

Ecological Monitoring of Pesticide Residues and Algae Tolerating Organic Pollution

Hanan A. Abd El-Gawad¹ and Salwa M. Abou El Ella²

¹Central Laboratory for Environmental Quality Monitoring (CLEQM), National Water Research Center (NWRC), El-Kanater, Qalubiya, P.O. Box 13621/6, Cairo, Egypt

²Channel Maintenance Research Institute (CMRI), National Water Research Center (NWRC), El-Kanater, Qalubiya, P.O. Box 13621/6, Cairo, Egypt.
salwaabouellella@yahoo.com

Abstract: Organochlorine (OCPs) and organophosphorus (OPs) pesticides measured in water, sediment and fish samples along River Nile (the outfall of drain into River Nile) and its branches (Damietta and Rosetta,) that receive runoffs from agricultural lands and industrial pesticides. The study aimed to investigate the toxic waste status in the environmental matrices using biological biomarker, included fish and algae. Qualitative and quantitative analysis of OCPs and Ops during winter closure period (December 2013 – March 2014) were carried out using developing analytical method. Due to possible toxicity and bioaccumulation tendency of the OCPs and OPs in fish, the levels of OCPs and OPs must be detected in the fish samples that could be health problems source in the future. The obtained data depicted the varieties toxic measurements that showed significant differences of OCPs and OPs concentration and algae capacities in water. The OCPs and OPs detected are consistent with the agricultural activities of the study area due to pesticide usage and industrial pesticides. The levels of OCPs and OPs in the studied area were still within safety margins compared to the permissible limits for water and sediment. The study clarified biomarkers included fish and quality of algae communities valuable tool in bio-monitoring pollution for toxic pollution in ecological monitoring.

[Hanan A. Abd El-Gawad and Salwa M. Abou El Ella. **Ecological Monitoring of Pesticide Residues and Algae Tolerating Organic Pollution.** *Nat Sci* 2014;12(7):1-12]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 1

Key words: Ecological Monitoring, Fish, Sediment, Algae, Pesticides, River Nile

1. Introduction

River Nile is the principal freshwater resource for Egypt, meeting nearly all demands for drinking water, irrigation, industry and others. Human activities, agricultural and pesticides industry resulted a large range of toxic contaminants into aquatic ecosystems and caused negative effects in the environment.

The combined toxicity of pesticides pollution is complex that gives large range of toxic molecules (pesticides and their metabolites) in streams and other water bodies. The monitoring system of pesticides based on quantitative indicators that focused on monitor pesticide concentrations into different environmental matrices (water, sediment and biota) according to their physical and chemical characteristics (Cruz *et al.*, 2014). They are rapidly absorbed and accumulated by the bottom sediment, plankton, algae, aquatic invertebrates, aquatic vegetation, fish and threat to the health and productivity of aquatic ecosystems (Ogunfowokan1 *et al.*, 2012).

The application of OPs and OCPs increased output of agricultural products and better life quality. OCPs are known to be harmful for human health and the environment due to their persistence, bio-accumulative nature and high lipid solubility. These factors led to the problems of bio-concentration, bio-

magnification in biota tissues and they “biomagnify” up the food chain (SEI, 2010). One such group of organochlorine pesticides has been classified as Persistent Organic Pollutants (POPs) (UNEP, 2010).

OPs cause environmental problems when present at high concentration due to their highly soluble in water. They depend on their rapid degradation, depending on their formulation, method of application, climate and growing stage of plant (Abou El-Ella, 2007 and Ramzy and Abd El-Gawad, 2012).

The greatest potential of chance adverse effects of pesticides is the contamination of hydraulic system; hence water is primary responsible matrix to transport pesticides from the application area to other locations and pesticide contamination of ground water is a global issue (Ló pez-Blanco *et al.*, 2005 and Abd El-Gawad, 2008).

Biological markers define and measure the effects of presence organic pollutants on aquatic system. Various aquatic organisms occur in rivers, lakes, seas and marines potentially useful as biomarkers of organic pollutants including fish, aquatic plants and algae (Rashed, 2001b). The principle of biomarker approach is the analysis of fish to measure their pesticides contents in order to monitor pesticides excess in their tissues. Algae are excellent biological markers of pesticides in water to

define health and water quality status along River Nile and their sediment (**Rashed, 2001a**).

Skillful water control of environmental and public health problems in Egypt is required to overcome devastating effects on the ecological balance of the aquatic environment and the diversity of aquatic organisms. This paper focuses on the quantitative detection of 18 organochlorine pesticides including six different POPs (HCB, O, P'- DDT, DDD, 4, 4'-DDT, and α -HCH and β -HCH) and δ -HCH in water samples from River Nile around agricultural fields. The study conducted to evaluate the pesticide residues of OCPs and OPs in fish, sediment and water from River Nile (the outfall of drain into River Nile) and its branches along agricultural fields.

2. Materials and Methods

2.1 Area Description

Environmental sampling is carried out during winter closure period, (Dec.2012 – March 2013). The area of focused on Ten locations along River Nile (the outfall of drain into River Nile) namely Aswan, Qena, El-Menia, Helwan, Shoubra, Delta Barrage. Two drains outfalls are El-Rahway and industrial pesticide of Kafr El-Zayat pesticides Production Company along Rosetta branch. Additionally, two locations of River Nile along Damietta branch at 1096 and 1180 Km from Aswan represented many activities (Fig.1)

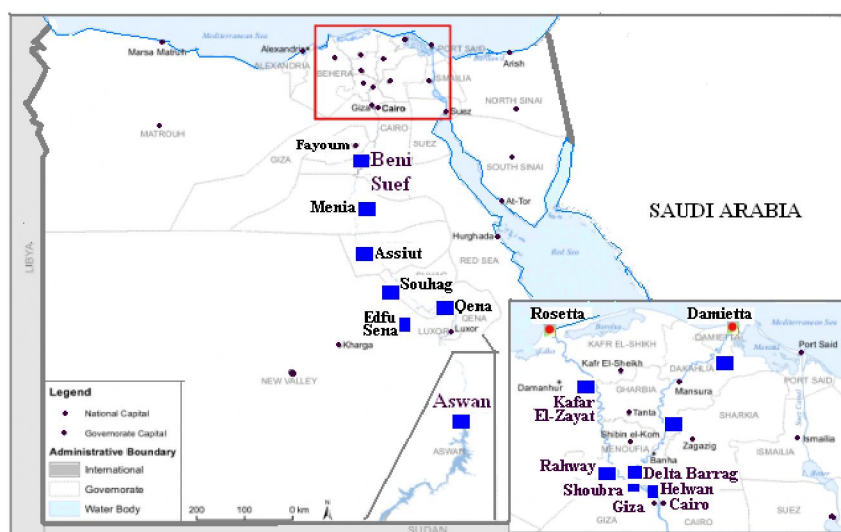


Fig.1 Study Location along River Nile and Nile Delta

2.2 Sampling

2.2.1 Water Samples

Water samples are collected directly from River Nile and its branches (Table 1) into pre-cleaned

brown glass bottles (1 liter) in an ice box. Care was taken not to disturb the surface of the soil layer while collecting the samples.

Table (1): Environmental Sampling

Sampling Site at River Nile		Code		
Location	Distance (KM) from Aswan	Water	Sediment	Fish
Aswan	10	R1	S1	F1
Qena	280	R2	S2	F2
El-Menia	685	R3	S3	F3
Helwan	902 - Industrial and high population	R4	S4	F4
Shoubra	938 - Industrial and high population	R5	S5	F5
Delta Barrage		DP	DPS	DPF
El-Rahway drain	Rosetta branch- 962- outlet drain	RB6	RBS6	RBF6
Kafr El-Zayat	Rosetta branch- 1156-outlet pesticides production	RB7	RBS7	RBF7
Damietta branch	1180- many activities	DB8	DBS8	DBF8
Damietta branch	1096 - many activities	DB9	DBS9	DBF9

The water samples were not filtered to separate suspended particles because the suspended particles, especially fat particles, could contain POPs in them. Samples were kept in ice box for quick transportation

and below 4°C until analysis. The concentration of OCPs and OPs evaluated according to international guidelines (Table 2).

Table 2 Permissible limits of OCPs and OPs Residues in Water (µg/l)

OPs	Australian limits	OCPs	Australian limits	CWQGs,2005
Ethoprophos	1	α - BHC	-	0.01
Cadusaphos	-	β - BHC	-	
Phorate	-	γ - BHC	-	
Diazinon	1	δ - BHC	-	
Chlorpyriphos methyl	-	Heptachlor	0.05	0.01
Chlorpyriphos	-	Hep. Epoxide		
Malathion	-	Aldrin	0.01	0.004
Fenitrothion	-	Dieldrin	-	0.0023
Fenthion	-	Endrin	-	
Fenthioate	-	PP- DDE	-	0.001
Profenophos	0.3	PP. DDD	-	
Fenamiphos	-	PP.DDT	0.06	
		γ - Chlordane	0.01	

Denis Hamilton and Stephen Crossley (2004) & (CWQGs), 2005

2.2.2 Bed Sediment

Sediment samples are collected from depths (0 - 25cm) using pre-cleaned stainless steel sediment sampler from the selective sites. Samples are taken in plastic bags and delivered immediately to the laboratory. The samples are air dried at room temperature (25°C), crushed and finely ground, sieved through (0.2 mm) sieve. The concentration of OCPs and OPs evaluated according to international guidelines (Table 3).

2.2.3 Fish

A total of 50 individual adult fishes, Nile Tilapia (*Oreochromis niloticus*) were collected from the studying locations and kept in ice boxes during transportation. The collected samples were transferred quickly to the laboratory and immediately dissected. Muscles tissues were removed then kept at 4°C till analysis within few days to monitor the toxic residues during the investigated study. The concentration of OCPs and OPs evaluated according to international guidelines (Table 4).

2.2.4 Algae

Various characteristics of algae production including species and diversity reflect the ecosystem conditions for water quality and organic pollution levels. Algae as bio-indicators were sampled by dipping a plastic container into the water and collecting 1 L for each point. All samples were preserved in 4% neutralized formalin solution and their volumes were adjusted to 50ml in the laboratory by settling and removing the supernatant (Abd El-

Gawad and Hammad, 2011). In case of phytoplankton samples identification, 30 L were filtered through 20 µm net mesh and fixed with Lugol's solution (APHA, 2012).

Sub-samples were filtered (Whatman GF/C membranes) and used to quantify chlorophyll-a using spectrophotometer DR-2010 - after acetone extraction (Golterman *et al.*, 1978). Algae identification were carried out by Sedgwick Rafter counting method (APHA, 2012) using an Olympus binocular compound microscope. Determination of phytoplankton diversity and density were expressed on organism/liter Cell/L, respectively.

Table 3 Permissible limits of OCPs and OPs Residues in Sediment

OCPs	CSQGs (µg/kg)	
	ISQG	PEL
Total HCHs	0.94	1.38
Total DDE	1.42	6.75
Total DDD	3.54	8.51
Total DDT	1.19	4.77
Aldrin		
Endrin	2.67	62.4
Endosulfan		
Dieldrin	2.85	6.67
Heptachlor	0.6	2.74
Methoxychlor		

ISQGs: Interim freshwater sediment quality guidelines (dry weight)

PELs: probable effect levels (dry weight) CSQGs,(2002)

Table 4 Permissible limits of OCPs and OPs Residues in Fish (ng/g)

OPs	CAC, 1999	OCPs	FDA, 1983	FDA, 1994	CAC, 1999	CAC, 1986
Ethoprophos	-	α - BHC	200			
Cadusaphos	-	β - BHC		100		
Phorate	-	γ - BHC			2000	
Diazinon	700	δ - BHC				
Chlorpyrifos methyl	50	Heptachlor				200
Chlorpyrifos	200	Hep. Epoxide				200
Malathion	-	Aldrin			200	
Fenitrothion	50	Dieldrin			200	
Fenthion	-	Endrin			100	
Fenthioate	-	PP- DDE				
Profenophos	50	PP. DDD				
Fenamiphos	-	PP.DDT			5000	
		γ - chlordane			50	

2.3 Extraction Procedure

2.3.1 Reagents:

The pesticide standards were purchased from Sigma-Aldrich with the purity of 99.8%. All other chemicals, solvents and reagents used in this study were of analytical grade. De-ionized water and free organic were used throughout the study.

2.3.2 Extraction of Residues - Water

A volume of 500 ml of each water sample was taken in a separating funnel and 50g of sodium chloride was added. The content was extracted three times with 50 mL of n-hexane. 30 g of anhydrous sodium sulphate was added to the combined extracts (EPA, 1992). The extracts were filtered and the water-free organic layer was taken in an evaporation flask. The volume was carefully reduced to about 0.5 – 1.0 mL by evaporation. The sample was cleaned up with 2 mL of 95 - 97% pure sulphuric acid saturated with cyclohexane. The mixture was left for separation and the upper phase was taken for analysis in a gas chromatograph.

2.3.3 Extraction of Residues - Bed sediment

Extraction of organochlorine pesticide residues from bed sediment samples procedure, as described by EPA, 1986 was used. Air-dried bed sediment (10 gm) with 25gm anhydrous sodium sulphate is taken to the extraction cell. Extract the vessel with a mixture of hexane and acetone (10:3). When the extraction is complete transfer the contents into separatory funnel with adding 200 ml of free organic water. Samples extracts are combined and concentrated by rotary evaporating to approximately 5 ml, add 50 ml of n-hexane and concentrate extract to approximately 2 ml (Leyva – cardoso *et al.*, 2003).

2.3.4 Extraction of Residues - Fish

Ten grams of fish muscles were homogenized with 20 g of anhydrous sodium sulfate with tissue homogenizer till a fine homogenate was obtained. The homogenate was extracted with 50 ml of n-hexane:

acetone (2:1) using HPLC grade. Extraction was carried out using orbital shaker for 2 hours, and then the extract was filtered through anhydrous sodium sulfate and evaporated till dryness, using rotary evaporator under vacuum at 40 °C. Blank trials for water, sediment and fish samples were carried out to remove any interfering materials coming from solvents used (Mosaad *et al.*, 2008).

2.3.5 Cleaning up of Sediment and Fish Samples

Cleaning up was carried out using 6g activated florisil (60-100 mesh) topped with 1 g anhydrous Na₂SO₄ then column was wet using 30 ml n-hexane then elution of sample was done with 200 ml of the following mixture dichloromethane : n-hexane: acetonitrile (50:48.5:1.5) (Mills *et al.*, 1972).

2.4 Analytical Procedure

Organochlorine pesticide residues are measured by subjecting samples to Finnigan Gas Chromatography (Hewlett Packard GC Model 6890), equipped with an Electron Capture Detector (ECD). The gas chromatograph condition: DB-17 capillary column (30m length x 0.32 mm internal diameter (i.d.) x 0.25 μ m film thickness). Operating temperatures: column temperature was programmed: initial oven temperature, 160 °C for 2 min., raised at 3° C/ min to 220 °C, then raised 15 °C to 270 °C and then held at 240 °C for 15 min. Injector temperature was 280 °C and detector temperature 320 °C with nitrogen carrier gas flow at 4 ml/min. All compounds were identified by their retention times compared to known standards.

Peak areas were used as the basis for quantification. Standard solutions of OCPs including: Alfa-BHC, Gamma-BHC, Beta-BHC, Delta-BHC, Endrin Keton, Endrin, Endrin aldehyde, Dieldrin, Aldrin, Alfa chlorodan, Gamma chlorodan, Heptachlor, Heptachlor epoxid, Methoxychlor, Endosulfan I, Endosulfan II, Endosulfan sulfate, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT were made from double distilled, deionized water. Each of the standards was

run thrice in the gas chromatograph to check whether the retention time was reproducible. After measuring the retention times of each standard, a mixture of standards was analyzed to verify whether all the retention times remained the same. In order to check the reliability of the experimental results, samples were spiked with 10 µg/l of the mixed standards and the resultant peak areas were compared with the calculated values of the analytes. The concentration in samples was expressed in µg/l.

Organophosphorus pesticide residues were determined by gas chromatograph, (Hewlett Packard GC. Model 6890) equipped with a flame photometric detector (FPD) with phosphorus filter. A fused silica capillary (DB-1701), column containing 14 % cyanopropylsilyoxane as stationary phase (30 m length x 0.32 mm internal diameter (i.d.) x 0.25 µm film thickness) was used for the separation in the GC.

The operating conditions of GC instrument included injector and detector temperature were 250 °C, initial oven temperature 175°C for 2 min, raised at 6°C/min., and then held at 240 °C for 15 min, the carrier gas nitrogen at 4 ml/ min and hydrogen and

air were used for the combustion at 75 °C and 10 ml/min., respectively.

3. Results and Discussion

The obtained data depicted the concentrations of chlorinated pesticides residues included POPs and organophosphorus residues concentrations in environmental matrices (water, bed sediment) and biomarkers included fish and algae. The result clarified the release of applying chemical control and their effect on aquatic organisms that presented in Tables (5-7) and graphically illustrated in Figs. (2-5).

3.1 Qualitative and Quantitative Analysis

3.1.1 OCPs in Surface Water

The data obtained from a broad study of 18 OCPs residue in water samples collected from environmental matrices along River Nile and its branches, drains outfall and industrial outfalls to detect the levels of organic contaminants and represent an challenge to advance our understanding of pollution in investigated sites as shown on Table (5) and valuable chromatogram of OCP residues in the standard sample is illustrated in Fig.(2).

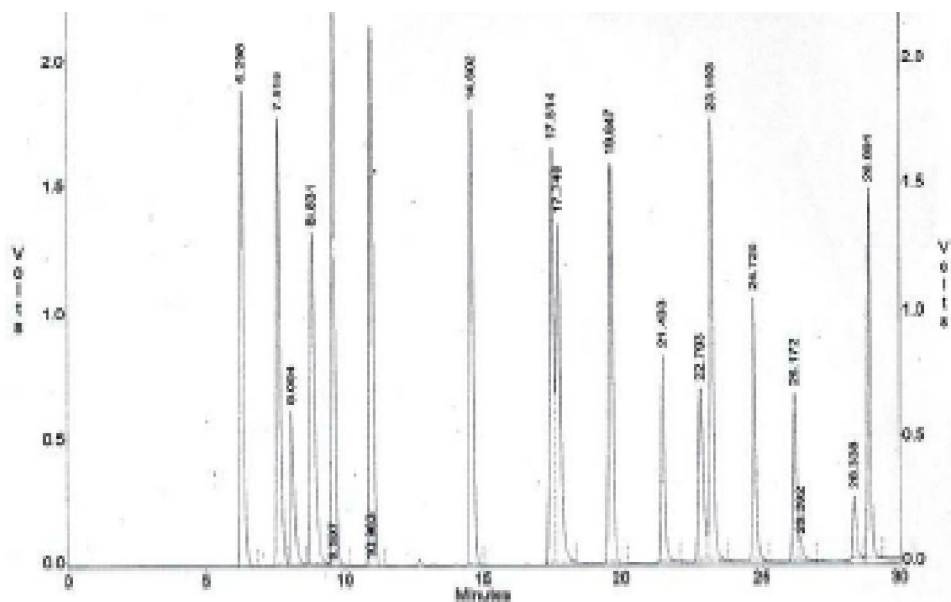


Fig. 2 Chromatograms of OCP Standard Residues

The obtained results revealed that the average levels of the detected organochlorine pesticide residues in investigated matrices were varied 7.9 to 95.4 µg/l in water at Qena, El-Menia, Helwan, Shoubra, Kafr El-Zayat industrial pesticides, and Damietta branch. Aswan and Delta barrage recorded OCPs residue was < 0.01µg/l (minimum detection limits of the methods).

In all analyzed water samples, twelve OCPs are detected (below MDL 0.01 µg/l), although they are

widely used in agricultural purposes. This can be attributed to the fast rate of degradation of this class of pesticides that was accelerated through the variation of climatic conditions in the study area. The obtained results agree with findings obtained by **Abd El-Gawad and Amer (2010)** and **Amer and Abd El-Gawad (2012)** in water of River Nile.

OCPs, especially DDT, were used intensively during past years; therefore, it is still detected with its metabolites (DDE and DDD) in irrigation, drainage

canals and in low concentrations in the River Nile and its branches. This can be related to the currently flushing processes, its use during the past years, low rate of application and attachment to the sediments along their flow as they are associated with solid phase or due to its low solubility and low photo-oxidation. The HCHs concentrations was 12.21 µg/l have lower values than DDTs' in water (Table 5) because of their differences in physico-chemical and biological properties, having HCHs a higher water solubility, vapor pressure, biodegradability, lower lipophilicity and particle affinity as compared to DDTs properties. The major component (77%) of the

commercial DDT is the P,P' isomer. It can be speculated from the results that commercial DDT is being used in almost all the sample sites. DDD was found in 5 samples and their concentrations ranged from 25.33 to 95.4 µg/l. P,P' DDE was found in Kafr El-Zayat industrial company and the concentrations was 15.33 µg/l and 9.15 µg/l for PP, DDT at El-Menia. Almost all the samples had at least one of the three isomers of DDT in them. The presence of these DDT isomers is an indication of the continued use of POPs in agriculture. The concentration of aldrin varied from 7.9 to 8.85 µg/l while endrin concentration ranged from 73 to 29.3 µg/l.

Table 5 Concentration of OCPs Residues in Environmental Matrices

OCPs	Matrices	R1	R2	R3	R4	R5	DP	RB6	RB7	DB8	DB9
α- BHC	Water ng/l	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment ng/g	ND	ND	ND	ND	ND	ND	ND	12.64	ND	ND
	Fish ng/g	ND	ND	ND	ND	ND	ND	ND	23.9	ND	ND
β- BHC	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	65	ND	359	ND	ND	ND	ND	ND	130
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
γ- BHC	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	86.87	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
δ - BHC	Water	ND	ND	ND	12.21	ND	ND	ND	ND	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	ND	ND	ND	ND	140	ND	370	215	209
	Fish	ND	ND	ND	ND	ND	ND	ND	206.6	ND	ND
Hep. Epoxide	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	152	ND	300	ND	ND	ND	ND	36	143
	Fish	ND	ND	ND	930	ND	ND	ND	ND	ND	75.2
Aldrin	Water	ND	ND	ND	ND	7.9	ND	ND	8.58	ND	ND
	Sediment	ND	ND	25	ND	ND	13	ND	17.5	99.33	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	109.3	ND	ND
Endrin	Water	ND	ND	ND	37	ND	ND	ND	ND	33	ND
	Sediment	ND	ND	ND	16	ND	ND	ND	ND	249	ND
	Fish	ND	ND	ND	76.66	ND	ND	ND	ND	124	ND
PP.DDD	Water	ND	28.2	57.1	48.2	ND	ND	25.33	95.4	ND	ND
	Sediment	ND	7	204	13.25	ND	ND	275	186	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
P,P - DDE	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	33	19.5	ND	277	ND	293	ND	20.3	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PP - DDT	Water	ND	ND	9.15	ND	ND	ND	ND	ND	ND	ND
	Sediment	730	ND	511	313	509	545	315	330	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
γ-chlordane	Water	ND	ND	ND	7.05	ND	ND	ND	ND	30.95	ND
	Sediment	ND	ND	145	55	ND	65.75	ND	110	345	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total OCPs	Water		28.2	66.25	104.46	7.9		25.33	103.98	63.95	
	Sediment	763	243.5	885	1333.25	509	1056.75	590	1133.31	944.33	482
	Fish				1006.66				339.8	124	75.2
Environment		763	271.7	951.25	2444.37	516.9	1056.75	615.33	1577.09	1132.28	557.2

Dieldrin, Endrin Keton, Endrin aldehyde, Alfa chlorodan, Methoxychlor, Endosulfan I, Endosulfan II, Endosulfan sulfate: ND in all samples

3.1.2 OCPs in Sediment

Pesticide leaching from the soil into drains, irrigation water and finally into the Nile and pose serious environmental and health risks. This depends on the type of pesticide, soil characteristics, hydro-geological conditions, climatic factors, agro-technical factors and human factors. The results of sediment sample analyses for the same study sites are shown in table (5). All the samples collected had at least one of the seven organochlorine pesticides present. No sample site showed the presence of all seven POPs pesticides, but Helwan sample (R4) showed the presence of six OCPs pesticides.

The minimum detection limits of the methods used for extraction of OCPs residue from bed sediment is 0.01 $\mu\text{g kg}^{-1}$. In all analyzed bed sediment samples, OCPs are detected (below the method detection limit 0.01 $\mu\text{g/kg}$). In general, the levels of OCPs in sediment are still within safety margins compared to the permissible limits for Canadian sediment quality guidelines in Table (3) (**Abdel Gwad and Rashed (2008)**). Among the analyzed organochlorine residues in sediment samples, α -BHC, β -BHC, Heptachlor, Aldrin, Hep. Eposide, γ -Chlordane, pp-DDE Endrin, pp.DDD, and PP-DDT were detected at levels ranging from ND to 12.64 ng/g for α -BHC, 65 to 359 ng/g for β -BHC, 86.87 ng/g at R7 for γ -BHC, 140 to 370 ng/g for Heptachlor, 36 to 300 ng/g for Heptachlor epoxide, 14 to 25 ng/g for Aldrin, 16 to 249 ng/g for Endrin, 7 to 275 ng/g for pp. DDD and 315 to 730 ng/g for pp. DDT and 55 to 345 ng/g for γ -chlordane. Monitoring program recorded DDTs residues in all studied samples except Qena and Damietta branch while the highest detected level of DDTs was at Aswan Dam.

3.1.3 OCPs in Fish

Organochlorine pesticides levels of all investigated sites in fish were below permissible levels according international limits except heptachlor was 203.6 ng/g at Kafr El-Zayat industrial pesticides. OCPs pollution at Kafr El-Zayat (aldrin), Helwan (endrin) and Damietta (endrin) along water, sediment and fish was attributed to the continued pesticides industry and use chemical control for agricultural pests.

Although concentration of POPs in most of the samples were within the regulatory limits, it must be emphasized that POPs are inherently unmanageable and they bio-accumulate in living species. Therefore, the acceptable standard for any POP in any sample should ideally be zero (**Li et al., 2006**).

3.2 Organophosphorus Pesticide in Environmental Matrices

3.2.1 OPs in Water

Concentrations of organophosphorus pesticides in studied locations are shown in Table (6).

Compounds identified included ethoprophos, diazinon, Chlorpyrifos methyl, chlorpyrifos, malathion, fenitrothion, fenthion, fenthioate, and profenophos. Tables (6) illustrate OP concentration in water samples for ten locations sites. The level of concentrations were 100 ng/l for Diazinon at Dommettia branch, 45 ng/l for Chlorpyrifos methyl at Kafr El-Zyat industrial pesticide company, 18 and 21 ng/l for fenthion at Helwan and Kafr El-Zyat industrial pesticide company, 24 ng/l for Fenthioate and 38 and 41 ng/l for profenophos in Helwan and Kafr El-Zyat industrial pesticide company.

3.2.2 Ops in Bed Sediment

Residues of OPs in sediments is marked that the concentrations of studied compounds were subject to considerable variation with value to sites. Table (6) showed the concentration values were 59 and 155 ng/g for Chlorpyrifos methyl in El-Menia and Kafr El-Zyat industrial pesticide company and 37 ng/g for fenthioate in El-Menia. The presence of chlorpyrifos methyl and fenthioate, in sediments samples could be attributed to the intense agricultural activity in the area and industrial pesticides. Most organophosphorus pesticides residues were non detected in water and sediment may be aspect to its rapid degradation, depending on their formulation, rate of application, method of application and climatic factors. These compounds are highly soluble in water and relatively short-lived in the environment.

3.2.1 OPs in Fish

In fish, bioaccumulation concentration (BC) from water through the gills, skin, and food is a possible route for organophosphorus (OP) pesticide to accumulate in tissue: the route depends mainly on their feeding preference, general behavior, and trophic level (**Abd El-Gawad and Ramzy, 2013**). The present study indicated OPs tendency for Chlorpyrifos methyl 159.15 to 83.28 ng/g, Chlorpyrifos 41.5 to 83.12 ng/g, Fenitrothion 100.8 to 212 ng/g, Fenthioate 45.2 to 109.6 and Profenophos 91.1 to 98.93 to bio-accumulate in Nile Tilapia (*Oreochromis niloticus*) while the other OPs were not detected (Table 6).

3.3 Biological Marker

In present investigation algal communities which are used as biomonitors of pesticides pollution in study area. Total 5 genera have been identified, among these 26 species belonged to Chlorophyceae, 25 species to Cyanophyceae and 20 species to Bacillariophyceae. Table (7) showed the list species of Chlorophyceae and the most pollution tolerant species of *Oocystis elliptica* (Hansgirg), *Oocystis parva* (Wittrock), *Pediastrum simplex* and *Scenedesmus opoliensis*. The obtained results agree with findings obtained by **Abd El-Gawad and Ramzy, 2013**.

Table (8) showed the list species of Cyanophyceae and the most pollution tolerant species of *Anabaena fertilissima* Forti, *Anabaena spiroides*, *Aphanocapsa elachistavar.conferta* and *Microcystis aeruginosa* Kutz.

Table (9) showed the list species of Dinophyceae and the most pollution tolerant species of *Peridinium umbonatum* (Woloszynska).

Table (10) showed the list species of Bacillariophyceae and the most pollution tolerant species of *Cyclotella meneghiniana* Kutz,

Cyclotella ocellata Pant, *Melosira granulata* (Her.) Ralfs, *Melosira granulata* var. *angustissima* (Her.) Ralfs and *Synedra actinostroides* Kutz.

The algal communities indicate that Helwan and Kafr El-Zayat were highly organically polluted. Table 11 depicted the varieties percentage of algae communities, total OCPs and OPs concentrations in environmental matrices. The toxic waste affect on biomarker included fish and algae species distribution and their difference.

Table (6) Concentration of OPs Residues in Environmental Matrices

OPs	Matrices	R1	R2	R3	R4	R5	DP	RB6	RB7	DB8	DB9
Ethoprophos	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cadusaphos	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Phorate	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diazinon	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	100
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlorpyriphos methyl	Water	ND	ND	ND	ND	ND	ND	ND	45	ND	ND
	Sediment	ND	ND	59	ND	ND	ND	ND	155	ND	ND
	Fish	83.28	159.15	ND	ND	ND	ND	ND	ND	ND	ND
Chlorpyriphos	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	83.12	73.78	ND	ND	ND	41.5	ND
Malathion	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fenitrothion	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	107	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	100.8	ND	ND	212	ND	ND	ND	ND	ND
Fenthion	Water	ND	ND	ND	21	ND	ND	ND	18	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fenthioate	Water	ND	ND	ND	ND	ND	ND	ND	24	ND	ND
	Sediment	ND	ND	37	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	109.6	ND	ND	ND	ND	ND	ND	45.2
Profenophos	Water	ND	ND	ND	38	ND	ND	ND	41	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	98.93	ND	ND	91.1	ND	ND	ND
Fenamiphos	Water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Sediment	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total	water				59				128		
	Sediment			96					155		
	Fish	83.28	259.95	109.6	182.05	285.78		91.1		41.5	45.2
Environmental		83.28	259.95	205.6	241.05	285.78		91.1	283	41.5	45.2

Table (7) List of Chlorophyceae Species in the Study Area

List of Chlorophyceae Species	R1	R2	R3	R4	R5	DP	RB6	RB7	DB8	DB9
<i>Actinastrumhantzchii</i> (lagerheim)	7	2	0	2	4	2	2	0	4	0
<i>Ankistrodesmusfalcutus</i> (Precott)	2	0	1	0	0	1	0	1	0	0
<i>Ankistrodesmusfusiformis</i> (Corda.)	1	0	1	3	3	0	0	1	2	1
<i>Chlorella vulgaris</i> (Beijerinck)	4	2	1	7	2	0	1	1	0	0
<i>Coelastrummicroborum</i> Naegeli	4	0	0	7	3	1	0	0	7	1
<i>Coelastrumsphaericum</i> (Naegeli)	1	0	5	1	2	0	0	2	1	1
<i>Dictyosphaeriumjurisii</i> (Hindak)	0	6	1	1	0	1	1	1	2	0
<i>Dictyosphaeriumpulchellum</i> (Wood)	0	2	1	0	2	0	2	1	2	1
<i>Golenkiniaradiata</i> W. & G. S. West	6	0	0	2	4	1	1	0	0	0
<i>Golenkiniapaucispina</i> W. & G. S. West	0	4	0	2	0	0	1	5	2	2
<i>Micractiumquadrisetum</i> (Fresenius)	0	2	1	1	2	0	3	0	3	0
<i>MicractiumPusillum</i> (Fresenius)	10	0	2	3	2	1	1	1	0	1
<i>Monoraphidiumcontortum</i> (Thuret)	0	1	2	2	0	0	0	2	2	1
<i>Oocystiselliptica</i> (Hansgirg)	9	10	0	17	3	2	5	6	0	2
<i>Oocystisparva</i> (Wittrock)	0	0	1	0	2	1	1	1	1	1
<i>Oocystissolitaria</i> (Wittrock)	4	2	1	10	8	2	2	1	1	2
<i>Pediastrumboryanum</i>	0	0	0	8	1	0	1	5	2	0
<i>Pediastrum simplex</i>	6	3	0	6	4	1	1	0	14	2
<i>Pediastrum simplex var. duodenarium</i>	4	0	0	0	4	1	1	5	0	1
<i>Scenedesmusacuminatus</i> (Hansgirg)	1	0	0	2	1	1	0	0	9	2
<i>Scenedesmusopoliensis</i> (Chodat)	18	21	1	17	21	2	7	16	0	6
<i>Scenedesmuscornis</i> (Ehrenberg)	0	0	2	3	1	0	0	3	2	1
<i>Scenedesmusquadricuda</i> G.M Smith	3	3	0	8	6	2	3	0	0	1
<i>Schroederiasetigera</i>	3	0	1	2	6	0	2	4	0	4
<i>Westellabotryoides</i> (West)	6	2	4	5	3	1	3	1	0	1
Sub-total	89	60	25	111	84	20	38	59	54	31
%	19.0	23.4	4.3	33.3	25.1	7.9	12.8	14.4	15.9	16.7

Table (8) List of Cyanophyceae Species in the Study Area

List of Cyanophyceae Species	R1	R2	R3	R4	R5	DP	RB6	RB7	DB8	DB9
<i>Anabaena unisporea</i> (Ralfs)	0	2	0	2	0	2	0	0	0	2
<i>Anabaena fertilissima</i> Forti	9	9	4	0	0	0	21	19	20	0
<i>Anabaena circinalis</i> Forti	29	3	3	0	0	6	0	3	7	6
<i>Anabaena constricta</i> (Szafer) Geitler	0	4	5	0	5	0	2	14	3	0
<i>Anabaena spiroides</i> (Ralfs)	27	21	0	0	31	65	140	2	13	60
<i>Anabenopsisarnoldii</i> (Wolosz.)	0	1	7	0	0	0	40	50	0	0
<i>Aphanocapsaelachistavar. conferta</i>	18	88	0	80	60	0	13	35	120	3
<i>Aphanocapsagrevillei</i> (Hansg.)	0	0	0	13	0	7	32	37	0	23
<i>Chroococcuscohaerens</i> (Breb.) Nag.	0	0	0	0	0	0	0	0	0	0
<i>Chroococcusdispersus</i> Lemmermann	1	0	4	0	0	0	5	2	0	2
<i>Chroococcusminutus</i> Nag. (Kutz.)	6	0	0	0	0	0	0	0	50	0
<i>Coelosphaeriumnaegelianum</i> Grunow	0	0	250	0	0	15	2	1	0	3
<i>Crucigeniatetrapedia</i> (Lemmer.)	0	2	21	0	0	18	0	0	0	0
<i>Cylindrospermopsisiraciboroskii</i> Wolosz.	0	0	0	0	0	43	0	0	0	0
<i>Gloeocapsa magma</i> (Itzigs.) Rabenh.	0	0	244	0	0	0	32	28	0	0
<i>Gomphosphariumkuetzingianum</i> (Nag.)	0	0	0	2	0	0	55	50	0	13
<i>Gomphosphariumlacustris var. compacta</i>	0	0	4	4	4	0	0	0	21	0
<i>Lyngbyalimnetica</i> Lemmer.	11	4	10	7	0	7	0	0	4	0
<i>Merismopediapunctata</i> (Meyen)	0	0	2	59	127	0	22	7	75	0
<i>Microcystisaeruginosa</i> Kutz.	275	210	195	2	15	29	59	95	0	10
<i>Microcystisflos-aquae</i> (Wittr.) Kirchner	90	0	0	1	1	0	28	0	0	14
<i>Myxosarcinaburmensis</i> (Geitler)	0	0	0	2	15	0	0	0	0	0
<i>Phormidiuminterruptum</i> Kutz.	0	0	70	5	0	0	0	0	0	0
<i>Phormidiumlaminosa</i> (Agardh.) Gomont	0	0	0	0	4	0	0	0	2	0
Sub-total	472	343	819	183	262	193	460	343	315	137
%	89.1	77.8	4.3	33.3	25.1	78.3	11.8	14.4	15.9	16.7

Table (9) List of Dinophyceae Species in the Study Area

List of Dinophyceae Species	R1	R2	R3	R4	R5	DP	RB6	RB7	DB8	DB9
<i>Ceratiumhirundinella</i> (O. F. Muell.) Dujardin	0	1	0	0	0	1	1	0	0	1
<i>Ceratiumlimneticum</i> (O. F. Muell.) Dujardin	1	0	0	0	0	0	0	0	0	0
<i>Peridiniumumbonatum</i> (Woloszynska)	1	2	0	1	0	0	0	1	0	0
<i>Peridiniumumbonatum</i> Stein.	0	0	0	0	0	0	1	0	0	1
Sub-total	2	3	0	1	0	1	2	1	0	2
%	0.1	0.8	0.6	0.6	0.0	0.4	0.3	0.2	0.6	0.0

Table (10) List of Bacillariophyceae Species in the Study Area

List of Bacillariophyceae Species	R1	R2	R3	R4	R5	DP	RB6	RB7	DB8	DB9
<i>Achnanthesminutissima</i> Kutz.	1	5	0	0	0	1	4	1	0	0
<i>Amphora ovalis</i> Kutz.	2	0	0	0	1	1	2	2	0	0
<i>Cocconeisplacentula</i> (Ehrenberg)	0	0	0	0	1	3	0	0	0	0
<i>Cyclotella meneghiniana</i> Kutz.	16	20	5	4	3	37	28	16	4	4
<i>Cyclotellaocellata</i> Pant.	8	9	7	25	33	15	10	16	1	24
<i>Gyrosigmaacuminatum</i> (W. Smith)	0	3	1	16	11	2	3	2	0	0
<i>Gyrosigmakutzingii</i> (W. Smith)	1	0	0	1	2	2	0	0	1	0
<i>Melosiragranulata</i> (Her.) Ralfs	175	170	200	131	1	155	265	195	185	70
<i>Melosiragranulata</i> var. <i>angustissima</i> (Her.) Ralfs	40	63	21	120	184	16	33	26	23	0
<i>Naviculadicephala</i> (Gregory)	1	0	1	15	6	1	0	2	1	0
<i>Naviculusilla</i> Weber	0	0	0	1	1	0	0	0	0	0
<i>Navicula pinnularia</i> (Gregory)	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia acicularis</i> W. Smith	1	1	1	1	0	1	1	0	0	0
<i>Nitzschiafrustulum</i> (Kutz.) Grun.	0	0	0	0	2	0	0	0	0	0
<i>Nitzschia kutzingii</i> Hilse	0	0	0	0	0	0	0	0	0	0
<i>Nitzschia palea</i> (Kutz.) W. Smith	1	3	0	0	0	4	4	0	0	1
<i>Syndraactinostroides</i> Kutz.	170	38	110	43	3	32	92	125	136	83
<i>Syndra ulna</i> (Nitzsch) Her.	0	0	2	16	61	6	1	1	1	1
<i>Syndra affinis</i> Kutz.	4	0	5	2	16	3	3	3	7	3
Sub-total	422	282	353	375	325	279	293	389	241	195
%	45.7	49	29.7	56.1	48.75	56.7	41.73	49.15	48.6	53.7

Table (11) Concentration of Toxic Waste in Environmental Matrices and Percentage of Algae Communities in The Study Area

List of Species	R1	R2	R3	R4	R5	DP	RB6	RB7	DB8	DB9
Total OCPs: <i>Water</i>		63.95	103.98	25.33		7.9	104.46	66.25	28.2	
<i>Sediment</i>	482	944.33	1133.31	590	1056.75	509	1333.25	885	243.5	763
<i>Fish</i>	75.2	124	339.8				1006.66			
Total OPs: <i>Water</i>				59				128		
<i>Sediment</i>			96					155		
<i>Fish</i>	83.28	259.95	109.6	182.05	285.78	91.1	ND	ND	41.5	45.2
Chlorophyceae %	19.0	23.4	4.3	33.3	25.1	7.9	12.8	14.4	15.9	16.7
Cyanophyceae %	89.1	77.8	4.3	33.3	25.1	78.3	11.8	14.4	15.9	16.7
Bacillariophyceae %	45.7	49	29.7	56.1	48.75	56.7	41.73	49.15	48.6	53.7
Dinophyceae %	0.1	0.8	0.6	0.6	0.0	0.4	0.3	0.2	0.6	0.0
Total Algae	78.15	81	87.1	98.95	143.3	66.6	153.9	151	38.9	123.3

4. Conclusion

Ecological monitoring was carried out for toxic waste along River Nile and its branches included drains outfall and industrial outfalls and Delta Barrage as reference point during winter closure period (December 2013 – March 2014). The presented data indicated most of organochlorine pesticide and organophosphorus residues in surface

water, bed sediment and fish samples are found below the detection limit. 0.01 µg/l, 0.01 ng/g and not detected for water, bed sediment and fish, respectively.

From the results, River Nile (the outfall of drain into River Nile) shows that the residues of organochlorine and organophosphorus pesticides exist in low concentrations in water, sediment and

bio-accumulated in majority Nile Tilapia (*Oreochromis niloticus*). These residues have apparently originated from the areas of intensive pesticide application in Helwan and Kafr El-Zayt industrial pesticides company along River Nile and Rossetta branch.

The natural processes of weathering are likely to result in the transfer of pesticide residues from site to another to the main course of the Nile, especially during the rainy season and hence their transport downstream. Increased contamination with residues is certain to adversely affect the fish population and difference of algae communities for the development of the fish feeding in this vital area. In order to keep the situation under control, it is essential that a system for the continuous monitoring of residues as key environmental components in the whole River should be established.

Recommendations

- Continuous monitoring of pesticide residues to detect any probable addition of organochlorine pesticide along Nile.
- Study suitable enzymatic treatment for individual industry, specially Kafr El-Zayat and Helwan area.

Acknowledgement

The authors gratefully Acknowledgement their colleges in Central Laboratory for Environmental Quality Monitoring (CLEQM) and Channel Maintains Research Institute (CMRI) for their valuable cooperation, providing all facilities, suggestions and financial assistance.

References

1. Abd El-Gawad H. A. and Rashed A., 2008. Tracing of Pesticide Residues Along Northern Delta: (Case Study), Scientific Bulletin, Faculty of Engineering, Ain Shams University, Vol.43, No.3, Sept. 2008.
2. Abd El-Gawad H. and Amer A., 2010. Environmental Indicators of Drainage Water and Water Quality, TECE. J. 2010, Vol.32, No.4, pp.76:85.
3. Abd El-Gawad H. and Hammad D., 2011. Ecological Monitoring of Organic Pollution in Urban Sewage Using Algae as Bioindicator: Case Study, TECE. J., Vol.33, No.1, pp.1:9
4. Abd El-Gawad H. and Ramzy E., 2013: Purification and characterization of toxic waste in the aquatic environment using common carp, *Cyprinus carpio*. Journal of Natural Resources and Development 2013; 03: 27-34.
5. Amer A. and Abd El-Gawad H., 2012 Rapid Bio-indicators Assessment of Macrobiotic Pollution on Aquatic Environment, Inter. Water Tech. J., 2012, Vol.2, No.3, pp.205:216.
6. APHA, 2012. Standard Methods for Examination of water and waste water, American public health Association 21th, New York.
7. Canadian Council of Ministers of the Environment (CSQGs), 2002. Canadian sediment quality guidelines for the protection of aquatic life: Summary tables. Updated. 2002. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.
8. Canadian Council of Ministers of the Environment (CWQGs), 2005. Canadian water quality guidelines for the protection of agricultural water uses: Summary table. Updated October 2005. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.
9. Codex Alimentarius Commission (CAC), 1986. Guide to codex recommendation concerning pesticide residues. 2nd Ed., Vol. XIII., CAC/PR, FAO/WHO, Rome, Italy.
10. Codex Alimentarius Commission (CAC), 1999. Pesticides in food: Maximum Residue Limits. Extraneous Maimum Residue Limits 2, Sep. 1999.
11. Cruz E., Bravo-Durán V., Ramírez F. and Castillo L., 2014. Environmental hazards associated with pesticide import into Costa Rica, 1977-2009. Journal of Environmental Biology (JEB), Vol. 35, Special issue, pp.: 43-55, January 2014.
12. Denis Hamilton and Stephen Crossley (2004): Pesticide residues in food & drinking water : human exposure and risks . John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England
13. EPA, 1986. Method 3500: Organic extraction and sample preparation.
14. Food and Drug Administration (FDA), 1983. Limits issued from Canada, FRG, Denmark, Sweden, United States and Thailand. Cited after Al- Safy (2000).
15. Food and Drug Administration (FDA), 1994. Organochlorine contaminants in egg and tissues of wood duck from Mississippi. Bull. Environ. Contam. Toxicol. 45: 870-875.
16. Golterman H., Clyma S., and Ohnstad M., 1978. Methods for Physical & Chemical Analysis of Fresh Waters. IBP Handbook 8. Blackwell Scientific Publication, Oxford, 1978, 213.
17. Leyva cardoso D.O., Ponce-Velez G., Botello A.V. and Diaz-Gonzalez, 2003. Persistent

- Organochlorine Pesticides in Coastal Sediments from Petacalco Bay, Guerrero, Mexico. *Bll. Environ. Contam. Toxicol.* 71:1244-1251.
18. Li J, Zhu T, Wang F, Qiu XH and Lin WL, 2006. Observation of Organochlorine Pesticides in the Air of the Mt. Everest Region. *Ecotoxicology and Environmental Safety*, 63: 33-34.
 19. López-Blanco C., Gómez-Á S., Rey-Garrote M., Cancho-Grande B. and Simal-Gándara J., 2005. Determination of carbamates and organophosphorus pesticides by SDME–GC in natural water. *Anal. Bioanal. Chem.*, Vol.383, pp.:557–561.
 20. Mills P.A., Bong B.A., Kamps L.R. and Burke J.A., 1972. Elution solvent system for florisil column cleans up in organochlorine pesticide residue analysis. *J.A.O.A.C.*, 55 (1):39-43.
 21. Mosaad M.N., Abd El-Gawad H.A. and Ramzy E.M., 2008. Toxicity and Bioaccumulation of Organophosphorus Insecticide, Profenofos in Aquatic Environment, *Journal of Environmental Science*, Ain Shams University, Cairo, Egypt, Vol.17, No.4, Dec. 2008.
 22. Ogunfowokanl A., Oyekunlel J., Torto N. and Akanni S., 2012. A study on persistent organochlorine pesticide residues in fish tissues and water from an agricultural fish pond. *Emir. J. Food Agric.*, Vol.24 (2), pp.:165-184.
 23. Ramzy E. and Abd El-Gawad H., 2012. Environmental Study of Toxic Effects on Aquatic Organism along Water Resources: Case Study. *Arab Council Journal*, Vol.3 (2), pp.:83-96
 24. Rashed M.N., 2001a. Cadmium and lead levels in fish (*Tilapia nilotica*) tissues as biological indicator for lake water pollution. *Environ. Monito. Assess.* Vol.68, pp.:75-89.
 25. Rashed M.N., 2001b. Monitoring of environmental heavy metals in fish from Nasser Lake *Environ. Intern.* 27-33.
 26. Salwa M. Abou El Ella, 2007. Pesticides Residues in Environmental Components of Nubia Lake, Sudan. *Egypt. J. Aquat. Biol. & Fish.*, Vol. 11 (4), pp.: 81-94.
 27. Stockholm Environment Institute (SEI), University of York. 2010. [online] Persistent Organic Pollutants Workshop. University of York, Stockholm, Sweden. Accessed on April 2011, Available: <http://www.york.ac.uk/sei/projects/completed-projects/organic-pollutants-workshop>.
 28. United Nations Environment Program (UNEP), 2010. Persistent Organic Pollutants. Accessed on April 2011, Available [online].: <http://www.chem.unep.ch/pops>.

5/23/2014