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ORGANOCHLORINE PESTICIDES (OCS) AND POLYCHLORINATED BIPHENYLS (PCBS) IN SEDIMENTS AND CRABS (*Chasmagnathus granulata,* DANA, 1851) FROM MANGROVES OF GUANABARA BAY, RIO DE JANEIRO STATE, BRAZIL

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Abstract

Organochlorinated compounds, seven indicator PCB congeners, DDT and its main metabolites, were determined in sediment and crab (*Chasmagnathus granulata*) samples collected from mangrove areas near Rio de Janeiro, Brazil. Samples were analysed according to the FAO/SIDA protocols using continuous non-polar solvent extraction and a conventional GC-ECD apparatus. The highest levels of total PCB congeners and total DDT metabolites in sediments (184.16 and 37.40 ng.g⁻¹d.w. respectively) and crab eggs (570.62 and 98.22 ng.g⁻¹d.w. respectively) were found at impacted mangroves. The higher PCB congeners than DDT metabolites levels suggesting a stronger industrial impact in this area. The results indicate that the population density of crab is negatively affected by sediment contamination that is reflected basically by the organochlorine content in the female eggs. The organochlorine concentration in eggs is more significant to evaluate or estimate an impact of these pollutants upon *C. granulata* population than the organochlorine concentration in sediment samples.

Introduction

Organochlorinated compounds (insecticides and PCB) have some important features as their hard biological decomposition, high lipid solubility and moderate chronic and acute toxicities. Once in the environment, these characteristics combined to low water solubility and easy

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adsorption to organic matter lead to bioaccumulation in fat tissues of living organisms with subsequent trofic magnification (Connell, 1987; Fasola et al., 1998; Monirith, 2002).

Among chlorinated pesticides, DDT is the most important one, being decisive in both agricultural pest and disease vector insects controls until the appearance of resistant lineages. DDT presents adverse effects on reproduction due to its estrogen agonistic and neurotoxic activities (WHO, 1979).

PCB consist of isomers mixtures with different chlorine atoms in their molecules. There are up to 209 PCB compounds and their chlorination degree is determinative in toxicity and biological activity. PCB are rapidly absorbed by gut mucosa and stocked in fat tissue (Torres, 1998).

Mangroves are one of the most important coastal tropical ecosystems due to the fact that are nursery and development area for many aquatic organisms and feed area for mammals, birds, reptiles and also for man. They are considered transition ecosystems (ecotones) between terrestrial, marine and freshwater environments. Normally, mangroves develop in low hydrodynamic locals, like the intertidal zone of estuaries and rivers until the influence of saline water could be detected. The mangrove flora presents tolerance to acid pH and high salinity levels, with mechanisms to salt excretion by leaves.

The hydrodynamic of these ecosystems favors the sediment deposition from land, especially the fine sedimentary fractions. In association to fine particles, can also occur organic matter deposition which, in association with water saturation of mangrove soil, cause oxygen concentration depletion. By this reason, many mangrove species present aerial roots for atmospheric oxygen absorption. This organic matter - accumulated and produced in the mangroves - give to this ecosystem an important role in marine ecosystem production, once mangrove can play a role of marine water enrichment by organic matter exportation. In consequence of these hydrodynamic conditions, mangroves can also concentrate pollutants, including here the PCBs and DDT (Peters *et al.*, 1997).

Chasmagnathus granulata Dana, 1851 (Figure 1) is a largely distributed crab species on the SW Atlantic Coast, ranging from Rio de Janeiro State until Argentina, in San Matias Gulf (Boschi, 1964; Melo, 1996 and 1998). Inhabits soft sandy or muddy estuary bottoms in which excavates, building burrows in the upper intertidal zone and can forages to the lower intertidal zone when feeding (Bond-Buckup *et al.*, 1991).

C. granulata is omnivorous and detritivorous, feeding from sediment (D'Incao *et al.*, 1990) and are perfectly adapted to aquatic or terrestrial habitats. Breeding period take place from October to February in Argentina (López & Rodriguez, 1998), but in the studied area ovigerous females were seen during all the year. Giménez & Torres (2002), point out that the first zoeal stage hatches in estuarine waters. Then, the zoea is carried to ocean, where develops into the subsequent zoeal stages and a Megalopa (Boschi *et al.*, 1967; Pestana & Ostrenski, 1995). After, the Megalopa enters the estuaries reaching the mangroves where completes the development and passes to live in the soil (Anger et al., 1994). Menone *et al.* (2004) studied the influence of *C. granulata* upon organochlorines in the sediment, concluding that this crab, with its excavating activity, is an important agent in bioturbation, causing a differential accumulation of organochlorines in salt marshes sediment in Argentina.

Organochlorine compounds were measured in tidal sediments and crab eggs (*Chasmagnathus granulata*, Dana, 1851) collected from mangroves located at Guanabara Bay (22°57′ and 22° 41′ S; 43°00′ W), Rio de Janeiro, Brazil. Two areas were sampled, one very industrialized and another inside a protected natural reservoir. Organochlorines are a class of toxic compounds included in the persistent toxic substances category that may exert detrimental effects in the

environment. They are considered priority pollutants and are included in the 1984 Stockholm Convention of the United Nations. In Brazil, the first restrictions on organochlorine pesticides began in the early 1970's, but final prohibition took effect in 1985. DDT was used until 1997 in vector control. Polychlorinated biphenyls were never produced in Brazil, but most of the transformer oils already in use may contain PCBs. Their introduction in the country was formally restricted in 1986.

In this work different classes of organochlorines were measured in tidal sediments and in crab eggs collected from two mangrove areas in Guanabara Bay, located near Rio de Janeiro City in Brazil. The purpose of this work is to verify if the OCs have a detectable effect upon crab population density.

Study area

Guanabara Bay (Figure 2) is a semi enclosed bay located in metropolitan Rio de Janeiro and receives large amounts of domestic and industrial effluents. A diverse industrial park with up to 6.000 industries is located on its shores, as well as two petroleum refineries and several petrochemical plants which discharge their effluents directly into the bay. The impacted area is located in the western side in Duque de Caxias City, whereas the preserved mangrove, located in Guapimirim City, is located in the eastern portion of the bay near Magé City.

Material and Methods

Samples

The densities of *C. granulata* population were estimated monthly during an year (may/2003-april/2004) at five sites (4 in the industrial area and 1 at the natural reservoir, used as control area) using wooden squares techniques $(1m \times 1m)$ from the number of crab holes in the mangrove mud. Five squares were placed in each site, totalizing $5m^2$ per site. The squares were placed randomly in a delimited area of $24m^2$ in each site. *Chasmagnathus granulata*, a small red crab that feeds basically on detritus found within the mud particles, is the most abundant and widely distributed crab in the region and is not harvested by man. However, other predators do exist in the area. Geographic coordinates of collection sites are: Duque de Caxias – P1- 22° 43'32"S and 43° 14' 17"W; P2 – 22° 43' 38"S, 43° 14' 20"W; P3 – 22° 43' 43"S, 43° 14' 22" W and P4 – 22° 43' 20"S, 43° 14' 13"W. Guapimirim - GP - 22° 41' 38"S e 42° 59' 58"W. Sites localization can be seen in Figs. 1 to 3.

A number of 25 ovigerous females were collected per site once in the initial time of study period inside the collection squares and carried out to laboratory, where the egg masses were removed from the abdomen and, after cleaning, weighted. After, egg masses were placed in the stove at 50° C for desiccation. Egg masses were weighted again to obtain the dry weight. Finally, the material was grinded and prepared for extraction.

Sediments were collected using an acetone rinsed metallic scoop. Eggs were collected from the crab female specimens. All samples were refrigerated in boxes with ice until the time of analysis (~2h). Five samples were collected in each site in a delimited area of $24m^2$.

Chromatographic procedures

Analysis was performed on a gas chromatograph from Shimadzu Co. (Japan) with an automatic injector, (AOC-17C), and an ⁶³Ni electron capture detector (ECD). Column temperature was programed from 100°C, held for four minutes and then, increased to 205°C at a rate of 5°C/ min. and held for 15 min. Then, the temperature was increased to the final temperature of 300° C at a rate of 2°C/min. Chromatographic column CBP-5 (SE-52/54). Injected volume, 2µl/ sample. Vector gas: ultra-pure hydrogen with a flow of 16,2 mL/min in column; make-up

columns: ultra-pure nitrogen with a flow of 35 mL/min. Injection: Splitless (30 seg.), 300°C. Detector temperature: 310°C.

Extraction, purification and fractioning

The sediment extraction was performed using a continuous hot sohxlet for 8h as adapted by Torres and co-workers in 1999 to the local conditions of the Brazilian Laboratory (Torres *et al.*, 1999). Each sample was weighted up to 1g (dry weight) and treated with differentiate polarity reagents to remove pollutants from studied material with greater efficiency. To each sample was applied 5ml of n-hexane, 5ml of acetone and 1ml of isooctane. After extraction, the upper layer was collected from each sample and evaporated to 1ml in a hot bath at 90°C.

Sediment sample purification was described by Japenga *et al.* (1987) and adapted by Torres (1998) for tropical soil and sediment. This process consists in remove humic acids and elemental sulfur which can make difficult posterior quantification of pollutants in chromatographic analysis. The extracting solvents were a mixture of n-hexane and cyclohexane (3:1). In order to remove sulfur that may cause strong interference in the ECD, a basic alumina column impregnated with sodium sulfite in an alkaline solution was used. This is a modification of the Jensen reaction, which used TBA (tetrabutylammonium) to precipitate thiosulfate in liquid: liquid extractions (Japenga et al, 1987).

After clean up, in order to reduce co-elution the extracts were fractionated using activated silica columns, the first fraction was eluted with n-hexane and recovered all of the PCBs (PCBs 28, 52, 101, 118, 153, 138 and 180) and some of the OCPs (HCB, Heptachlor, Heptachlor-epoxide, Aldrin, p,p'-DDE and part of o,p'-DDE). The second fraction contained G-HCH, lindane, alpha-endosulfan, dieldrin, endrin and p,p'-DDD, p,p'-DDT and part of the o,p'-DDE). The total chromatographic run time was 25 minutes.

The crab egg samples were subjected to a similar extraction procedure but now the extracts were mixed with concentrated sulfuric acid in order to destroy interfering fat according to the FAO/SIDA protocol (Jensen, 1983). The upper layer was then collected and passed in an activated sodium sulfide column (2g). The final volume was reduced to 0.5ml to which octachloro-naphtalene (OCN) in iso-octane was added as an internal standard for quantification purposes. Fractioning methods used for egg analysis are the same employed for sediment samples. Detection limits of the method were calculated as $3 \times \text{Std Desviation}$ and varied from 0.12 ng.g^{-1} to 3.4 ng.g^{-1} for PCB congeners and 0.06 ng.g^{-1} to 1.8 ng.g^{-1} for DDT and its metabolites.

Statistical Analysis

The results were statistically evaluated using the software STATISTICA 6.0 to generate a principal component analysis (based on correlations) of the observed annual average crab density, facing the sediment and egg concentration of organochlorines. Is considered that organochlorine concentration in eggs reflects the concentration in female gonads and is useful for revealing an elimination via of these pollutants from crab's body. Nonparametric ANOVA (Kruskal-Wallis) was used to verify significant influence of collection sites upon crab density, and cluster analysis (based upon Euclidean distance) was performed for comparing crab densities between collection sites using the software MVSP.

Results and Discussion

C. granulata density and organochlorine concentrations

The obtained results are presented in Table 1 and Figures 3 and 4. Table 1 describes the density of the crab holes in the different sampling points of *C. granulata* in Duque de Caxias and

Guapimirim from May, 2003 to April, 2004. It is clear that in site number 4, the density is lower than all of the other points while site GP (control) presents the highest crab density. Box plot (Figure 3) presents results that confirm this evidence, where P4 has the lowest median and GP, the highest. The Kruskal-Wallis ANOVA obtained (H=34,95 df=4 p=0,00) for factor "site" was highly significant (Chi-Square = 20,66667, df = 4, p = 0004), which indicates that the site of collection has an influence upon crab density. This agrees with cluster analysis results (Figure 4), which indicates that especially sites P4 and Guapimirim are very distant from the others while P1 and P2 are the most similar and P3 stay in a intermediate condition in concern to crab density. One can assume that these results are due to the organochlorinated compounds present in sediment, which can influence negatively crab population density, or the bioaccumulation occurring in the area or both.

Table 2 shows the distribution of the contaminants among the different collection sites in dry weight. Sites 2 and 3 are more contaminated than the other, but at site number 3 the presence of higher chlorinated congeners is more evident. Guapimirim site presented the lowest levels of sediment organochlorine concentrations. PCBs have higher concentrations in Guanabara Bay mangrove sediments than have DDT and metabolites, suggesting a stronger industrial impact in the area.

Table 3 and Figure 5 clearly indicates that the bio-concentration of the pollutants is occurring in the region once the organochlorine concentrations in eggs are grater than that in the sediment except for site three. The PCB concentration in eggs can be as high as 668,84 ppb on a dry basis in the eggs of the females collected in site number four. Thus, the eggs may be viewed as a unique form of excretion of these contaminants from the crab's body. Other available study with benthic organisms (decapod crustaceans) along the Sao Paulo coastal area, showed that the total PCB and DDT levels were lower than 20 ppb (dry weight), one order of magnitude lower than the present results (Gorni & Weber, 2004). These results agree with that found by Menone *et al.* (2000) and Menone *et al.* (2004) reported also high concentrations of OCs in *C. granulata* in comparison to sediment concentration. Guapimirim site presented the lowest levels of egg organochlorine concentrations.

There seems to have no direct correspondence between soil concentration and egg concentration of organochlorines in the study area (Figure 5), especially for PCBs. This maybe due to *C. granulata* bioturbation, which influences organochlorines distribution in consequence of its excavating activity as could be detected by Menone *et al.* (2004) in salt marshes of Argentina. This activity cause a concentration of organochlorines inside crab beds, increasing bioaccumulation. As a result, one should consider crab movement in the habitat, and the interaction of crab organism to the whole area, which have a differential organochlorine distribution. Site four is located in an area used for various industrial and domestic residuals disposal for many decades and appears to be the most contaminated site of all studied. Another possible influence in this difference is soil features in the different sites, as organic matter content or vegetation which can cause different bioavailability of organochlorine to crab organisms Menone *et al.* (2000). This indicates that sediment is not a good indicator for evaluate organochlorine impact upon crab density.

As can be seen in Figure 6, the PCA showed that the most important force that explains or drives the distribution of the crabs, seems to be the egg PCB and DDT contamination. The most important factor (1) which explains 61,69% of the variation among samples, has a high significant negative contribution from DDT and PCB congeners concentrations in eggs, while crab density has the same contribution to factor 1 but on the positive axis of the factor. This indicates that the highest concentration of these pollutants in the soil corresponds to the smallest *C. granulata* population density. On the other hand, DDT and PCB congeners concentrations in the soil appear to have no relation to crab density, once they have an important negative

contribution in factor 2 (explaining only 32,58% of variation) in which crab density has an inexpressive contribution. This indicates that sediment is not a good indicator for evaluate organochlorine impact upon crab density, considering the obtained results of this work.

Bioconcentration is a very important process. It enables chemicals to attain body burdens in organisms that are high enough to trigger adverse ecotoxicological effects. Hydrophobic compounds are very toxic because their high bioaccumulation properties result in critical or lethal body burdens in organisms already at low environmental ambient concentrations.

Clarck *et al.* (1986) explained that PCBs adsorb to organic matter, accumulating in the sediment and contaminating ecological food chains. Nimmo *et al.* (1971) demonstrated PCBs bioaccumulation by crabs of the genus *Uca* from sediment and Marinucci & Bartha (1982) say the same for detritus. When preyed by birds, mammals or fishes, crabs can transmit these compounds to terrestrial or aquatic food chains (Montague, 1980). Tatem (1986) reported bioaccumulation of Aroclors 1242 and 1254 by shrimp *Macrobrachium rosembergii* (De Man, 1879) from contaminated sediment in laboratory conditions. Mattig *et al.* (1997) reported several organochlorinated compounds in the crabs *Carcinus maenas* and in the shrimp *Crangon crangon.* Heptaclor-epoxi, dieldrin, endosulfan, clordane, DDT and metabolites and HCH was detected in *C. granulata* from Argentina (Menone *et al.*, 2000). Borga *et al.* (2002) report DDTs, PCBs, Chlordane, HCB, and HCH in amphipods from Artic Ocean and Biais *et al.* (2003) detected PCBs, DDTs and metabolites, endosulfan, hepataclor-epoxide, HCHs and HCB in tissues of *Gammarus lacustris* (Amphipoda).

Metabolites as DDE and DDD in the eggs shows that *C. granulata* can metabolize DDT. They can also be eliminated by feces being more hydrophilic and carried to ocean (Menone *et al.*, 2004).

The PCA results report an obvious fact: the accumulated compounds have a direct effect to crab physiology, what cannot be expected for contaminants in the soil. Mayer *et al.* (1977) demonstrated in acute toxicity tests that crustaceans are inside the group of organisms more sensible to Aroclor 1248 (PCBs mixture). In spite of, the low concentrations of the organic pollutants in environment, chronic effects of bioaccumulation will have, prior, high significance.

Because of agricultural and industrial activities aquatic and coastal environments are increasingly contaminated with various kinds of pollutants, many of which can interfere with hormonal signaling in invertebrates. An important effect which has claimed attention of researchers is the endocrine disruption or environmental signalization (McLachlan, 2001) in which organochlorines and other chemicals, but especially organochlorines can affect crustacean molting. Weis & Mantel (1976) detected an stimulating effect of p,p'-DDT in *Uca pugilator* molting, and Fingerman & Fingerman (1977) reported the same for Aroclor 1242 in relation to molting of the same species.

Zou & Fingerman (1997a) reported that endosulfan delay the molting in *Daphnia magna* (Cladocera). The same authors (Zou & Fingerman, 1997b) observed the same effect in relation to Aroclor 1242, PCB-29 and diethyl phthalate. Zou & Fingerman (1999a, 1999b) studied the mechanism of molting disruption concluding from initial results with *Uca pugilator* that it results from the disturbance to th Y-organ-ecdysteroids receptor axis by pollutants. These effects due to the influence of these compounds in the interruption of molting process produce non-directly detectable consequences in natural populations.

In the studied area the results demonstrated that site P3 is the most contaminated site in the impacted mangrove and P4 the less contaminated one in the same place, whereas site GP in the protected area was the less contaminated of all. This is probably due to the fact that P3 is

the nearest site to an old PCB reservoir in the mangrove area near an industrial plant, while P4 the most distant and GP is localized in a cleaner and protected reserve. But surprisingly, the highest values of organochlorinated egg concentrations were detected in P4, probably a consequence of *C. granulata* activity dinamics in the mangrove. As demonstrated by PCA, the concentrations in eggs reflect more efficiently the negative impact of organochlorines upon crabs and their population density.

In spite of crab density could also be affected by other chemicals as PAH's and metals, or by predators action, in the present work one important correlation between egg contamination and crab abundance was described for the first time for a tropical mangrove in Brazil.

Conclusions

Chasmagnathus granulata density was higher where its egg organochlorine concentration was presented lower. However the Organochlorine bioaccumulation is occurring in the mangrove studied area, in spite of be not detected a directed correlation between egg and sediment concentrations. Furthermore, the presence of organochlorines in eggs indicates that they maybe the unique form of excretion of these contaminants from crabs' body. The organochlorine concentration in eggs is more significant to evaluate or estimate an impact of these pollutants upon *C. granulata* population than is the concentration in the sediment.

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Figure 1. *Chasmagnthus granulata* (male) collected in Duque de Caxias mangrove, RJ Brazil

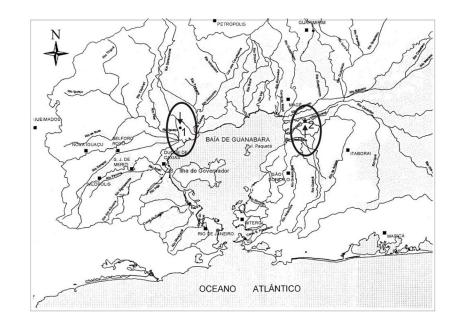


Figure 2. Study Areas in Guanabara Bay mangroves, RJ, Brazil

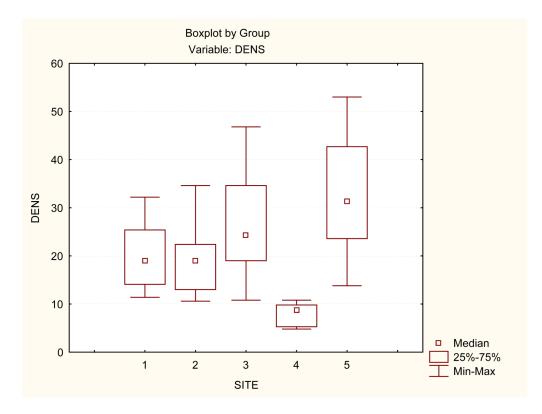
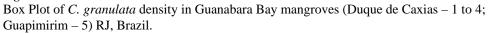


Figure 3.



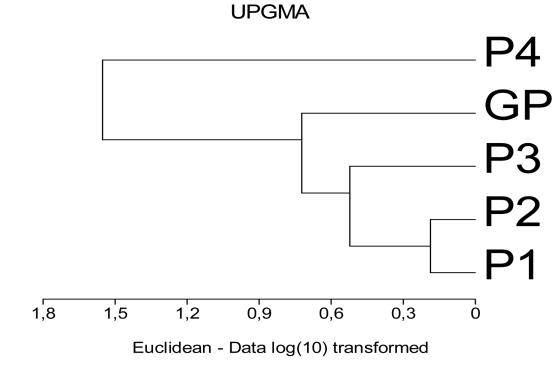


Figure 4.

Cluster analysis of the crab's holes density in Duque de Caxias (P1 to P4) and Guapimirim (GP) mangroves, RJ, Brazil

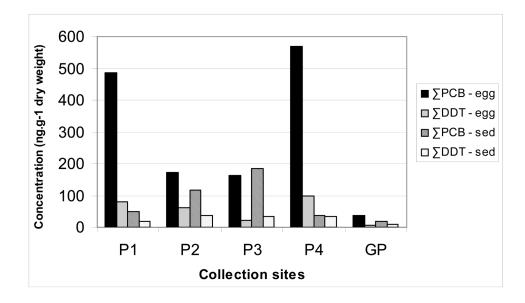
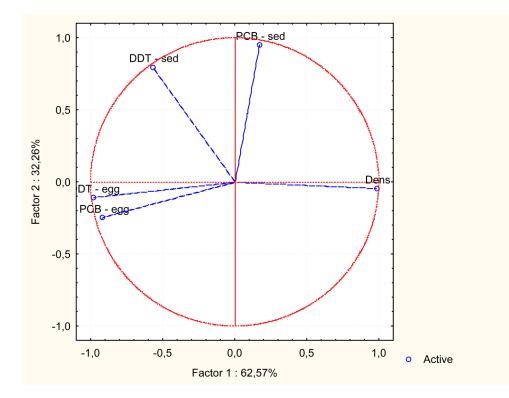
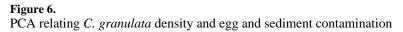


Figure 5.

Organochlorine concentrations in *C. granulata* eggs and in mangrove sediment $(ng.g^{-1} in a a dry weight basis)$, RJ state, Brazil





e of five	DEV	7.4	7.1	11.6	2.3	12.5
) (average	AVG	20.0	19.0	27.1	7.9	33.0
mirim (GF	APR	15.2	14.8	22.2	9.0	39.0
and Guapi	MAR	13.2	14.2	23.0	5.8	13.8
Table 1 Average density of <i>C. granulata</i> holes (holes/m ²) from Duque de Caxias (P1, P2, P3 and P4) and Guapimirim (GP) (average of five squares/site)	FEB	12.0	11.6	14.4	4.8	20.2
(P1, P2, P.	JAN	11.4	10.6	10.8	4.8	18.8
le Caxias (DEC	15.0	11.8	17.4	4.8	27.0
Table 1 rom Duque o	NOV	22.8	20.6	25.8	8.8	30.2
es/m ²) fro	OCT	17.4	19.8	20.6	9.2	32.5
holes (hol	SEP	21.2	18.2	38.8	7.0	37.6
granulata	AUG	31.6	26.2	46.8	10.8	53
sity of <i>C</i> .	lut	32.2	34.6	45.4	10.6	49.6
erage dena 1ares/site)	JUN	28	23.8	29.6	10.4	46.4
Av	MAY	20.4	21.8	30.4	8.6	28.4
		P1	P2	P3	P4	£

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PCB-28 n.d. PCB-28 n.d. PCB-52 0.02 PCB-101 5.09 PCB-118 3.84 PCB-138 1.661 PCB-153 1.661 PCB-153 1.775 PCB-180 1.7.03 PCB-180 1.7.03 PCB-190	46.87 31.03			
	31 03	1.44	0.33	5.29
	00.10	1.2	1.16	2.93
	14.96	13.84	0.63	0.95
	15.83	69.68	n.d.	7.55
	0.24	34.28	11.71	0.34
	6.12	31.88	3.53	0.77
	0.99	31.84	17.03	n.d.
	116.04	184.16	34.39	17.83
	n.d.	0.16	0.46	0.60
	3.98	0.18	0.44	0.50
	4.23	0.07	n.d.	0.47
o,p'-DDE (F1) n.d.	7.32	3.94	0.38	0.34
	5.95	5.25	0.29	0.92
	4.39	2.78	0.36	0.66
	0.67	2.04	0.80	0.64
	n.d.	0.27	1.16	n.d.
A-ENDOSULFAN 2.25	1.30	0.94	1.16	0.68
	2.59	0.24	2.32	0.28
	0.61	1.37	5.67	1.40
	3.50	1.53	16.05	1.72
	0.47	1.29	1.20	2.12
	2.39	12.40	3.75	0.28
cong	37.40	32.46	34.04	10.61
TOTAL OCS 69.86	153.44	216.62	68.43	28.44

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	Ρ1	P2	P3	P4	GP
PCB-28	48.55	31.01	17.82	38.45	n.d.
PCB-52	n.d.	19.70	8.97	n.d.	21.63
PCB-101	29.08	19.03	43.37	51.43	2.92
PCB-118	39.69	8.67	16.93	35.38	9.87
PCB-153	151.03	35.36	26.22	187.24	2.58
PCB-138	76.05	17.05	18.15	77.41	1.37
PCB-180	142.49	41.94	31.25	180.71	n.d.
FOTAL PCB	486.89	172.76	162.71	570.62	38.37
HCB	n.d.	n.d.	1.42	n.d	n.d.
HEPTACLOR	0.47	28.38	1.57	n.d	0.43
ALDRIN	0.37	5.09	n.d.	0.59	n.d.
),p'-DDE (F1)	n.d.	n.d.	1.79	n.d	n.d.
HEPTA-EPOX	n.d.	5.48	6.69	4.92	0.43
p,p'-DDE	n.d.	2.73	3.27	n.d	4.55
3-HCH	5.78	1.52	1.58	2.42	n.d
A-ENDOSULFAN	9.50	2.93	n.d.	14.90	n.d
DIELDRIN	7.07	5.26	3.36	5.19	n.d
ENDRIN	9.31	1.84	1.58	18.08	1.12
,p'-DDD	n.d.	8.63	n.d.	52.12	n.d
0,p'-DDE (F2)	35.95	n.d.	n.d.	n.d	n.d
p,p'-DDD	10.35	n.d.	n.d.	n.d	n.d
,p'-DDT	n.d.	n.d.	0.38	n.d	n.d
FOTAL DDT cong	78.80	61.86	21.64	98.22	6.53
FOTAL OCS	565.69	234.62	184.35	668.84	44.90