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**Evidence of Micropollutants in Sediment and Mud Clams
(*Polymesoda erosa*) from One of Mangrove Biodiversity Hotspots
in Indonesia**

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ABSTRACT

This study aims to explore persistent microorganic pollutants (Poly chloro byphneyls (PCBs) and Organochlorine Pesticides (OCPs)) in sediment and mud clams (*Polymesoda erosa*) in Segara Anakan Lagoon (SAL). In addition, polycyclic aromatic hydrocarbons (PAHs) in the mud clams were evaluated their risk consumption and Bioaccumulation factor in sediment. Concentrations of 7 PCBs in the sediment were found in the range 0.11 ± 0.05 to $2.63 \pm 0.1 \mu\text{g.kg}^{-1}$ sed.dw. OCPs levels were found in the range of 0.2 ± 0.05 and $2.9 \pm 0.01 \mu\text{g.kg}^{-1}$ sed.dw. In *P. erosa*, 16 Polycyclic Aromatic Hydrocarbons (PAHs) (USEPA) were found in the range from 103.34 ± 2.01 to $1348.31 \pm 18.21 \mu\text{g.kg}^{-1}$ tissue dry weight (dw), PCBs content was found only in one station at concentration $2.34 \pm 1.72 \mu\text{g.kg}^{-1}$ tissue dw and OCPs content was found in the range 0.87 ± 0.03 to $24.79 \pm 1.30 \mu\text{g.kg}^{-1}$ tissue dw. The disturbance matrices have been evaluated with the appropriate samples according to the extraction yield and yield analysis. Several guidelines evaluate the quality of sediment on PCBs and OCPs (i.e. pp-DDE). The consumption evaluation shows that this mud clam *P. erosa* has been contaminated of PAHs and not recommended for human consumption. They were potentially as site-specific bioaccumulation in sediment for organic micropollutants which receives from any untreated sewage, industrial waste discharge into the canal system of river of Segara Anakan Lagoon.

Keywords : Segara Anakan Lagoon (SAL), *Polymesoda erosa*, surface sediments, PAHs, PCBs, OCPs

Introduction

The Segara Anakan Lagoon is located on the southern coast of Central Java, close to the Indian Ocean and Nusakambangan island. The Segara Anakan area has geographic coordinates $07^{\circ}34'29.42''$ - $07^{\circ}47'32.39''$ and $108^{\circ}46'30.12''$ - $109^{\circ}03'21.02''$ [1, 2, 3]. This lagoon is connected to several rivers, such as Citanduy, Cibeurem, Cikujang, Cikonde, Kayu Mati, Ujung Alang, Dangkal, Kembang Kuning, Sapuregel, and Donan. Among these rivers, the Donan River is located close to several petroleum and mining industries, such as Cilacap Industrial areas and Tanjung Intan Port as well as close to the Cilacap urban area [4]. This lagoon is also the largest remaining mangroves expanse in Java, which accommodating a lot of living spaces for coastal

species [4, 5, 6]. The supply of fresh water that enters the aquatic environment is very rich in organic matters as the source of nutrition for aquatic biota, but there are also pollutants and sediments that come from anthropogenic activities. The sediment contains inputs from deforestation, volcanic eruption, major anthropogenic activities, and materials that are potential to act as environmental degrading agents. The bioavailability of organic micropollutants adsorbed by sediment is directly related to the solubility of compound. Organic micropollutants that are adsorbed into the sediment are only slightly bioavailable and often contains high concentrations of adsorbed pollutants [7]. All of these substances have several common characteristics. Their octanol/water partition coefficients are always very high, meaning that they have lower water solubility and higher lipophilicity indicating their adsorption along the food web and accumulation in animal tissues in transporting organic micropollutants [7, 8, 9]. Nonpolar organic micropollutants such as Polycyclic aromatic hydrocarbons (PAHs) are the by-products of incomplete combustion of organic material, such as wood, petroleum, and coal. In chemical terms, PAHs are a large group of diverse organic compounds that contain two or more fused aromatic rings ranging from the two-ring naphthalene and naphthalene derivatives to complex ring structures containing up to 10 rings [10]. PAHs dissolve well in fats, bind to particles, and accumulate in organisms and the environment. PAHs enter the environment mainly through the air as a result of industrial and natural thermal processes. Many PAHs are carcinogenic, mutagenic, or toxic for reproduction. Due to their chemical, biological stability, and their potential to bioaccumulate, they are persistent in the environment and accumulate in organisms [11]. The term persistent refers to the fact that the substances remain in the environment for a long time and are difficult to decompose.

Two examples of Persistent organic pollutants (POPs) are Polychlorinated biphenyls (PCBs) and Organochlorine pesticides (OCPs) that are usually found to be toxic and accumulate biologically in wildlife [12]. **The Stockholm Convention [12]** was released by the United Nations Environment Programme (UNEP) to identify and prohibit the utilisation and release of these substances into the environment. POPs have a high organic carbon partition coefficient (K_{oc}) associated with particulates and organic matter that can be suspended or dissolved as transport chemicals or contaminants in aquatic ecosystems [13]. PCBs are produced along with commercial products containing complex mixtures of congeners with different levels of chlorination on their chemical and physical properties. Their properties like stability, low

flammability, lipophilicity, and electrical insulation make them persistent in the marine ecosystem. Their wide range of applications include dielectric fluids used in capacitors or transformers, plasticizer, adhesives, sealants, paints, and additives in pesticides.

PCBs are toxic to fish, kill them when they reach certain high doses and cause spawning failure at lower doses. The researchers also linked PCBs to reproductive failure and immune system suppression in various wild animals, such as seals and minks. Consumption of PCB-contaminated rice oil in Japan in 1968 and in Taiwan in 1979 caused pigmentation of the nails, mucous membranes, and swelling of the eyelids, along with fatigue, nausea, and vomiting [14]. Most countries no longer use OCPs, such as hexachlorobenzene, pp'-DDE, pp'-DDT, pp'-DDD, aldrin, dieldrin, endosulfan-1, and heptachlor for agricultural use as they are banned worldwide [12]. POPs like PCBs and OCPs have been shown to exhibit potentially harmful effects on human and the environment [15].

The supply of fresh water that enters the aquatic environment is very rich in organic matters as the source of nutrition for aquatic biota, but there are also pollutants and sediments that come from anthropogenic activities [3, 16, 17]. Surface sediments provide an ideal natural archive for regional marine pollution, means of integrating input and combined processes of deposition, runoff, water mixing, and sedimentation [18]. Accelerated sediment deposition is a major environmental phenomenon monitored, as a temporary or long-term risk results from many classes of anthropogenic contaminants sinking [19]. Even small amounts of pollutants play an important role in the physiological processes of an organism. Problems often arise when pollutant concentrations exceed the organism's capability to neutralize them. This may lead to cell damage or even death [20, 21].

P. erosa is known to inhabit in the mangrove mud flats, implying that they live under varying environmental stresses [22, 23] covering high salinity (7 to 22 ppt) and low pH. Mud clams, also known as mangrove clams (*P. erosa*) (Solander, 1786), are widely distributed across Indo-Pacific coastal areas, including Segara Anakan, Cilacap. Mature mangrove clams of *P. erosa* (Solander, 1786) can reach up to 11 cm in shell size [24]. These clams are mostly consumed by local people who live close to coastal areas, including the community around the Segara Anakan area [24]. These clams are sessile organisms and thus experience the adverse effect of environmental changes due to the input of pollutants. Mud clams were chosen as the object of this study for reasons, such as its common use as bio-indicator for pollution, ability to accumulate pollutants in

their tissues without reaching the point of death, abundance, long life span, big size, ease in handling, tolerance to brackish water and freshwater, and shows a positive correlation between pollutant concentrations in the environment and effects on body tissues [25]. Several studies related to this lagoon have been and are still being conducted to explore and evaluate the organic pollutant content. **Dsikowitzky et al. [26]** reported the presence of organic contaminants, such as several hydrocarbons (n-alkanes and polyaromatic hydrocarbons) in sediments and some aquatic organisms. **Syakti et al. [3]** found the distribution and quantification of hydrocarbons content (i.e. n-alkanes, PAHs) in sediment in Segara Anakan Lagoon. However, monitoring of microorganic pollutants, especially for persistent compounds such as PCBs and OCPs in sediment and *Polymesoda erosa* along with the risks posed to the site are still needed to evaluate the baseline pollutant content regularly. In other hand, no information about hydrocarbons aromatic compounds (PAHs) content in this species of mud clams based on different activities from their cultivation area. PAHs can associated in the lipid tissue of living organism and sediment. The risk of consumption and bioaccumulation factor for this species were needed to evaluate as preliminary study in this site. The occurrence of POPs in this lagoon were need to Baseline data provide a starting point for making comparisons with the current data. If such information is not available, assessing or observing future environmental impacts will be more difficult and less accurate [18].

This study aims to explore persistent microorganic pollutants (Poly chloro byphneyls (PCBs) and Organochlorine Pesticides (OCPs)) in sediment and mud clams (*Polymesoda erosa*) in Segara Anakan Lagoon (SAL). to evaluate the risk consumption and Bioaccumulation factor in sediment of polycyclic aromatic hydrocarbons (PAHs) in the mud clams. Then to assess the ecotoxicological risk of 7 PCBs and OCPs (i.e. pp'-DDE) in sediment using the Sediment Quality Guidelines (SQG). Several studies about analysis of organic pollutants in mussels have been published and can be use as a comparison to the results obtained in this study from *Polymesoda erosa* mud clam samples.

Materials and Methods

Study Area

Seven surface sediment sampling stations (Station 2, 7, 8, 9, 10, 11, and 12) and four mud clam sampling stations, *P. Erosa*, represent different zones such as petroleum refinery sites (P1 and

P2), the urban areas (Kampung Laut village, KL), and the mangrove zones (M) taken from Segara Anakan Lagoon area (**Figure 1**). The GPS sites of the sampling locations and sediment characterisation shown in **Table 1**.

Table 1. GPS sites of the sampling locations and sediment characterisation

Station	Parameters				Notes
	Salinity (ppb)	Coordinate	Depth (m)	Granulometry (μm)	
SA-2	5	07°39'44.160" S 108°50'57.060" E	2	Sand (> 1000)	Many gastropods, color: brown
SA-7	2	07°40'46.260" S 108°47'24.420" E	3	Sand (>1000)	Many gastropods, color: brown
SA-8	5	07°40'51.780" S 108°47'40.500" E	3	Sand (>1000)	Color: brown
SA-9	5	07°41'34.140" S 108°47'27.420" E	10	Sand (>1000)	Color: brown, black
SA-10	8	07°42'30.420" S 108°53'44.580" E	6	Sand (>1000)	Color: brown, black
SA-11	4	07°43'3.420" S 108°56'8.460" E	1	Gravel, granules (>2000)	Color: black
SA-12	4	07°43'3.600" S 108°57'53.880" E	3	Gravel, granules (>2000)	Color: black

The sediment sampling stations are located at the same sites as several research sites published by Syakti et al. [3] to complete the baseline references with other compounds organic pollutants content in Segara Anakan sediments. They analyzed PAHs in surface sediments as well as their potential origins and found that the sources of hydrocarbons in those stations were generally biogenic, including both terrigenous and marine, with an anthropogenic pyrolytic contribution (petrogenic and biogenic combustion) [3]. Station 7, 8, and 9 are located close to the sea area and downstream from several rivers. Meanwhile, stations 2, 10, 11, and 12 are predominantly covered by mangrove (Figure 1).

Sampling of Sediment

Quantum GIS map software has been used to draw maps and mark the zones around the sampling sites. A global positioning system (GPS, Garmin Etrex Summit HC, Kansas City, USA) was used to mark sampling target at different sites. Seven surface sediment (0 - 5 cm) were collected

(Figure 1) using a Van Veen stainless steel grab sampler. A composite sample for each site was created by mixing 3 sub-samples with the same quantities. Then the sediment was mixed immediately in an aluminium container and stored in a cool box. The cool box minimizes microbial degradation during transport to the laboratory. Samples stored at low temperature (18°C) before treatment in the laboratory. The wet sediments subsequently freeze dried [19] using Operon, Myo Gimpo City, South Korea at -50°C and 8 mbar, filtered with a 200 µm stainless steel sieve and homogenized, stored for a week before analytical treatment.

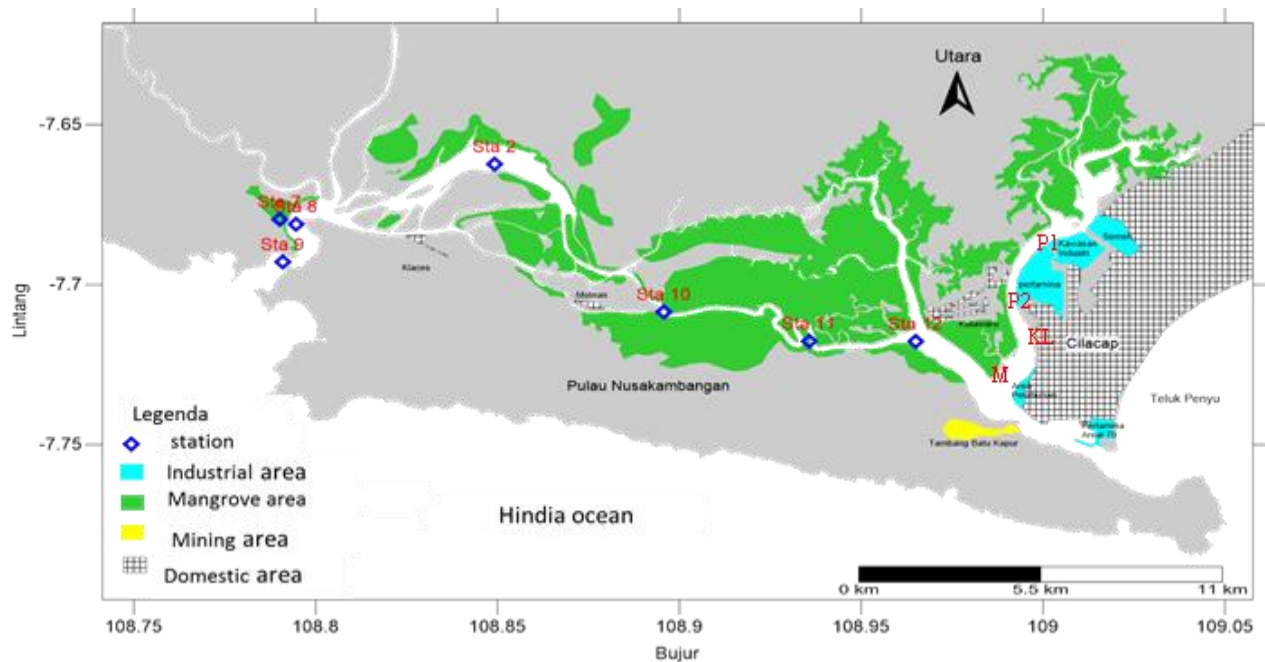


Figure 1. Seven sediments sampling sites (Station 2, 7, 8, 9, 10, 11, 12) and four clams sampling sites, *Polymesoda erosa* (mangrove zones (M)), petroleum refinery site 1 (P1), petroleum refinery site 2 (P2), domestic area (Kampung Laut)

Sampling of Mud Clams, *P. erosa*

Mud clams samples were collected at four sampling stations (5-20 cm depth) (Figure 1). A composite sample for each sampling station was made by mixing together the same quantity of each Mud clams sub-sample (n=10). The length of clam is about 4-9 cm with their cockles and its diameter about 3 cm. The cockles of mud clams are removed from their clam to reduce water for 1 hour and covered with aluminium foil to avoid air contamination under vacuum. Their wet mass was weighed and then freeze-dried at -50°C and 8 mbar for 48 hours. The body condition

index (BCI) is a measure of the physical condition of the mussels and calculated as dry weight of soft tissue (dried at -50°C until the weight remaining constant, obtained for all samples within 48 hours) divided by dry weight of the clams. The dry mass was weighed on a microscale (Perkin-Elmer AD2Z) then cut and crushed into powder manually with a mortar for several days. The dry tissue was filtered, weighed, homogenized, and stored at room temperature before analysis.

Chemicals

Several solvents used were acetone (ACET), dichloromethane (DCM), acetonitrile (ACN), and n-heptane (HEPT) (GC SupraSolv grade (Merck, Darmstadt, Germany)). HCl (37 %) of the pure grade (Fisher Scientific, Leicestershire, UK). Each replacement standard deuterated of PCB-101- d_3 to calibrate 7 PCBs standard mix solutions and 9 organochlorines solutions (pp'-DDE, pp'-DDD, pp'-DDT, hexachlorobenzene, aldrin, dieldrine, endosulfan 1, heptachlor, and heptachlor epoxide) and several mixtures of commercial standard solutions are PAH Mix 25, US EPA 16 PAH mix solutions (Dr. Ehrenstorfer Laboratories, Augsburg, Germany). PCB mix 3 contains 7 PCBs mix solutions (CIL CLUZEAU, Andover, MA, USA). Dichloromethane is used to dilute standard working solutions for PAHs and HEPT is used for dilution of PCBs and OCPs. Commercial alumina (1 gram and 5 gram) and SPE dispersive tubes (1 gram and 5 gram) were used to optimize the purification method of mussels. Both were purchased from Agilent fournisseur, France. Cu powder (> 230 mesh) from VWR International and Merck (Darmstadt, Germany) for washing the sediments.

Extraction of PCBs and Organochlorine pesticides (OCPs) in Sediment

Based on the modification method reported by Kanzari et al. [15], PCBs and OCPs extracted with ASE (Accelerated Solvent Extraction) using 33 mL thimble cells with filter fibers at the base of cell, ca. 3 grams of alumina are added at the base of the cell, then 10 grams of sediment ca. 10 grams and 3 grams of Cu powder (which has been diluted by HCl 1:4, v:v) were added, then the cell completed with fontainebleu sand (**Figure 2**). The solvent extraction with 150 mL HEPT:ACET (1:1) was performed at 100°C and 500Psi for 2 cycles [15], then evaporated at turbovap under nitrogen stream until final volume of 0.5 mL obtained. The analysis of PCBs and OCPs were performed by gas chromatography-triple quadrupole mass spectrometry (GC-QQQ/MS) system. Villaverde et al. [27] use this purification method by adsorption

chromatography using various composition of silica:alumina with two successive elutions. Consequently, the method was modified that use the alumine inside the ASE instrument as extraction and purification method simultaneously then evaporate before analysis.

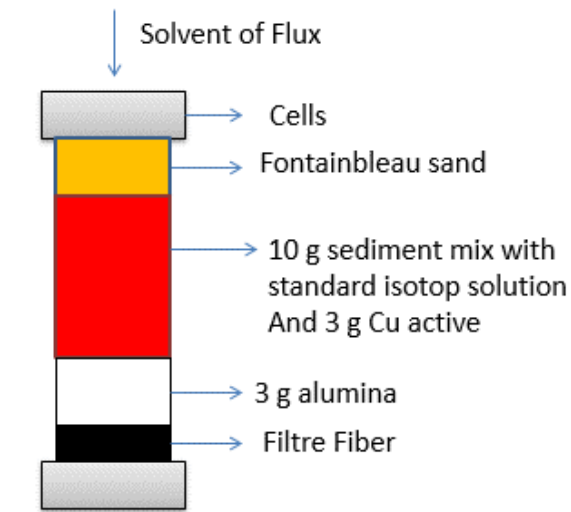


Figure 2. The content of thimble cells for ASE extraction method

Extraction and Purification of PAHs and POPs (PCBs and OCPs) for Mud Clams

QUECHERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) is an extraction and clean up method for marine tissue analysis using Agilent 700 [28]. It has been proved as a popular extraction of pesticides for multi-residues method and it is more sensitive due to the cleaner nature produced compare to soxtec method [29]. 2 gram of homogenized mud clam's dry tissue placed into a QUECHERS extraction tube [28]. 50 μL of each respective internal standard deuterated ($10 \text{ mg}\cdot\text{L}^{-1}$) were added into sample. The samples vortexed for 30 seconds, then evaporated for 30 minute. 13 mL of deionized water and 2 ceramic homogenizers added into a tube contains the mussel's dry soft tissue sample and the tube then vortexed for 1 minute. 15 mL of extraction solvent (1% acetic acid in acetonitrile) added and vortexed for 1 minute. The tube then centrifuged for 5 minutes at 2683 g, then it cooled in a freezer at -20°C for 30 minutes. The acetonitrile layer has been transferred to a centrifuge tube contains 1 gram of anhydrous sodium sulphate, shaken by hand for 1 minute then placed in a freezer at -20°C overnight. 10 mL of clear extract evaporated with turbovap to 0.5 mL, then 20 mL of DCM added into the extract and evaporated to 0.5 mL. Finally, ca. 10 mL HEPT added then evaporated to 0.5 mL.

The purification was carried out using a solid phase extraction method with selected SPE tube commercial that had been tested and optimized with different elution conditions. 2 types of

commercial SPE tube were tested with different composition (1 gr and 5 gr of alumina, 1 gr and 5 gr of SPE dispersive tube). In addition, several compositions of eluent have been tested. They are DCM:HEPT (9:1) for 1 gr of alumina, 5 gr of alumina using DCM:HEPT (6:4). For SPE dispersive tube with HEPT and then eluted with DCM. The SPE tube conditioned with 10 mL of DCM and 20 mL of HEPT. The concentrated extract transferred to the SPE tube and eluted with 13 mL of selected eluent, then the extract evaporated to 0.5 mL and the purification process was repeated. Finally, it was evaporated to 0.5 mL. The analysis of the extract was performed using gas chromatography-mass spectrometry, Agilent 7000 triple quadrupole GC-QQQ/MS system.

Lipid were extracted by homogenizing with the $\text{CHCl}_3/\text{CH}_3\text{OH}$ solution (1:2, v/v) (10g wet weight) in 6 h at 30°C. Then filtered and the residue was repeatedly extracted with CHCl_3 (20 mL) a second time. After extraction, the extracts were mixed and separated with added H_2O . The lower layer was evaporated and dissolved in CHCl_3 and stored -5°C, the total lipid content was obtained. Fatty acids, corresponding FAME (fatty acid methyl esters) were analyzed with GC-MS and resulted spectra were matched with Fatty acid mass spectra with the NIST library. GC-MS analysis initiated at 160°C and then increased at 2°C/min to 240°C which was kept constant for 20 min, injector and detector temperatures were 250°C [30, 31, 32].

Gas Chromatography-mass Spectrometry (GC-QQQ/MS)

The extracts from ASE (sediments) and QUECHERS (mud clams tissue) methods diluted in 1 mL DCM, then analysed using GC-QQQ/MS (Gas Chromatography Triple Quadrupole Mass Spectrometry; Agilent 7000, USA). The analysis of parameters conducted with QQQ-Quantitative and QQQ-Qualitative analysis agilent technologies software. The chromatographic conditions use DB5MS column (30 m x 0.25 mm i.d. x 0.25 μm film thickness) in the splitless (30s), the GC oven is temperature-programmed from 80 °C (isotherm 2 min) to 300 °C at 12 °C.min⁻¹, then held for 5 minutes until run time 25.33 min. Flow rate of the carrier gas of helium maintained constant at 3 mL.min⁻¹ and the PSS injector programmed from 80°C (0.1 min) to 250°C (200°C.min⁻¹), then held for 10 minutes. The temperature reduce to 60°C (200°C.min⁻¹) to minimize the solvent peak.

The mass spectrometer is operated in positive electron impact ionisation (EI) mode (70 eV) and scanned in both full scan (FS) and selected ion monitoring (SIM) modes for aromatic hydrocarbons (PAHs) analysis in mud clams. We analyzed 7 indicators PCBs mix (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180) and 9 organochlorines

compounds (pp'-DDE, pp'-DDD, pp'-DDT, hexachlorobenzene, aldrin, dieldrine, endosulfan 1, heptachlor, and heptachlor epoxide) in sediment and mud clams samples. Then mass analysis have been scanned optimal in product ion mode (PIM) at certain energy collision then scanned in Multiple Reaction Monitoring (MRM) mode. All molecular targets are compared with those corresponding against molecular weight from mass spectra library, matching retention time parameters, and mass fragmentation profiles against corresponding standard solutions either their deuterated standards [33]. The analysis of 16 PAHs analysis use the 16 US-EPA priority PAH deuterated internal standards mix in various concentration, from 0.5 pg.μL⁻¹ to 1000 pg.μL⁻¹, then PCBs and Organochlorine pesticides deuterated individual standards (PCB-101-d₃ m/z; pp'-DDE-d₄ m/z, and pp'-DDT-d₄ m/z) in various concentration, from 5 pg.μL⁻¹ to 1000 pg.μL⁻¹ [33]. The summarize qualification and quantification ions for SIM mode shown in **Table 2** and the transition for MRM mode shown in **Table 3**.

Table 2. MRM (Multiple Reaction Monitoring) mode of PCBs and OCPs

Compounds	Quantification transition			Confirmation transition		
	Precursor ion (amu)	Product ion (amu)	Collision Energy (V)	Precursor ion (amu)	Product ion (amu)	Collision energy (V)
Window 1 (11,5 to 15,6 minute)						
Aldrin	298.0	→ 263.0	8	263.0	→ 191.0	30
Hexachlorobenzen	283.9	→ 248.8	25	283.9	→ 213.9	35
Heptachlore	271.1	→ 237.0	10			
PCB 28	256.0	→ 186.0	26	258.0	→ 186.0	26
PCB 52	289.9	→ 220.0	28	291.9	→ 222.0	28
Window 2 (15.6 to 25 minute)						
Dieldrin	262.8	→ 193.0	30	262.8	→ 191.0	15
Endosulfan 1	240.9	→ 205.9	10	240.9	→ 169.9	20
Heptachlor epoxy	352.7	→ 262.9	20	352.7	→ 316.9	20
ppDDD	237.0	→ 165.0	20	235.0	→ 199.1	8

pp'DDD d4	242.9	→	173.1	20			
pp'DDE	248.0	→	176.0	30	246.0	→	211.0 20
pp'DDT	237.0	→	165.0	20	235.0	→	199.1 20
pp'DDE d4	253.9	→	184.2	30			
PCB 101	323.9	→	253.9	28	325.9	→	255.9 28
PCB 101 d3	328.7	→	258.9	20			
PCB 118	323.9	→	253.9	28	325.9	→	255.9 28
PCB 138 and 153	357.0	→	287.9	28	359.8	→	289.9 28
PCB 180	393.8		323.9	30	395.8	→	325.9 30

Table 3. SIM (Selected Ion Monitoring) mode of PAHs

Target compounds (standard marquer)	Qualification m/z (amu)	Quantification m/z (amu) (standard marquer)
Naphtalene (d_8)	127	128 (136)
Acenaphtylene (d_{10})	-	154 (162)
Acenaphtene (d_{10})	-	152 (160)
Fluorene (d_{10})	-	166 (176)
Phénanthrene / Anthracene (d_{10}/d_{10})	174/ 174	178 (188)
Fluoranthene / Pyrène (d_{10}/d_{10})	200/200	202
Benzo(a) anthracene / Chrysene (d_{12}/d_{12})	226/226	228 (240)
Benzo(b)Fluoranthene Benzo(k)fluoranthene Benzo(a)pyrene ($d_{12}/d_{12}/d_{12}$)	250	252 (264)
Indéno (1,2,3) cd pyrene / Benzo(g,h,i)pérylene (d_{12}/d_{12})	276/ 274	276 (288)
Dibenzo(a,h)anthracene (d_{14})	274	278 (292)

Quality Controls

All concentrations data were based on dry weight (dw) and the analysis had replications. Sample matrix evaluation has been tested with appropriate samples to determine the analysis and extraction yields in the range of 80 to 120% for selected molecular targets. The detection limit evaluated for 16 PAHs was $0.05 \mu\text{g.kg}^{-1}$ dw, while for PCBs and OCPs in real samples was $0.5 \mu\text{g.kg}^{-1}$ dw and the quantification limit relatively was $< 5 \text{ pg.}\mu\text{L}^{-1}$. The detection limits for PAH, PCBs and OCPs were determined as the content giving rise to a peak with a signal/noise ratio (S/N) of 3. Calibration curves were built using the analyte/internal standard peak area ratio, for the GC-MS analysis in SIM or MRM mode versus analyte concentration. Each calibration graph was established for each compound at 10 increasing concentrations of the analyte with range 1-1000 $\text{pg.}\mu\text{L}^{-1}$ (1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 $\text{pg.}\mu\text{L}^{-1}$) in 1 mL solution. To optimize the analytical protocol, sediment samples were spiked with $10 \mu\text{g.kg}^{-1}$ each deuterated standard solution at a concentration of PCB 101-d₃, pp'-DDD-d₄ and pp'-DDE-d₄ and OCPs mix. Three experimental replicates for each concentration level were evaluated performing samples analysis. Performing analysis method such linearity performance of coefficient (R^2) must be equal or greater than 0.990, Relative standard deviation of response factors must be less than 20%, Mean recovery, measured in the range 60-120% and precision, expressed as reproducibility $\leq 35\%$. Those parameters are accordance with the Commission Decision 2002/657/EC (European Commission, 2002) concerned the performance of analytical methods. Procedural blank was added in sequential running program before running of environmental samples to minimize coelution and matrix interference in column contamination [3, 34, 35].

Result and Discussion

The gravimetric EOM (Extractable Organic Material) data and mineralogy of sediments given in **Table 4**. Most of sediment samples in Segara Anakan Lagoon sampling sites were silt loam (silt percentage was more abundance than clay and sand). Sedimentary organic carbon fraction depends on the size of the sediment particle which able to export Organic materials to the ecosystem in comparison to the silt and clay sediment substrates. Sampling stations were near from effluent of Cibereum and Citanduy rivers. Extractable organic material (EOM) from

different input (i.e. river, lagoon and ocean) may be also give different standard deviation. The EOM was ranged from 480- 960 mg.kg⁻¹ dw.

Table 4. Extractable organic material (EOM) and mineralogy of sediments

Station	EOM (mg.kg ⁻¹ dw)	Sand (%)	Silt (%)	Clay (%)	Mineralogy
2	880	7	35	58	Silt clay loam
7	740	38	55	7	Silt loam
8	630	13	74	13	Silt loam, loam, sandy loam
9	520	43	50	7	Silt loam
10	460	5	80	15	Silt loam
11	620	46	47	7	Loam, sandy loam
12	440	19	68	13	Silt loam

Stations 2,7, 8,10, 11: near effluent of citanduy and cibeureum rivers; Station 9 near from seawater. P1,P2, mangrove and Kampung Laut = sampling sites of *Polymesoda erosa* .EOM = Extractable organic matter.

PCBs and OCPs Content in Sediments

The three PCBs (CB 28, CB 52, and CB 101) and one OCPs (pp'-DDE) were found in the sediments. Total PCBs concentrations were found in the range of 0.11 to 2.63 µg.kg⁻¹ sed.dw. In addition, pp'-DDE concentrations were found in the range of 0.2 to 2.9 µg.kg⁻¹ sed.dw. PCBs and OCPs contents in sediment are summarized in **Table 5**. Samples collected from SA-2 had the highest PCBs content compared to other stations. This site is close to mangrove forests. The unique properties of sediment near from mangrove, can being reduced, anaerobic and rich in organic matter pollutants from inland freshwater drainage and tidal flush tend to accumulate in these sediments [36, 37]. Spatial variations in the physico-chemical and biological properties of mangrove ecosystems are significant because of the heterogenous composition well as human interference and also as living habitat of biota [38]. The differences in sediment composition such

as its grain-size distribution and organic carbon content would affect concentrations of PCBs [39]. In future research, it is necessary to analyse another matrix like mangrove vegetation. Mangrove ecosystems naturally function as anthropogenic and industrial pollutants. Mangrove ecosystems in specific numerous aspects (i.e. carbon and nutrients cycles, sediment characteristics, tidal conditions) are expected to affect the speciation and bioavailability of contaminants [40]. The growth in industrialization and uncontrolled anthropogenic pressure on virgin mangrove patches has increased in recent years. Mangroves are under severe stress condition due to reclamation and urbanization, and several valuable resources including *P. erosa* are at a risk of death mainly due to habitat destruction [41, 42]. However, mangrove ecosystems adapt by acting as natural pollution sinks [43]. Mangrove soil/sediment is usually fine-grained, water-logged, and receive allochthonous organic matter from terrigenous sources [44, 45]. Chemical contaminants can be present in mangrove ecosystems from overlying water and solid phases, such as sediment or suspended particulate matter and biota [44]. Several stations that provide high concentration in PCBs and OCPs samples were SA-7, 8, and 9 (especially for 7 PCBs and pp'-DDE content) which located close to the Indian ocean and cross sections of several rivers from urban areas forming estuaries. The concentration of PCBs and OCPs in surface sediment shown in **Table 5**.

Table 5. The concentrations of PCBs and OCPs in surface sediment ($\mu\text{g}\cdot\text{kg}^{-1}$ sed.dw \pm SD) from Segara Anakan Lagoon (stations SA-2 to SA-11) (n=3)

Compounds	SA-2	SA-7	SA-8	SA-9	SA-10	SA-11	SA-12
PCB 28	0.45 \pm 0.08	0.51 \pm 0.07	0.5 \pm 0.03	< LOD	0.14 \pm 0.02	0.27 \pm 0.09	<LOD
PCB 52	0.7 \pm 0.1	0.9 \pm 0.1	< LOD	0.11 \pm 0.05	< LOD	<LOD	<LOD
PCB 101	1.48 \pm 0.13	< LOD	< LOD	<LOD	<LOD	<LOD	<LOD
Σ PCBs	2.63 \pm 0.1	1.41 \pm 0.17	0.5 \pm 0.03	0.11 \pm 0.05	0.14 \pm 0.02	0.27 \pm 0.09	<LOD
pp'DDE	<LOD	2.9 \pm 0.01	0.2 \pm 0.05	2.85 \pm 0.04	<LOD	2.87 \pm 0.11	<LOD
Σ OCPs	< LOD	2.9 \pm 0.01	0.2 \pm 0.05	2.85 \pm 0.04	<LOD	2.87 \pm 0.11	<LOD

LOD : Limit of Detection

In general, estuaries are able to accumulate pollutants at high concentration in sediment and they potentially able found the degradation products of pollutants structure such as pp'-DDE. This

compound able to degraded product as pp'-DDT. In the initial stage of the reaction, the aliphatic chain of DDT can be subject to a dehydrochlorination to form DDE or a hydrodechlorination to form DDD. The pp'-DDE was found in rivers and estuairies before being passed into the ocean. They were found in station 10 and 11 but it more below than limit of detection in station 12. This case was same from station 2, 7, 8, through to station 9 near from ocean is lower than station in the river (**Figure 1**). But Station 2, 10, and 8 have low level of pp'-DDE because these stations are close to the ocean where the pollutants compounds have degraded. We conclude that at stations near to the ocean, pp'-DDE and PCBs content accumulates. The standard deviation of the PCBs and pp'-DDE concentration levels was ± 0.2 , meaning that the repeated concentration values have a confident limit of 0.2. Distribution of PCBs and OCPs in surface sediments from Segara Anakan Lagoon (SAL) shown in **Figure 3**

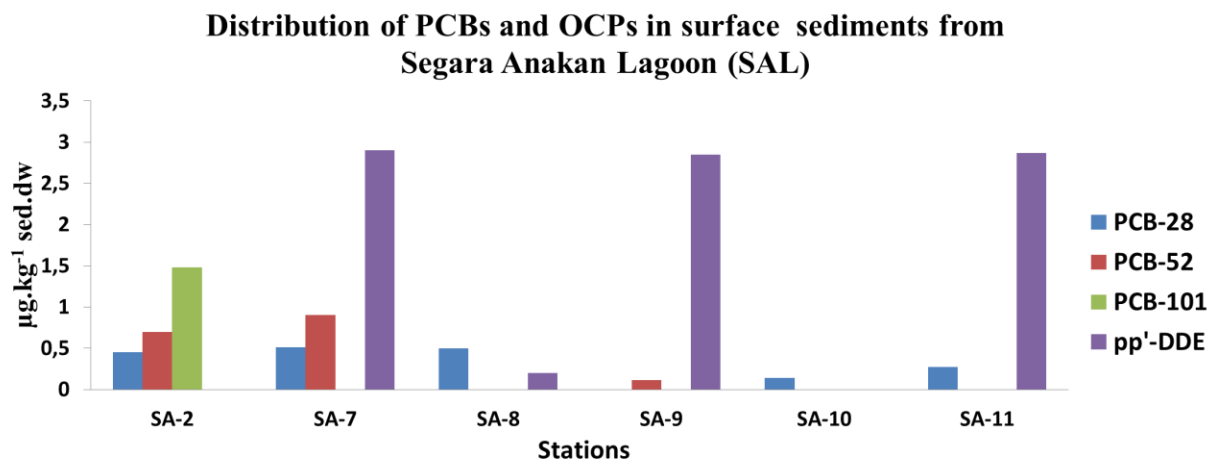


Figure 3. Distribution of PCBs and OCPs in 7 stations from Segara Anakan Lagoon (SAL)

Station 2, 9 and 11 (SA-2, 9 and 11) were near the crosssection of river branch and agricultural activities near from mangrove forest and also the slowly flow stream. That were possibility the compounds were adsorbed in sediment. Several studies can compare the level of PCB contamination in sediments from the Segara Anakan Lagoon which is higher than another several location in the Jakarta Bay (since 2011) 2-9 $\mu\text{g.kg}^{-1}$ dw and Tokyo Bay 37-47 $\mu\text{g.kg}^{-1}$ dw [45], Hauveane River, France 27.2-36.4 $\mu\text{g.kg}^{-1}$ dw [15]. It is lower than in Mazaleh Lake 125-330 $\mu\text{g.kg}^{-1}$ dw, Mariout Lake 4-304 $\mu\text{g.kg}^{-1}$ dw, Nil River, Egypt 53-1500 $\mu\text{g.kg}^{-1}$ dw[46], Cortiou Marseille 1,2 – 190.6 $\mu\text{g.kg}^{-1}$ dw [47], Paranagua Bay, Brazil 56-189 $\mu\text{g.kg}^{-1}$ dw [48], and Lenga Estuary, Chili 28-13791 $\mu\text{g.kg}^{-1}$ dw[19]. In Jakarta Bay, pp'-DDE is the highest organochlorines

level 1-7 $\mu\text{g.kg}^{-1}$ dw [48], while others are lower than Jakarta Bay level, such as Hauveane River, France 0.06-5.48 $\mu\text{g.kg}^{-1}$ dw [15] and Bothnian Bay, Baltic Sea 0.48-1.1 $\mu\text{g.kg}^{-1}$ dw [50].

Ecotoxicology of Sediments

The Sediment Quality Guidelines (SQG) have been used in various applications for assessing sediments quality and ecological risk, also aiming to produce sediment quality remediation [15, 51, 52]. Several studies have used this assessment to protect sediment-dwelling organisms in freshwater ecosystems. They differ in different categories, treshold effect levels (TELs), effect range low values (ERLs), probable effect levels (PELs), and effect range median values (ERMs) [51]. PCBs assessment was used to combine ERM/ERL values between 22.7-180.0 $\mu\text{g.kg}^{-1}$ sediment dw [53]. ERL sediments that have concentrations of adverse toxicological effects can be detected with values of 180 $\mu\text{g.kg}^{-1}$ dw and ERM values representing concentrations under adverse effects (22.7 $\mu\text{g.kg}^{-1}$ sediment dw). The sediment collected from all stations indicate that the PCBs content is below the ERM and there is no possible risk for short term period (**Figure 4**), but there can be a potential risk in the environment for long period of time.

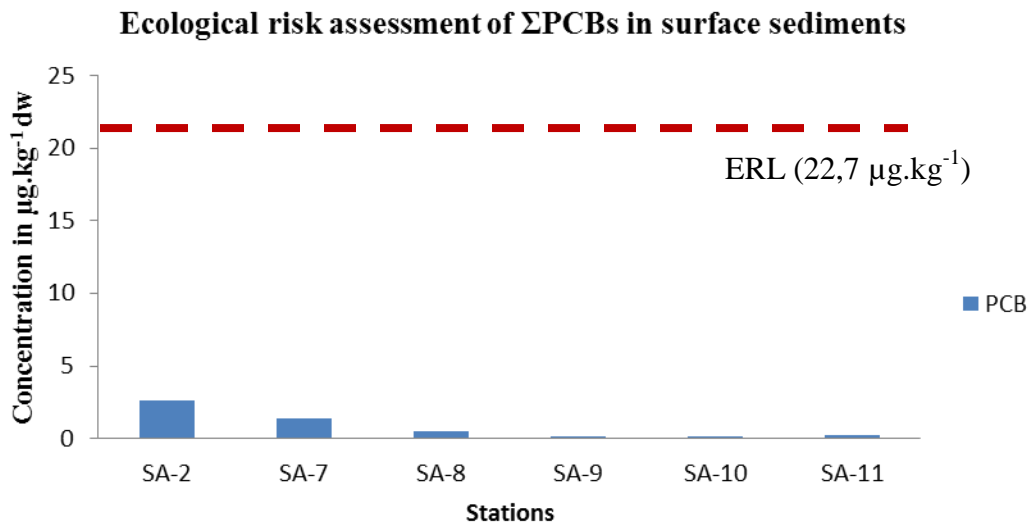


Figure 4. Sediment Quality Guidelines (SQG) of PCBs) in surface sediments Segara Anakan Lagoon, Indonesia ($\mu\text{g.kg}^{-1}$ sed.dw)

Meanwhile, the $\Sigma pp'$ -DDE assessment has three considerable parameters, such as TELs ($1.45 \mu\text{g.kg}^{-1} \text{ dw}$) meaning under potential effect; ERLs ($2 \mu\text{g.kg}^{-1} \text{ sediment dw}$) can be a potential side effect that can be detected, and PELs ($6.75 \mu\text{g.kg}^{-1} \text{ sediment dw}$) present a possible risk to the environment and biota. Station 7, 9, and 11 present a potential risk to the environment due to pp' -DDE level is higher than TELs level, but still lower than PELs (**Figure 5**). In addition, Station 2 and 8 are still lower than TELs. From this data, pp' -DDE has more potential risk to the environment and biota that found in sediments than PCBs. Station 12 was obtained lower than limit of detection, the current was very swift in that site. The sampling site (SA 12) is still trace into ocean through the current, the current from Station 12 is not arrived from industrial and domestic area. Potentially the PCBs and OCPs were degraded through to ocean because the previous stations have lower content or the pollutants potentially have been adsorbed into living biota or sediment in mangrove forest

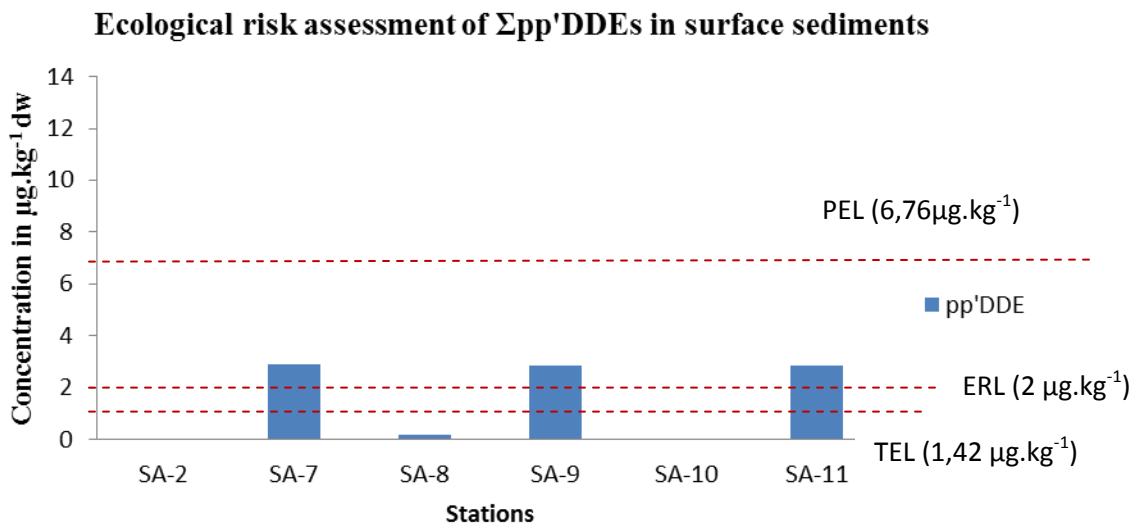


Figure 5. Sediment Quality Guidelines (SQG) of DDEs in surface sediments Segara Anakan Lagoon, Indonesia ($\mu\text{g.kg}^{-1} \text{ sed.dw}$)

PAHs, PCBs, and OCPs Content in *P. Erosa*

The purification performance from QUECHERS method has been optimized. The extraction and analysis yields have been simulated by adding several standard solutions combined with standard deuterated mix solution into several types of SPE tubes (SPE derivatives 1 g and 5 g; SPE tube 1

g, 5 g alumina). 1 gr of alumina SPE tube was selected for purification method based on highest recovery result ranged from 84-109%. The tube was eluted with eluent DCM:HEPT (9:1) obtained has high performance compared to other SPE tubes. The PAHs level is in the range 103.34±2.01 to 1348.31±18.21 as shown in **Table 6**.

Table 6. Concentrations of PAHs , PCBs and pp'-DDE from *P. erosa* ($\mu\text{g.kg}^{-1}$ tissue dw±SD) (n=3)

Compounds	Mangrove	P1	P2	Kampung Laut
Naphthalene	11.12±2.08	13.78±2.24	14.49±0.64	169.49±16.16
Acénaphtylene	40.14±2.71	74.67±0.90	25.17±5.84	89.61±39.94
Fluorene	8.74±0.52	10.31±0.14	7.48±0.10	12.25±2.6
Phénanthrene	74.10±0.11	48.94±2.91	52.89±4.91	90.50±16.69
Anthracene	103.46±2.95	< LOD	< LOD	< LOD
Pyrene	<LOD	< LOD	< LOD	6.83±0.30
Chrysene	< LOD	55.20±7.44	1.76±0.50	146.85±14.47
Benzo(a)Anthracene	< LOD	90.35±5.41	< LOD	< LOD
Benzo(k)Fluoranthene	<LOD	30.33±5.91	<LOD	< LOD
Benzo(a)pyrene	7.84±1.84	8.18±2.28	1.55±0.12	839.61±37.85
Indéno(1,2,3cdi)pyrene	< LOD	308.99±17.70	< LOD	< LOD
Benzo(ghi)perylene	<LOD	122.69±10,97	< LOD	< LOD
Σ PAHs	245.40 ± 1.70	763.44±5.59	103.34±2.01	1348.31±18.21
LMW	237.56	147.7	100.03	361.85
HMW	7.84	615.74	3.31	993.29
LMW/HMW	30.30	0.23	30.22	0.36
An/ Σ 178	0.42	0	0	0
Fluo/ Σ 202	0	0	0	0
Bz(a)Anth/ Σ 228	0	0,62	0	0

IndP/Σ276	0	0,72	0	0
Σ PCBs	<LOD	nd	< LOD	2.34±1.72
pp'-DDE	0.87±0.03	1.76±0.05	0.92±0.11	24.79±1.30

nd = not detected ; LOD: Limit of Detection

Four sites were found to be contaminated with different amounts of PAHs. The structure of PAHs lends them to analysis in the more sensitive, SIM mode, because they commonly ionize without significant fragmentation under typical electron ionization conditions, resulting in strong molecular ion signals. This makes it easy to convert an existing semivolatile compound analysis, like US EPA 8270D, that is typically run in full scan mode, to a SIM analysis that is able to detect even lower levels of PAHs [54]. The PAHs found at all stations are naphthalene, acenaphthylene, fluorene, phenanthrene, and benzo (a) pyrene. The total PAHs identified from 103.34±2.01 to 1348.31±18.21 $\mu\text{g.kg}^{-1}$ tissue dw. The Kampung Laut site has the highest PAHs levels with 6 types of PAHs (naphthalene, acenaphthylene, fluorene, phenanthrene, chrysene, and benzopyrene) (**Table 6**). P1 and P2 have different levels of PAHs. P1 has higher PAHs level and has more types of PAHs than P2 (**Table 2**). P1 has 10 types of PAHs while there are only 6 types of PAHs that found in P2. Based on the location, P1 and P2 were located near from petrochemical industrial zone, while P2 is near from the mangrove sites (**Figure 1**). P1 has more types of PAHs than P2, Mangrove, and Kampung Laut sites. **Table 6** also shows the low concentration of low molecular weight (LMW) PAHs in mud clams compared to the higher molecular weight (HMW) PAHs, possibly due to the greater bioavailability of the more water-soluble HMW PAHs or related to a partial biotransformation of the higher molecular weight PAHs [15, 55, 56, 57]. Ratio LMW/HMW > 1 indicates the source of petroleum, while < 1 comes from pyrolytic activities (combustion wood, vegetation) [55, 56, 57]. However, LMW concentration were found to be higher than HMW of PAHs in the clams in Mangrove and P2. These values indicate that PAHs with 2 and 3 rings are abundant in this sites, while in P1 and urban area (Kampung laut) site have high levels of PAHs > 3 rings level. Based on **Figure 1**, there are still higher of HMW PAHs in P1 and Kampung Laut sites and degraded to small molecular weights and only the remaining PAHs in 2 or 3 rings is found in P2 and Mangroves sites. Based on the An/Σ178, Bz(a)Anth/Σ228 and IndP/Σ276 ratios from **Table 6**, They were indicate that PAHs sources of P1 and mangrove are related to pyrolytic activities (< 0.2) . LMW/HMW ratio was shown that P1 and KL station ratio related to pyrolytic activities while P2 and mangrove stations were petroleum sources. The

different sources of P1 and P2 were indicated from characteristic activities near from those sites. P1 was near from petrochemical combustion process, and agricultural areas while P2 was near from mangrove, urban areas sites and transportation track the fisheries to mangrove forest from their village. These evidence suggest that PAH contamination in the clams might from petroleum and petrogenic sources depending the dominant activities near from sampling sites

In addition, plastic waste can be ingested by aquatic organisms and then release several POPs (such PAHs) that found from the coastal waters of Cilacap coastal area [58]. Several studies of micropollutants organic analysis in the mussels are available. **Isobe et al. [59]** published the distribution of 19 PAH compounds at several locations in South East Asia in *P. viridis* with a concentration range of 11-1133 $\mu\text{g.kg}^{-1}$ tissue dw. Based on l'AFSSA (Agence Francaise de Sécurité Sanitaire des Aliments [60], a risk evaluation can be proposed by the sum of 6 PAHs (benz(a)anthracen, benzo(b+j)fluoranthen, benzo(k)fluoranthen, benzo(a)pyren, dibenzo(a,h)antracen shown in **Table 7**.

Table 7. Concentrations of 6 PAHs from *P. erosa* ($\mu\text{g.kg}^{-1}$ tissue dw)

<i>Polymesoda erosa</i>	Σ 6 HAPs ($\mu\text{g kg}^{-1}$ tissue dw)
Station	
P1	560.54
P2	1.55
Mangrove	7.84
Kampung Laut	839.61

The limit value for consumption of seafood contaminated with PAHs is 0.2 mg.kg^{-1} tissue dw or 200 $\mu\text{g.kg}^{-1}$ tissue dw (Avis de AFSSA (l'Agence française de sécurité sanitaire des aliments [60]). This can apply to crustaceans, the contaminated mollusks. In this case, the *P. erosa* at P1 and Kampung Laut station has been contaminated and this mud clams are not recommended for consumption. Meanwhile the mud clams in P2 and Mangrove station are still safe for consumption.

From this study, mud clams samples from Kampung Laut, Pertamina 1 and Mangrove stations were exceeded the EU regulatory limits of Maximum levels of 5 $\mu\text{g kg}^{-1}$ dry weight tissue for Benzo[a]pyrene. In addition, Kampung Laut station was higher than 30 $\mu\text{g kg}^{-1}$ dry weight tissue for the sum of 4 PAHs, i.e. (Benzo (a) pyrene, Chrysene, Benzo (a) anthracene, and Benzo (b) fluoranthene) as Bivalve mollusk type issued by Commission Regulation (EU) No 835/2011.

We analyzed 7 indicators PCBs mix (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180) and 9 organochlorines compounds (pp'-DDE, pp'-DDD, pp'-DDT, hexachlorobenzene, aldrin, dieldrine, endosulfan 1, heptachlor, and heptachlor epoxide) in sediment and mud clams samples. In clams, the PAHs pollutants were dominantly founded in all clams stations. we found pp'-DDE in all stations at small concentrations ranged from 0.87 to 24.79 $\mu\text{g.kg}^{-1}$ tissue dw and $\sum\text{PCBs}$ founded in fisheries village, urban activities (Kampung Laut station) with 2.34 $\mu\text{g.kg}^{-1}$ tissue dw (Table 6) and for other stations found below than limit of detection and not detected.

Index BSAF (Bio-Sediment Associated Factor) describing bioaccumulation of sediment-associated organic compounds into tissues of ecological reseptors [61] with the formula:

$$\text{BSAF} = \frac{\text{Co} / \text{fl}}{\text{Cs} / \text{fsoc}} \quad \dots (1)$$

Co was the chemical concentration in the organism ($\mu\text{g}/\text{kg}$ wet weight), fl was the lipid fraction of the organism (g lipid/g wet weight), Cs was the chemical concentration in surface sediment ($\mu\text{g}/\text{kg}$ wet weight) and fsoc was the fraction of the sediments as organic carbon (g organic carbon/g dry weight), from Extractable organic material (EOM). This index was described the bioaccumulation of sediment contaminants in aquatic environment may determined coordinated organism and surficial sediment samples under spatial conditions in which recent loadings of the chemicals to aquatic ecosystem [62]. Total lipid content from clams tissue were investigated in 4 stations of *Polymesoda erosa* as a model at different sources or activities at their sites to indicate the pollutants content near from their cultivation habitat. In general, the lipid in marine clams contains hydrocarbon and wax, triacylglycerol, free fatty acids, sterol, polar lipid and monoalkyl diacylglycerol. The presence of sterols in clams is also consistent with the ubiquity of the lipid content in marine organisms. The total lipid content was obtained from Blight-Dyer extraction technique [31] at 1.7 to 1.9 of wet weight. The effect of seasonal variations can affect on the concentration levels of PAHs in sediment as their habitat of the mud clams. During spring season due to enhanced migration of dust particles from ground surface to wastewater treatment streams influence the behaviour of sediment movement [34, 63].

The BSAF index of PAHs from mud clams in KL (Kampung Laut) station was highest value reached until 3.423 ± 0.421 . For Pertamina 1, Mangrove and Pertamina 2 stations were different because the availability of mud clams at each sites. Their BSAF index respectively, 1.532 ± 0.341 , 0.824 ± 0.135 and 0.511 ± 0.231 . Depending the non-polar organic chemical for lipid and sediment organic carbon, the BSAF, under specific conditions, should be in the range of 1 to 2 [63]. The BSAF index of PAHs from mud clams in 4 stations of Segara Anakan lagoons were ranging 0.511 to 3.423. Those values were still higher than other type clams (*Meretrix lyata*) which found in Soai Rap estuary -Ho Chi Minh City (0.429-1.974 [64] and manila clams from Mid Western coast of Korea (0.02-1.04 [65] Based on study area with different activities, Mud clams. *P. erosa* as potentially as site-specific bioaccumulation in sediment for organic micropollutants which receives from any untreated sewage, industrial waste discharge into the canal system of river of Segara Anakan Lagoon.

Conclusion

Pollutant organic persistent compounds (i.e. PCBs and OCPs) have been found in sediments from Segara Anakan Lagoon. The concentration range for 7 PCBs is from 0.11 to $2.63 \mu\text{g.kg}^{-1}$ sed. dw and the total of OCPs range is from $0.2 \pm 2.90 \mu\text{g.kg}^{-1}$ sed. dw. In the case of mud clams (*Polymesoda erosa*), the concentration range of 16 PAHs (US-EPA) is from 103.34 ± 2.01 to $1348.31 \pm 18.21 \mu\text{g.kg}^{-1}$ tissue dw, PCBs content was found in fisheries village station at concentration $2.34 \pm 1.72 \mu\text{g.kg}^{-1}$ tissue dw. and OCPs (i.e. pp'-DDE) content varies from 0.87 ± 0.03 to $24.79 \pm 1.30 \mu\text{g.kg}^{-1}$ tissue dw. The consumption evaluation shows that this mud clam has been contaminated and not recommended for human consumption. The ecotoxicological evaluation, based on the international Sediment Quality Guidelines (SQGs), shows that the samples collected from most stations have an environmental risk between low and medium-low, except for Station 7, 9, and 11 which have a higher ecological risk for marine organisms. This data can serve as a baseline reference for future assessment as part of an integrated watershed management program, including biodiversity, freshwater ecology, and economic activities in the study area. Our findings are a valuable recommendation for *P. erosa* as a site-specific bioindicator for all types of biomonitoring program including community effect (type 1), pollutant concentrations in indicator species (type 2), pollutant effects (type 3), and genetically-based resistance to pollution (type 4).

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