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Biodegradation of Selected Chlorinated Pesticides Contaminating Lake Mariut Ecosystem

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Abstract: Water pollution with chlorinated pesticides is one of the most serious environmental problems due to their high persistence as a result of the slow biodegradation. Residue levels of organochlorine compounds including (P, P' - DDT, γ -HCH and DDE) and cyclodiene components (aldrin, endrin) in the water and sediments of Lake Mariut, Alexandria (brackish water) were analyzed and determined by capillary gas chromatography. Bacterial isolates collected from sediments of such lake were identified and investigated for their ability to biodegrade the selected pesticides. Water and sediment samples were collected from six different sites in the main basin of Lake Mariut and also through three successive seasons, summer, autumn and winter 1996-97. Bacterial isolates were identified and subjected to two concentrations: 0.05 and 50 ppm of the investigated pesticides to study the interaction between pesticides and bacteria. Results showed that lindane, aldrin, P,P' - DDT and endrin were present in the water and sediments of Lake Mariut at very high levels with residue levels significantly higher in sediments compared to water samples. Seasonal and spatial variation of their distribution in the water and sediments were observed. Biodegradation results showed superior ability of the isolated bacteria to decompose the investigated pesticides with very high efficiency reaching 100% for most of them. Results also revealed selective ability among the tested bacteria for biodegradation of different pesticides especially at the lowest concentrations.

Key words: Chlorinated pesticides, bacteria, biodegradation

Introduction

Microbial metabolism of pesticides has been extensively reviewed (Hill and Wright, 1978; MacRae, 1989; Somasundaram and Coats, 1990; Cork and Krueger, 1991; Linn *et al.*, 1993; Aislabie and Lloyd, 1995). Fate of pesticides in the environment is affected by microbial activity while their rate of biodegradation is widely varied. Some pesticides are easily biodegradable while others are recalcitrant. DDT (1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane) and dieldrin (chlorinated hydrocarbons) and their residues remain in the environment more than 20 years after application and are known to be accumulated into food chains. Other group of pesticides such as organophosphorus are more readily biodegradable while atrazine and simazine are slowly biodegradable and may be leached from soil to ground water where they pose a threat to drinking water supplies (Kookana and Aylmore, 1994).

Biodegradation of pesticides is determined by two groups of factors, the first relates to microbial consortium and the optimum condition for their survival and activity while the second relates to the chemical structure of the pesticides. Factors related to microorganisms including the presence and number of appropriate microorganisms, the contact between microorganisms and the substrate (pesticide), pH, temperature, salinity, nutrients, light quality and intensity, available water, oxygen tension and redox potential, surface binding, presence of alternative carbon substrates and alternative electron acceptors. The second group of factors including chemical structure, molecular weight and functional groups of the applied pesticides, their concentration and toxicity and their solubility in water (Aislabie and Lloyd, 1995). Organic pesticides including chlorinated hydrocarbons enter surface water from application for control of aquatic

weeds, trash fish, aquatic insects, percolation and runoff from agricultural lands, drift from aerial and land application, discharge of industrial wastes from manufacturing and formulating plants and wastewater from clean up of equipment used for pesticide application (Faust and Aly, 1964).

Among the organic pesticides, it is well known that chlorinated hydrocarbons are the most persistent pesticides in the environment (with a half life time over 20 years) and the most important according to their production and field application (Abo El-Amayem *et al.*, 1979). Toxicity of chlorinated hydrocarbons and their accumulation in aquatic biota have been known for years. Because of their low solubility in water, they tend to be absorbed in the aquatic organisms and into sediment particles. Therefore, sediments act as reservoir for such organic pollutants. The environmental impact of chlorinated pesticides on aquatic life fall into two categories: Direct toxicity by lowering dissolved oxygen levels and production of odor and taste in the edible fish and shellfish (WHO, 1984).

Lake Mariut, south Alexandria, Egypt suffered in the recent decades from intensive pollution as a result of a continuous discharge of huge amounts of agricultural wastewater that contains large concentration of the washed pesticides and fertilizers in addition to domestic and industrial wastewater. Previous studies on L. Mariut proved the availability of chlorinated pesticides which increased with time indicating their accumulation and cycling in such environment (Abo El-Amayem *et al.*, 1979; Abd El-Aal, 1981; Macklad *et al.*, 1984a; El-Sebae *et al.*, 1984; Badawy and El-Dib, 1984). The main objective of the present study was to isolate a naturally occurring microorganisms with a high ability for biodegradation of organic pollutants to be used for

bioremediation of the highly persistent chlorinated pesticides, thus provide a low-cost and naturally renewable method for removing organic pollutants from contaminated media.

Materials and Methods

Study area: Lake Mariut (Fig. 1) is the smallest of the four Delta lakes of Egypt. It is closed, brackish, very shallow lake where the depth rarely exceeds 120 cm. It is situated to the south of Alexandria at latitude $31^{\circ}10' N$ and longitude $29^{\circ}55' E$. The lake area is divided by artificial dykes into four basins: the main basin; the fish farm; the south western basin and the north western basin (Wahby *et al.*, 1978). The current samples were collected from the main basin of the lake. It is an extremely fertile and highly productive water body, one where advanced state of eutrophication threatens its usefulness to man. It receives heavy industrial untreated wastewater, domestic primary treated wastewater and agricultural wastewater. The overflow from the lake is discharged directly to the sea through El Mex pumping station via El Umum Drain. This basin is suffering much, at present, from the intensive pollutants entering it through all kinds of discharges.

Sampling: Six defined samples sites (Fig. 1) in the lake were selected in relation to known sites of effluents discharge including agricultural (El Qalaa Drain), domestic and industrial during summer, autumn 1996 and winter 1996-97. Because of the shallowness of L. Mariut, only subsurface water samples were collected. Samples for bacterial analysis were collected in pre-sterilized glass bottles (100 ml). For pesticides, samples of 1 liter were collected in pre-acid washed polyethylene bottles. Sediments were collected from the same sites where water were collected. About 250 g wet weight were collected using a sediment scooper which had a provision for guarding the sediment against leaching during vertical hauling. Sampling technique for water and sediments followed the standard procedure.

Bacterial identification: Bacteria were isolated from sediments particles by resuspension (by vortexing) of 1 g in sterilized distilled water and then inoculation onto nutrient agar medium (NA) after appropriate dilution. Purification of bacterial isolates was done after a series of culturing and reculturing. Identification of bacterial isolates took place first by categorizing them into two groups by gram stain followed by classical biochemical reaction (Senath *et al.*, 1989) according to the schemes of Le Chevallier *et al.* (1980) for the identification of Gram-positive and Gram-negative bacteria.

Biodegradation assays: Bacterial isolates were inoculated in 100 ml nutrient broth medium (NB), incubated at $37^{\circ}C$ under 120 rpm. Cultures were left for 4.5 hrs. to get into early log phase of growth without suppression. Then three different chlorinated pesticides (P,P'-DDT, hexachlorobenzene and endrin), selected based on their availability in L. Mariut, were added at two concentrations; lower one of 0.05 ppm and higher one of 50.0 ppm. Bacteria were left in contact with pesticides mixture for 48 hrs in addition to control samples without any microorganisms. Ten ml of each of the 48 hrs cultures were drawn aseptically by a sterile pipette, centrifuged at 6000 rpm for 5 min to harvest bacterial cells. Bacterial palletes were discarded and the supernatants were filtered through 0.22 μm Millipore membrane filter to retain the rest of bacteria. Then pesticides residues or their metabolites in the clear supernatants, after biodegradation were extracted,

cleaned up and determined using gas chromatography. The same procedure was repeated every 24 hrs for another 3 days to investigate the effect of the contact time on pesticides biodegradation.

Determination of chlorinated pesticides residues

Extraction and cleanup

Water: Twenty five ml of 25% sodium sulphate was added to 250 ml of each water sample and extracted two times with 50 ml methylene chloride. The extract was filtered through anhydrous sodium sulphate column, dried and dissolved in 50 ml of n-hexane. Cleanup was carried out using small column packed with silica gel and prewashed with n-hexane where 0.5 ml from the sample and 0.5 ml of n-hexane were added to the column followed by partitioning using hexane, benzene and acetone. The solvent was evaporated and concentrated using a micro-syringe column fitted on a concentrator tube and the volume was adjusted to exactly 2.0 ml (Ernst *et al.*, 1974).

Sediments: A 50 g sample of the sediment was dried and Soxhlet extracted with 200 ml n-hexane overnight (4-5 cycle/hr). The extracts were dehydrated with anhydrous sodium sulphate and concentrated in a Kuderna-Danish evaporator to a suitable volume of 2 ml in n-hexane. Clean of the resulted extracts was done on Florisil column and washed with 25 ml of n-hexane. Clean of the resulted extracts was done on Florisil column and washed with 25 ml on n-hexane then partitioned with solvent system (hexane, hexane-methylene chloride and methylene chloride). The solvent extracts were combined in a separatory funnel and dissolved with 2 ml n-hexane (Keller and Bidleman, 1984; Duursma *et al.*, 1974).

Biological samples: Clear supernatants with the remaining residues of the tested pesticides were washed three times with 5 ml methylene chloride in a separatory funnel. The lower phase was collected and filtered through anhydrous sodium sulphate. The extract were evaporated and dissolved in 2 ml with n-hexane and appropriate aliquots were injected in Gas chromatography.

Determination: An appropriate aliquot was injected in gas chromatography equipped by Hewlett Packard 5890 series II capillary with electron capture detector (GC-ECD). Column, HP-5 (crosslinked 5% pH Me silicon) 30 m X 0.32 mm X 0.25 Mm film thickness, made in USA). The injection took place at oven temp. $200^{\circ}C$, injection temp. $250^{\circ}C$ and ECD temp. $300^{\circ}C$. The concentration of each compound was calculated by comparing its total area with the total area of standard detected insecticides.

Results and Discussion

Residue levels of chlorinated pesticides at L. Mariut Water:

Table 1 represents residue levels of chlorinated pesticides as detected in the water of L. Mariut's main basin at the selected sites. Lindane, aldrin, P,P'-DDT and endrin were detected in the water at very high levels ranged between 0.00162 and 22.317 ppm with averages of 3.30, 2.52, 0.070 and 0.014 ppm for aldrin, lindane, endrin and P,P'-DDT respectively. Distribution of chlorinated pesticides in the water of the main basin showed clear and significant spatial and seasonal variation during the study period. Seasonally very clear general trend for all the detected pesticides was noticed. It was represented by proportional increase in the residue

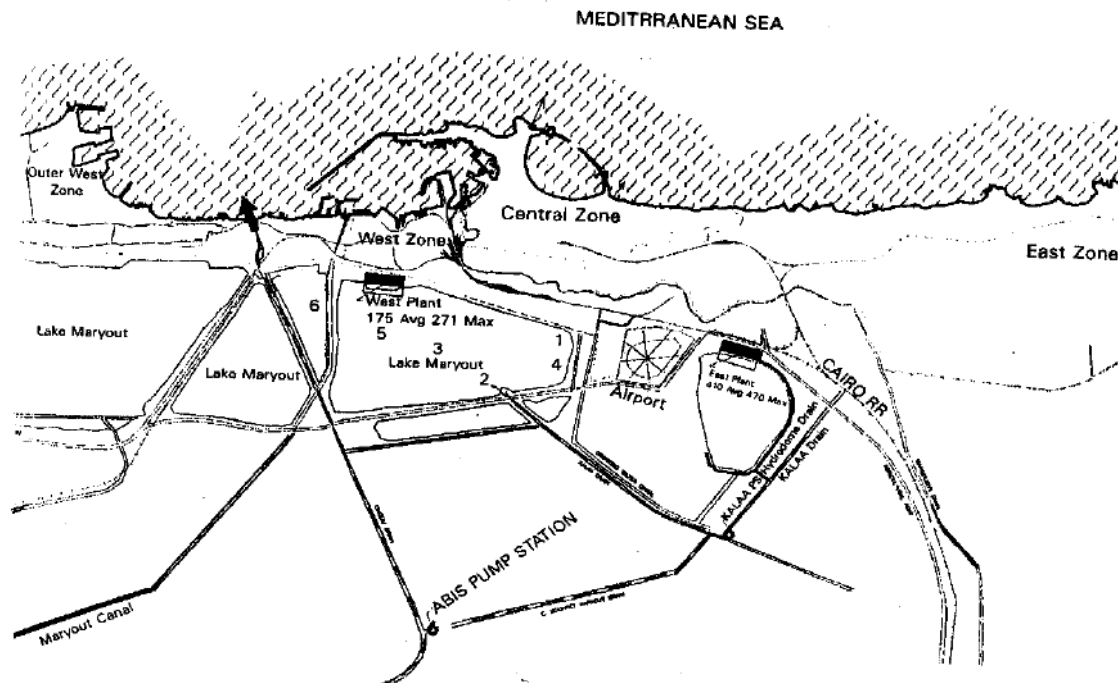


Fig. 1: Study area and sampling sites

Table 1: Residue levels of Chlorinated Pesticides in the water of Lake Mariut during the period Oct. 96-Jan 97

Season	Site	Pesticides	Conc. (ppm)
Autumn (Oct. 96)	1	Lindane	0.08596
		Aldrine	0.49928
			0.00162
	2	Lindane	0.80426
		Aldrine	0.77300
			0.01625
	3	P,P'-DDT	0.01625
		Endrine	0.06550
			0.20000
	4	Aldrine	0.20000
			0.15850
			0.120000
(Nov. 96)	6	Lindane	0.120000
		Aldrine	0.34030
			0.93132
Winter (Dec. 96)	2	Lindane	0.93132
		Aldrine	0.54890
			1.55940
(Jan, 97)	2	Aldrine	1.55940
		Lindane	0.68572
		P,P'-DDT	0.03089
	2	Lindane	10.416000
		Aldrine	22.31700
		P,P'-DDT	0.00629

levels of all the investigated pesticides with time from Oct. 96 towards Jan. 97 (Table 1). This trend is attributed to two main factors; microbial population (occurrence and number of appropriate degrading microorganisms) and the temperature. During warm months in autumn, microbial activities of the degrading bacteria increased and led to the decomposition and removal of such pesticides, thus relatively low levels were recorded. While in the colder months, decreasing temperature decreased both bacterial density, bny limiting their growth and their metabolic activities including degradation processes (Sharma and Azeez, 1988; Greene and Darnall, 1998).

Table 2: Residue levels of chlorinated pesticides in the sediments of lake mariut during Summer and Autumn 1996

Session	Site	Pesticides	Conc. (ppm)
Summer (Aug. 961)	2	Lindane	12.83500
		Endrine	0.02610
			8.69700
	4	Lindane	9.75450
		P,P'-DOT	0.04270
			2.00210
Autumn (Nov. 96)	5	Lindane	2.00210
		Aldrine	2.03400
		P,P'-DDT	0.016911
	6	Lindane	5.32720
			12.58112
			0.01698
	2	Lindane	12.58112
		Endrine	0.01698
		Lindane	9.69980
	3	Lindane	9.69980
		P,P'-DDT	0.00652
			12.83350
4	4	Lindane	12.83350
		Aldrine	18.03000
		Endrine	0.01869
	5	P,P'-DDT	0.01987
		Lindane	9.99128
		P,r-DDT	0.10298
6	6	Aldrine	5.72900

Spatially, water at site 2 in the front of El-Oalaa Drain which transfers huge amounts (500,000 m³/day) of agricultural wastes (nutrients, fertilizers and pesticides) characterized by the highest levels of pesticides. These levels decreases elsewhere away from this outfall. Thus, only samples at site 2 were collected furing the followed months (Nov., Dec., 1996 and Jan., 97).

Sediments: Table 2 represents residue levels of chlorinated pesticides in the sediments of L. Mariut during summer and autumn 1996. Lindane, endrine, P,P'-DDT and aldrine were

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Table 3: Bacteria Isolated from the sediments of lake mariut during Summer and Autumn 1996

Bacteria species	Isolation site	Granin strain	Glucose fermentation	Lactose fermentation	Oxidase	Indole	Methyl Red	Gitrate utilization (Simmon)	Nitrate reduction	Motility	Tween 20 (deg.)	Growth At 42°
<i>Pseudomonas</i>												
<i>Paucimobills</i>	1	-ye	+ l-	+/-	+ ve	-ve	-ve	-va	-ve	+ ye	-ve	-ve
<i>P. aeruginosa</i>	1	-ye	+/-	+/+	+ ve	-ve	-ve	+ve	+ve	+ ve	+ ve	-ve
<i>P. mallei</i>	2	-ve	+1-	+1-	+ ve	-ve	-ve	-ve	-v8	+ ve	+ ve	-ve
<i>P. pseudomallei</i>	2,3,5	-ye	+1-	+1-	+ ye	-ve	-ve	+ve	-ve	+ ve	+ ve	+ve
<i>P. picketun</i>	5	-ve	+/-	-1-	+ ve	-ve	-ve	+ve	-v8	+ ve	+ ve	-ve

Table 4: Biodegradation of selected chlorinated pesticides by different bacterial species isolated from sediments of lake Mariut at the initial concentration of 0.05 ppm

Bacterial species	Isolation site	Exposure time (days)	Compound (remained)	Conc. (ppm)	Removal Efficiency (RE%)
<i>Pseudomonas</i>	1	2	-	0.0	100% of all the tested compounds
<i>panucimobills</i>		3	-	0.0	
		4	Endrin	0.0305	39.0
		5	P,P'-DDT	0.00177	96.5
<i>P. aeruginosa</i>	1	2	Lindane	0.0061	87.8
			P,P'-DDT	0.0079	84.2
		3	Lindane	0.001382	97.2
		4	Endrin	0.0047	90.6
		5	-	-	100% of all compounds
<i>P. mallei</i>	2	2	Lindane	0.00138	97.2
			Endrin	0.00370	85.4
		3	Endrin	0.00702	85.9
		4	Endrin	0.0005	99.0
		5	-	-	100% of all compounds
<i>P. pseudomallei</i>	2,3,5	2	-	0.0	100% degradation of all compounds
		3	-	0.0	
		4	-	0.0	
		5	-	0.0	
<i>P picketti</i>	5	2	Endrin	0.00131	97.4
		3	Endrin	0.00324	95.3
		4	Endrin	0.00402	92.0
		5	Endrin	0.00625	87.5

Table 5: Biodegradation of selected Chlorinated Pesticides by different Bacterial Species Isolated from sediments of faker mariut at the highest Concentration of 50 ppm

Bacterial Species	Isolation site	Exposure time (days)	Compound (remained)	Conc. (Ppm)	Removal Efficiency (RE%)
<i>Pseudomonas</i>	1	1	P,P'-DDT	0.0028	99.99
<i>Paucimobilis</i>			Endrin	0.0065	99.98
			DDE	0.0222	99.96
			Lindane	0.6114	98.78
		2	P,P'-DDT	0.00	100.0
			Endrin	0.00	100.0
			DDE	0.0081	99.98
			Lindane	0.6468	98.71
		3	P,P'-DDT	0.2657	99.47
			Endrin	0.4405	95.12
			DDE	0.00	100.0
			Lindane	3.0874	93.83
		4	P,P'-DDT	0.00	100.0
			Endrin	1.104	97.79
			DDE	0.00	100.0
			Lindane	1.882	96.24
		5	P, P'-DDT	0.00	100.0
			Endrin	0.00	100.0
			DDE	0.00	100.0
			Lindane	1.4266	97.15
		6	P, P' -DDT	1.3744	97.25
			Endrin	6.3261	87.35
			DDE	0.00	100.0
			Lindane	1.0791	97.84
<i>P. aeruginosa</i>	1	1	P,Pr-DDT	0.00	100.0
			Endrin	0.00	100.0
			DDE	0.00	100.0
			Lindane	0.1040	99.79
		2	P,P'-DDT	0.0010	99.99
			Endrin	0.0092	99.98
			DDE	0.0484	99.90

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<i>P. aeruginosa</i>	1	3	Lindane	0.7754	98.45		
			P,P'-DDT	0.00	100.0		
			Endrin	0.00	100.0		
	4	4	DDE	0.00	100.0		
			Lindane	0.4858	99.03		
			P, P'-DDT	0.00	100.0		
		5	5	Endrin	2.2070	95.59	
				DDE	0.00	100.0	
				Lindane	3.3034	93.39	
	6	6	P,Pr-DDT	0.00	100.0		
			Endrin	0.00	100.0		
			DDE	0.00	100.0		
6		6	Lindane	1.4266	97.15		
			P,P'-DDT	1.3744	97.25		
			Endrin	6.3261	87.35		
<i>P. mallei</i>	2	1	DDE	0.0452	99.90		
			Lindane	1.0791	97.84		
			P,P'-DDT	0.00	100.0		
		2	2	Endrin	0.8744	98.25	
				DDE	0.0095	99.98	
				Lindane	0.495	91.90	
	3	3	P,P'-DDT	0.0075	99.98		
			Endrin	0.00	100.0		
			DDE	0.00	100.0		
		3	3	Lindane	0.651	98.70	
				P,P'-DDT	0.5768	98.65	
				Endrin	3.6771	92.65	
	4	4	DDE	0.00	100.0		
			Lindane	5.1434	89.71		
			P,P'-DDT	0.0011	99.99		
		4	4	Endrin	0.00	100.0	
				DDE	0.0130	99.97	
				Lindane	1.1543	97.69	
<i>P. mallei</i>	2	5	P,P'-DDT	0.00	100.0		
			Endrin	0.00	100.0		
			DDE	0.0122	97.98		
		5	5	Lindane	1.5564	96.89	
				P,P-DDT	0.00	100.0	
				Endrin	1.8145	96.37	
	<i>P. pseudomallei</i>	2,3,5	1	DDE	0.00	100.0	
				Lindane	1.6486	96.70	
				P,P'-DDT	0.00	100.0	
			2	2	Endrin	0.02423	99.52
					DDE	0.0030	99.99
					Lindane	2.527	94.95
3		3	P,P-DDT	0.00	100.0		
			Endrin	3.779	92.44		
			DDE	0.3606	99.28		
		3	3	Lindens	4.7265	90.55	
				P,P-DDT	3.3165	93.37	
				Endrin	7.2171	85.57	
4	4	DDE	0.0066	99.99			
		Lindane	2.1424	95.72			
		P,P'-DDT	0.00	100.0			
	4	4	Endrin	2.1792	95.64		
			DDE	0.00	100.0		
			Lindane	2.5864	94.83		
5	5	P,P'-DDT	0.00	100.0			
		Endrin	1.030	97.94			
		DDE	0.00	100.0			
	6	6	Lindane	2.2659	95.47		
			P,P'-DDT	0.00	100.0		
			Endrin	2.2287	95.54		
<i>P. pickettii</i>	5	1	DDE	0.0195	99.96		
			Lindane	1.1201	97.79		
			P,P'-DDT	0.00	100.0		
		2	2	Endrin	0.00	100.0	
				DDE	0.00	100.0	
				Lindane	0.4680	99.06	
	2	2	P,P'-DDT	4.2484	91.50		
			Endrin	8.7894	82.42		
			DDE	0.640	99.87		

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3	Lindane	13.3281	73.34
	P,P'-DDT	0.00	100.0
	Endrin	0.00	100.0
	DDE	0.0037	99.99
4	Lindane	0.2039	99.59
	P,P'-DDT	0.00	100.0
	Endrin	4.2695	91.46
	DDE	0.00	100.0
5	Lindane	0.00	100.0
	P,P'-DDT	0.00	100.0
	Endrin	4.9634	90.10
	DDE	0.0214	99.95
6	Lindane	3.7256	92.55
	P,P'-DDT	0.00	100.0
	Endrin	3.3201	93.36
	DDE	0.0254	99.95
	Lindane	0.8133	98.37

detected in the sediments at much higher levels compared to their levels in the overlying water. Levels of chlorinated pesticides ranged between 0.00652 and 18.03 ppm with a regional average of 5.4883 ppm. Lindane was the most detected compound in the sediment at the different sites with a distribution frequency of 90% followed by P,P'-DDT (50%), endrin and aldrin (30% distribution frequency in the analyzed samples). Also, Lindane had the highest levels in the sediments of L. Mariut cornered with other compounds with an average of 9.302 ppm followed by aldrin (mean = 8.598); P,P'-DDT (mean = 0.038) and finally endrin with the lowest levels (mean = 0.021). The order of magnitude of these compounds in the water was slightly different and was as follows aldrin > lindane > endrin > P,P'-DDT. As in the water, distribution of chlorinated pesticides detected in the sediments showed highly significant seasonal and spatial variations. Seasonally, all the detected pesticides except for endrin showed remarkable increase in their levels during autumn compared to their levels during summer. This could be attributed to biodegradation and removal of these compounds at the high temperature during summer by microbial action which subsequently decreases with decreasing temperature towards autumn and winter. Spatially, the same trends, as for water, was observed with sites 2,3 and 4 near El-Qalaa Drain were characterized by the highest levels of the detected pesticides. Sites 5 and 6 in the western zone still had high levels of the detected compound but relatively lower than detected in the eastern zone.

Comparison between residue levels of the detected compounds in the water and sediments of L. Mariut revealed clear and significant increase in the levels detected in the sediments compared to those in the water. Levels of Lindane, P,P'-DDT and aldrin in the sediments were respectively 3.69, 2.71 and 2.6 folds higher than their levels in the overlying water. However, endrin showed the opposite trend with 3.33 fold increase in the water compared to sediments level. These results are in agreement with and supported by the results obtained by (Abo El-Amayem *et al.*, 1979) where levels of the detected organochlorine compounds in the sediments were much higher than those detected in the water. The tendency of chlorinated pesticides to be accumulated in the sediments could be attributed to their high persistence and low water solubilities (Saleh *et al.*, 1980). Thus they tend to be uptaken by aquatic organisms; bound to suspended particulate and colloidal matter and adsorbed onto sediments particles. So sediments act as a reservoir for such pollutants in the aquatic environments and could be very important source of

organochlorine compounds to the overlying water depending on microbial action.

Comparing residue levels of the detected chlorinated pesticides in the water and sediments of L. Mariut with previous studies confirmed remarkable increases in their levels with time with few exceptions. For example, Lindane and P,P'-DDT were detected during winter 1979 as 0.089 ppm respectively compared to 11.276 and 0.04312 ppm respectively during autumn 1996. This means 126.7 fold increase for Lindane and 0.48 fold decrease in the level of P,P'-DDT.

In the water, levels of lindane, P,P'-DDT were monitored in L. Mariut during 78-79 by (Abo El-Amayem *et al.*, 1979; Saad *et al.*, 1982). Levels of these compounds were approximately 0.00196 and 0.00013 ppm respectively compared to 2.52 and 0.014 ppm for lindane and P,P'-DDT respectively in the present study. This indicated a highly significant increase estimated 1285.7 and 107.7 folds increase in the levels of these compounds respectively. These huge levels of the very toxic and highly persistent chlorinated pesticides detected in L. Mariut water and sediments far exceeding the environmental limits of such compounds in surface water of 0.00005 ppm recommended by federal Committee of water Quality Criteria. Moreover, they are on the other extreme with the statement by (Belluck, 1981) who reported that concentration of chlorinated pesticides in water is usually in ppt range. Not only the levels of the detected pesticide were varied but also their types indicating a change in the applied compounds. For example, (Macklad *et al.*, 1984b), reported that HCH, DDE, DDD and DDT were the major detectable chlorinated pesticides in fish samples from L. Mariut which indicated their availability in the surrounding water. While, during 1985-86, HCH, DDE, Heatachlor epoxide were the major detected compounds (Macklad *et al.*, 1990). During the present study lindane, aldrin, P,P'-DDT and endrin were the major detected chlorinated insecticides in both the water and sediments of L. Mariut.

Identification of bacterial isolates: Table 3 illustrates the bacterial taxa identified during the present study. Eight morphologically different bacterial isolates were collected and isolated from sediments at the different sites. Identification of these isolates confirmed that they are all different species belong to the genus *Pseudomonas*. The dominance of this genus at all the sampling sites in the highly contaminated soil confirmed and reflected its high resistance not only to pesticides but also to heavy metals and other toxic chemicals, all of which find their last destination at the bottom sediment.

The mervlies resistance and superior potentiality of *Pseudomonas* for biodegradation of toxic organic pollutants and as a biosorbent of heavy metals are extensively proved by many authors (Appaiah and Karanth, 1991; Lindqvist and Enfield, 1992; Zboinska *et al.*, 1992; Barriault and Sylvestre, 1993; Yanze-Kontchou and GSchwind, 1994; Mandelbaum *et al.*, 1995; Aislabbie and Llyoed, 1995; De Souza *et al.*, 1998) and many more. Thus, the occurrence of *Pseudomonas* with different species in L. Mariut's water and sediments under such huge levels of organic pollution is expected and logical.

Biodegradation of selected organochlorine compounds: Based on their availability in the water and sediments of L. Mariut, three different compounds were selected including P,P'-DDT, endrin and lindane for biodegradation assays. Five species of *Pseudomonas* dominating the highly contaminated sediments of L. Mariut were subjected to two elevated concentrations (0.05 and 50 ppm) of the selected pesticides for different exposure times (Table 5).

At the lowest pesticides concentration (0.05 ppm): Table 4 represents the biodegradation of selected compounds at their lowest concentration by *Pseudomonas* spp. Generally all species showed high ability for biodegradation of the highly toxic and persistent compounds with removal efficiencies ranged between 39 and 100%. They also exhibited selective behavior depends on bacterial species and the organic compound. *P. pseudomallei* isolated from the highly polluted sites 2, 3 and 5 showed superior ability for biodegradation with 100% removal of all the tested compounds at a very fast rate where none of them were detected in the growth medium through out the course of the experiment. *P. paucimobilis* isolated from site 1 in the polluted zone comes next in the order of biodegradation magnitude with complete degradation and removal of the tested compounds after 2 and 3 days. At the fourth and fifth exposure days, about 60% of endrin and only traces of DDT were detected in the medium that could be a release from dead biomass. *P. aeruginosa* and *P. mailei* isolated from sites 1 and 2 respectively showed the same behavior where they needed longer intact time (5 days) with different compounds to achieve their maximum degradation (100% RE). *P. pickettii* from site 5 had high ability for degrading P,P'-DDT and lindane with a very high efficiency (100%) in a short time (2 days). While endrin was removed with high efficiency ranged between 87.5 to 97.4%. In conclusion, the identified bacteria showed excellent ability for biodegradation of the tested compounds.

At the highest pesticides concentration (50 ppm): It was very surprising that higher biodegradation efficiencies were recorded for all the tested compounds by the bacterial species at the highest tested concentration. DDT has the highest removal rate by almost the five species with almost 100% biodegradation RE followed by endrin with RE ranged between 60.5 and 100% while lindane had RE ranged between 73.3 and 100%. These results provide a very promising tool for decontaminating polluted aquatic environments from the most dangerous organic pollutants.

Many bacteria able to degrade pesticides have been isolated and identified. Among these species *Pseudomonas* exhibited high for degrading different kind of pesticides as for phenaxy compounds, triazine compounds (Mandelbaum *et al.* 1995), organophosphates (Serdar *et al.*, 1982) and carbamates (Chaudhry and Ali, 1988). Present results confirmed and supported these studies and proved that

Pseudomonas spp. are very effective bacterial species in degrading chlorinated pesticides and can be efficiently used in bioremediation processes for that purposes.

References

- Abd El-Aal, A.A., 1981. Studies on pesticide residues, occurrence of pesticides in Egyptian lakes. M.Sc. Thesis, Faculty of Agriculture, Alexandria University.
- Abo El-Amayem, M., M.A. Saad and A.H. El-Sebae, 1979. Water pollution with organochlorine pesticides in Egyptian lakes. Proceedings of the International Egypt Germinal Seminar on Environmental Protection from Hazardous Pesticides, March 24-29, 1979, Alexandria, Egypt, pp: 94-108.
- Aislabbie, J. and G.J. Lloyd, 1995. A review of bacterial degradation of pesticides. *Aust. J. Soil Res.*, 33: 925-942.
- Appaiah, K.A. and N.G.K. Karanth, 1991. Insecticide specific emulsifier production by hexachlorocyclohexane utilizing *Pseudomonas* strain. *Biotechnol. Lett.*, 13: 371-374.
- Badawy, M.J. and M.A. El-Dib, 1984. Residues of organochlorine pesticides in fish from the Egyptian delta lakes. *Environ. Int.*, 10: 3-8.
- Barriault, D. and M. Sylvestre, 1993. Factors affecting PCB degradation by an implanted bacterial strain in soil microcosms. *Can. J. Microbiol.*, 39: 594-602.
- Belluck, D.A., 1981. Pesticides in the aquatic environment. Ph.D. Thesis, University of Illinois, Urbana-Champaign.
- Chaudhry, G.R. and H.D. Ali, 1988. Bacterial metabolism of carbofuran. *Applied Environ. Microbiol.*, 54: 1414-1419.
- Cork, D.J. and J.P. Krueger, 1991. Microbial transformations of herbicides and pesticides. *Adv. Applied Microbiol.*, 36: 1-66.
- De Souza, M.L., L.P. Wackett and M.J. Sadowsky, 1998. The *atzABC* genes encoding atrazine catabolism are located on a self-transmissible plasmid in *Pseudomonas* sp. strain ADP. *Applied Environ. Microbiol.*, 64: 2323-2326.
- Duursma, E.K., M. Marchand and D. Vas, 1974. Chlorinated hydrocarbon residues in biota, sediments and water collected from the Ligurian sea (No. IAEA-163). UAEA, Activities of the International Laboratory of Marine radioactivity, 1974 report. IAEA, Monaco.
- El-Sebae, A.H., M.A. El-Amayem, I. Sharaf and M. Massoud, 1984. Factors affecting acute and chronic toxicity of chlorinated pesticides and their biomagnification in Alexandria region. Proceeding of the Med. Pol. Meeting on Toxicity and Bioaccumulation of Selected Substances in Marine Organisms, Revinj, Yugoslavia, November 5-9, 1984, FAO. UNEP.
- Ernst, W., R.C. Schaefer, R.C. Goerke and G. Eder, 1974. Preparation of marine animals for determination of PCB, DDT, DDE, HCH and HCB. *Anal. Chem.*, 46: 358-363.
- Faust, S.D. and O.M. Aly, 1964. Water pollution by organic pesticides. *J. Am. Water Works Assoc.*, 56: 267-279.
- Greene, B. and D.W. Darnall, 1998. Temperature dependence of metal ion sorption by spirulina. *Biorecovery*, 1: 27-41.
- Hill, I.R. and S.J.L. Wright, 1978. *Pesticide Microbiology*. Academic Press, London.
- Keller, C.D. and T.F. Bidleman, 1984. Collection of airborne polycyclic aromatic hydrocarbons and other organics with a glass fiber filter-polyurethane foam system. *Atmos. Environ.*, 18: 837-845.

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- Kookana, R.S. and L.A.G. Aylmore, 1994. Estimating the pollution potential of pesticides to ground water. *Soil Res.*, 32: 1141-1155.
- Le Chevallier, M.W., R.J. Seidler and T.M. Evan, 1980. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. *Applied Environ. Microbiol.*, 40: 922-930.
- Lindqvist, R. and C.G. Enfield, 1992. Biosorption of dichlorodiphenyltrichloroethane and hexachlorobenzene in groundwater and its implications for facilitated transport. *Applied Environ. Microbiol.*, 58: 2211-2218.
- Linn, D.M., T.H. Carski, M.L. Brusseau and F.H. Chang, 1993. Sorption and Degradation of Pesticides and Organic Chemicals in Soils. SSSA Special Publication No. 32, Soil Science Society of America, USA.
- MacRae, I.C., 1989. Microbial Metabolism of Pesticides and Structurally Related Compounds. In: *Reviews of Environmental Contamination and Toxicology*, Ware, G.W. (Ed.). Springer, New York, pp: 1-87.
- Macklad, F., Y. Halim, A.K.L. Sebae and M. Barakat, 1984a. Monitoring of chlorinated pesticides in fish samples from lake Mariout and Alexandria hydrodrome. *Bulletin of High Institute of Public Health*, No. 14, pp: 161.
- Macklad, F.A., K. El-Sebae and M. Barakat, 1984b. Monitoring of chlorinated pesticides in fish samples from lake Maryout and Aklexandria hydrodrome. *The Bulletin, HIPH*, 14, No. 2.
- Macklad, M.F., M. Abu El-Aamayem, S. El-Mahdy and M. Aabbas, 1990. Chlorinated insecticides levels in the irrigation and drainage syste of Alexandria region. *Comm. Sci. Dev. Res.*, 31: 83-94.
- Mandelbaum, R.T., D.L. Allan and L.P. Wackett, 1995. Isolation and characterization of a *Pseudomonas* sp. that mineralizes the s-triazine herbicide atrazine. *Applied Environ. Microbiol.*, 61: 1451-1457.
- Saad, M.A., M.A. Elamayem, A.H. El-Sebae and I.F. Sharaf, 1982. Occurrence and distribution of chemical pollutants in Lake Mariut, Egypt. *Water Air Soil Pollut.*, 17: 245-252.
- Saleh, F.Y., G.F. Lee and H.W. Wolf, 1980. Selected organic pesticides, occurrence, transformation, and removal from domestic wastewater. *J. Water Pollut. Control Fed.*, 52: 19-28.
- Senath, P.H.A., N.S. Mair and M.E. Sharpe, 1989. *Bergey's Manual of Systematic Bacteriology*. Vol. 2, Williams and Wilkins, London.
- Serdar, C.M., D.T. Gibson, D.M. Munnecke and J.H. Lancaster, 1982. Plasmid involvement in parathion hydrolysis by *Pseudomonas diminuta*. *Applied Environ. Microbiol.*, 44: 246-249.
- Sharma, R.M. and P.A. Azeez, 1988. Accumulation of copper and cobalt by blue-green algae at different temperatures. *Int. J. Environ. Anal. Chem.*, 32: 87-95.
- Somasundaram, L. and J.R. Coats, 1990. Influence of Pesticide Metabolites on the Development of Enhanced Biodegradation. In: *Enhanced Biodegradation of Pesticides in the Environment*, Rcke, K.D. and J.R. Coats (Eds.). American Chemical Society, Washington, D.C..
- WHO., 1984. *Guidelines for Drinking Water Qualit*. Vol. 2, World Health Organization, Geneva, pp: 33-39.
- Wahby, S.D., S.M. Kinawy, T.I. Tabbakh and M.A. Abdul Moneim, 1978. *Estuar. Coastal Mar. Sci.*, 7: 17-22.
- Yanze-Kontchou, C. and N. Gschwind, 1994. Mineralization of the herbicide atrazine as a carbon source by a *Pseudomonas* strain. *Applied Environ. Microbiol.*, 60: 4297-4303.
- Zboinska, E., B. Lejczak and P. Kafarski, 1992. Organophosphonate utilization by the wild-type strain of *Pseudomonas fluorescens*. *Applied Environ. Microbiol.*, 58: 2993-2999.