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Determination of Organochlorine Pesticide Residues in Aquatic Systems and Organisms in Upper Sakarya Basin, Türkiye

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Pesticides are poisons which are used to control or destroy unwanted organisms, especially those which have economic implications. Since they are intrinsically biocidal they are particularly important when they enter flowing waters in significant quantities. Aquatic ecosystems may be contaminated by organochlorine insecticides used in crop protection. These pesticides reach aquatic ecosystems by direct application, spray drift, aerial spraying, erosion and run off from agricultural land, by discharge of effluents from factories and in sewage. Organochlorine insecticides are now less widely used than previously, because of a number of disadvantages including environmental persistence, bioaccumulation and their toxic action upon the nervous systems (Haider and Inbaraj 1986; Hellawell 1988). Also, entry of these pesticides into the aquatic ecosystem will adversely affect many non target organisms including fish and birds (Dutta et al. 1993; Ayas et al.1996). These effects may be acute, resulting in mass mortality or chronic, involving changes in survival, growth and reproduction (Kocan and Landolt 1989). Many researchers have reported that organochlorine pesticides and their degradation products have more toxic effects on animals (Barlas 1994; 1997), and played important role in the population declines of waterbirds (Gilbertson 1988; Fox et al. 1991). Usage of OC pesticides have been prohibited since 1980 in Türkiye, but it was obvious that Sakarya river or basin was contaminated by a variety of pesticides.

The present work was carried out to determine some organochlorine pesticide residues in water, sediment and fish tissue, especially in adipose tissue in upper Sakarya river basin.

MATERIALS AND METHODS

After a preliminary study, eight stations were selected in upper Sakarya river, Türkiye to determine some organochlorine pesticide residues in water and sediment samples, whereas five stations were selected for the fish samples.Water and sediment samples were collected in October 1995 and February, May, and August of 1996 from the stations (1, 2, 3, 4, 5, 6, 7, 8) indicated in figure 1. These samples were collected 0.5 m below the water surface in 1 L precleaned glass bottles and kept at -18 °C. The upper 10 cm of sediments were collected with Ekman sampler in a sterile 250 mL glass bottles. Four *Cyprinus carpio* were collected from each of five stations (1, 3, 4, 5, 6). As much as it was possible, similar sized fish samples were

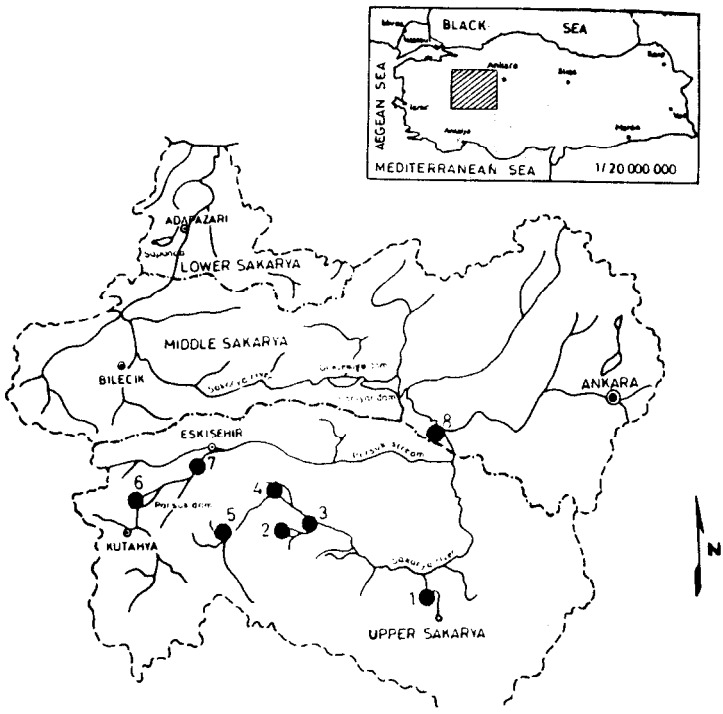

Figure 1. Map showing the location of the study area in Sakarya basin Türkiye, and sampling stations.

collected from the selected stations. Each fish was measured (cm), tagged, and placed on ice and later frozen until they were analysed. Water, sediment and fish samples were taken to laboratory in Ankara. Organochlorine extraction of water samples was carried out according to Zweig method (1972). In this method, 900-950 mL water sample was extracted with 25 mL n-hexane into a 1 L separatory funnel for three times. The combined extracts were dried over anhydrous sodium sulfate (0.5g). The extracts were evaporated at 50 °C to 1-2 mL for the determination of water samples. Sediment samples were extracted with n-hexane using Soxhlet equipment for 4 hours (Sodergren and Wartiovaara 1988). At the laboratory, each fish was weighed (g) and the skin was removed with steel knife and steel tweezers from muscle, in the middle of the fish. Then a sub sample was cut from the muscle of about 10 g and this sample was pureed in a Waring blender. These procedures were done according to manual of methods in aquatic environment research by FAO (1983). The pureed fish samples were extracted with petroleum benzene in 150 mL using Soxhlet equipment for 8 hours. The extracts were evaporated using a rotary evaporator, at 50 °C to constant weight for the determination of extractable lipid. Mixture standards of organochlorine pesticides were used for identification and measurements in GC. The extract was cleaned on a partially deactivated Florosil column. Organochlorine pesticides and metabolites (including o,p'DDT, p,p'DDE, p,p'DDD, o,p'DDD, heptachlor, heptachlor epoxide, aldrin, dieldrin endrin α-BHC and β-BHC) were analysed with a Chrompac-SFC Instrument 138 A with a Nickel Electron Capture detector automatic sampler, digital processor and 4% SSE- 30/60 % QF-capillary column. The column temperatures were programmed at 270 °C.

Nitrogen at a flow rate of 40 mL/mm was used as a carrier gas. Duplicate injections (0.8 µL) of each sample were analysed. All residues are expressed as µg /g. Minimum, maximum and geometric means were used to express residue levels. According to this method, the detection limit is 0.0001 µg /g. Values below detection limits were assigned as a Not-Detectable (ND).

Also, bioconcentration factors of these pesticides were calculated according to Branson et al. (1975) method. A student t-test was used statistically analysis of organochlorine pesticide residues in different stations and seasons (Sümbüllüoglu and Sümbüllüoglu 1987). The 0.05 level of significance for probability was used as the criterion of statistical significance.

RESULTS AND DISCUSSIONS

Organochlorine pesticides were the first synthetic insecticides to be developed and of these, DDT and BHC are probably the best known. Following their introduction in 1940s, they were used widely with considerable benefits for human health through the destruction of vectors of disease and protection of crops. Widespread and sometimes indiscriminate use of these insecticides, largely because of their low acute toxicity to man and their very powerful effects on insect pests and vectors, led to what proved to be serious environmental problems.

In this study, analyses were carried out on samples from different localities and organisms in upper Sakarya basin. Occurrence and concentrations of organochlorines varied among stations and seasons. Organochlorine residues in water samples were shown in Table 1. In October organochlorine concentrations in water sample were generally higher than other seasons (February, May and August) especially β-BHC, aldrin endrin heptachlor, DDT as their metabolites (DDE, DDD, heptachlor epoxide, dieldrin). Also, DDE is the compound that appeared at highest concentrations in August (Table 1). The average amount of extractable DDE in water samples was 1.117 µg/g (range ND-3.661 µg/g mean) in August. OC pesticide residues were measured very high quantities than their solubility in water. These high quantities may be due to high level of turbidity and other particulate material in water because the water samples were extracted without filtration. Lindane was observed in water samples and sediments collected in October but not afterwards. In sediment samples, all of the OC residue levels were detected higher, from the water quality criteria for protection of the fresh water fish and aquatic life. Especially heptachlor epoxide (7.892 µg/g mean in October), α-BHC (2.551µg/g mean in August), dieldrin endrin o,p'DDT, p,p'DDT, o,p'DDD, p,pDDE were detected at high levels in sediment samples than water and fish samples (Table 2).

In fish samples, DDT and it's metabolites p,p'DDE, o,p'DDT, p,p'DDT, p,p'DDD, o,p'DDD (mean concentrations ranging from 2.454, 1.74, 1.474, 1.262, 1.199 µg /g mean, respectively) were detected at high levels in October. Also, heptachlor epoxide which is the degradation product was found greater in adipose tissue in the same mounth (mean concentrations 3.635 µg/g). These levels were significantly higher than in fish collected there in February, May and August. In February

dieldrin heptachlor epoxide and o,p'DDT residue level in adipose tissue (mean concentration 1.351µg/g, 2.412 µg/g and 1.295 µg/g respectively) were established at higher level than in May and August. The bioconcentration factors of these pesticides were shown in Table 3.

Table 1. Seasonal organochlorine residues (µg /g wet weight) in water samples from upper Sakarya river basin, between October 1995 to September 1996.

Organochlorine Pesticides	October 1995 (min-max) mean n: 8	February 1996 (min-max) mean n: 8	May 1996 (min-max) mean n: 8	August 1996 (min-max) mean n: 8
α - BHC	ND-1.816 0.226	ND-5.523 1.558	ND-4.300 1.878	ND-4.555 1.877
β - BHC	ND-1.656 0.298	ND	ND	ND
Lindane	ND-1.109 0.183	ND	ND	ND
Aldrin	ND-0.871 0.239	ND	N.D.-0.745 0.093	ND
Dieldrin	ND-5.533 1.346	ND	ND-0.092 0.045	ND-0.618 0.117
Endrin	0.26.-7.964 2.738	ND-0.272 0.092	ND-0.53 0.162	ND-0.418 0.063
Heptachlor	ND-4.44 2.555	ND	ND-2.002 0.325	ND
H.epoxide	ND-1.674 0.441	ND	ND-0.103 0.112	ND
$_{O,P'}$ DDT	ND-4.808 1.013	ND-0.185 0.068	ND-0.373 0.096	ND-0.014 0.001
$_{P,P'}$ DDT	ND -1.93 1.879	N.D.-0.157 0.069	ND-0.826 0.175	ND-0.460 0.086
$_{O,P'}$ DDD	0.177-6.21 1.765	ND -0.272 0.093	ND -0.549 0.168	ND
$_{P,P'}$ DDD	ND -6.219 1.676	ND -0.182 0.071	ND -0.126 0.04	ND- 0.338 0.042
$_{P,P'}$ DDE	ND-2.396 0.825	ND	ND-0.745 0.382	ND-3.661 1.117

ND: Not Detectable
n: number of samples

In this study, all of the organochlorine pesticide residues were detected at a higher degree as stations 6, 7 and 8 than others, because these areas consist in many pollutant factors such as discharge of effluents from factories, agricultural and industrial wastes. Five different organochlorine pesticides and their degradation products were detected in sediment, water and fish tissue in upper Sakarya basin. It was established that dieldrin was greater than aldrin and heptachlor epoxide was

greater than heptachlor. This is a usual situation, because, heptachlor is metabolised to heptachlor epoxide, isodrin is metabolically converted to endrin and aldrin is converted to its enoxide analogue dieldrin by mammals, soil microorganisms, plants and insects (Matsumura 1985). Although, in this study aldrin and heptachlor were measured high level in fish tissue than their converted products. Generally the ratio of dieldrin to aldrin in fish is 10:1, but cath fish having 1440 µg/kg dieldrin, 0.10 µg /kg aldrin was detected (Murty 1985). These results support our findings.

Table 2. Seasonal organochlorine residues (µg/g wet weight) in sediment samples from upper Sakarya river basin between October 1995 to September 1996.

Organochlorine Pesticides	October 1995 (min-max) mean n: 8	February 1996 (min-max) mean n: 8	May 1996 (min-max) mean n: 8	August 1996 (min-max) mean n: 8
α - BHC	ND-0.618 0.269	ND -4.818 1.778	ND -3.648 1.943	ND-3.395 2.551
β - BHC	0.31.-0.495 0.1	ND -0.429 0.061	ND -0.278 0.034	ND -1.929 0.385
Lindane	0.539-2.24 0.728	ND	ND	ND
Aldrin	ND -0.846 0.13	ND -0.702 0.1	ND -2.087 0.26	ND -1.292 0.351
Dieldrin	ND -2.555 1.893	ND-1.065 0.228	ND -0.281 0.135	ND -0.896 0.153
Endrin	0.01.-6.357 1.603	ND -3.457 0.511	ND -5.411 1.750	ND -0.286 0.169
Heptachlor	ND-0.903 0.265	ND-4.254 1.076	ND-3.754 0.622	ND
H.epoxide	ND-14.953 7.892	ND-15.604 2.501	ND-14.092 4.521	ND -1.442 1.31
$_{O,P'}$ DDT	ND -2.676 0.239	ND -1.846 0.199	ND	ND -5.374 0.703
$_{P,P'}$ DDT	0.46-2.81 1.228	ND -1.128 0.273	ND -0.629 0.22	0.279-1.35 0.656
$_{O,P'}$ DDD	ND -2.997 1.142	ND-0.946 0.253	ND -0.852 0.159	ND -0.389 0.168
$_{P,P'}$ DDD	ND-1.666 0.992	ND -2.085 0.386	ND -0.622 0.151	ND -0.249 0.102
$_{P,P'}$ DDE	0.92-2.086 1.925	ND -2.135 0.966	ND-2.070 0.581	0.446-1.45 1.240

ND: Not Detectable
n: number of animals

In this study, the average amount of DDE was established to higher degree than other DDT metabolites in sediment and fish adipose tissue except February and August. On the contrary, DDT was found greater concentrations than DDE in water samples except in August. Many authors have concluded that p,p'DDE was detected

to a higher degree than DDT in soil sea bird tissue and their eggs (Barret et al. 1985; Hernandez et al. 1988; Fasola et al. 1987). However, some researchers have concluded that DDT, aldrin and heptachlor have been found in higher quantities than their converted products (Ayas et al. 1996), although the usual route of metabolism of DDT by insects appears to be through DDE. Unfortunately the residue levels of some DDT metabolites seem to be in very unusual proportions, indicating that standarts may have been mixed up. Also, these results may be due to still using OC pesticides and other environmental factors in the study area. In the sediments of Lake Michigan, Choi and Chen(1976) reported that p,p'DDE constituted 61 to 71%, and p,p'DDD formed 10-16.6% of the total DDT.

Table 3. Seasonal organochlorine residues (µg/g wet weight) in fish (*Cyprinus carpio*) tissue from upper Sakarya river basin between October 1995 to September 1996.

Organochlorine Pesticides	October 1995 (min-max) mean n: 20	February 1996 (min-max) mean n: 20	May 1996 (min-max) mean n: 20	August 1996 (min-max) mean n: 20	BCF
α - BHC	ND-1.293	ND.-0.914	ND-0.693	0.04-3.502	
	0.387	0.217	0.234	1.713	0.721
β - BHC	ND -0.775	ND	ND -0.293	ND -1.286	
	0.216		0.095	0.257	0.883
Lindane	ND-0.35	ND	ND	ND	
	0.07				0.095
Aldrin	ND -1.571	ND	ND	ND	
	0.254				0.265
Dieldrin	ND -3.871	0.13-2.355	ND -0.2	ND-0.079	
	1.409	1.351	0.04	0.015	0.739
Endrin	ND-8.122	ND -0.49	ND -0.128	ND	
	1.884	0.032	0.049		0.525
Heptachlor	ND -1.184	ND	ND -1.450	ND -7.686	
	0.332		0.417	1.537	1.06
H.epoxide	1.17-7.686	ND -4.827	0.09-0.978	ND -2.384	
	3.635	2.412	0.344	0.793	3.629
$_{O,P'}$ DDT	ND -4.805	ND -5.193	ND	ND -1.533	
	1.74	1.298		0.474	6.384
$_{P,P'}$ DDT	0.515-3.74	ND -0.706	ND -0.295	1.214-3.82	
	1.474	0.18	0.014	2.104	6.848
$_{O,P'}$ DDD	ND-3.547	ND -0.546	ND	ND -1.274	
	1.199	0.146		0.797	0.761
$_{P,P'}$ DDD	ND -3.526	ND -1.587	ND -0.229	ND -1.526	
	1.262	0.604	0.074	0.529	5.924
$_{P,P'}$ DDE	ND -5.027	ND -0.94	ND	ND -.1.562	
	2.454	0.218		1.225	1.072

ND: Not Detectable
n: number of samples (four samples were analysed from each stations)
BCF=Bioconcentration factor

In general, insecticide adsorption to the soil or sediment was inversely related to the water solubility of that compound. For example, DDT has very low solubility,

thereby being more strongly adsorbed than dieldrin. The greater the water solubility, the greater the mobility. For instance, lindane, which has a high solubility, is more mobile than many other OC compounds. Also, endrin aldrin, and dieldrin are slightly more mobile than DDT as they are more soluble than DDT (Murty 1985). Due to more solubility of lindane, it was detected only in October at a low degree and it was not observed in other mounts. In this study, organochlorine concentrations in water, sediment and fish adipose tissue samples were detected at a higher degree in October and in August than other seasons. Seasonal variation in residue concentration is related to the season of application on land, and the transport to the environment, as well as the condition of fish, extent of feeding, stage of reproduction, and the like. Also, the level of pesticide residues depends on water temperature, pH, organic matter content in sediments, load of particulate matter and depth of the water body. In upper Sakarya river, the temperature was measured very high, especially at stations 2 and 3 because, the area of these stations were comprised within the boundary of the origin of the Sakarya river consisting of hydrothermal water (Barlas 1999). Organochlorine insecticides have a high degree of persistence in soil and sediment. The toxicity of DDT to fish is quite high, the 96h LC_{50} values range from about 1-30 µg/L. In order to protect freshwater aquatic life, with regard to bioaccumulation potential, the EPA criterion is 0.001 µg/L (Train 1979). Conditioned reflexes, learning behaviour in fish and behaviour of invertebrates are all affected by exposure to DDT (McNichel and Mackay 1975). The toxicity of BHC is similar to that of DDT but its stability is less, making it less hazardous to wildlife.

Results of this study, α-BHC and β-BHC residues were detected higher in water, sediment and fish adipose tissue. Similarly, heptachlor epoxide and DDT metabolites, especially DDD and DDE residues were detected higher in fish adipose tissue. It was seen that OCs were highest on the top of the food chain in Sakarya river.Toxic substances which occur at such low concentrations in water at to pose no threat through direct toxicity, may, if absorbed, be accumulated in food chains and affect aquatic organisms and their predators. This bioaccumulation is often observed with pesticides. In this study, bioconcentration factor was calculated. This factor depends on chemical structure of compound and its solubility in lipids and water. It was seen that OC pesticides especially, DDT metabolites (DDD, DDE) were accumulated in fish adipose tissue. It is known that OCs have lipophilic properties for this reason, pass preferentially from water to fat tissue. OCs could be effective on reproductive success to bird eggs (Ohlendorf and Marois 1990).

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