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Polycyclic aromatic hydrocarbons (PAHs) in mangrove clam (*Geliona erosa*) from Guimaras, Philippines five years after oil spill

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Abstract. The study intends to determine polycyclic aromatic hydrocarbons (PAHs, by-products of fossil fuel burning) levels in mangrove clam (*Geliona erosa*) five years after the sinking of Motor Tanker Solar I in Guimaras Island, Philippines which resulted in oil spill in 2006. Clams and sediment were collected along affected coastal barangays of Guimaras and analyzed for sixteen (16) priority PAHs pollutant. In general, clams contained 41.7-77.8% low molecular (three- and four- ring) aromatics of the total 16PAHs. Phenanthrene was the major component measured in clams ranging from 0.6-45.6 ng g^{-1} with the presence of benzo(a)pyrene (BAP) (0.2-2.5 ng g^{-1}). Results indicated that oil residues from the 2006 incident still persisted in the marine environment. Total 16 PAHs in sediment showed temporal variation and they were mostly higher molecular weight aromatics at a range of 10-489 ng g^{-1}; low molecular weights were at a range of 2.3-30.0 ng g^{-1}. BAP levels in clams were far below the public health level of concern (2.71 ± 0.784 µg kg^{-1}) established by NOAA/FDA. In general, mangrove clams were exposed to lower molecular weight PAHs (7.0-63.7 ng g^{-1}) while high molecular weight PAHs (8.9-389 ng g-1) were more bioavailable to sediments which also showed temporal variation.

Key Words: polycyclic hydrocarbons, phenanthrene, Philippine oil spill, *Geliona erosa*, benzo(a)pyrene.

Introduction. The sinking of Motor Tanker Solar I, 16 nautical miles southeast of Guimaras Island, Philippines in August 11, 2006, was one of the largest oil spills in the country with the recorded biggest damage affecting 439.6 ha of mangrove, 58 ha of seaweed farm, and 367.3 ha of fish ponds (NSCB 2006). This type of marine pollution always affects the coastal waters, inter-tidal zones, and mangrove areas as well as their aquatic organisms. However, the most important issue relates to the public health concerns over the oil-contaminated seafood resource. Bunker oil, as one of the petroleum products, contains organic components such as polycyclic aromatic hydrocarbons (PAHs) and the monoaromatic hydrocarbons (MAHs). Of more than hundred PAHs, US Environmental Protection Agency (US EPA 1996) and World Health Organization (WHO) identify sixteen (16) priority pollutants due to their acute toxicity to aquatic organisms and carcinogenic potential to human (Tuvikene 1995; Yender et al 2002).

Among the fishery resources used as food, bivalve mollusks are highly susceptible to oil spill contamination. These filter-feeding mollusks are exposed to both water and suspended particulate materials and take up a full range of PAHs, especially lipophilic PAHs that tend to concentrate in lipid-rich tissues of the mollusks (Eisler 1987; Moffat & Whittle 1999). In general, waterborne PAHs are taken up relatively more rapidly than do sediment-associated PAHs. While bivalve mollusks rapidly accumulate these hydrocarbons, they have little capacity for their metabolism and excretion (Eisler 1987; Samiullah 1985). Also, being sessile and attached, their tissue concentrations of PAHs are a reflection of levels in their immediate surroundings. A significant positive relationship between the PAH bioaccumulation by the bivalves and the sediment PAH concentrations has been documented (Simpson et al 2006). It is for these reasons that bivalves such as mussels and oysters are commonly used as indicator species in PAH bioaccumulation and metabolisation pattern studies according to pollution source (Bustamante et al 2012; Pikkarainen 2004; Zuloaga et al 2009) or accumulation trends of shellfish affected by oil spill (Vinas et al 2009).

Mangrove clams (*Geliona erosa*) are widely distributed along the mangrove swamps and provide a sustainable fishery resource for the coastal communities of Guimaras Island as food. A month after the incident, significant PAH level range in shellfish were recorded to be 38.0–3,102 ng g^{-1} and 128–236 ng g^{-1} in Luzaran and Taklong, respectively, both located in the municipality of Nueva Valencia, Guimaras (Uno et al 2009). After three years, the sum total of all 16 PAHs level range was 3.71-25.39 ng g^{-1} wet weight in mangrove clams recorded for the period of March 2008-January 2009 (Hidalgo et al 2010). Benzo(a)pyrene (BAP) in sediments was recorded at 2.3-19.3 ng g^{-1}, levels at which the mangrove clams could readily assimilate and accumulate in their tissues through time.

The present study intends to determine status of PAH levels in mangrove clams from oil-spill affected areas of Guimaras Island 5 years after the incident with concern on the uptake of BAP from its habitat.

Material and Method. Mangrove clams and sediment were collected from the hardly hit oil-spill affected coastal barangays of Nueva Valencia, Guimaras (Table 1) for three sampling periods were conducted (May 2011, November 2011 and May 2012).

Table 1

Sites and GPS location of collected mangrove clams (*Geliona erosa*) and sediment samples in the municipality of Nueva Valencia, Guimaras

Sampling site (Sitio/Barangay)	*GPS coordinates*	
	Latitude	*Longitude*
Sitio Dungca-an, Lucmayan	N 10.47022^{o}	E 122.50153^{o}
Sitio Talabahan, San Roque	N 10.42566^{o}	E 122.51028^{o}
Sito Alaman, La Paz	N 10.42244^{o}	E 122.51517^{o}
Sitio Pototan, La Paz	N 10.41182^{o}	E 122.51468^{o}

Sample collection, handling and treatment were conducted using the protocols of NOAA (2002) with some modifications. About 5 kg of bivalve samples were collected, packed in aluminum containers and kept at 0-4^{o}C during transport. Upon arrival at the laboratory, meat and intravalvular contents were separated from the shell, homogenized and kept at -20^{o}C until analysis. Soil/sediment samples were obtained from a depth of 30 cm using a 1.5 m sediment core sampler. Approximately 200 g were contained in a clean aluminum screw-capped glass bottle and kept at chilled temperatures prior to analysis.

PAH analysis. Sediment and bivalve tissue samples were packed in a cool box containing frozen gel packs and analyzed at the Research and Analytical Service Laboratory (RASL) of the National Science Research Institute, Manila Philippines for the 16 priority PAHs namely acenaphthylene (ANY), acenaphthene (ANP), fluorine (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLU), pyrene (PYR), benz(a)anthracene (BAA), chrysene (CRY), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzo(e)pyrene (BEP), benzo(a)pyrene (BAP), indenopyrene (IDP), dibenz(a,h)anthracene (DBA), benzo(ghi)perylene (IDP). PAHs in sediment were determined using GC/MS (Selected Ion Monitoring) following extraction with methanol, saponification, liquid-liquid portioning, florisil chromatography and solid phase extraction in silica gel (US EPA 1996). Mangrove clams samples were initially saponified with alcoholic sodium hydroxide then purified by solvent partition and column chromatography prior to PAHs analysis using GC/MS (AOAC 2002). Total PAHs correspond to the sum of the 16 parent compounds.

Results and Discussion. An important aspect of the environmental fate of PAHs is their accumulation by aquatic animals. Total 16 PAHs in mangrove clams fluctuated from 10.3 to 65.2 ng g^{-1} wet weight with an observed increase in level during the November sampling period (Table 2). In general, clams contained 41.7-77.8% low molecular (three- and four- ring) aromatics of the total 16 PAHs. Phenanthrene (three-ring) was the major PAH (0.6-45.6 ng g^{-1}) and some high molecular (five- and six-) aromatics including benzo(a)pyrene (BaP). These levels indicated that oil residues from the 2006 incident still persisted in the marine environment. Total 16 PAHs in sediment showed temporal variation and that composition was mostly higher molecular weight aromatics at levels from 10-489 ng g^{-1} and while the low molecular species at level range of 2.3-30.0 ng g^{-1} (Table 3). PAHs are slightly soluble in water and their solubility decreases with increasing molecular weight (ATSDR 1995). Thus, PAHs concentrations in the water column comprised mostly of water-soluble, low molecular hydrocarbons. In undisturbed water, bivalve mollusk can passively uptake the dissolved fraction and be enriched with these PAHs. The mussel's abundance of low molecular weight PAHs is due to its better bioavailability for the organism to ingest through its gills than the high molecular weight PAHs (Palma-Fleming et al 2004). This general pattern of accumulation is also observed in mussel (Namiesnik et al 2008) and oysters (Ramdine et al 2012) in PAH-contaminated marine environment.

Table 2

Polycyclic aromatic hydrocarbons(PAHs) content (ng g^{-1}, wet weight) of mangrove clams (*Geliona erosa*) in oil-spill affected areas in Guimaras, Philippines

PAHs	*Sampling sites*											
	Dungca-an, Lucmayan			*Alman, La Paz*			*Talabahan, San Roque*			*Pototan, La Paz*		
	May -11	*Nov -11*	*May -12*	*May -11*	*Nov -11*	*May -12*	*May -11*	*Nov -11*	*May -12*	*May -11*	*Nov -11*	*May -12*
Three- and Four- rings												
Acenaphthylene	4.0	0.8	2.1	4.0	*	2.5	1.1	*	2.5	2.0	*	1.1
Acenaphthene	*	*	*	*	*	1.9	0.6	*	2.1	0.6	*	1.5
Fluorene	1.5	1.7	1.6	*	1.2	2.6	1.2	2.1	4.4	0.9	4.1	3.6
Phenanthrene	8.2	9.9	0.6	*	7.1	1.0	7.0	45.6	*	7.0	43.8	1.6
Anthracene	0.4	2.5	*	*	*	0.2	0.5	3.1	10.0	*	3.3	*
Fluoranthene	3.5	1.4	1.0	3.0	3.9	1.1	4.3	9.2	*	2.4	7.0	0.5
Pyrene	1.0	2.3	2.1	*	3.1	2.6	2.3	3.7	0.9	0.6	2.9	1.4
Benz(A)anthracene	*	0.2	*	0.2	0.4	*	0.5	0.5	1.8	*	0.4	0.3
Chrysene	0.8	*	0.8	4.0	0.5	0.9	0.8	0.5	6.8	0.7	0.4	0.4
Five- and Six- ring												
Benzo(b)fluoranthene	0.6	0.3	*	0.8	0.4	*	*	*	0.4	*	*	0.3
Benzo(k)fluoranthene	*	*	*	*	*	*	0.2	*	*	*	*	*
Benzo(e)pyrene	*	*	*	0.5	*	*	*	0.2	*	0.2	0.3	*
Benzo(a)pyrene	*	0.2	2.1	*	1.3	2.5	*	0.3	1.1	*	0.2	*
Indenopyrene	*	*	*	*	*	*	0.3	*	*	1.4	*	*
Dibenzanthracene	*	*	*	*	*	*	*	*	*	*	*	*
Benzo(ghi)perylene	*	*	*	*	0.7	*	*	*	*	*	*	*
TOTAL	20.0	19.2	10.3	12.5	18.5	15.3	18.8	65.2	30.0	15.8	62.4	10.7

*<EMDL (Estimated Method of Detection Limit) = <0.02 ng g^{-1}

Fluctuation in levels of PAHs in clams and sediment was probably influenced by perturbation and resuspension of sediment particulate materials caused by weather-related disturbances. Wet season in the Philippines normally starts from June until October or November with occasional tropical typhoons. During these conditions, dissolved fraction and suspended particulate materials from sediment becomes available and readily assimilated by the bivalve. Results of the present study showed a general increase in PAHs levels; the high molecular aromatic species in clam including BAP were at measurable quantities of 0.2-2.5 ng g^{-1} in November 2011 (Table 2). Considering that the uptake of BAP by clam was of concern, the levels recorded were relatively below the maximum permitted level (2.71+0.784 µg kg^{-1}) established by NOAA/FDA (2002) to pose health risk to its consumers.

Table 3

Polycyclic aromatic hydrocarbons(PAHs) content (ng g^{-1}, dry weight) of sediments in oil-spill affected areas in Guimaras, Philippines

PAHs	*Sampling sites*											
	Dungca-an, Lucmayan			*Alman, La Paz*			*Talabahan, San Roque*			*Pototan, La Paz*		
	May -11	*Nov -11*	*May -12*	*May -11*	*Nov -11*	*May -12*	*May -11*	*Nov -11*	*May -12*	*May -11*	*Nov -11*	*May -12*
Three- and Four- rings												
Acenaphthylene	*	*	1.0	2.7	0.8	*	0.4	*	*	3.0	*	*
Acenaphthene	3.0	*	8.0	1.3	*	*	*	*	9.0	*	*	7.0
Fluorene	10.0	2.5	2.0	3.6	2.0	0.8	*	0.9	1.9	2.0	2.9	0.9
Phenanthrene	4.0	*	14.0	6.0	*	3.0	2.8	*	10.0	10.0	*	3.7
Anthracene	4.0	2.5	5.0	7.0	1.8	3.1	2.2	1.4	3.8	8.3	6.1	3.8
Fluoranthene	4.5	3.4	5.0	15.0	4.8	3.0	4.3	1.1	2.7	40.0	3.2	4.0
Pyrene	5.4	5.2	5.0	36.0	13.7	4.0	7.1	*	2.4	60.0	21.5	6.0
Benz(A)anthracene	2.9	2.4	3.0	25.0	3.7	7.0	3.0	1.4	2.0	50.0	5.8	11.0
Chrysene	3.1	1.7	2.0	32.0	4.2	3.0	3.3	1.1	1.4	50.0	4.5	2.7
Five- and Six- ring												
Benzo(b)fluoranthene	9.8	5.0	*	47.0	0.6	*	7.7	1.8	0.5	90.0	10.1	5.0
Benzo(k)fluoranthene	*	0.7	1.0	*	0.9	11.0	*	0.2	0.8	*	1.5	4.9
Benzo(e)pyrene	1.7	0.5	1.0	17.0	2.9	4.0	2.8	0.3	1.2	29.0	3.5	4.0
Benzo(a)pyrene	*	2.1	7.0	32.0	8.0	6.0	*	0.7	3.1	60.0	89.1	7.0
Indenopyrene	2.5	1.4	2.0	30.0	3.4	8.0	1.0	0.3	1.6	50.0	4.1	7.0
Dibenzanthracene	*	*	*	*	3.1	*	*	0.4	*	*	*	*
Benzo(ghi)perylene	3.2	1.1	3.0	39.0	3.3	9.0	4.8	2.7	2.5	60.0	10.8	10.0
TOTAL	54.1	28.5	59.0	293.6	53.1	61.9	39.3	12.2	42.9	512.3	163.1	76.9

*<EMDL (Estimated Method of Detection Limit) = <0.02 ng g^{-1}

Sediment associated-PAHs, mostly high molecular hydrocarbons, usually tend to concentrate in lipid-rich tissue of the animal (Moffat & Whittle 1999; Massara Paletto et al 2008) and their deposition is correlated with fat content (Cortazar et al 2008). PAHs accumulation also depends on the change in lipid content during reproductive cycle of the organism. Bivalves display a yearly cycle in the assimilation of contaminants due to changes associated with their reproductive cycle and lipid content (Massara Paletto et al 2008). It is probably for this reason that the concentration of high molecular PAHs in clams in the present study were minimal despite the relatively high content found in sediments.

Results of the present study showed that environmental and biological factors could influence the uptake and composition of PAHs in clams. PAHs in the environment can dissipate through time and are naturally subjected to a variety of processes in an aquatic environment. Their rate of degradation varies depending on factors such as availability of oxygen, water temperature, and presence of oil-degrading microbial species inherent to site characteristics (Samiullah 1985). While previous studies studies (Hidalgo et al 2010; Pahila et al 2010) of Guimaras oil spill have reported decreases in PAHs in sediments, the present study recorded increases in PAHs levels. Sediment levels in Sitio Dungca-an and Sitio Talabahan were lower but still reflected minimal increases throughout the sampling period than those of Sitio Alman and Sitio Pototan in which sediments contained relatively higher levels of PAHs (Table 3). PAHs are ubiquitous in nature and can be produced at elevated concentration in areas of significant wood-burning activities. PAHs produced through the incomplete combustion of fuels such as wood and coal can easily be transported through air and subsequently deposited in soil and water (Palanikumar et al 2012; Thorsen et al 2004). We observed that the sites were in close proximity to coastal communities that made use of wood as fuel for cooking. Other more major factors such as seepage, industrial and municipal wastewater, urban and suburban surface run-off, and atmospheric deposition can contribute to aquatic contamination of PAHs (Eisler 1987; Tamamura et al 2007). It would, therefore, be interesting to recognize whether PAHs detected are related to the particular oil spill. Hydrocarbons of petrogenic source tend to show higher proportions of alkyl-substituted to parent compounds while the pyrogenic PAHs show higher proportions of the parent compounds (Moffat & Whittle 1999). Crude oil contains very low concentrations of the high molecular weight PAHs (e.g 4-5 ring compounds such as chysene and benzo(a)pyrene) that are associated with combustion by-products (Yender et al 2002). These differences in PAH abundance are key components in identifying its source. While the analysis of PAHs in mangrove clams and sediment was limited to the 16 priority PAHs in this study, we could not altogether rule out other sources of PAHs other than the oil spill in 2006. This information is very helpful for monitoring studies to analyze alkylated PAH homologues for oil fingerprinting purposes.

Conclusions. Mangrove clams generally still contain low levels of PAHs priority pollutant comprising mostly of water-soluble low molecular aromatics while sediment have high molecular components five years after the incident. Although PAHs known to have mutagenic or carcinogenic properties were detected in the bivalve, levels were below maximum allowable limit.

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