBC) determined by using aseptically ten grams of sample from different places and homogenized with 90 ml of sterile saline solution. Standard plate count nutrient agar medium was used[32]. Salmonella, an important measure of the fish meat safety as regards of human public health was also determined using S-S agar medium as reported by[33].Yeast and mould count was determined using potato dextrose agar by[34]. The results were expressed as $\log_{10}$cfu /g sample.

Statistical analysis

Values of heavy metals and total bacterial count were analyzed statistically using the Standard Error (Mean ± SE) as reported by[35].

Results and Discussion

1- Heavy metals concentrations in silver carp and catfish fleshand effect of boiling, frying and grilling on them

Heavy metals; lead (Pb), cadmium (Cd), manganese (Mn), copper (Cu), and zinc (Zn) concentrations in raw and cooked flesh of silver carp and catfish obtained from first Lake of Wadi El-Rayan Lakes were determined and the obtained results were tabulated in Table 1.

Pb concentrations were 0.312and 0.204 (ppm, wet weight) of raw investigated silver carp and catfish samples, respectively. These results were lower than the permissible limit set by Egyptian Organization for Standardization[36]and Food and Agricultural Organization/World Health Organization[37] (2 ppm). Silver carp species showed a more accumulation of lead than catfish species, these observations are mainly due to the different fish habitat and the influence of the surrounding ecosystem status. Also, the obtained results were lower than reported by[38] that 1.03 (ppm, dry weight) in *Tilapia spp.* from Wadi El-Rayan 1st Lake, while higher than reported by[39], who found that Pb in *Tilapia nilotica* and *Claries lazera* fish samples from Wadi El-Rayan Lakes during winter were 0.003 and 0.006 (ppm). This results were supported by[40] that the average concentrations of lead in 50 fish tissues samples of *Tilapia nilotica* collected from Wadi El-Rayan was 0.157 ppm. With regard to the effect of traditional cooking methods; Pb concentrations were decreased from 0.312 and 0.204 ppm of raw silver carp and catfishflesh to 0.108 and 0.085 by boiling and to 0.188 and 0.150 ppm by grilling, but on the contrary increased to 0.365 and 0.276 by frying method, respectively. The decrease of Pb concentrations in boiled fish samples is higher compared with the grilled samples. The reduction in trace metals concentrations as affected by cooking methods may be due to the release of these coagulated proteins bounded with metals, while the increase in metals may be related to decrease in the moisture content that occur during cooking[18]. These results agreed with[41] in grilling of Nile tilapia and mullet fish fillets from Wadi El-Rayan first Lake but differed in the frying, where the Pb concentrations were 0.415 and 0.196 (ppm, w.w.) for raw Nile tilapia and mullet fish fillets decreased to 0.373 and 0.145 in grilled and to 0.388 and 0.185 in fried samples, respectively. Also, these results were almost consistent with reported by[42] indicated that the concentrations of lead in sardine and tuna were higher in fried samples and lower in grilled as compared to the raw samples while, [43]reported that Pb in fresh Nile and farm tilapia fish were 0.205 and 0.152 (ppm, ww), respectively decreased to 0.130 and 0.143 ppm after frying. In the same direction, [44]found that the boiling was presented the greater reduction for Pb concentration of *Clarias gariepinus* when compared with the other cooking methods; frying and charcoal-grilling.

Cd concentration in raw silver carp flesh was 0.045 ppm w.w., increased to 0.060 ppm by frying and decreased to 0.023 by grilling, while undetected (UD) any concentrations of Cdin boiled samples. In catfish flesh, the Cd levels UD in raw and boiled samples, while detected by frying (0.030, ppm)and grilling (0.008, ppm). These results were lower than the permissible limit set by[36,37] (0.5 ppm). In the same trend, [38] demonstrated that the content of Cd was UD in *Tilapia spp* from Wadi El-Rayan Lakes and[39]found that Cd concentrations were 0.003 and 0.002 ppm of *Tilapia nilotica* and *Claries lazera* from Wadi El-RayanLake. These results of Cd concentrations in our study were agreement with found by[41]who reported that the concentrations of Cd in raw Nile tilapia was 0.058 ppm, not affected by frying (0.058 ppm) and decreased to0.034 ppm by grilling, while in raw mullet fish fillets Cd levels were UDin boiled sample but detected by 0.021 and 0.053 ppm in grilled and fried samples, respectively.[44]reportedthat concentration of cadmiumin raw *Clarias gariepinus* was 8.8 mg/kg reduced to 5.7, 2.8 and 2.2 mg/kg in charcoal-grilled, fried and boiled samples. Also ,this result is in accordance with the notes by[45,46].

The same trend of Pb and Cd was found in Mncontent, the Mn of fish samples was increased by frying but not for the boiled and grilled fish samples. Mn concentration of fresh silver carp flesh was 0.185 ppm w.w., increased to 0.290 ppm by frying and decreased to 0.033 and 0.155 of the boiled and grilled fish samples. Also, Mn level of fresh catfish was 0.118 ppm increased to 0.224 ppm by frying, while decreased to 0.022 pm and 0.105 pm by boiling and grilling. These obtained data were lower than the permissible limit set by[37] (2-9 ppm) and agreement with reported by[39]that determined Mn by 0.038 and 0.009 ppm for *Tilapia nilotica* and *Claries lazera* fish samples from Wadi El-Rayan Lakes. Moreover, it could be noticed that silver carp meat contains a higher concentration of Mn than catfish samples. These results are in accordance with reported by[41,47]found that the Mn content of raw African catfish was 0.29 mg/kg increased in fried samples to 0.40 mg/kg and decreased to 0.25 mg/kg in grilled fish samples.

Cu concentrations in investigated raw silver carp flesh (0.023 ppm) and catfish (0.076 ppm)decreased by frying to 0.018 and 0.058 ppm and by grilling to 0.011 and 0.032 ppm respectively, while UD in boiled samples of two fish species. All detected Cu levels were very lower than the permissible limit set by[36,37] (20 ppm). Also, it could be noticed that catfish species showed a more accumulation of Cu than silver carp species and the reduction in the metal concentrations by boiling was much greater than on grilling and frying. Thus, the given results were agree with[39]who recorded that copper levels were 0.014and 0.011 ppm in *Oreochromis niloticus* and *Claries lazera* fish from Wadi El-Rayan Lakes, while were lower than that recorded by[48]of farmed carp muscle (0.33 ppm). With regarding the effect of traditional cooking methods on Cu, these results were agreement with reported by[47]for raw catfish fillets that contains 2.15 mg/kg Cu concentration decreased to 2.10 and 1.07 mg/kg of fried and grilled samples. Also, [41]for mullet fishfillets from Wadi El-Rayan first Lake that contains 0.051 ppm Cu decreased to 0.016 and 0.021 ppm of grilled and fried samples. However, our results not agree with[45]whoreported that Cu concentrations of boulty and boury were 0.37 and 0.76 ppm increased to 0.48 and 0.89 ppm of fried samples, but increased to 0.38 and 0.78 ppm in grilled samples, respectively.

Zn concentrations of examined raw silver carpand catfish flesh were 0.440 and 0.621 ppm, respectively. These results were lower than reported by[28] who found the concentrations of Zn in *Tilapia sp.*and *Mugile sp.* From Wadi El-Rayan 1st Lake were 3.41 and 3.10 ppm, respectively. Concerning effect boiling, frying and grilling; it was noticed that Zn concentrations of raw silver carpfleshwas0.440 increased to 0.522 ppm in fried samples, while decreased to 0.166 and 0.386 ppm for boiled and grilled samples, respectively. Also, the same trend was noticed in catfish flesh that was 0.621 ppm in raw catfish meat increased to 0.658 of fried samples, while decreased to 0.296 of boiled samples and 0.400 ppm of grilled samples. Zn content of investigated catfish samples were higher than that of silver carp and Zn concentrations of all investigated raw and cooked silver carpand catfish samples were lower than the permissible limit set by[36,37] (40 ppm). The results agree with[41]who found that Zn concentration were 0.221 and 0.086 ppm in raw Nile tilapia and mullet fish flesh from Wadi El-Rayan 1st Lake increased to 0.263 and 0.116 ppm by frying but decreased to 0.089 and 0.038 ppm by grilling.

Therefore, it could be concluded that the concentrations of all determined heavy metals in examined raw and cooked silver carp and catfish flesh from Wadi El-Rayan 1st Lake were lowerthan the permissible limit set by[36,37].Boiling was the highest method for reduction all determined heavy metals followed by grilling, while the frying cause increased most of them, therefore the cooking by boiling followed by grilling methods were found higher appropriate for human consumption than frying method for reducing the dangerous effect of heavy metals.

Table 1 Effect of traditional cooking methods on heavy metals concentrations (ppm, w.w.) of silver carp and catfishflesh from Wadi El-Rayan 1st Lake

Item	Silver carp flesh samples				Catfish flesh samples				MPL** (ppm)
	Raw	Boiled	Fried	Grilled	Raw	Boiled	Fried	Grilled	
Lead (Pb)	0.312 ± 0.110	0.108 ± 0.006	0.365 ± 0.101	0.188 ±0.005	0.204 ± 0.015	0.085 ± 0.008	0.276 ± 0.012	0.150 ± 0.006	**2**
Cadmium (Cd)	0.045 ± 0.003	UD*	0.060 ± 0.009	0.023 ± 0.010	UD	UD	0.030 ± 0.006	0.008 ± 0.000	**0.5**
Manganase (Mn)	0.185 ± 0.060	0.033 ± 0.005	0.290 ± 0.070	0.155 ± 0.060	0.118 ± 0.010	0.022 ± 0.005	0.224 ± 0.050	0.105 ± 0.006	**2-9**
Cooper (Cu)	0.023 ± 0.003	UD	0.018 ± 0.006	0.011 ± 0.001	0.076 ± 0.009	UD	0.058 ± 0.011	0.032 ± 0.007	**20**
Zinc (Zn)	0.440 ± 0.120	0.166 ± 0.019	0.522 ± 0.118	0.386 ± 0.020	0.621 ± 0.206	0.296 ± 0.081	0.658 ± 0.180	0.400 ± 0.110	**40**

UD*: undetected. MPLs: Maximum permissible limits as reported by EOS, 2005 and FAO/WHO, 1999**.

2- Organochlorine pesticides residues(OCPs) concentrations in silver carp and catfish and effect of boiling, frying and grilling on them

Concentrations of organochlorine pesticide residues (OCPs) were investigated in raw and cooked (boiled, friedand grilled) silver carp and catfish flesh from Wadi-El Rayan 1st Lake and the results were tabulated in Table (2). It was found that thirteen components of OCPs in raw and cooked fish products were detected in two fish species, they are p,p'-DDD, p,p'-DDE, p,p'-DDT, endosulfan-I, endosulfan-II, endosulfan-sulfate, heptachlore, endrin, endrin aldehyde, α-HCH, β-HCH, γ-HCH and δ-HCH. The raw flesh of catfish contains higher levels of p,p'-DDD, p,p'-DDE, p,p'-DDT, endosulfan-I, endosulfan-II, endosulfan-sulfate, heptachlore, endrin, endrin aldehyde by values of 0.015, 0.058, 0.007, 0.022, 0.040, 0.012, 0.020, 0.053 and 0.026 (ppm,w.w.), respectively than raw silver carp flesh that contains the same compounds by values 0.009,0.033, 0.005, 0.013, 0.026, 0.008, 0.006, 0.025and 0.008 mg/kg, respectively. However, raw silver carp flesh was higher of α-HCH (0.005), β-HCH(0.052), γ-HCH(0.018) and δ-HCH(0.032 mg/kg) than raw catfish flesh that contain UD (undetected), 0.048, 0.008, and 0.009 mg/kg, respectively of the same compounds.

Also, the study revealed that the dominant compounds in raw catfish and silver carpflesh were p,p'-DDE (0.058 and 0.033) and endrin (0.053 and 0.025 mg/kg)respectively, while, the lowest levels were observed in α-HCH (UD and 0.005) and p,p'-DDT (0.007 and 0.005 mg/kg). However, the concentrations of all detected OCPs in raw and cooked fish samples of two fish species were low, and did not exceed the maximum permitted levels that reported by[49,50].The concentration of OCPs in fish depends on environment conditions, level of exposure, nature of the pesticide and its solubility, and the fish species as they may differ in metabolism and its ability to excrete the compounds[51].

These results were agreement with[41]in some detected compounds and less in another compounds for raw Nile tilapia and mullet fish fillets from Wadi El-Rayan 1st Lake where a ranges of detected components (ppm, ww) were 0.017-0.028 4,4'-DDD, UD-0.032 4,4'-DDE, 0.065-0.126 endosulfan-I, 0.188-0.244 endosulfan-II, 0.003-0.021 endosulfan-sulfate,UD-0.043 endrin, 0.021-0.052 β-HCH, 0.037- 0.067 γ-HCH and 0.014-0.0.043 δ-HCH. Also, our results were lower than that found by[52] in Nile tilapia, blue tilapia, nango tilapia and bayad catfish collected from local market at El- Kanater El-Khairia city, Egypt that revealed the concentrations of p,p'-DDE, p,p'-DDD, p,p'-DDT, Heptachlor, α-HCH, β-HCH, γ-HCH and δ-HCH compounds were ranged 20-30, 33-68, 13-51, 15-27, 14-75, 28-67, 47-97 and 150-82 (ng/g ww) in raw fish fillets respectively, while endrin, endrinaldhyde, endosulfan I, endosulfan II, endosulfan sulfate in the same fish species were below than our detectable data. On the other hand, our results were higher than all organochlorine pesticides residues levels (<0.002-0.01μg/kg) that detected by[38] in flesh of *Tilapia spp.* from Wadi El-Rayan Lakes.

On the other hand,concerning the influence of cooking methods on these OCPs in raw flesh of two fish species, it could be noticed that the concentrations of most detected pesticide residues in raw flesh ofsilver carp and catfish were decreased by different cooking methods Table (2) and there was a difference in the loss percent of OCPs in every cooking method (Table 3). The biggest loss of OCPswas observed for samples that cooked by boiling followed by fried and grilled samples, the average of total losswere 80.2 and 78.3% in boiled silver carp and catfish samples, 60.6 and 54.3% of fried samples and39.0 and 32.1% of grilled samples, respectively. The biggest loss was observed forp,p'-DDT, Heptachlore, Endrin aldehyde and α-HCH compounds, however, some OCPs compounds did not loss (NL) such as γ-HCH and δ-HCH during frying or grilling, but the boiling process cause a decrease in all OCPs compounds in two fish species. Thus, on the bases of these obtained results, it was found that silver carp samples that cooked by different cooking methods (boiling,fryingand grilling) were higher in the percentage loss of OCPs than cooked catfish samples. Further decrease of organochlorine pesticide residuesby boiling and frying was due to more loss of fats and oils with hot water and high heat treatment caused that OCPs were somewhat drained out with this lost fats and oils and in oil dripped by grilling[53].Lipophilic OCPs are associated with the fat portion of foods. Thus, cooking methods that release or remove fat from the product, will tend to reduce the total amount of OCPs in the cooked food. Also, the reduction level in OCPs residues of cooked fish muscles was depending upon the nature and solubility of pesticide itself, fish species and the method of cooking[54]. Finally, it can be recommended that boiling is an appropriate method of cooking followed by frying and grilling methods for reductionorganochlorine pesticides residues (OCPs)from fish cooked products and improve fish fleshquality for human consumption, thus, this study was considered a consumer guide to cooking techniques which can reduce dietary PCBs exposure from fish consumption.

The similar results were reported by[55], who found that OCPs concentrations (α-HCH, γ-HCH and Endrin) in *Tilapia nilotica* and *Clarias lazera* fish collected from fish markets in Gharbia, Egypt were decreased after frying, grilling and boiling; by 85, 79 and 80% respectively by frying, and 87, 52 and 100%by grilling, respectivelyin *Tilapia nilotica*; while, in *Clarias lazera* samples the levels decreased by 100, 54 and 83%, respectively by frying and 100, 50 and 100, respectively by boiling. Also,[56] investigated the effect of grilling on concentrations of OCPs in raw *Oreochromus nilotica* collected from Manzala Lake, wild Nile River "El-Ryiah El-Tawfiky", El-Abbasa private fish farm and Gesrbahr el-baar agricultural drainage and the results revealed a great reduction in OCPs concentrations by ratio of 11% to 100% by grilling depending upon the type of pesticide residue and variation in localities.[57]reported that pesticide residue (B-HCH) in *Catlacatla* fish sample was reduced by many processing methods; deep frying caused maximum reduction by 45.4% , steaming by 15.2%, microwave oven by 21.2 and oven baking by 18.2%. While, [41]found that some OCPs compounds of four fish species obtained from Wadi El-Rayan Lakes were decreased and another were slightly increased by frying and grilling processes, however, frying process was more effective in reduction of some OCPs components than grilling.

Table 2 Organochlorine pesticide residues concentrations (ppm, ww) in raw and cooked silver carp and catfish from Wadi El-Rayan 1st Lake

Item	Silver carp samples				Catfish samples				MPL* (ppm)
	Raw	Boiled	Fried	Grilled	Raw	Boiled	Fried	Grilled	
p,p'-DDD	0.009	UD*	0.004	0.007	0.015	0.008	0.012	0.015	**0.5**
p,p'-DDE	0.033	0.010	0.018	0.025	0.058	0.024	0.040	0.055	**0.5**
p,p'-DDT	0.005	UD	UD	0.003	0.007	UD	UD	UD	**0.5**
Endosulfan-I	0.013	0.008	0.010	0.011	0.022	0.009	0.012	0.021	**0.3**
Endosulfan-II	0.006	0.005	0.018	0.020	0.020	0.013	0.015	0.020	**0.3**
Endosulfan-sulfate	0.008	UD	UD	0.005	0.012	0.003	0.007	0.011	**0.3**
Heptachlore	0.006	UD	UD	UD	0.020	UD	0.014	0.008	**0.3**
Endrin	0.025	0.009	0.011	0.019	0.053	0.012	0.022	0.030	**0.3**
Endrin aldehyde	0.008	UD	UD	UD	0.026	UD	0.008	0.020	**0.3**
Alpha(α)-HCH	0.005	UD	UD	0.003	UD	UD	UD	UD	**0.3**
Beta(β)-HCH	0.052	0.009	0.015	0.031	0.048	0.005	0.010	0.026	**0.3**
Gamma(γ)-HCH	0.018	0.010	0.018	0.018	0.008	UD	0.005	0.008	**0.3**
Delta(δ)-HCH	0.032	0.012	0.030	0.032	0.009	0.005	0.009	0.009	**0.3**

UD*: undetected. MPLs: Maximum permissible limits as reported by EPA (2007) and FAO/WHO (2007).**

Table 3 Effect of cooking methods onorganochlorine pesticide residues concentrations(ppm, w.w.) of silver carp and catfishfleshfrom Wadi El-Rayan 1st Lake

Item	Percent loss of pesticide residues after cooking methods (%)					
	Silver carp samples			Catfish samples		
	Boiled	Fried	Grilled	Boiled	Fried	Grilled
p,p'-DDD	100.0	55.5	22.2	46.6	20.0	NL*
p,p'-DDE	69.7	45.5	24.3	58.6	31.0	5.2
p,p'-DDT	100.0	100.0	40.0	100.0	100.0	100.0
Endosulfan-I	38.5	23.0	15.4	59.0	45.5	4.5
Endosulfan-II	80.8	30.8	23.0	67.5	62.5	50.0
Endosulfan-sulfate	100.0	100.0	37.5	75.0	41.7	8.4
Heptachlore	100.0	100.0	100.0	100.0	60.0	30.0
Endrin	64.0	56.0	24.0	77.3	58.5	43.4
Endrin aldehyde	100.0	100.0	100.0	100.0	69.2	23.0
α-HCH	100.0	100.0	40.0	100.0	100.0	100.0
β-HCH	82.7	71.2	40.0	89.6	79.2	52.4
γ-HCH	44.4	NL	NL	100	37.5	NL
δ-HCH	62.5	6.25	NL	44.4	NL	NL
Average total loss (%)	80.2	60.6	39.0	78.3	54.3	32.1

NL* : Not loss.

3- Microbiological aspects of silver carp and catfish flesh obtained from Wadi El-Rayan 1st Lake and effect of boiling, frying and grilling on them

Fish is one of the most perishable food, mainly due to the action of microorganisms occurring on the surface of the newly caught fish. In fish technology, the microorganisms control has a very important role in maintaining the high quality of the final product which must be safe for consumer's health, in conformity with International and Egyptian Standard Specifications and Legislations of food[58]. As shown in Table 4, it could be observed that the total bacterial count (TBC) for raw silver carp and catfish flesh were 3.44 and 4,85 (log_{10}cfu/g sample), respectively. The low number of TBC in raw flesh of investigated two fish species indicated to high safety and within the permissible limits which not exceeding than 10^6 cell /g fresh flesh as reported by[59] and agreement with found by[60] who found that the total plate count of fresh fish < 10^4 cells/g, sub fresh 10^4 -10^6 and deteriorated fish > 10^6 cells/g sample. Also, these results were lower than reported by[38] who found that TBC were 2.11x 10^5cfu/g and 2.02 x 10^5cfu/g sample in raw *Tilapia spp.* from the eastern and middle parts of the first Lake ofWadi El-Rayan Lake, respectively. Also, it could be noticed that raw and cooked catfish flesh samples were higher of TBC than silver carp samples

Concerning the effect of different cooking methods, TBC was sharply reduced in all cooked fish samples. TBC of raw silver carp was 3.44 decreased to 0.90, 0.98 and 1.12 (log_{10}cfu/g sample) of fried, grilled and boiled samples respectively, while TBC of rawcatfish flesh was 4.85decreased to 1.16, 1.35 and 1.68,respectively. This reduction occurred in TBC may be due to thermal processing during applied cooking methods. Therefore, the highest reduction was observed in fried samples as compared with raw fish samples followed by grilled and boiled fish samples. These results were agreement with found by[61] who found that total viable count (TVC) of raw tilapia and mullet fish flesh were 2.35 and 2.01 (log_{10}cfu/g sample) respectively, decreased to 1.95, 2,10 and 2.15 of tilapia fish flesh and to 1.70, 1.72 and 1.95 log_{10}cfu/g sample of mullet flesh by frying, grilling and boiling, respectively.

On the other hand, results in Table 4 showed that neither *salmonella sp.* nor yeast and mould were detected in investigated raw and cooked both silver carpand catfish flesh samples, indicted to that the flesh of silver carp and catfish obtained from Wadi El-Rayan 1st Lake was a good safe microbiologically and better quality for human consumption.

Table 4 Effect of traditional cooking methods on microbiological aspects (log_{10}cfu/g sample)of silver carp and catfish flesh from Wadi El-Rayan 1st Lake

Item	Silver carp samples				Catfish samples			
	Raw	Boiled	Fried	Grilled	Raw	Boiled	Fried	Grilled
Total bacterial count (TBC)	3.44 ± 0.210	1.12 ± 0.051	0.90 ± 0.016	0.98 ± 0.009	4.85 ± 1.002	1.68 ± 0.035	1.16 ± 0.010	1.35 ± 0.050
Salmonellacount	ND	ND	ND	ND	ND	ND	ND	ND
Yeast & Mold counts	ND	ND	ND	ND	ND	ND	ND	ND

ND :Not detected.

Conclusion

In our study, the obtained results evident that all determined heavy metals, detected organochlorine pesticides residues (OCPs) and total bacterial count (TBC) in raw and cooked flesh of silver carp and catfish obtained from Wadi El-Rayan 1st Lake were lower than the permissible limits set by International and Egyptian Standard Specifications and Legislations of food. This indicate that the heavy metals, pesticide residues and microbial count do not pose a health risk to the consumers of fish from the areas studied. Cooking methods; boiling was the highest method for reduction all heavy metals and OCPs followed by grilling in the case of reduce heavy metals or followed by frying in the case of reduce OCPs. Frying was the highest method for reduction of TBC followed by grilling and boiling. Therefore, the traditional cooking methods (boiling,fryingand grilling) were an appropriate methods for reducing the dangerous effect of investigated contaminants. Thus, the flesh of silver carp and catfish obtained from Wadi El-Rayan 1st Lake was a good safe and better quality for human consumption.

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