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Effect of microwave and halogen oven on polychlorinated biphenyl (PCBs) residues in Tilapia fish

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Abstract

The stability and high bioaccumulation coefficients of polychlorinated biphenyl (PCBs) in aqueous organisms, may constitute a threat for the health of consumers. Thus, it is important to determine concentrations of PCBs in *Oreochromis niloticus* (Tilapia), and estimate the effect of cooking by microwave and halogen oven on concentration changes of PCBs. This study was done to investigate the PCBs residues in 50 samples of *Oreochromis niloticus* (Tilapia) fish which were purchased from different fish markets in Cairo governorates, Egypt. Samples were examined with Agilent gas chromatograph GC. The results indicated the presence of Polychlorinated biphenyls in the examined fresh raw fish samples with an incidence of 18 %. The positive samples contain the different congeners of PCBs as 52, 31, 101, 105, 138 and 128 in the raw fish meat. The levels were not exceeded the maximum allowable concentration according to **(US.FDA, 2001)**. There was a significance differences at ($P<0.05$) between PCBs residue in original sample and its residue after microwave and halogen cooking. Halogen cooking method was more effective than microwave in reducing the PCBs residues and it can be used to enhance the nutritional value of fish products and promote good health.

Keywords: *Microwave, halogen oven, polychlorinated biphenyl (PCBs) residues, Tilapia fish.*

Introduction

Fish is considered one of the most important food stuffs, they are the cheapest source of animal protein in many countries. Fish have protein of high biological values as they contain essential amino acids and good source of minerals such as calcium, phosphorus, iron and trace elements like Iodine, as well as, vitamins, in addition, to the content of polyunsaturated fatty acid **(Sedik *et al.,* 1989).**

Polychlorinated biphenyls (PCBs) are synthetic compounds, once widely used as coolants and insulating fluids in the production of transformers and capacitors, and also as hydraulic fluids, plasticizers, additives in paints, adhesives, Lubricants, plastics and pesticides. PCBs are known to cause adverse effects on human health and the ecosystem and because of properties such as toxicity, high environmental persistence, solubility in fats, the ability to transfer along the trophic chains and long-range transport to regions where they have never been used or produced, they have been classified within the framework of the Stockholm Convention as persistent organic pollutants (POPs), (**Stockholm Convention on Persistent Organic Pollutants, 2009).**

Polychlorinated biphenyls (PCBs) are one groups of organic pollutants found ubiquitously in various environmental samples including sediments in lakes, rivers and estuaries. Determination of their concentrations involves extraction, preconcentration or volume reduction, chromatographic clean-up and instrumental analysis (**Aceves *et al.,* 1988).**

They are persistent in the environment as they are very stable against chemical and microbiological degradation. Due to their lipophilic properties, they accumulate in the food chain and are stored in fatty tissues. **(EFSA, 2012).**

The concentration and distribution of PCBs in fish are controlled by many factors including the species, the lipid content in body, age, size, gender, growth rate, and food choice **(Ashley *et al.,* 2000)**. In general, the position in the food chain or feeding habits of the organism play an important role in the accumulation of the persistent organic pollutants (POPs). Species situated at higher trophic levels are tending to accumulate the most POPs, including PCBs **(Zhou *et al.,* 1999 and Gunnarsson and Skold, 1999).**

The maximum allowable concentration set by US Food and Drug Administration (FDA), USA for total PCBs in fish and shellfish is 2000 ng/ g wet weight **(US.FDA, 2001)**. It should be mentioned that no maximum levels for non dioxin like (ndl) PCB in feed and food have been set in the European Union, so far. According to Commission Regulation (EC) No. 199/2006 (199/ 2006 EC, 2006), maximal levels based on cumulative risk assessment and relative toxicity regarding 2, 3, 7, 8-TCDD, have been given only for dioxin like (dl) PCBs. The maximum level of 100 ng/g fresh weight for the sum of six ndl PCBs in fish has been proposed by the European Commission (EC) draft regulation **(AFSSA, 2007).**

Several analytical techniques had been used to determine the contents of PCBs such as GC–MS **(Abb *et al.,* 2010; Dmitrovic *et al.*, 2002 and Görel Manav *et al.*, 2018),** HPLC **(Chu *et al.*, 2003)** GC–ECD **(Johansen *et al.,* 1994)** and gas chromatography coupled to triple quadrupole mass spectrometry (GC-QqQ-MS/MS) using selected reaction monitoring (SRM) **(Bolaños *et al.,* 2007).**

Numerous studies on both laboratory mammals human and provide strong evidence of the toxic potential of exposure to PCBs. The health effect associated with PCBs include liver, thyroid, dermal and ocular changes, neurodevelopmental changes, immunological alterations, reduced birth weight, reproductive toxicity and cancer. **(Woff *et al.,* 2000; Ibeto *et al.,* 2019).**

Fish consumption is a possible source of PCBs accumulation in humans muscle. It is possible that the accumulation of these contaminants reduces fish quality in the hatchery and undermines their survival after release, resulting in increasing the financial costs to aquacultures **(Botaro *et al.,* 2011).** Microwave oven and halogen cooker is widespread in Arabian and European households, where working women spend less time preparing meals, as well as ready-to-eat foods now available everywhere. Therefore, the goal of this study was to investigate the levels of PCBs residues in fish samples and the effects of microwave and halogen cooking methods on PCBs levels in fish samples aiming to guide consumers in cooking techniques which can reduce dietary PCBs exposure from important fish species consumption.

Materials and Methods

Fish samples:

Fifty random samples of *Oreochromis niloticus* (Tilapia) fish were collected from different fish markets in Cairo governorates. Each sample was kept in a separate sterile plastic bag and transferred to the laboratory in an insulated ice box as quickly as possible.

Fish samples preparation:

All fish samples were washed with tap water, manually dissected and rinsed with tap water, then distilled water. Each fish muscle was sliced into three parts, the first was kept raw, the second part was cooked by microwave for 3-5 minutes for each side using standard household (Sharp R-770AR) and the third part was cooked by halogen oven at 180ºC for 3-5 min for each side using halogen oven (Usha 3514I), cooking methods were applied on positive samples only.

Extraction of PCBs from fish samples:

Method used for extraction was recommended by (**UNEP/IOC/IAE, 1989 and IOC, 1993)** which involved the following steps: Fish sample (10 g of wet weight) was placed in ceramic mortar, anhydrous sodium sulfate (30 g) was added and the mixture was well homogenized. The mixture was transferred to a precleaned extraction thimble and the dehydrated tissue was extracted with 200 ml of 50% methylene chloride in n-hexane for 8 hours in a Soxhlet apparatus cycling 5-6 times per hour. The extracted solvents were concentrated with rotary evaporator to about 1 ml.

Clean Up techniques:
Fish extracts were cleaned and fractionating using 20 g of 0.5% deactivated florisil topped with 1 g anhydrous sodium sulfate in order to avoid resuspension of the top layer when pouring solvents into the column then the column washed with 50 ml n-hexane, before the sample loaded. The first fraction (F1) eluted by 70 ml of n-hexane contained PCBs congeners. The fraction was transferred to rotary vacuum evaporator adjusted at 35ºC and evaporated until the volume reached 2-3 ml. The final extract was transferred quantitatively by rinsing with aliquot of the organic solvent into a concentrator tube and evaporated to dryness. The residue was dissolved in 2 ml of n-hexane and transferred into autosampler vial for GC-ECD

Quantitative Determination of PCBs:
The extracts were concentrated and injected into GC (Aglient 6890) equipped with a ^{63}Ni 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 id., 0.25 µm film thickness. The oven temperature was programmed from an initial temperature 160 (2 min hold) to 240ºC at a rate of 5ºC/min and was maintained at 240ºC for 20 min. Injector and detector temperature were maintained at 260 and 320ºC, respectively. Nitrogen was used as a carrier at flow rate of 3 ml/ min. The retention time, peak area and peak height of the sample were compared with those of the standards for quantization.

Statistical methods:
The data obtained from level of PCBs in fresh samples and that processed by microwave and halogen oven were analyzed by one –way ANOVA using the SPSS statistical package program, and difference among the individual means were compared using LSD range test **(SPSS, 2007).**

Results

Table (1). Statistical analytical result of Polychlorinated biphenyls (PCBs) recovered from Fresh *Oreochromis niloticus* samples (n=50):

Fresh *Oreochromis niloticus* samples			
+ve samples		-ve samples	
No.	%	No.	%
9	18	41	82

Table (2). Incidence of different congeners of Polychlorinated biphenyls (PCBs) in *Oreochromis niloticus* (Tilapia fish) (expressed as ppb) (n= 50):

Types of PCBs	+ve samples		Mean*± SE
	Number	%	
PCBs 28	0	0	ND
PCBs 52	5	10	17±0.2
PCBs 31	8	16	24.8 ±0.2
PCBs 70	0	0	ND
PCBs 101	4	8	7.4 ±0.07
PCBs153	0	0	ND
PCBs 118	0	0	ND
PCBs 105	9	18	44.5 ±0.13
PCBs 138	5	10	4.74±0.05
PCBs 128	6	12	6±0.06
PCBs 180	0	0	ND

*Mean of positive samples.

Table (3). Incidence of different congeners of Polychlorinated biphenyls (PCBs) in positive samples (n= 9):

+ve samples	PCBs 52	PCBs 31	PCBs 101	PCBs 105	PCBs 138	PCBs 128
1	-	+ve	-	+ve	+ve	+ve
2	+ve	+ve	+ve	+ve	-	+ve
3	-	+ve	+ve	+ve	+ve	-
4	-	+ve	-	+ve	+ve	+ve
5	+ve	+ve	-	+ve	-	+ve
6	-	+ve	-	+ve	-	+ve
7	+ve	+ve	-	+ve	-	+ve
8	+ve	+ve	+ve	+ve	+ve	-
9	+ve	-	+ve	+ve	+ve	-
Total	5	8	4	9	5	6

Table (4). Statistical analytical results of the effect of microwave on PCBs residues (expressed as ppb) recovered from +ve fish samples:

Types of PCBs	Before cooking			Microwave cooked fish			
	Min.	Max.	Mean ± SE	Min.	Max.	Mean±SE	Reduction%
PCBs 52	2	37	17±0.2 [A]	1.1	19.9	9.2±0.4[a]	45.9%
PCBs 31	4	41	24.8 ±0.2[A]	2.1	21.3	15.5±0.7[a]	37.5%
PCBs 101	1.5	17	7.4 ±0.07[A]	0.6	6.8	2.95±0.9[a]	60%
PCB 105	27	71	44.5 ±0.13[A]	14	36.9	23.3±0.4[a]	47.6%
PCBs 138	1.7	9	4.74±0.05[A]	0.39	2.1	1.1±0.2[a]	76.8%
PCBs 128	2	13	6±0.06[A]	1.2	7.7	3.54±0.7[a]	41%

There are significant difference at ($p < 0.05$) between means having the same capital and small letter in the same raw.

Table (5). Statistical analytical results of the effect of halogen oven on PCBs residues (expressed as ppb) recovered from +ve fish samples:

Types of PCBs	Before cooking			Halogen oven cooked fish			
	Min.	Max.	Mean ±SE	Min.	Max.	Mean ±SE	Reduction%
PCBs 52	2	37	17±0.2[A]	1	18.5	8.5±0.4[a]	50%
PCBs 31	4	41	24.8 ±0.2[A]	1.9	20.1	14.6±0.8[a]	41.1%
PCBs 101	1.5	17	7.4 ±0.07[A]	0.63	7.1	3.1±0.9[a]	58.1%
PCB 105	27	71	44.5 ±0.13[A]	11.9	31.2	19.6±0.4[a]	56%
PCBs 138	1.7	9	4.74±0.05[A]	0.34	1.8	0.95±0.2[a]	79.9%
PCBs 128	2	13	6±0.06[A]	1.1	7	3.24±0.7[a]	46%

There are significant difference at ($p < 0.05$) between means having the same capital and small letter in the same raw.

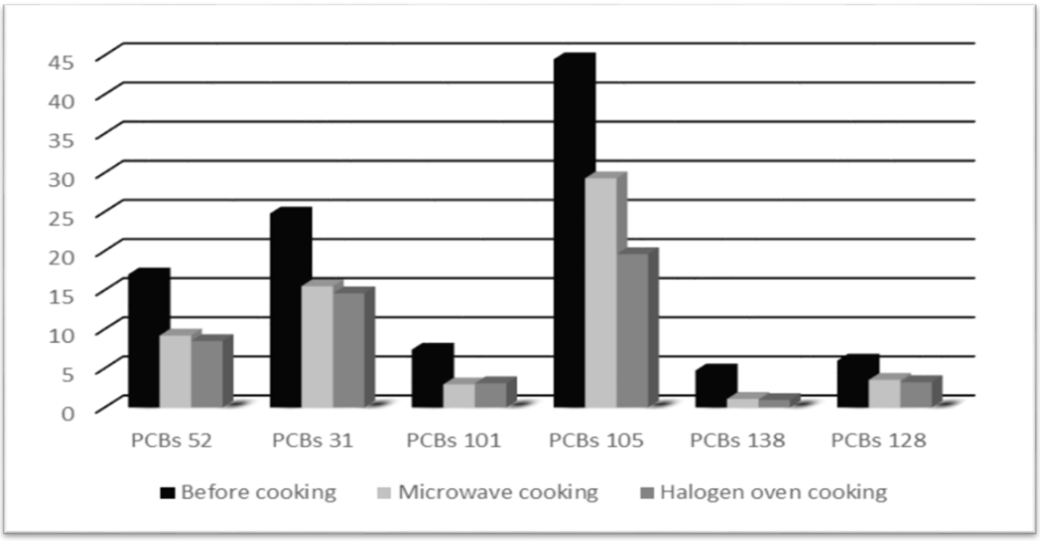

Fig. (1): Reduction effect of thermal processing (microwave and halogen oven) on PCBs levels (expressed as ppb) recovered from positive *Oreochromis niloticus* samples:.

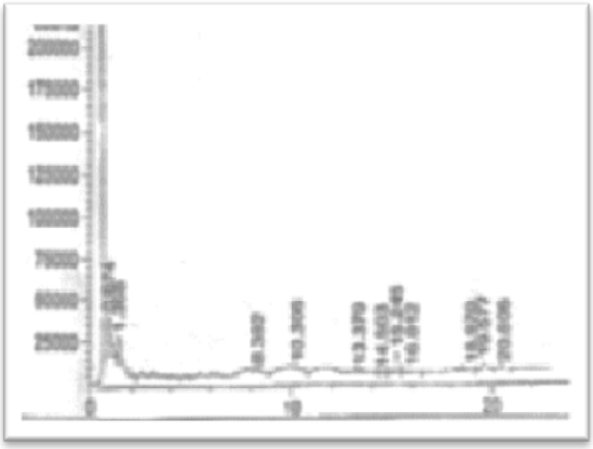

Fig. (2): Negative result of PCBs

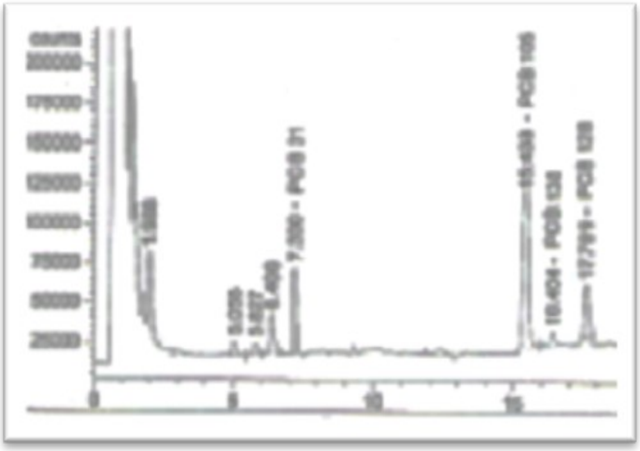

Fig. (3) : Positive result of PCBs

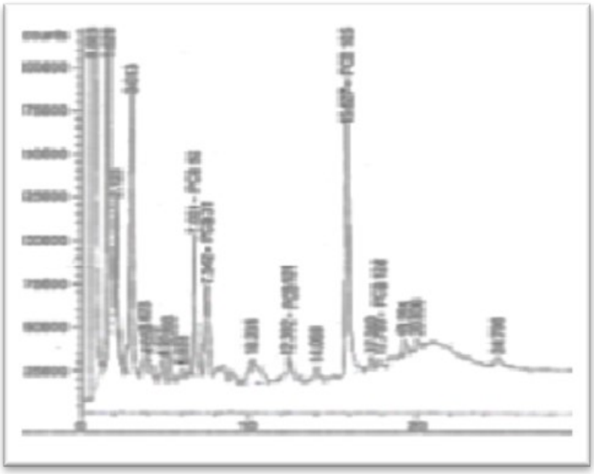

Fig. (4): Positive result of PCBs

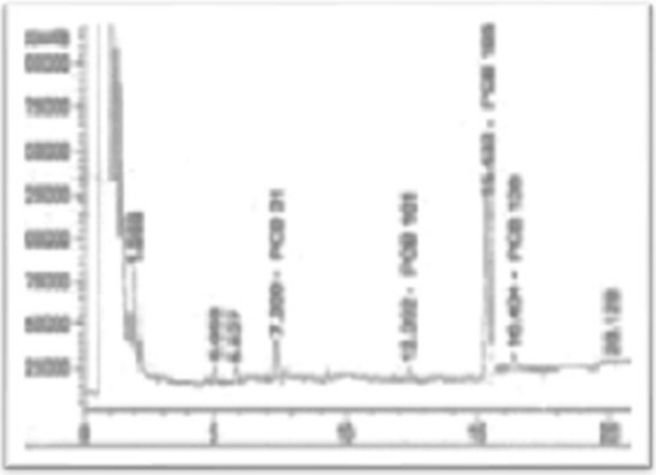

Fig. (5): Positive result of PCBs

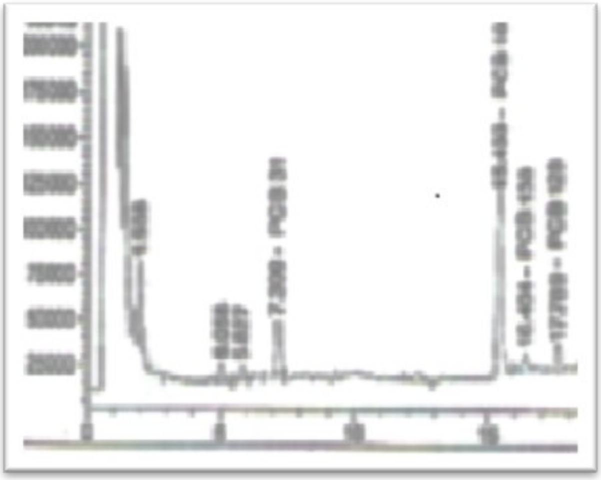

Fig. (6): Positive result of PCBs

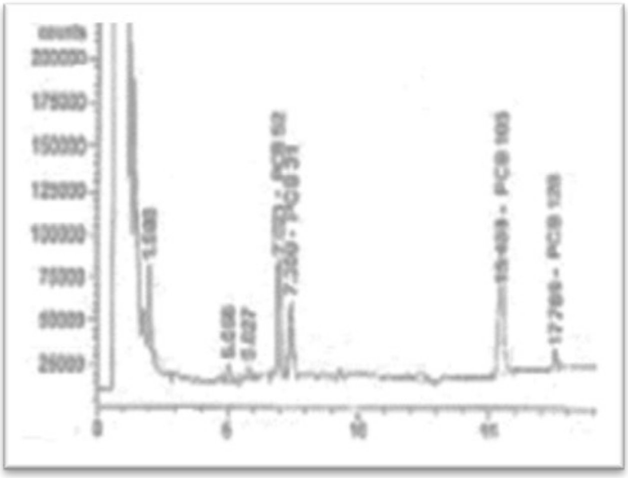

Fig. (7): Positive result of PCB

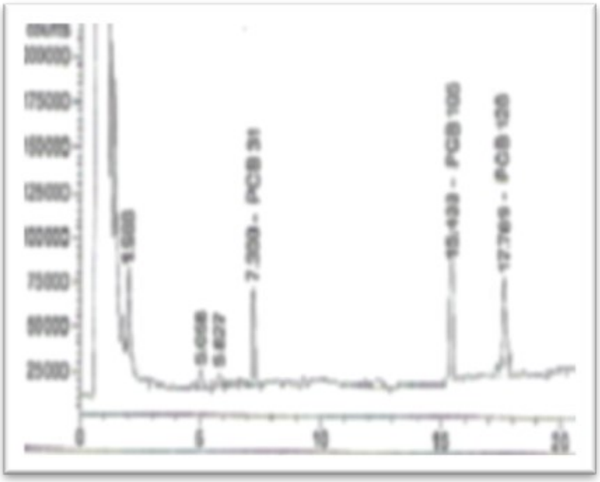

Fig. (8): Positive result of PCBs

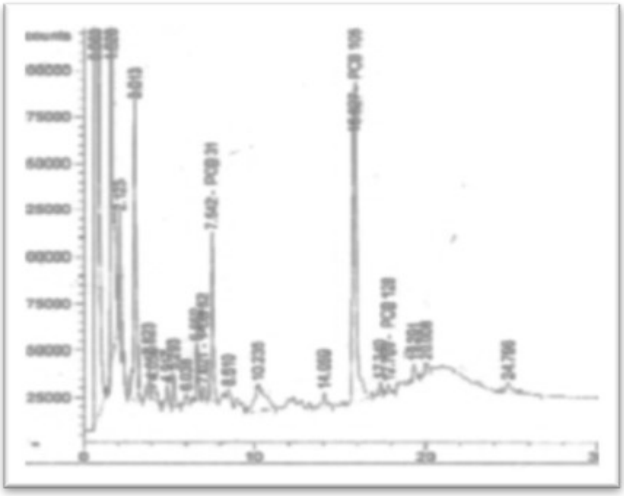

Fig. (9): Positive result of PCBs

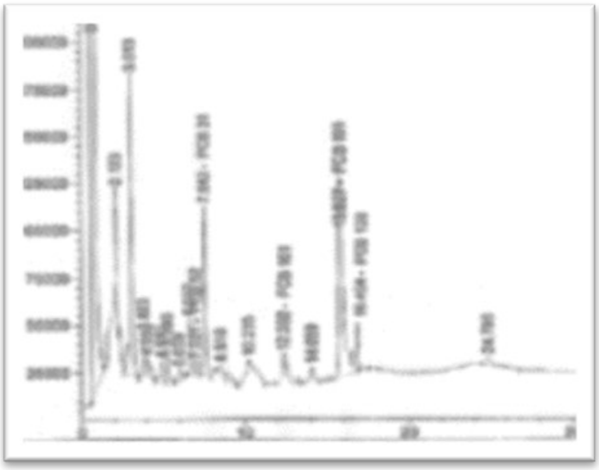

Fig. (10): Positive result of PCBs

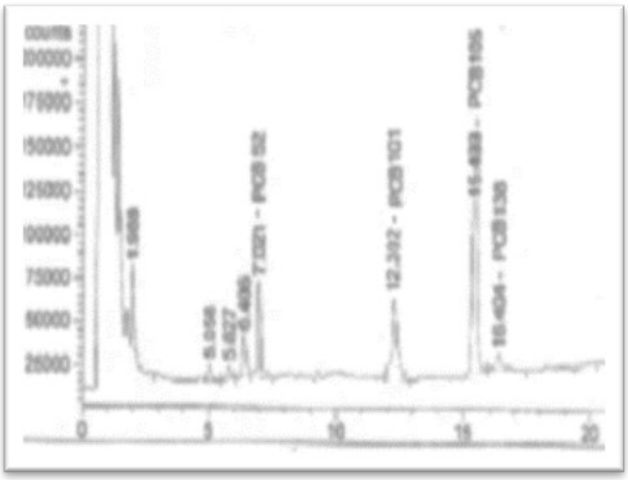

Fig. (11): Positive result of PCBs

Discussion

In developing countries, environmental protection laws have not been enforced, industrial and domestic wastes are dumped indiscriminately into water bodies. These wastes have been reported to contain toxic and hazardous substances including heavy metals, pesticides and PCBs. Aquatic organisms can accumulate hydrophobic compounds like PCBs and reach concentrations considerably higher than those of the surrounding waters. Therefore, mussels and fish are suitable and commonly used for monitoring of these chemical residue **(OSPAR, 2004).** Chemical contaminants in fish were considered as an important source of human exposure to chemicals. Assessments of the fish consumption pathway need to adjust the concentrations of the chemical to account for reduction in PCBs that can occur during cooking.

The assessment of polychlorinated biphenyl residues levels in the examined Tilapia fish samples were showed in (Table 1). Polychlorinated biphenyls detected in the examined fresh samples with an incidence of 18 % (9 samples) but not detected in 82% (41samples).This result was less than the results recorded by **Salem and Aid, (2011) and Nasr *et al.,* (2009).**

Tables (2 and 3) showed the incidence of different congeners of Polychlorinated biphenyls (PCBs) in examined fish samples These results indicated the presence of PCBs congeners' 52, 31, 101, 105, 138 and 128 in 10, 16, 6, 18, 10 and 12%of examined fish samples. Also, the result illustrated that, the highest concentration of PCBs congeners in fish samples was PCB congener no. 105 which represented (44.5±0.13ppb), followed by PCB-31 (24.8±0.2 ppb), PCB-52 (17 ±0.2 ppb), PCB-101 (7.4±0.07ppb), PCB- 128 (6±0.06ppb), then PCB-138 (4.74±0.05 ppb), whereas other congeners (28, 70, 153, 118 and 180) were not detected in all samples.

While **Yahia and ELSharkawy, (2013)** detected the PCB congeners(52, 118 and 138) in cat fish and (28 and 52) PCB congeners in Tilapia collected from Assiut city, **Nasr *et al.,* (2009)** who found (70, 101, 118, 44, 52 and 180) congeners in fish samples collected from different locations in Monofia governorate, Egypt.

On studying, the effect of cooking on PCBs residual levels in the examined *Oreochromis niloticus* sample which showed in (Tables 4 and 5) and (Fig.1). The reduction % in PCBs congeners (52, 31, 101, 105, 138 and 128) in microwave treated samples were 45.9, 37.5, 60, 47.6, 76.8 and 41 % respectively. While, reduction % in PCBs congeners (52, 31, 101, 105, 138 and 128) in halogen oven treated samples were 50, 41.1, 58.1, 56, 79.9 and 46 % respectively. The obtained results showed high reduction in PCB levels found in halogen -cooked fish compared to microwave-cooked fish and PCBs residues in fish tissues showed an obvious variation in their loss The lowest losses occurred in the case of PCB 31 in microwave treated samples (37.6%) and in halogen oven treated samples (41%) while the greatest losses were found for PCB 138 (77 and 80%) in microwave and halogen oven treated samples respectively. Therefore, from the present data it could be concluded that there were significance differences at ($p < 0.05$) between PCBs residue in original raw samples and its residue after microwave and halogen oven treated. The environmental fate and behavior of PCBS congeners, as well as their selective accumulation in living organisms, result from physicochemical properties of the compounds, the lower chlorinated congeners (PCB 28) and (PCB 52) have higher vapour pressures and water solubility than the more highly chlorinated congeners, such as (PCB 138) and (PCB 180), which have higher lipophilicity **(Falandysz *et al.,* 2002 and Mackay *et al.,* 2006).** Reduction of PCB residue levels in processed food usually results from fat leakage (together with dissolved PCBs) during culinary and technological treatments, and partly also from PCBs codistillation with water vapour during thermal processing **(Zabik *et al.,* 1996; Ciereszko & Witczak, 2003).** The effect of both frying and grilling in Mugil cephelus and Sardine fish who concluded that cooking has potential significant decrease PCBs concentration in fish tissues, the decrease was observed to be ranged from 13.6 to 100%, **(Salem and Aid, 2011).** While the obtained result by **(Ciereszko & Witczak, 2003)** revealed that

PCB residue losses in fried carp meat can be up to 50% of the initial concentration in raw meat. PCBs cooking losses also depended mainly on changes in the initial lipid concentration in the fish, heat processing temperature and duration **(Sherer and Price, 1993).** As the fish are mostly eaten in the form of processed products, the influence of different methods of cooking on changes in the levels of toxic compounds in final products is of great importance and may be helpful for appropriate processing (**Witczak and Ciereszko, 2006).**

Conclusion and recommendation

From the present data it could be concluded that the Polychlorinated biphenyls detected in the examined fresh samples with an incidence of 18 %. The positive samples contain the different congeners of PCBs as 52, 31, 101, 105, 138 and 128 in marketed *Oreochromis niloticus* Egyptian fish. The mean values of total congeners were below the permissible limit according to **(US.FDA, 2001)**. There was a significance differences at ($P<0.05$) between PCBs residue in original sample and its residue after microwave and halogen cooking. Halogen cooking method was more effective than microwave in reducing the content of persistent PCBs. Studies are needed to guide consumers in cooking techniques which can reduce dietary PCBs exposure from fish consumption and to provide toxicological estimates with more accurate PCBs exposure estimates. Accurate monitoring of chemical residue levels in food and food products is essential to assure the safety of the food. Further studies should be done on efficient degradation process of PCBs, particularly because these compounds are extremely resistant to chemical, photochemical and biological degradation. Due to PCBs have lipophilic properties and stored in fatty tissues trim away fatty areas before cooking to allow fat to drain off.

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