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Effect of Grilling on Pesticides Residues in *O. niloticus* Muscles

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A B S T R A C T

Thirty six random healthy *Oreochromusnilotica* (*O. niloticus*) collected from Manzala Lake, wild Nile River "El-Ryiah El-Tawfiky", El-Abbasa private fish farm and Gesrbahr el-baar agricultural drainage (9 fish from each source). Concentrations of organochlorine pesticide residues (OCPs) were investigated in both raw and grilled fish (dry heat treatment). Fish musculature samples were analysed for detection the effect of grilling on level of organochlorine pesticides residues. Examination revealed that, samples collected from El-Ryiah El-Tawfiki recorded the highest concentration of organochlorine pesticide residues in raw *O. niloticus* followed by Manazla Lake, El-Abbasa fish farm and Gesrbahr el-baar agricultural drain, respectively. Application of grilling revealed a great reduction in organochlorine pesticides residues concentrations by ratio of 11% to 100% depending upon the type of pesticide residue, its sensitivity to heat and locality of fish collection. In some cases thermal treatment showed unexpected results represented by appearance of some residues which not detected in raw samples. This study concluded that fish from some water resources in Egypt is highly contaminated with organochlorine pesticide residues which greatly supposed to come from agricultural contaminated water. Moreover, grilling is a good and highly recommended method to reduce organochlorine residues with uneven degrees in fish muscles before consuming.

Keywords: Organochlorine pesticide residues, *O. niloticus*, fish muscles, grilling heat treatment.

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1. INTRODUCTION

Pesticide defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. Pests can be insects, mice and other animals, unwanted plants (weeds), fungi, or microorganisms like bacteria and viruses. In Egypt, as in many other agricultural countries, pesticides are widely used to control harmful pests, mainly, cotton, maize and rice. Nearly all these chemicals are readily soluble in plant oils and waxes; this common property places them all under suspicion as food contaminants.

Environmental pollution by pesticides residues is a major environmental concern due to their extensive use in agriculture and in public health programs. Pesticides are applied by hand, and large mechanical sprayers, during pesticide application, variable amounts of these toxic substances found their way to adjacent vegetation, wild life, soil and water including rivers, lakes, ponds, and other water bodies as organisms which contribute to the food of fish (Saker and Gaber, 1992), so ecosystem including fish will be contaminated with pesticides. In this way the impact of pesticides is felt far beyond the designated target area that only 5 % of the pesticides reach the target pests, while about 95 % of the used pesticides end up in other parts of the environment.

Pesticide degradation in soil generally results in a reduction in toxicity; however, some pesticides have breakdown products (metabolites) that are more toxic than the parent compound (USDA, 1998). Organo-chlorine pesticides are major group of pesticides. They are used in agricultural, public health, industry and the household. They are widespread environmental contaminants in the global system, because of their properties, where it is characterized by its stability in both fresh and salt water and its resistance to photo degradation**.**

They are poorly hydrolyzed and slowly biodegraded in environment. Therefore, these compounds are persistent in food chains and are readily accumulated in animal tissues. Fish absorb these compounds directly by water through gills or by ingesting contaminated food. The tendency to accumulate in body tissues, its long persistence and the acute health risks of OCPs, have raised concerns about possible human health impacts due to low, but chronic, exposure from dietary intake. Organochlorine pesticides have been considered as 'endocrine disrupting chemicals' and carcinogenic substances (Lemaire *et al.*, 2004). Certain cooking processes could reduce or increase the levels of chemical contaminants in foods; it seems that the influence of cooking on the levels of these contaminants depends not only on the particular cooking process, but even more on the specific food item. In general terms, cooking procedures that release or remove fat from the product should tend to reduce the total concentrations of the organic contaminants in the cooked food as recorded by (Domingo, 2011).

2. MATERIAL AND METHODS

2.1. Collection of samples:

Thirty sex random apparently healthy fish samples representing *O.niloticus*about 200-500g b\w. The fish obtained from 4 areas El-Manzala Lake (Dakahlya governorate), Gesr Bahr El baar agricultural drainage (Kaluobyia governorate), private fish farm in El-Abbassa (Sharkeya governorate) and El-Ryiah El-Tawfiki (Kaluobyia governorate) (9 of each). They were transport without sexing to animal health research institute toxicology department either alive or freshly dead.

2.2. Analytical procedures

2.2.1. Sample preparation and residue analysis

A- Fish samples were prepared, and the pesticide residues were extracted according to the methods described by UNEP/IOC/IAE (1991). Samples are heat treated by grilling at 65C for 10 minutes then stored in aluminum foil and kept in deep freezer then prepared according to UNEP/IOC/IAE (1991), and analyzed.

2.2.2. Sample cleanup

The extracted samples were cleaned and fractionated according to the method which described by UNEP (1988). The residue was dissolved in 2 ml of n-hexane and transferred into an autosampler vial for GC-electron capture detection (ECD) analysis. The extract was concentrated and injected into GC (Aglient 6890) equipped with a 63Ni ECD, a split/splitless injection inlet, capillary column capability, and a 7683A autosampler. Chemistation software was used for instrument control. GC analysis was conducted on a HP-5MS (Aglient, Folsom, CA) capillary column of 30 m, 0.25 mm i.d., and 0.25-μm film thickness. The oven temperature was programmed from an initial temperature of 160 (2-min hold) to 240 °C at a rate of 5 °C/min and was maintained at 240 °C for 20 min. Injector and detector temperatures were maintained at 260 and 320 °C, respectively. Nitrogen was used as a carrier at flow rate of 3 ml/min.

3. RESULTS

Fig. (1) Showed the OCPs residues in *O. niloticus* before heat treatment from the four

Table (1): pesticide residues (ppb) in *O. niloticus* samples before and after heat treatment.

Pesticide residue	Manzala lake			El-Reiah El-Tawfeki			El- Abbasa farm			Agricultural drain		
	Before	After	DR	Before	After	DR	Before	After	DR	Before	After	DR
α BHC	6±0.81	UD	100%	3 ± 0.7	UD	100%	UD	UD	UD	UD	UD	UD
β BHC	70±7.1	7±0.8*	90%	80 ± 0.8	60 ±0.9*	25%	UD	UD	UD	UD	UD	UD
γ Chlordane	90±11	80±14	11%	20 ± 1.1	10 ±1.4*	50%	UD	UD	UD	UD	UD	UD
HCB	60±10	55±12	8%	50 ± 3.7	UD	100%	UD	UD	UD	UD	UD	UD
Heptachlor	3±0.42	5±0.72	-67%	3 ± 1.4	UD	100%	0.2±0.166	0.1±0.09	50%	UD	UD	UD
Aldrin	60±4.8	20±7.9	67%	20 ± 3.2	20 ± 1.4	0%	UD	UD	UD	UD	UD	UD
Diladrin	UD	30±4.3	-100%	UD	UD	UD	UD	UD	UD	UD	UD	UD
DDE	30±3.2	10±3.8*	67%	20 ± 3.4	30 ±3.2*	-50%	UD	UD	UD	UD	UD	UD
DDD	UD	UD	UD	170 ± 21	UD	100%	UD	UD	UD	30±2.3	9±3.4	70%
ENDRIN	UD	UD	UD	80 ± 12.1	UD	100%	UD	UD	UD	UD	UD	UD
Heptachlor epoxide	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD

Before = before heat treatment after = after heat treatment DR= Depletion Rate

Table (2) Organochlorine pesticide residues (ppb) in *O. niloticus* samples from Manzala lake (n=9).

Pesticide	Before heat treatment				After heat treatment				Depletion
	Incidence	Min	Max	mean±se	Incidence	Min	Max	mean±se	
α BHC	6/9	1.8	8.5	6±0.81	0/9	-	-	UD	100%
β BHC	8/9	41.6	82.6	70±7.1	7/9	0.31	1.16	7±0.8*	90%
γ Chlordane	9/9	71.5	102.5	90±11	8/9	61.7	69.8	80±14	11%
HCB	6/9	43.5	73.9	60±10	6/9	31.6	71.9	55±12	8%
Heptachlor	6/9	2.9	5.2	3±0.42	5/9	2.8	6.4	5±0.72	-67%
Aldrin	8/9	49.4	72.7	60±4.8	7/9	13.7	29.8	30±7.9	67%
Diladrin	-	-	-	UD	3/9	21.2	41.3	30±4.3	-100%
DDE	8/9	21.8	39.7	30±3.2	8/9	5.6	18.7	10±3.8*	67%

Table (3) Organochlorine pesticide residues (ppb) in *O. niloticus* samples from El-Reiah El-Tawfeki (n=9).

Pesticide	Before heat treatment				After heat treatment				Deplition
	Incidence	Min	Max	mean±se	Incidence	Min	Max	mean±se	
α BHC	6/9	2.1	4.9	3±0.7	UD	-	-	UD	100%
β BHC	7/9	62.3	97.5	80±0.8	6/9	53.4	69.4	60±0.9*	25%
ɣ Chlordane	6/9	12.7	32.8	20±1.1	4/9	6.7	15.8	10±1.4*	50%
HCB	7.9	41.7	68.9	50±3.7	UD	-	-	UD	100%
Heptachlor	6/9	0.65	4.8	3±1.4	UD	-	-	UD	100%
ALDRIN	5/9	16.2	28.7	20±3.2	4/9	12.7	26.8	20±1.4	0%
DDD	4/9	152	193.5	170±21	UD	-	-	UD	100%
DDE	6/9	16.7	28.8	20±3.4	6/9	28.9	38.9	30±3.2*	-50%
ENDRIN	7/9	63.7	102.4	80±12.1	UD	-	-	UD	100%

Table (4) Organochlorine pesticide residues (ppb) in *O. niloticus* samples from El-Abbasa private fish Farm (n=9).

Pesticide	Before heat treatment				After heat treatment				Deplition
	min	max	mean±se	incidence	min	max	mean±se	incidence	
Heptachlor	0.15	0.27	0.2±0.166	5/9	0.02	0.13	0.1±0.09	3/9	50%

Table (5) Organochlorine pesticide residues (ppb) in O.niloticus samples from Gesrbahr el-baar agriculture drainage (n=9).

Pesticide	Before heat treatment				After heat treatment				Deplition
	Min	Max	Mean±SE	Incidence	Min	Max	Mean±SE	Incidence	
DDD	24.8	36.8	30±2.3	8/9	8.2	10.5	9±3.4*	6/9	70%

UD undetectable

* Significant at P < 0.001 comparing with control using t-student test.

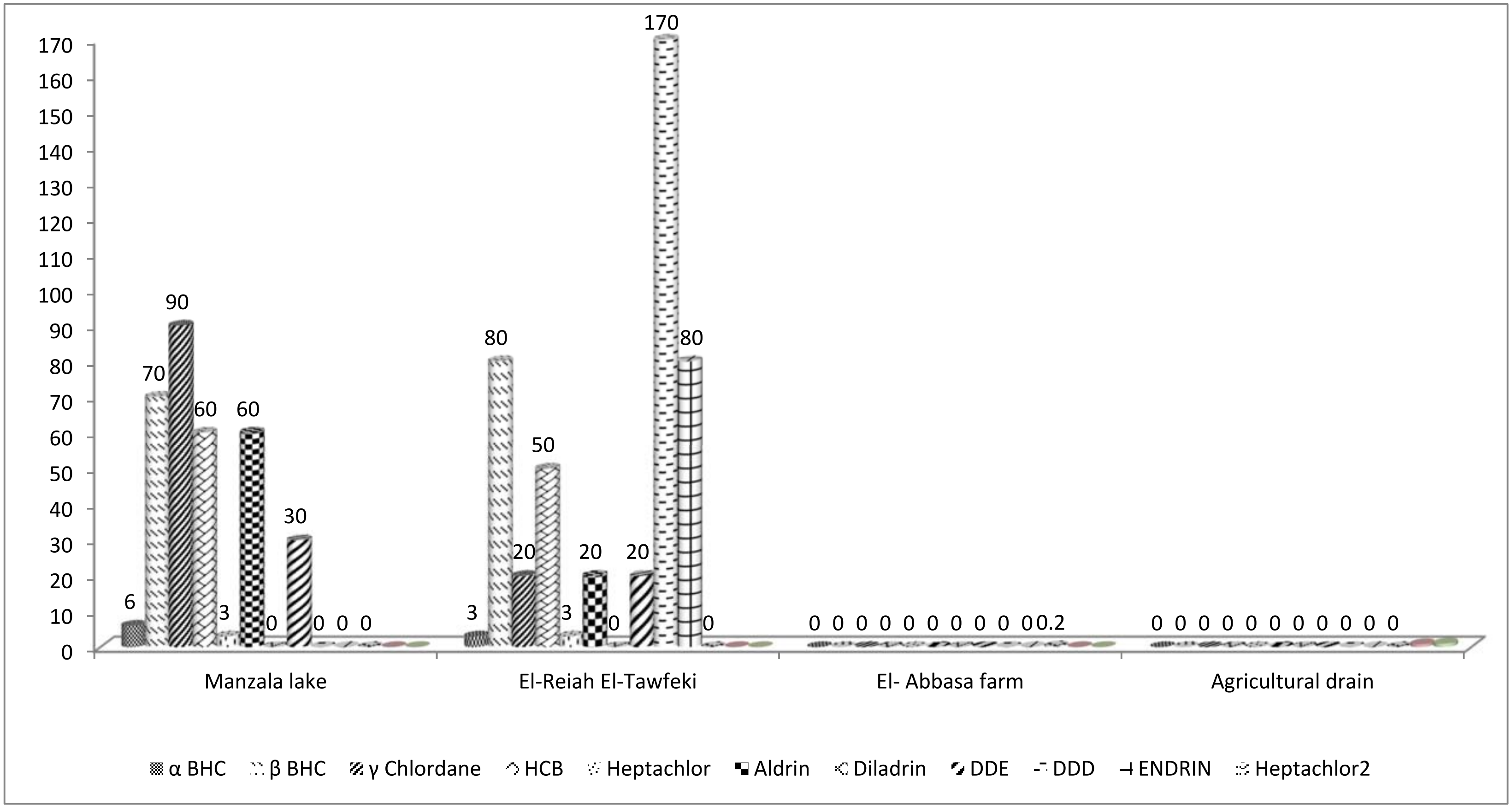

Fig. (1): pesticide residues in *O. niloticus*samples from different localities before heat treatment.

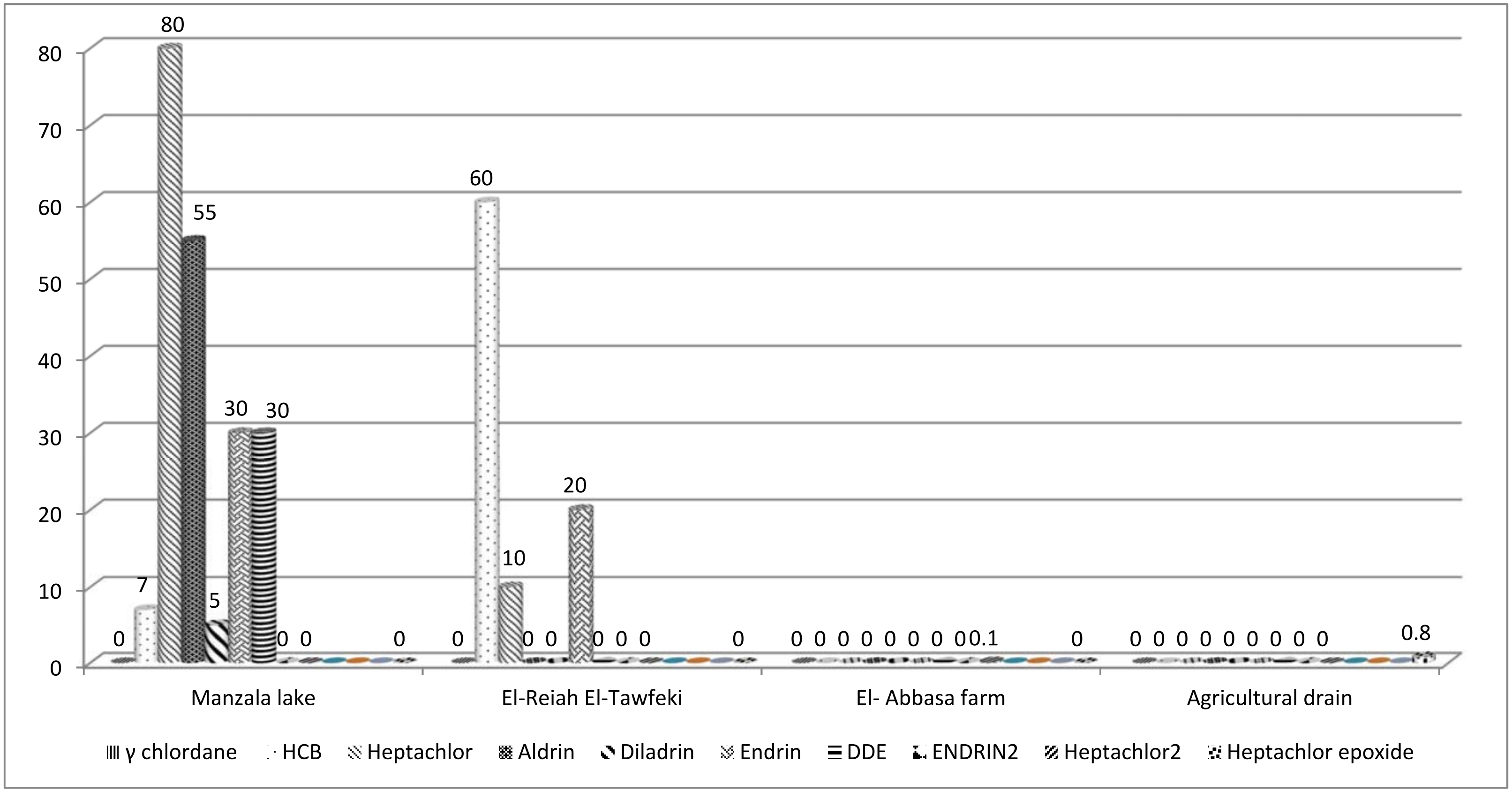

Fig. (2): pesticide residues in *O. niloticus*samples from different areas after heat treatment.

localities of collection. α BHC recorded the highest concentration in samples of Manzala lake (6± 0.81 ppb) and lowest concentration was found in El-Ryiah El-Tawfiki samples (3±0.7 ppb); β BHC recorded the highest concentration in El-Ryiah El-Tawfiki samples (80 ± 0.8 ppb) and lowest was in Manzala Lake samples (70±7.1ppb); γ Chlordane showed the highest concentration in samples collected from Manzala Lake (90 ± 11ppb) and lowest in El-Ryiah El-Tawfiki (20±1.1ppb); HCB highest concentration was detected in samples collected from Manzala Lake (60±10 ppb) and lowest concentration detected in El-Ryiah El-Tawfiki samples (50±3.7ppb); Heptachlor recorded the highest concentration in samples collected from El-Ryiah El-Tawfiki (3±1.4ppb) and lowest concentration in Abbasaa fish farm samples (0.2±0.166ppb); Aldrin showed the highest concentration in Manzala Lake (60±4.8ppb) and lowest concentration was in El-Ryiah El-Tawfiki samples (20±3.2ppb); Dialdrin failed to be detected in raw samples of *O. niloticus*; DDE recorded the highest concentration in Manzala Lake samples (30±3.2ppb) and the lowest concentration was in El-Ryiah El-Tawfiki (20±3.4ppb); DDD was only found in El-Ryiah El-Tawfiki samples with concentration of (170±21ppb); Endrin was found only in El-Ryiah El-Tawfiki samples with concentration of (80±12.1ppb); Heptachlor epoxide was found only in Gesrbahr el-baar agriculture drain samples with concentration of (1±0.6ppb).

Table (1) showing results of *O. niloticus* samples collected from Manzala Lake before and after heat treatment which revealed eight organochlorine residues from which α BHC not detected after heat treatment, while β BHC and DDE showed significant decrease. On the other hand, heptachlor recorded increasing in concentration after heat treatment and Dialdrin could be detected only in heat treated samples.

Table (2) recorded ten OCPs residues could be detected in *O. niloticus* samples from El-Ryiah El-Tawfiki showing: α BHC, β BHC, heptachlor, DDD and endrin disappeared after heat treatment; β BHC, γ Chlordane and DDE showed significant decrease, while DDE increased after heat treatment.

Table (3) revealed that heptachlor was the only OCPs could be detected and reduced after grilling by 50%.

Table (4) show results of OCPs residues of *O. niloticus* collected from Gesr Bahr El-baar agricultural drain including DDD which showed significant decrease after heat treatment.

Fig. (2) Show a comparison of OCPs residues in *O.niloticus* from different localities after heat treatment, where depletion rates varied after grilling from 11% to 100%, while others increased or even detected after grilling depended on type and sensitivity of pesticide residue and variation in localities.

4. DISCUSSION

Organochlorine pesticide is one of highly persistent pesticides (M'Anampiu 2011). The illegal and widespread application of OCPs in Egypt over years released several effluentes into the ecosystem, which has led to severe damage to untargeted organisms, including humans.

Results showed in Fig. (1) Revealed variations in both types and concentrations of OCPs residues according to the locality of collecting samples.

Concerning Manzala Lake samples, it revealed presence of α BHC, β BHC, γ Chlordane, HCB, Heptachlor, Aldrin and DDE with different concentration ranging from 3±0.42 ppb for heptachlor to 90±11 ppb for γ Chlordane. These results were closely similar to some authors and differed in their levels from high to low concentration such asBadawy and Wahaab

(1997) who evaluated environmental pollution problems in Manzala Lake and its main drains by measuring the levels of organochlorine insecticides, lindane, HCB, DDT and its metabolites were detected in all fish samples. The highest concentration of DDTs was 101.45 ng/g wet weight, while some disagreed with Yamashita *et al*. (2000) who recorded that samples of fish collected from River Nile and Manzala lake during 1993-1994 for organochlorine pesticides analysis, DDE was the most predominant pesticide residue (7.6 to 67 ng/g wet wt.).

Concerning to samples collected from El-Ryiah El-Tawfiki, results are lower than those recorded by Abou-Arab*et al*. (1995) who studied the distribution of organochlorine pesticides in random fish samples collected from (El-Malek-El-Saleh and Manzala lake). Data showed that DDT and its analogues were predominant in fish samples collected from Manzala Lake. On the other hand, total DDT followed by heptachlor were predominant in fish samples collected from the river Nile

Concerning to fish samples collected from El-Abbasa fish Farm, results are lower than those recorded by Abd El-Kader *et al*. (1988) who detected α - BHC, lindane, chlordane, endrin and p,p- DDT in tilapia muscles collected from fish ponds at Al-Sharkia Governorate ,Egypt in levels ranged from 0.705 to 1.764 , 3.200 to 12.000, 1.445 to 3.132 , 0.753 to 2.898 and 1.853 to 4.392 µg/kg wet weight, with mean concentrations of 1.309 , 8.152 , 2.297 , 1.532 and 2.959 µg/kg wet weight, respectively.

Concerning to fish samples collected from Gesrbehr el-baar Agriculture drainage, results are higher than those recorded byAbd El-Halim *et al.* (2006) who reported that the residue levels of pesticide residues in fish tissues samples from drainage canal surrounding a pesticide factory at Damietta Governorate were varied, ranging from undetectable to 62 ppb for HCH isomers during spring 2000. The highest residue level of o,p′-DDD was found to be 9 ppb in samples collected from site 2 (Mogawra) during winter 2000. However, o,p′-DDT was detected only in samples collected in spring 2000 at levels of 1 and 2 ppb. This difference may be attributed to difference of pollution rate between the two drains.

Concerning the effect of grilling on level of OCPs residues concentration of α BHC and HCB couldn't be detected after heat treatment (Reduction rate 100%). This result is supported by results recorded by Gergis (1993) who examined the effect of heat treatment on OCPs found in fish samples. Results revealed that no pesticide residues could be detected in positive samples after being heat treated.

β BHC recorded its highest reduction rate (90%) after grilling of samples taken from Manzala Lake and also recorded reduction rate (25%) in samples taken from El-Ryiah El-Tawfiki. These results are nearly similar to what recorded by Zabik*et al.* (1995) who reported that the level of pesticides in walleye and white bass fish are low and that cooking reduces the levels of contaminants by 1/4 to 1/3.

γ Chlordane recorded a significant decrease (50%) in samples from El-Ryiah El-Tawfiki. This result is nearly similar to what recorded by Zabik *et al.* (1995) who reported that, cooking significantly reduced the DDT complex, dieldrin, hexachlorobenzene, chlordane, toxaphene, heptachlor epoxide.

Few significant differences were found among cooking methods. Average losses of pesticides from the salmon ranged from 30 to 41%. Similar average percentage losses were found for carp, ranging from 30 to 35% for the DDT complex, chlordane complex, while the losses of HCB and dieldrin were greater than 40%.

Heptachlor showed 3 different values after grilling according to locality of sample. It showed an increase in samples from Manzala Lake, this result is nearly similar to what recorded by De Boer *et al.* (2013)

who reported that the concentrations of organochlorine pesticides (OCPs) increased in eel fish after frying. The effect of boiling is negligible. This shows that preparation methods for eel as food product do not help in bringing OCPs concentrations down to safe values. In samples collected from El-Ryiah El-Tawfiki, heptachlor couldn't be detected. This result is similar to what recorded by Gergis (1993).Heptachlor in samples collected from El-Abbassa fish farm showed a decrease (50%).This difference may be due to variation in fish age and size which affect the fat ratio which affect the residue level after heat treatment.

Aldrin showed a reduction rate of 67% after grilling of samples from Manzala Lake. this result may be due to its oxidation giving dieldrin, while in samples from El-Ryiah El-Tawfiki, it showed nearly no change so dieldrin not detected in the same area.

These results are similar to what explained by UNEP (1990) which explained that, Aldrin is an alicyclic chlorinated hydrocarbon and is therefore less resistant to oxidation than the aromatics. It is being rapidly converted to the epoxide form (dieldrin). DDE Showed a significant decrease (67%) in samples from Manzala Lake while in samples from El-Ryiah El-Tawfiki.DDD and Endrin recorded its highest reduction rate (100%) in samples from Manzala lake after grilling those results are similar to those recorded by Gergis (1993).

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