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DETERMINATION OF THE BIOACCUMULATION FACTORS OF ORGANOCHLORINE PESTICIDES (OCPs) AT SOME SPECIES OF BIVALVE MOLLUSKS IN SOAI RAP ESTUARY - HO CHI MINH CITY

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Abstract. Deposited sediments contain organochlorine pesticide chemicals which can pollute surface water as well as aquatic ecosystems. In this study, the accumulation of organochlorine pesticide chemicals in the bivalve mollusk group including Pacific oyster (*Crassostrea gigas*, Thunberg, 1793), green mussel (*Perna viridis*), blood scallop (*Anadara granosa*)), white clam (*Meretrix lyrata*) and pearl oyster (*Pinctada maxima*) at Soai Rap estuary, Ho Chi Minh city was selected as subject of this research because of their high bioaccumulation capacity; their sedentary life, organic residue filter feeding habit and the ability to clean the lagoon environment. The pesticide chemicals were analyzed on GC-ECD system. Result of this research shows the different levels of accumulation in meat tissues of 5 species but they are lower than the allowable value of MARD 193:2004 - The bivalve mollusk harvest zone - Conditions to ensure food hygiene and safety. The results of calculating the Bio-Accumulation Factor (BAF) and Biota Sediment Accumulation Factor (BSAF) of the five species have shown that the bioaccumulation of organochlorine pesticides (OCPs) of bivalve mollusks in the area is natural tendency. BAF and BSAF of *Crassostrea gigas* were 56.672 and 0.429, *Perna viridis* were 66.730 and 0.608, *Anadara granosa* were 123.884 and 1.974, *Meretrix lyrata* were 52.060 and 0.489, *Pinctada maxima* were 115.176 and 1.076, respectively.

Keywords: Estuary, bioaccumulation factor, biota sediment accumulation factor, organochlorine pesticide, bivalve mollusks, OCPs.

INTRODUCTION

The estuary is a place where the watersheds of fresh and salt water are intertwined. It has the abundance and diversity of phytoplankton, zooplankton, aquatic animals and plants, living and developing in both bottom and surface layers. However, it is also one of the places with a high risk of environmental pollution, a high accumulation of many toxic compounds in which the plant protection chemicals are one of the main groups of pollutants influencing water quality as well as quality of seafood products. The plant protection chemicals accumulate in the environment and had acute effect on aquatic ecosystems, most notably the large amounts of pesticides present in the environmental components in general and estuaries in particular. The organochlorine pesticides (OCPs) are natural or synthetic chemicals that are capable of causing endocrine disruption, growth inhibition, high toxicity, persistence in water and sediment, are capable of bioaccumulation and have significant adverse effects on ecosystems and human health through food chains [1]. This compound is used for a variety of purposes in agriculture, forestry and fisheries. It increases accumulation in sediments of estuaries and coastal areas because OCPs have a strong affinity for suspended particulate matter, low water solubility and high hydrophobicity, slow decomposition in the environment.

Biological accumulation (Bioaccumulation) is the final accumulation of pollutants in an organism from all sources of infertility that occurs in the nature environment (soil, water and air) [2], it is a term that describes the direct uptake process of the plant protection chemicals into the organism from water through tissues or grills and influenced by consumption of food [3, 4]. The bioaccumulative potential of OCPs in organisms has been investigated and documented by several authors [2, 3, 5–11]. According to Duong et al., (2013) and Le et al., (2015), the OCPs distribution in coastal waters of the Northern Vietnam has seasonal characteristic, that in rainy season is higher than in dry season and the OCPs accumulation in surface sediments does not change significantly between sampling periods [6, 7]. The OCPs accumulation is also involved in global atmospheric transport, OCPs and PCBs can be deposited permanently or temporarily during migration [10, 11]. The content of OCPs and PCBs in molluscs species is a parameter reflecting sediment pollution in South-West coastal through Biota Sediment Accumulation Factor (BSAF) [3]. The other studies have also reported that the OCPs accumulation in the tissues of organisms is higher than the permitted limits for quality of marine products [8, 9].

However, most of studies have not evaluated the correlation between OCPs accumulation in biological tissue (BAF) and in sediment (BSAF). Therefore, the aim of this paper is to determine the OCPs bioaccumulation in five bivalve molluscs species that is popularly cultured in the study area, including Pacific oyster (*Crassostrea gigas*, Thunberg, 1793), green mussel (*Perna viridis*), blood scallop (*Anadara granosa*), white clam (*Meretrix lyrata*) and pearl oyster (*Pinctada maxima*) at Soai Rap estuary - Ho Chi Minh city. These species have high bioaccumulation, sedentary life and filter feeding habit, they are species specific to sediment surface in water bodies and are often chosen as model organisms in environmental toxicology and ecological risk studies [12–14]. The study area is the receiving area of domestic and industrial waste water, a part of municipal solid waste and hazardous waste, water from agricultural production containing pesticides and fertilizer which seriously threaten the quality of river water [15]. In addition, the river also supplies water and also receives waste from aquaculture activities in Long An, Tien Giang provinces and Ho Chi Minh city. Evaluation of bioaccumulation indicators will contribute to providing the scientific information on the impact of OCPs on the environment in general and the ecosystem in particular, providing the scientific basis for the control of sediment's quality, control of food safety, avoidance of impacts on aquaculture activities and application of environmental management measures in the study area.

MATERIALS AND METHODS

Sample preparation

Biological sampling. 5 bivalve mollusk species were collected randomly from May to November 2017. The specimens were collected with commercial size, sampled and preserved in accordance with Vietnamese Standard 5276:1990 (ISO 7828:1995) [16, 17]. The sampling area is the Soai Rap estuary belonging to the Sai Gon-Dong Nai river system, downstream Nha Be river (from Nam Hiep Phuoc-Nha Be to the Dong Tranh bay, fig. 1). The sampling area is subjected to river-sea interaction, which is influenced by the semi-diurnal tide regime of the East Sea with large amplitude [18]. 8 samples of Pacific oyster (*Crassostrea gigas*, Thunberg, 1793), white clam (*Meretrix lyrata*) and pearl oyster (*Pinctada maxima*) were taken; 13 samples of green mussel (*Perna viridis*) and blood scallop (*Anadara granosa*) were taken.

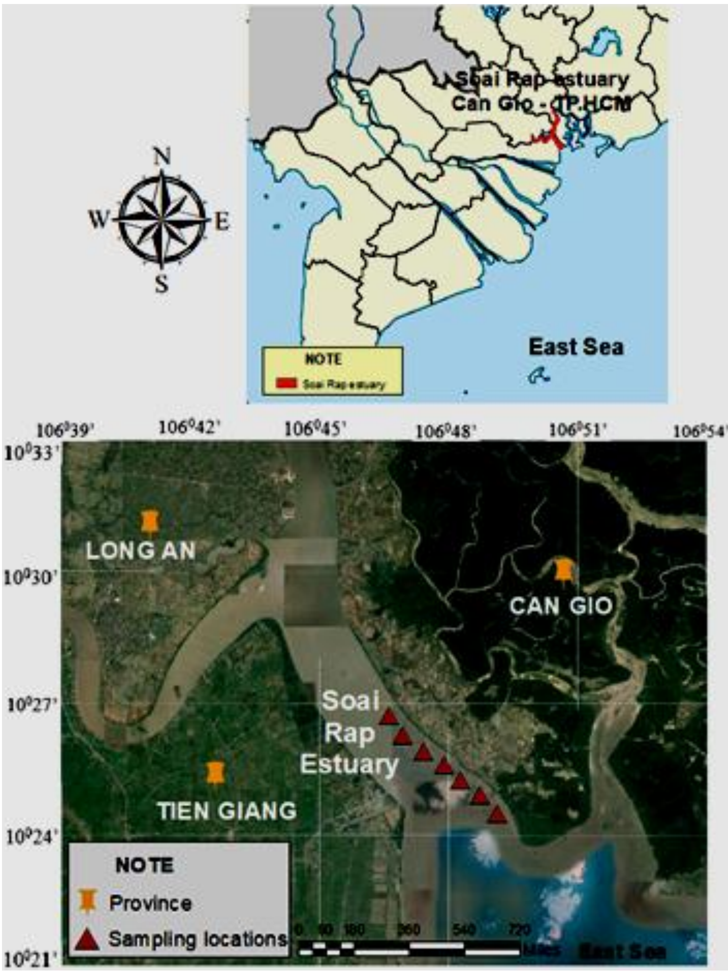

Fig. 1. The Soai Rap estuary and sampling areas

Sediment sampling. The sediment samples were collected at the same sites with the biological samples by Ekman clam shell style. The Ekman's area is 0.036 m^2, the sediment samples were collected at the surface layer varying from 0 to 10 cm, mixed well, put into the polyethylene bags and packed by aluminum foil, preserved in a box with ice gel and transported to the laboratory. After the sample was dried, milled, sieved by a sieve with the hole size of 0.63 µm, the samples would be analyzed of OCPs content. The samples were stored at 4^{o}C and defrosted to room temperature before analysis and determination of drying coefficient according to Vietnamese Standard 6648:2000 [19].

The method for determination of OCPs content. The specimens of the mollusk were ground well by a dedicated machine and dried with anhydrous Na_2SO_4. 20 g dry sample would be ultrasonically extracted and centrifuged three times with n-hexane/acetone (1:1). The extract solution was concentrated to obtain about 5 ml and transferred through gel permeation chromatography column to remove the fat, amine,... present in the extract sample. Then, it was cleaned by column silica gel 2 g.

Rinsing the OCPs with n-hexane (3 × 15 ml), the rinsing solution is concentrated to 1 ml; adding the internal standard substance and standard norm to 1 ml with n-hexane [2, 20, 21]. The biological specimens were assimilated by anhydrous Na_2SO_4 before extraction. The total content of OCPs in the sample was determined by Varian GC-450 gas chromatography, Electron Capture Detector (ECD) in the Department of Ecological Toxicology - University of Lige (Belgium) and Department of Environmental Analysis, Industrial University of Ho Chi Minh city.

Calculation of BAF. Bioaccumulation Factor is the ratio of the chemical concentration in an organism to the chemical concentration in the water environment [4].

$$BAF = C_B / C_W$$

Where: BAF- Bio Accumulation Factor is calculated by experimental data, the value of coefficient calculated in wet weight; C_B is the chemical concentration in organism (mg/kg dry weight); C_W is the chemical concentration in the water (mg/l).

Calculation of *BSAF*. Biota-Sediment Accumulation Factor (*BSAF*) is the ratio of the chemical concentration in an organism to the chemical concentration in the sediment [21]. *BSAF* of bivalve molluscs was determined by the following equation:

$$BSAF = \frac{c_0 / f_l}{c_s / f_{soc}}$$

Where: BSAF- Biota Sediment Accumulation Factor is calculated by experimental data; c_0 is the organochlorine concentration (ng/g wet weight); f_l is the biota lipid concentration (fraction by weight) (ng fraction of lipid/g wet weight); c_s is the sediment organochlorine concentration (ng/g dry weight); f_{soc} is the fraction of organic carbon in the sediment (ng fraction of organic carbon/g dry weight).

Statistical analysis. All experiments were done in triplicate and the data were calculated as mean ± SD (standard deviation) and drawn by the software SPSS 20.0 and Sigmaplot 12.5. Statistical significance was accepted at a level of $\rho < 0.05$.

RESULTS AND DISCUSSION

The OCPs content in biological samples. The content of OCPs in biological samples is shown in table 1, the analyzed values are compared with the Branch standard 193:2004 - The bivalve mollusk harvest zone - Food hygiene and safety conditions. The results in table 1 show that the content of OCPs in the meat tissue of the five mollusks has been distributed differently according to the weight and the range of fluctuation is rather large, however it is still below the Branch standard [22]. This shows that the level of exposure with the plant protection chemicals of the research subjects is low and the quality of food safety is still ensured. In table 1, total OCPs content in the Pacific oyster *Crassostrea gigas* is lowest with an average value of 9.297 ng/g and that in the blood scallop (*Anadara granosa*) is highest with 34.108 ng/g wet weight. The OCPs of other three species are recorded respectively, 12.076 ng/g in the white clam (*Meretrix lyrata*), 13.345 ng/g in the green mussel (*Perna viridis*) and 19.212 ng/g in the pearl oyster (*Pinctada maxima*). Fig. 2 shows that the OCPs content is relatively low in samples and varies from 0 to 6.122 ng/g. The endosulfan content in the oyster's tissue is significantly different, with the highest value of 14.482 ng/g. The total content of DDTs in the five species was higher than the total content of HCHs, heptachlor, aldrin, dieldrin, endrin, with the value of DDTs varying from 3.588 to 9.524 ng/g. The content of aldrin in the clam sample is lowest with the value of 0.011 ng/g, the content of eldrin obtained in the oyster sample is 0.040 ng/g. The analysis of ANOVA shows that the total content of OCPs in mollusk species is different, the content of OCPs in mussel is significantly different compared with the remaining species ($\rho < 0.0001$).

The present results are different from some previous studies [3, 8, 23]. The research data of Yatawara et al., (2015) in San Jorge bay in Patagonia showed that the total content of OCPs was much lower and varied from 0.004–

0.005 ng/g to 0.21 ng/g for the samples that were collected in autumn season (mean is 0.06–0.09) and about 0.22 ng/g (mean is 0.12–0.08) for samples that were collected in spring season, ΣOCPs had no significant difference among the two seasons [8]. The results of Yatawara were in agreement with the data of Daniela et al., (2008) in the Piracicaba river Basin, Brazil [23]. The total content of OCPs in bivalve mollusks was very low in comparison with the data that is recorded in this study, the OCPs value varied from 0.125 to 0.263 ng/g in the rainy season and that in the dry season was 0.041 to 0.293 ng/g. In contrast, Choi et al., (2014) found that the total content of OCPs (ΣDDTs and ΣHCHs) in Manila clams on the West coast of the Republic of Korea was higher and varied from 13.7 to 73 ng/g [3]. As the tide rises, the mollusks with the organic residue filter feeding habit will peer out of the sand and depress the tentacles into bottom water layer for filtering food [5]. The major foods of this group are organic humus, algae and microorganisms in the bottom sludge layers, so there is significant correlation between the content of accumulation in their tissue and the concentration of OCPs in sediment in the area where they live [5]. In order to compare the level of OCPs accumulation of different mollusk species, it is necessary to assess the BAF and BSAF. The following is a calculation of the BAF and BSAF of this study.

Table 1. The content of OCPs in mollusk species from the study area (ng/g wet weight)

Mollusk species	**The content of OCPs in biological tissue (ng/g)**							
	HCHs	DDTs	Heptachlor	Aldrin	Dieldrin	Endrin	Endosulfans	Σ OCPs
Oyster (n = 8)	2.702 ± 0.897	3.588 ± 2.716	0.484 ± 0.329	0.771 ± 0.705	0.077 ± 0.082	0.032 ± 0.038	1.642 ± 1.283	9.297 ± 4.571
Clam (n = 8)	2.826 ± 0.948	3.589 ±1.204	3.063 ± 2.853	0.011 ± 0.012	0.216 ± 0.233	0.165 ± 0.183	2.206 ± 1.521	12.076 ± 4.715
Scallop (n = 13)	4.805 ± 2.666	6.083 ± 6.161	3.516 ± 2.448	1.713 ± 1.717	1.227 ± 1.731	2.283 ± 2.220	14.482 ± 10.936	34.108 ± 14.059
Pearl (n = 8)	5.645 ± 2.994	6.122 ± 4.891	1.360 ± 0.891	0.399 ± 0.473	0.511 ± 0.563	0.585 ± 0.565	4.591 ± 3.361	19.212 ± 9.915
Mussel (n =13)	2.218 ± 2.209	5.251 ± 4.478	2.309 ± 2.810	0.168 ± 0.269	0.202 ± 0.199	0.259 ± 0.377	2.937 ± 3.160	13.345 ± 9.511

Note: n is the number of samples collected at the study sites.

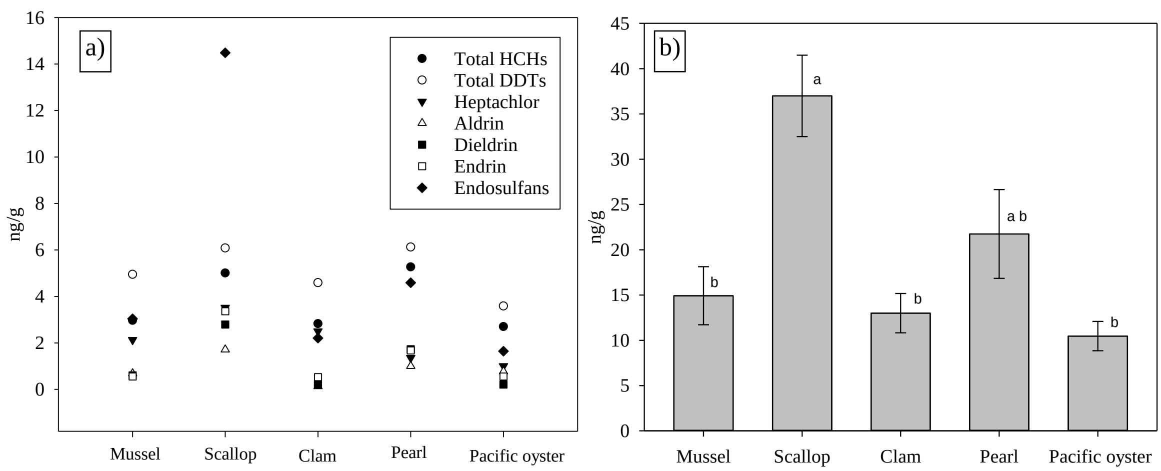

Fig. 2. The graph shows the distribution characteristics (a) and the proportion of OCPs content (b) in mollusk species in the study area

Assessment of OCPs accumulation in sediment of mollusk species in the study area. The Biota Sediment Accumulation Factor (BSAF) is a valuable parameter to determine the bioaccumulation of lipophilic compounds, which is primarily associated with tissue lipid and sediment organic carbon. The content of OCPs in biological samples tends to vary with the characteristics of each species and the sediment environment of the biological sampling species. The results of BSAF analysis in table 2 show that the content of OCPs in the sediment in the Soai Rap estuary - Ho Chi Minh city varies from 0.429 ng/g to 1.974 ng/g and is lower than Vietnamese Standard 43: 2012/MONRE - National technical regulation on sediment quality [26].

Table 2. Biota Sediment Accumulation Factor (BSAF) of mollusk species in the study area

Mollusk species	BSAF (ng/g)							
	HCHs	DDTs	Heptachlor	Aldrin	Dieldrin	Endrin	Endosulfans	Σ OCPs
Oyster (n = 8)	0.551 ± 0.897	0.612 ± 0.392	0.285 ± 0.267	0.591 ± 0.410	0.136 ± 0.142	0.026 ± 0.033	0.326 ± 0.238	0.429 ± 0.182
Clam (n = 8)	0.620 ± 0.232	0.491 ± 0.295	0.677 ± 0.601	0.009 ± 0.010	0.258 ± 0.361	0.296 ± 0.507	0.913 ± 0.918	0.489 ± 0.262
Scallop (n = 13)	1.197 ± 0.767	1.356 ± 1.440	1.877 ± 2.001	3.304 ± 4.191	1.862 ± 2.802	1.912 ± 1.728	7.985 ± 7.354	1.974 ± 0.944
Pearl (n = 8)	1.485 ± 0.943	1.023 ± 0.961	0.837 ± 0.786	0.826 ± 1.008	0.965 ± 0.939	0.657 ± 0.442	0.962 ± 0.895	1.076 ± 0.760
Mussel (n =13)	0.502 ± 0.510	0.898 ± 0.857	0.819 ± 1.073	0.137 ± 0.224	0.171 ± 0.185	0.328 ± 0.489	1.064 ± 1.457	0.608 ± 0.501

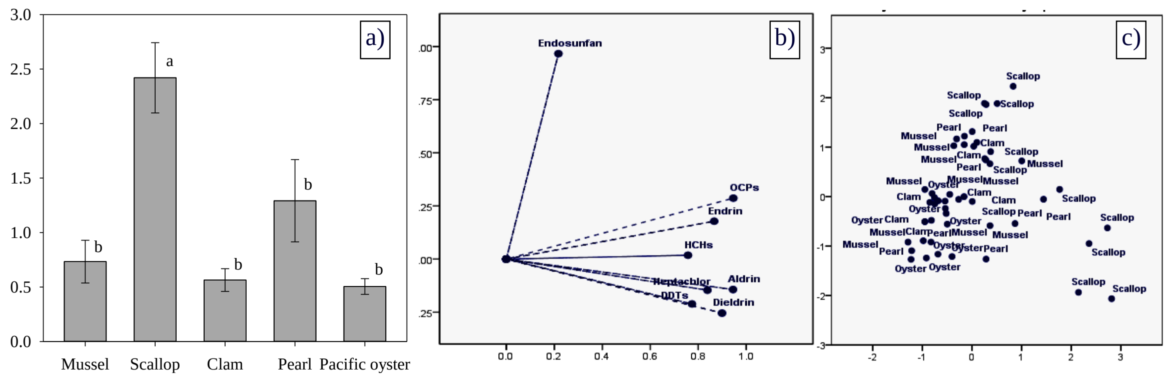

Fig. 3. The graph shows the rate of OCPs (a) and trends of OCPs distribution by substances (b) and individual mollusk species (c) in sediments at the study area

The analysis results in table 2 and fig. 3 show that the bioaccumulation ability of OCPs for sediment environment is very low, varies from 0.430 to 1.974 ng/g. The BSAF values range from 1.197 to 7.985 ng/g in the blood scallop; 0.027–0.612 ng/g in Pacific oysters; 0.657–1.485 ng/g in pearl oyster; 0.009–0.913 ng/g in white clam and 0.138–1.014 ng/g in green mussel. The organochlorine pesticide varies in species, BSAF in Pacific oysters is lowest (0.429 ng/g), the highest one is in scallop (1.974 ng/g) and distinct from the rest of species ($\rho<0.000$) (fig. 3a, 3c). The BSAF of endosulfans in the scallop is highest with 7.985 ng/g and BSAF of aldrin in Pacific oysters is lowest with value of 0.009 ng/g (fig. 3b). According to Choi et al., (2014), the BSAF index of OCPs in the Manila clam on the West coast of the Republic of Korea has the mean value of 0.35 ng/g (ranging from 0.02 to 1.09), approximately 7 times lower than the highest value of this study [3]. In contrast, the BSAF value in gray mullet (*Mugil cephalus*) (3.8-4 ng/g) and shrimps

(*Metapenaeus* sp.) (5.3-8.2 ng/g) living in Mai Po swamp, Hong Kong (the South of China), is approximately 4–8 times higher than the recorded value of mollusk species in Soai Rap estuary [27]. This may be because the study area receives untreated sewage, industrial and municipal wastes combined with sudden spills that are discharged directly into the canal system of river. The flow of floods and rain bearing many toxic impurities has significantly contributed to increase of pollutants at Soai Rap estuary.

Assessment of the level of OCPs accumulation in study species. The results of calculating the BAF of OCPs in the study species including Pacific oyster (*Crassostrea gigas*, Thunberg, 1793), green mussel (*Perna viridis*), blood scallop (*Anadara granosa*), white clam (*Meretrix lyrata*) and pearl oyster (*Pinctada maxima*) are shown in table 3.

Table 3. BAF value of the mollusk species at study area

Mollusk species	Length (cm)	Σ OCPs in organism (ng/g)	Σ OCPs in sediment (ng/g)	BAF
Pacific oyster (*Crassostrea gigas)*	11.83	9.297	0.429	56.672
White clam (*Meretrix lyrata)*	4.63	12.076	0.489	52.060
Blood scallop (*Anadara granosa)*	6.29	34.108	1.974	123.884
Pearl oyster (*Pinctada maxima)*	6.62	19.212	1.076	115.176
Green mussel (*Perna viridis)*	5.81	13.345	0.608	66.730

The results in table 3 show that the biological accumulation has transferred from water and sediment environment into aquatic animals and the value of BAF (OCPs) in bivalve mollusks at the study area is natural tendency. The OCPs accumulation of pearl oyster (*Pinctada maxima*) and blood scallop (*Anadara granosa*) has the highest concentrations with BAF value of 123.884 and 115.176, respectively, because these species live in tidal areas, cling in the coral reef under the sea surface at some meters, and are distributed in the windless soft mudflats where the water flows slowly. The Pacific oysters (*Crassostrea gigas*) live in areas where the water flows slowly, sticking to rocks, stones, trunk,... far from the waste sources so the content of OCPs obtained in tissue is lowest and therefore, BAF also has the lowest value of 52.060 [5, 10]. The white clams (*Meretrix lyrata*) and blue mussel (*Perna viridis*) live in the tidal area, bury in the sand and have the similar distribution law in the tidal areas [5, 28], the BAF value of the two species is close to each other at 56.672 and 66.730, respectively.

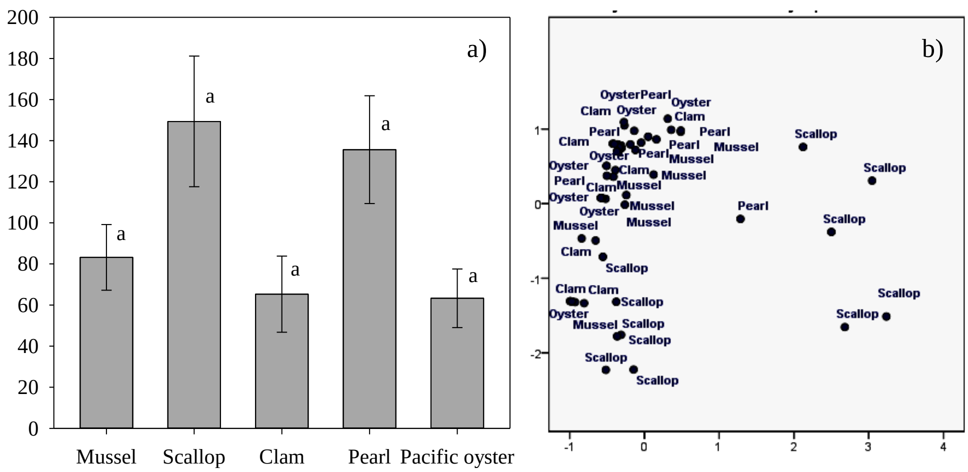

Fig. 4. BAF (a) and trend of OCPs distribution of mollusk species in Soai Rap estuary - HCM city

ANOVA statistical analysis (fig. 4a) shows that BAFs of OCPs are not affected by species (ρ = 0.0125 < 0.05). The BAF (OCPs) values are rather low, ranging from 52.060 to 123.884. The different species accumulate OCPs differently (fig. 4b), the blood scallop has the highest BAF value of 123.884, the lowest one recorded in the clam is 52.060. The green mussels, clam and oysters have a BAF value less than 100, according to the CPA standard (GreenScreen, 2016), this value is very low (the CPA standard is BAF ≤ 100). BAF (OCPs) in blood scallop and pearl oysters has the low BAF value of 100 < BAF < 500 (values in these samples are 123.884 and 115.176, respectively). Similar results have been found in some other studies [4, 6]. The data observed by Duong et al., (2013) in coastal waters of the Northern Vietnam showed that BAF (OCPs) value in clam (*Meretrix lyrata*) ranged from 32.78 to 75.69 ng/g [6]. According to a report by Arnot (2006) [4], the OCPs monitoring values in clam samples in California and San Francisco from 1980 to 1996 showed that the BAF of clam *M. californianus* for OCPs varied from 2.9 ng/g (for lindane) to 15.7 ng/g (for p'p-DDD). Therefore, this study shows the table of BAF (OCPs) value in bivalve mollusk species which are aquacultured or distributed in Soai Rap estuary - Ho Chi Minh city. The analysis results provide information on OCP residues in both sediments and bivalve mollusks. It also offers some recommendation about using these species for daily food, avoiding the risk of OCPs accumulation in the body.

CONCLUSION

The study investigated, analyzed and calculated the OCPs, BAF and BSAF values of five bivalve mollusk species, those of Pacific oyster (*Crassostrea gigas*) were 9.297, 56.672 and 0.429; those of the blue mussel (*Perna viridis*) were 13.345, 66.730 and 0.608; those of the blood scallop (*Anadara granosa*) were 34.108, 123.884 and 1.974; those of the white clam (*Meretrix lyrata*) were 12.076, 52.060 and 0.489; those of the pearl oyster (*Pinctada maxima*) were 19.212, 115.176 and 1.076, respectively.

The results of calculating the bioaccumulation factor (BAF) and Biota Sediment Accumulation Factor (BSAF) of the five species have shown the bioaccumulation of organochlorine pesticides (OCPs) of bivalve mollusks in the area that is natural tendency and lower than the allowable value of MARD 193:2004 - The bivalve mollusk harvest zone - Conditions to ensure food hygiene and safety. The results also showed the potential risk of plant protection chemicals and seafood unsafety in the study area because OCPs can cause endocrine disorders with very low content.

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