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CHLORINATED HYDROCARBONS (PESTICIDES AND PCBS) IN SOME MARINE ORGANISMS AND SEDIMENTS IN AN EXPERIMENTALLY POLLUTED ECOSYSTEM IN THE LAGOON OF STRUNJAN (NORTH ADRIATIC) AND ITS SURROUNDINGS

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INTRODUCTION :

In order to evaluate the effects of municipal waste-waters on communities, one of two experimental basins, each of 63 m^2 surface and with average depth of 0.6 m, in the lagoon of Strunjan (North Adriatic) was treated with 300 l of primary settled sewage daily (about 1,5 population equivalent), transported from the sewage system of Piran, while the other was kept clean as a control (Fig. 1). The average composition of the seawage is presented in Table 1.

Table 1 : Average composition of primary settled sewage from the town of Piran

Organic-N	6,5 mg/l
NH_4-N	7,2
NO_3-N	0,1
Organic-P	0,3
Ortophosphate	12,5
Particulate-N	9,4
Particulate-P	11,4
Particulate-C	23,0

In this work the organochlorine content, such as pesticides and PCBs, in mussels (*Mytilus galloprovincialis*), crabs (*Carcinus mediterraneus*), and holothuria (*Holothuria* sp.) was analysed. In addition to the available living organisms in both basins, chlorinated hydrocarbon content in the sediments of both basins, as well as those in their surroundings was determined (Fig. 1). The purpose of this work was also to determine the level of chlorinated hydrocarbons in sediments of the surroundings, so the analyses were extended to sediments in the area within about 14 nautical miles offshore (Fig. 1). Only a qualitative analysis of the particulate matter in the sewage was carried out.

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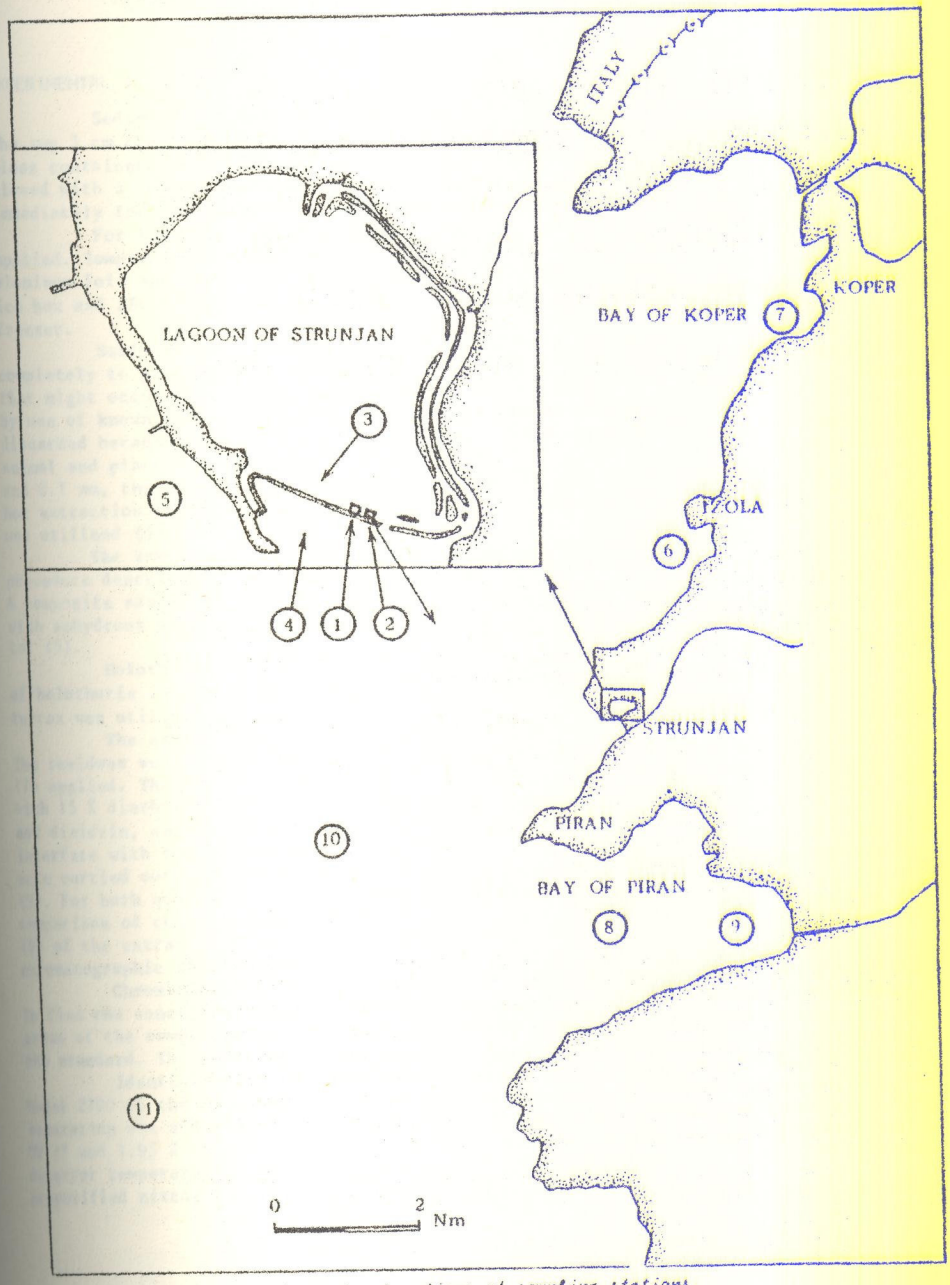

Figure 1 - Locations of sampling stations.

EXPERIMENTAL :

Sediment samples were taken with a gravity core sampler (i.d. 35 mm). The top 5 cm fraction was taken for analytical purposes, and was transferred to a glass container. After covering the top with aluminum foil, the container was closed with a screw cap and placed in an ice box. If the samples were not treated immediately for analysis, they were stored frozen at -25°C.

For the sampling of mussels, crabs and holothuria the same procedure was applied. Samples were taken, washed with normal sea-water, measured, wraped in aluminum foil and placed in polyethylene containers. The samples were kept in an ice box and after transporting them to the laboratory, they were transferred to a freezer.

Sediment samples were dried at 110°C, taking care not to dry them completely to prevent some chemical reactions and/or transformations of pesticides that might occur (2). After homogenization of the sediments, they were fractionated by use of known aperture sieves. The fraction which was greater than 0.4 mm was discarded because most of this fraction was a mixture of small-fish and other animal and plant residues. The rest was divided into two fractions : on between 0.4 and 0.1 mm, the second less than 0.1 mm. For the extraction of the sediments, the hot extraction technique, which is a well established method for soil analysis, was utilized (4).

The sample preparation of mussels and crabs was done by following the procedure described elsewhere (5), and only the edible parts were used for analysis. A composite sample of each species was prepared and after homogenization mixed with anhydrous Na_2SO_4. The samples were extracted with hexane in a Soxhlet extractor (5).

Holothuria were washed with dilute sulfuric acid (2 %). The homogenization of holothuria is rather difficult, and thus the cold extraction method with ultraturrax was utilized (7).

The extracts from all samples were concentrated in a rotavapor to dryness. The residues were dissolved in hot hexane and the FDA florisil clean-up procedure (7) applied. The florisil column was eluted first with 6 % (by volume) and then with 15 % diethyl ether in petrol ether. For the confirmation of aldrin, total DDT and dieldrin, and to be sure that probably naturally occurring compounds which interfere with the PCB chromatographic pattern were eliminated, hydrolysis reactions were carried out. For this purpose ethanolic hydrolysis in 2 % KOH was performed (5). For both qualitative and quantitative determination of PCBs (Fig. 2), the comparison of chromatograms was done mostly after hydrolysis and acidic oxidation (8) of the extracts. This was necessary because pesticides interfere with the PCB chromatographic pattern (3).

Chromatographic peaks were confirmed by comparison with standards (Fig. 2). To find the concentrations of the chlorinated hydrocarbons in the samples, the areas of the sample peaks were compared to the area of the corresponding peaks in the standard. The areas were calculated by the triangulation method.

Identification of chlorinated hydrocarbons was accomplished on a Varian Model 2700 GL chromatograph equipped with an electron capture detector (Ni^{63}). The separation was achieved on a 10 foot x 2 mm coiled glass column packed with 1.5 % OV 17 and 1.95 % OV 210 on Varaport 30 (80 - 100 mesh). The injector, column and detector temperatures were 250°, 230° and 280°C, respectively. The carrier gas was prepurified nitrogen with a flow rate of 20 ml/min.

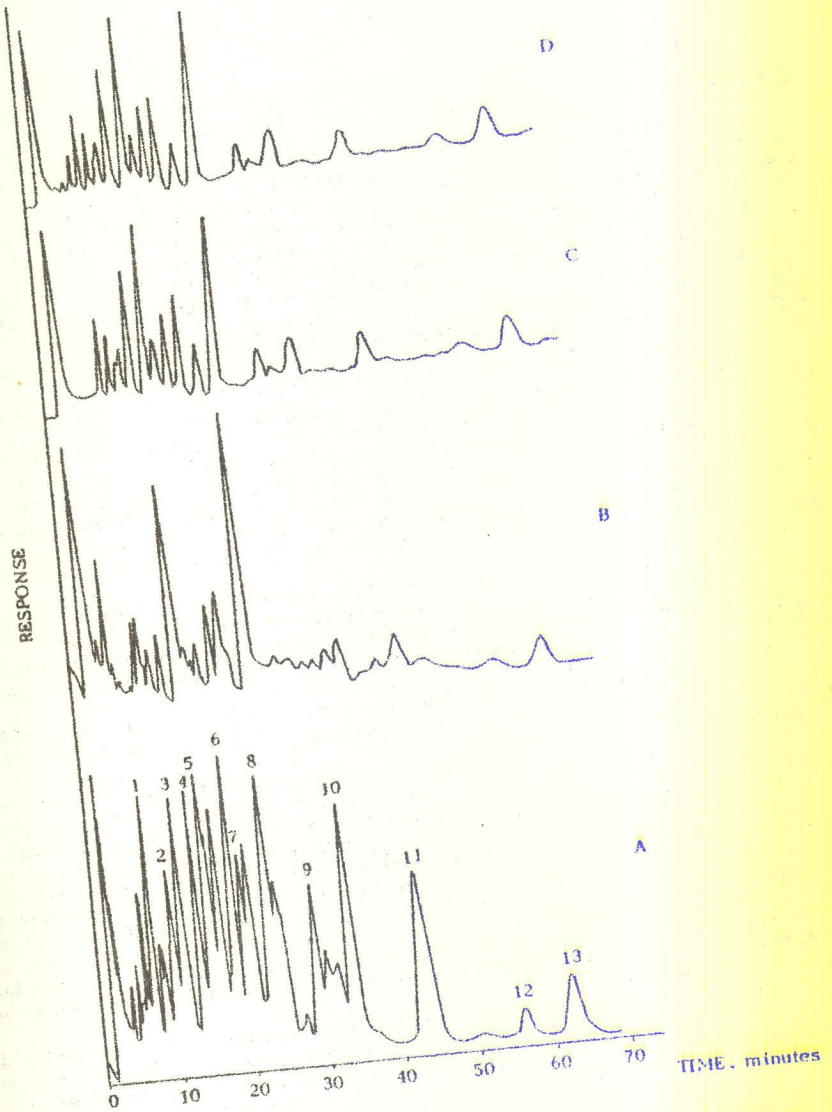

Figure 2 - Gas chromatograms :
A. Standard mixture of pesticides and PCBs,
B. 6 % diethylether-petroleum ether fraction obtained from florisil clean up,
C. The same fraction after alkaline hydrolysis,
D. After hydrolysis and acidic oxidation.

The retention time of organochlorine pesticides are numbered 1 to 13 :

1, aldrin
2, PCB
3, o,p'-DDE
4, p,p'-DDE
5, dieldrin - o,p'-DDD
6, o,p'-DDT
7, p,p'-DDD
8, p,p'-DDT
9, 10, 11, 12 and 13, PCB

RESULTS AND DISCUSSION :

The concentrations of chlorinated hydrocarbons in the sediment of the polluted basin are evidently much higher than in the clean one (Table 2). The sum of the DDT and DDT derivatives is approximately three times higher in the polluted than the values obtained in the clean basin. The same is true for PCBs. About the same trends were found also for organisms (Table 2), actually for deposit-feeding holothurians and filtrating mussels, while for the fast moving crab who is predator and scavenger this was not the case.

Table 2 : Chlorinated Hydrocarbon content of marine organisms (ng/g fresh tissue)

Chlorinated Hydrocarbon	Crab		Mussel		Holothurai	
	Clean basin	Polluted basin	Clean basin	Polluted basin	Clean basin	Polluted basin
α-HCH	5	7	15	10	11	14
β-HCH	2	7	3	30	-	8
γ-HCH	21	34	-	-	-	63
Aldrin	16	35	-	25	12	60
p,p'-DDE	42	39	17	80	-	28
p,p'-DDD	40	57	20	41	3	28
p,p'-DDT	20	32	10	70	-	25
1221-1242(a)	8	50	21	28	4	31
1254-1260(b)	17	23	11	29	18	20
$\frac{\Sigma DDT}{\Sigma PCB}$	4	2	1	6.5	0.1	1.4

(a) The given values are the sum of Aroclor 1221 and 1242
(b) The given values are the sum of Aroclor 1254 and 1260.

The high concentration of chlorinated hydrocarbon in the sediment of the polluted basin is due to the seawage discharge, since such a high value was not found at the other stations. The chlorinated hydrocarbon content of sediments from sampling stations 3. - 11. (Table 3) did not show great differences, but there is an increase from the open sea to the near-shore area as expected. The results from station 7. were the highest. These samples were taken in the Bay of Koper, which shore is the location of the largest industrial centre of the area. The waste water of the town and industrial effluents which enter the Bay are the main sources of the high concentrations of chlorinated hydrocarbons in marine sediments confirming that sewage discharge is one of the major route of entry for organo-chlorine pesticides to the nearshore marine environment. Our results for DDT and PCB concentrations in sediments are higher than those reported by Picer et al (8) in the South Adriatic offshore of Dubrovnik, and in the North Adriatic offshore of Porec.

From Table 3 another conclusion on the effect of particle size can be drawn. The pesticide and PCB content of the smaller size sediment samples was higher than of the larger ones. This is expected since total surface area increases with decreasing size which consequantly causes an increase in the adsorption of chlorinated hydrocarbons (1).

Table 3 : Chlorinated Hydrocarbon content of Sediments (ng/g dry weight)

Sampling Point (a)	Particle Size	Lindane(b)	Aldrin	ΣDDT	ΣPCB	$\frac{\Sigma DDT}{\Sigma PCB}$
1(Polluted)	Coarse(c)	18	55	104	97	1
1(Polluted)	Fine (d)	37	83	228	50	4.6
2 (Clean)	Coarse	10	0.5	0.4	1	-
2 (Clean)	Fine	28	5	24	44	0.6
3	Coarse	78	18	7	1	-
3	Fine	90	33	33	8	4
4	Coarse	27	6	17	-	-
4	Fine	42	14	25	-	-
5	Fine	18	20	57	67	0.9
6	Fine	5	11	50	96	0.5
7(e)	Fine	14	57	110	201	0.6
8	Fine	32	85	52	37	1.5
9	Fine	23	78	48	39	1.3
10(f)	Fine	21	40	29	55	0.5
11	Fine	-	30	27	43	0.7

(a) The sampling points are given in Fig. 1
(b) The given values are the sum of the α-,β-,γ-, and δ- isomers
(c) Particle size between 0.4 and 0.1 mm
(d) Particle size equal or less than 0.1 mm
(e) More than 90 % of the sediment has a size less than 0.1 mm
(f) Shell fish waste content of the sediments obtained from sampling points 8, 10 and 11 was approximately 30 %

Aldrin distribution in the samples is random. This randomness can be explained as originating from local sources, since aldrin is used for agricultural purposes. The results for dieldrin are not included in the tables because it was not possible to identify them owing to the overlap of o,p'-DDD peak with dieldrin (Fig. 2), which both disappear on subsequent oxidation.

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SUMMARY

Chlorinated hydrocarbons (pesticides and PCBs) were analysed in certain species and sediments in two experimental basins located in the lagoon of Strunjan (North Adriatic). One of the basins was daily regularly receiving sewage transported from the town of Piran, while the other basin was serving as an undisturbed control. The chlorinated hydrocarbon content in crabs in both basins was the same. Concentrations of the same residues in mussels, holothuria and sediment from the polluted basin were found to be at least twice that from the clean one.

Chlorinated hydrocarbons were analysed in the sediments all along Slovenian coast, too (14 Nm of Eastern part of the Gulf of Triest, Yugoslavia). A gradual increase in organochlorine content was found in the sediments from the open sea towards the coast. The highest concentrations were detected in the Gulf of Koper in the vicinity of local sewage discharge.

RESUME

Les hydrocarbures chlorés (les pesticides et polychlorobiphényles) ont été analysés sur certaines espèces et sédiments dans deux bassins expérimentaux situés dans la lagune de Strunjan (Adriatique Nord). Un des deux bassins a reçu chaque jour 300 litres d'eau usée transportée de Piran, l'autre bassin a servi de contrôle. La teneur en hydrocarbures chlorés des crabes des deux bassins est identique (dans les limites des erreurs expérimentales). Les concentrations de ces mêmes substances dans les moules, les holothuries et les sédiments du bassin pollué sont deux fois plus fortes que dans le bassin de contrôle.

Les analyses d'hydrocarbures chlorés ont été effectuées de même sur les sédiments le long de la côte de Slovénie (sur 14 Nm de la partie Est du Golfe de Trieste). On a remarqué une augmentation progressive de la teneur en organochlorés dans les sédiments de la haute mer vers la côte. Les plus hautes concentrations ont été trouvées dans le Golfe de Koper près de l'émissaire local des eaux usées.

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