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Organochlorine Pesticides in Green Mussel, *Perna viridis*, from the Cienfuegos Bay, Cuba

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Abstract The green mussel, *Perna viridis,* was used to measure bioaccumulated levels of organochlorine pesticides in the marine environment of Cuba. Samples were collected in the Cienfuegos Bay between January and December 2010. The organochlorine pesticides (i.e. DDT, Dieldrin, Chlordane, Endosulfan, HCB, Aldrin, Heptachlor and Lindane) were quantified by gas chromatography. The sum of all organochlorine pesticides in *P. viridis* was 6.31 ng g^{-1}. The concentration ranged from 3.53 to 4.42 ng g^{-1} dry weight (dw) for DDTs (i.e. sum of pp' DDT, pp' DDD, op' DDE and pp' DDE); 1.7–1.9 ng g^{-1} dw for Dieldrin; 0.17–0.20 ng g^{-1} dw for Chlordanes; 0.14–0.16 ng g^{-1} dw for Endosulfan; 0.11–0.17 ng g^{-1} dw for HCB; 0.07–0.11 ng g^{-1} dw for Aldrin; 0.046–0.054 ng g^{-1} dw for Heptachlor and 0.035–0.039 ng g^{-1} dw for Lindane. These levels can be considered as low when compared to reported values from similar studies conducted elsewhere in the world. The concentrations of all organochlorines residues detected in this study fell below the EU Maximum Residue Limits.

Keywords Organochlorine pesticides · *Perna viridis* · Cienfuegos Bay · Cuba

Organochlorine pesticides (OCPs), due to their chemical persistence and hydrophobicity, have a tendency to accumulate up the food chain. Therefore, human exposure to these pollutants occurs mainly from ingested food. In Cuba, the use of pesticides has been reduced with the decline of farming activities over several decades. Ever since 1990, the use and import of all pesticides included in the Stockholm Convention has been prohibited. However, from the national inventory of chlorinated pesticides in disuse, there are in Cuba about 9 tons of stockpile of obsolete pesticides, principally: DDT, Heptachlor, and toxaphene (Abo Balanza 2005).

Even now, the information on the status of OCPs in many Caribbean coastal areas is scarce (Fernandez et al. 2007). For Cuba, only some old data on pesticide soils contamination from the most agro-developed province of Cuba is available (Dierksmeier 1996), and little data exist from the International Mussel Watch (Sericano et al. 1995).

The Cienfuegos Bay, situated in the southern central part of Cuba is a semi-enclosed bay connected to the Caribbean Sea by a narrow channel of 3 km long. This bay plays an important role in the industrial development of Cuba. The northern basin suffers most from the anthropic impact of the sewage discharge of Cienfuegos city (150,000 habitants), from the industrial area (thermoelectric power plant, fertilizer industry, oil refinery) and from the outflow of the Salado and Damuji rivers who drain an intense agricultural area developed within the last 40 years. The southern basin is subject to a smaller degree of anthropic pollution originating from the Caonao and Arimao rivers.

Recently, Tolosa et al. (2010) published the spatial distribution of various organochlorinated compounds in sediments from the Cienfuegos Bay. The concentrations reported appeared to be relatively low by global standards and only sediments impacted by the Cienfuegos domesticated wastage waters and the thermoelectrical power

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wastage waters show higher concentration than the sediment quality guidelines for DDTs as set by NOA. Relatively higher $\sum$ DDT concentrations and high DDT/(DDE + DDD) ratios on two sites near the outfalls of the city indicate a current DDT usage, probably linked to public health emergencies.

In order to assess the current status of heavy metals, radionuclide, OCPs residues on the marine environment, a mussel watch program has been established along the coastal area of Cuba by the Environmental Study Center since 2005, with the support of the International Atomic Energy Agency (IAEA).

The monitoring of traces of toxic substances in the aquatic environment using green mussel (*Perna viridis*) as a biological indicator is common, mainly due to advantages such as a wide geographical distribution, static and therefore easy sampling, tolerance to a wide range of salinity and comparatively long life-span (Boonyatumanond et al. 2002; Liu and Kueh 2005; Sivalingam et al. 1982). The green mussel, *P. viridis*, is a filter-feeding bivalve commonly found in Asia–Pacific waters. This invasive species was first seen in the Cienfuegos Bay in 2005, near the oil refinery. A year later, it was reported in the whole coastal area, colonizing and displacing the native species. At present *P. viridis* constitutes a food source and their cultivation is evaluated as a management alternative.

In consideration of the above, the present study measured OCPs in soft tissues from specimens of *P. viridis* collected in the coastal waters of the Cienfuegos Bay, to assess the contamination status in this ecosystem. In addition, a comparison of our results with those from other environments is included in order to evaluate the relative significance of the contamination. Our results were evaluated against International standards to identify the potential public health risks.

Materials and Methods

Twenty specimens of *P. viridis* were collected from one sample station on the coastline of the Cienfuegos Bay between January and December 2010. Mussels were collected in April, August and December from floating structures and the shore defence walls of the Calicito port. After collection on site, samples were transported in polyethylene bags packed in ice to the laboratory for processing.

The sample size and homogenization of samples was consistent with other studies conducted elsewhere using mussel as a bioindicator (Villeneuve et al. 1999). As the size of the mussel is known to be an important factor determining the level of pollutants bioaccumulated, only mature individuals in the size range of 8–10 cm were collected. The gender of the bivalve is not a factor affecting the organochlorine and heavy metal contents in green mussel tissues (Phillips and Muttarasin 1985), and thus gender was not taken into account in the present study. In the laboratory, soft tissues were removed from shells and rinsed free of impurities before homogenization in a stainless steel blender to form a single batch sample. Homogenates were frozen and stored at −20°C prior to freeze drying.

The extraction and clean-up of the OCPs was similar to those previously described by de Mora et al. (2010). To perform the OCPs analyses, a mixture containing known amounts of PCB 29, PCB 198, ε-HCH and Endosulfan Id_4 was added to each sample as internal standard. Samples were extracted in a microwave oven for 20 min. at 115°C with a mixture of hexane/acetone. These extracts were concentrated in a rotary evaporator to about 10 mL and then subjected to a clean-up process in order to remove lipids; the extracts were then purified with sulphuric acid. Sample extracts were further evaporated to exactly 1 mL under a gentle stream of dry nitrogen gas. The extracts were then cleaned-up and separated in three fractions by chromatography using partially deactivated Florisil packed column. The first fraction, eluted with *n*-hexane, contained HCB, DDE, Heptachlor, Aldrin and PCBs. The second fraction eluted with *n*-hexane/$MeCl_2$ mixture (70:30) mainly contained DDTs and HCHs, while the third fraction eluted with pure MeCl2 contained Endosulfans, Dieldrin and Endrin. All fractions were evaporated to 1 mL under a gentle stream of dry nitrogen gas.

The OCPs in the extracts were analyzed using a Hewlett Packard HP6890 gas chromatograph equipped with a ^{63}Ni micro Electron Capture Detector ($\mu - $ ECD) and a splitless injector, two different programme temperatures and columns (HP5 and DB5) were used.

Individual organochlorine pesticides were identified either by the relative retention time using the internal standard as a reference or by analyzing the sample on two columns of different diameter polarity.

Quantification of the contaminants was based on the internal standard method using the HP-GC ChemStation. Quality assurance procedures were performed by co-analyzing the reference material mussel tissue homogenate IAEA-142 together with appropriate blanks (Villeneuve et al. 2004). Concentrations of the analytes are expressed in dry weight (dw) basis (ng g^{-1}). Relative standard errors of analyte concentrations based on replicate determinations of the same reference material are usually around 10 %.

Results and Discussion

The Table 1 shows the individual organochlorine pesticides and their respective mean, minima and maxima concentrations.

Table 1 Concentrations of organochlorine pesticide in *P. viridis* from the Cienfuegos Bay (ng g^{-1} dry weight)

Compounds	Jan–April	May–Aug	Sep–Dec	Media	Min	Max
Aldrin	0.11	0.036	0.059	0.068	0.036	0.110
Chlordane	0.204	0.173	0.155	0.177	0.155	0.204
Cis Chlordane	0.056	0.043	0.041	0.047	0.041	0.056
Trans Chlordane	0.074	0.067	0.078	0.073	0.067	0.078
Trans Nonachlor	0.074	0.063	0.036	0.058	0.036	0.074
$\sum$ DDT	4.42	3.536	3.81	3.922	3.536	4.420
pp' DDE	2.4	1.9	2.0	2.100	1.900	2.400
pp' DDD	0.70	0.52	0.59	0.603	0.520	0.700
pp' DDT	0.45	0.32	0.34	0.370	0.320	0.450
op DDE	0.12	0.086	0.10	0.102	0.086	0.120
op DDD	0.62	0.41	0.52	0.517	0.410	0.620
op DDT	0.13	0.3	0.26	0.230	0.130	0.300
Dieldrin	1.9	1.5	1.7	1.700	1.500	1.900
Endrin	<0.008	<0.010	<0.007			
Heptachlor	0.054	0.046	0.039	0.046	0.039	0.054
HCB	0.17	0.057	0.13	0.119	0.057	0.170
HCH						
Lindane	0.037	0.039	0.028	0.035	0.028	0.039
α HCH	<0.012	0.010	<0.012			
β HCH	0.074	0.056	0.094	0.075	0.056	0.094
δ HCH	<0.015	<0.014	<0.016			
Endosulfan	0.157	0.112	0.16	0.144	0.112	0.164
α Endosulfan	<0.003	<0.003	<0.002			
β Endosulfan	<0.004	0.041	0.064	0.053	0.041	0.064
Endosulf.sulfate	0.15	0.071	0.10	0.107	0.071	0.150

In all samples, 24 organochlorines were detected. However, organochlorine pesticide residues such as Heptachlor, Heptachlor epoxide, Endrin, Endosulfan-isomer and HCH-isomer were lower than the detection limit.

The sum of all OCPs in *P. viridis* was 6.31 ng g^{-1}. The concentrations of the pesticide residues ranged from 0.028 to 4.42 ng g^{-1}. The concentration ranged from 3.53 to 4.42 ng g^{-1} dw for DDTs (i.e. sum of pp' DDT, pp' DDD, op' DDE and pp' DDE); 1.7–1.9 ng g^{-1} dw for Dieldrin; 0.17–0.20 ng g^{-1} dw for Chlordanes; 0.14–0.16 ng g^{-1} dw for Endosulfan; 0.11–0.17 ng g^{-1} dw for HCB; 0.07–0.11 ng g^{-1} dw for Aldrin; 0.046–0.054 ng g^{-1} dw for Heptachlor and 0.035–0.039 ng g^{-1} dw for Lindane.

The highest concentration of 4.42 ng g^{-1} was measured for $\sum$ DDT in January–April, while the lowest concentration of 0.028 ng g^{-1} was measured for Lindane in the September–December sampling period. All pesticide residues were distributed as follows: $\sum$DDT > Dieldrin > Chlordane > Endosulfan > HCB > Aldrin > Heptachlor > Lindane (see Fig. 1), and differences among the concentrations of these elements were not observed during the whole sampling period.

The detection of these organochlorines in the *P. viridis* samples indicates the presence of those chemical compounds in the waters of the Cienfuegos Bay, even though Aldrin, Chlordane, DDT, Dieldrin, Endrin, Lindane and Heptachlor are among the banned pesticides by the Environmental Protection Agency of Cuba, since 1990.

The Fig. 2 shows the distribution of Aldrin, DDT, Heptachlor, HCB and Lindane concentrations in *P. viridis* versus the concentration of these elements in the sediments of the Cienfuegos Bay, reported by Tolosa et al. (2010). The result shows higher concentrations of OCPs in *P. viridis* samples than in the sediments, demonstrating the capacity of these organisms to accumulate the organic compounds present in the sediments and its potential as a biological indicator of traces of toxic substances. Indeed, the re-suspension of pollutant sediments can be considered an important source of contamination for the marine organisms in the Cienfuegos Bay.

The highest concentrations of OCPs were measured for $\sum$ DDT. Those pesticides had been used in Cuba since 1950 on a variety of agricultural, crops and for the control of human disease vectors. The largest agricultural use of

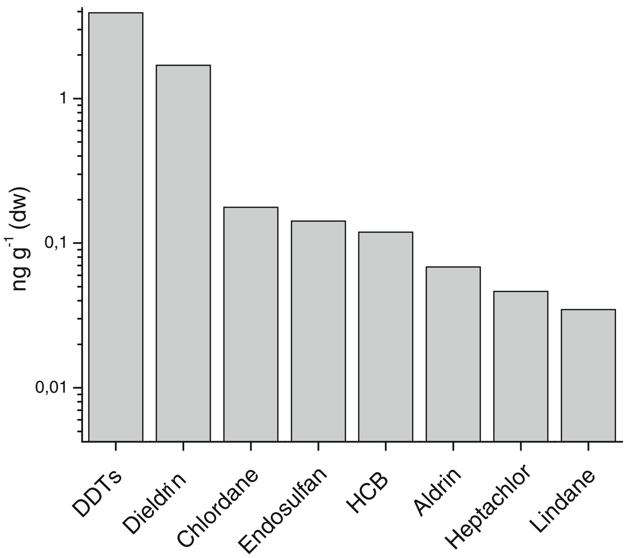

Fig. 1 Distribution of pesticide residues in *P. viridis* from the Cienfuegos Bay

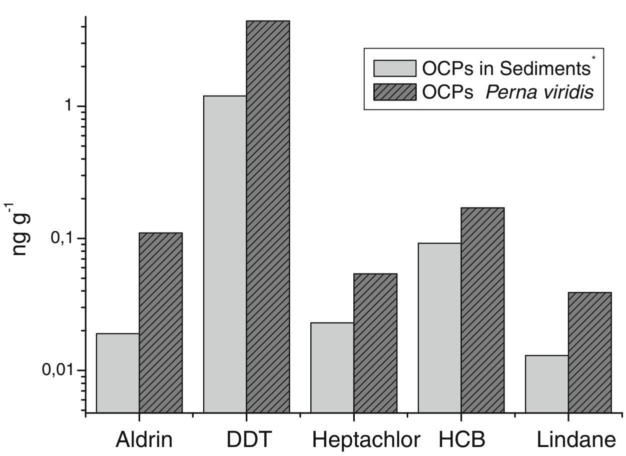

Fig. 2 Pesticide residues in *P. viridis* and sediments from the Cienfuegos Bay [* Reported by Tolosa et al. (2010)]

DDT had been on rice production before its ban in 1989. However, Tolosa et al. (2010) found residues of various organochlorinated compounds in sediments from the Cienfuegos Bay. Relatively higher ∑ DDT concentrations and high DDT/(DDE + DDD) ratios on two sites near the outfalls of the city indicate a current DDT usage, probably linked to public health emergencies.

The pesticide DDT gradually degrades to DDE and DDD through biological and photochemical transformations under both aerobic and anaerobic conditions (Thomas et al. 2008). The DDT/(DDE + DDD) ratio is usually used to know whether DDT input has occurred recently or not (Doong et al. 2002). Ratios of DDT/(DDE + DDD) <0.8 show that DDTs have been subjected to long term

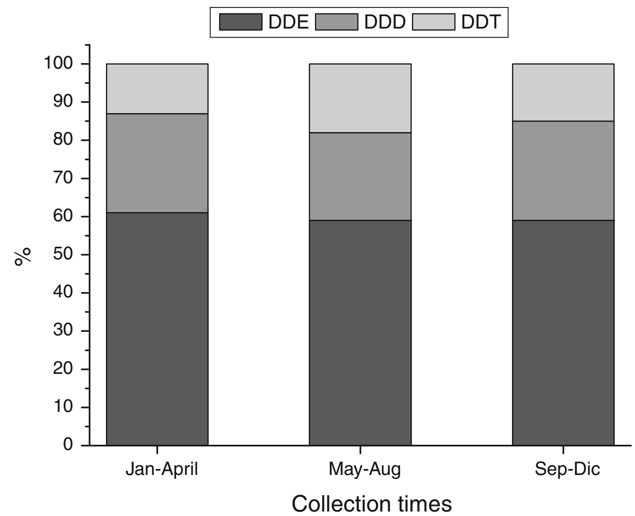

Fig. 3 Percentage of DDT, DDE and DDD in *P. viridis* from the Cienfuegos Bay

Table 2 A comparison of organochlorine pesticides concentration in *P. viridis* from the Cienfuegos Bay and other countries (ng g^{-1} dry weight)

Location	∑ DDT	Chlordane	HCB	Reference
Cienfuegos, Cuba	3.5–4.4	0, 177	0, 119	This study
Singapore	2.6–54	3.1–15		Sivalingam et al. (1982)
Hong Kong	13–36	2.2–8.2	5.3	So et al. (2005)
Philippines	0.2–4.2	0.15–9.5		Tanabe et al. (2000)
India	0.9–40	0.01–1.9	0.38	Tanabe et al. (2000)
Thailand	1.2–3.8	0.01–1.4	0.1	Cheevaporn et al. (2005)

weathering. In this study, the ratios DDT/(DDE + DDD) were in the range of 0.15–0.24 suggesting that DDTs contaminating marine biota in the study areas were old. The Fig. 3 shows the relative percentages of DDT, DDE and DDD, highlighting that DDT does not exceed 18 % of the ∑ DDTs. The low concentration of DDT compared to its metabolite DDE is an indication that there might not be recent input of DDT in the sampling area.

The levels of organochlorine pesticide residues in green mussel samples recorded in the present study were compared with those of other studies (Table 2). It may be concluded that the present results are lower than the organochlorine residue levels found in the same species from other sites in the world.

The concentrations of all the organochlorines residues detected in this study fell below the EU Maximum Residue Limits (MRL) of 50 µg kg for fresh fish. The Maximum Residue Level, is the maximum concentration of pesticide residue accepted by the European Union (EU) in a food product which might not represent a hazard to the health of the consumer. Hence, the contamination from theses organic pollutants in the Cienfuegos Bay is not particularly worrying in terms of human health and ecosystem perspectives. These results contribute to the sparse regional database of organochlorinated compounds in Cuba and the Caribbean marine environment.

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