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Biomonitoring of Mercury Contamination at Petroleum Production Platforms in the Gulf of Thailand using Transplanted Green Mussel, *Perna viridis*

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Abstract

Assessment of mercury contamination was conducted using transplanted green mussels (*Perna viridis*). Mussels were first exposed to $HgCl_2$ at 0.5, 1.0, 2.5, and 5.0 nmol/L for 8 weeks at laboratory conditions. The result showed that Hg level in the water decreased rapidly, while Hg in mussels increased coincidentally with the applied doses. After 8 weeks the Hg, levels in tissue were a thousand-fold higher than that in the water. Mussels were then transplanted to 3 petroleum production platforms for field study. The result revealed that survival and growth rates of transplanted mussels at all 3 stations were in close to each other but significantly lower than that from the reference site. Hg concentrations in the tissues of transplanted mussels ranged from less than 0.010 to 0.173 µg/g, and Hg concentrations in mussel tissues from all stations were significantly increased within 2 months, while Hg levels in mussel tissues from reference site were not changed. Hg levels of transplanted mussels increased with increasing depths of the water. The transplanted mussels showed no signs of any physical anomalies, indicating that transplanted mussels could be maintained for up to 3 months in an un-natural habitat, such as petroleum production platforms, where food is much less abundant.

Keywords: mercury; *Perna viridis*; transplanted mussel; biomonitoring

1. Introduction

Mercury (Hg) is far more toxic to marine organisms than any other heavy metal. Even very low levels of Hg have chronic effects on living organisms (Langston, 1990; Boening, 2000). Researches have shown that mercury can be a threat to the health of people and wildlife in many environments that are not obviously polluted (Neff, 2002; Pandey and MacRae, 1991; Aunaas *et al*., 1991; Devi, 1995).

In the Gulf of Thailand, one of the main sources of Hg release is the offshore petroleum operations which have been conducted continuously for almost 30 years (DMF, 2010). During the process, produced water is by far the largest volume of waste stream associated with offshore oil and gas production. In the past few years, the levels of Hg detected from the surrounding areas of oil and gas processing platforms were not significantly increased, and the ranges of Hg levels measured were still lower than the standard. However, the levels of Hg in sediment from different platforms vary vastly. The value at one platform was between 0.015 and 0.020 µg/g whereas it was between 0.02 and 5.01 µg/g at another location (Pornsook *et al*., 2010). In addition, Hg levels in the sediment collected from central production platform were higher than sediment from the distance radius points. The results reveal a trend of decreasing Hg levels in sediments with increasing distance from the platform. This indicates that Hg can accumulate further in sediment and if the discharge continues, perhaps finally Hg can go beyond the prescribed standard while Hg level in water is still below standard (1 µg/g sediment dry weight). Therefore, comprehensive monitoring programs for determining Hg contamination in marine organisms in the vulnerable areas surrounding the oil and gas processing platforms are needed.

In recent years, active biomonitoring has increasingly gained attention as a potential field approach for many pollution monitoring programs. Mussels are widely used as species for toxicological studies because of their high accumulation of many toxicants including heavy metals (Andersen, 1996; ZOHEÏR, 2009).

A small number of mussels have been successfully used as transplanted bioindicators (Bainy *et al*., 2000; Kim *et al*., 2008; Maria *et al*., 2009; Giarratano *et al*., 2010; Resgalla *et al*., 2010; Rodríguez *et al*., 2010). The green mussel, *Perna viridis*, is probably the most widespread species of bivalves in seawater of Thailand. For these reasons, they are the most suitable marine organisms for monitoring of contamination levels in Gulf of Thailand.

In this study, the accumulation of Hg was monitored in green mussels both in the laboratory and in field studies. Mussels were collected from a less polluted location and transported to the laboratory and subsequently transplanted to 3 petroleum production platforms located in the middle of the Gulf of Thailand in order to evaluate the usefulness of green mussel as bioindicator for determining the exposure and accumulation of Hg in the Hg contaminated areas.

2. Materials and Methods

2.1. Test organisms

Green mussels, *P. viridis* (8–10 cm), were collected from areas not polluted by Hg (Trad Bay at Trad Province) and transported to the facility for laboratory study. The mussels were acclimated for 1 week (30‰ salinity, pH 8.0 and temperature 27ºC). They were fed daily with unicellular algae (*Chaetoceros* sp.) and brine shrimp during acclimation. Ten per cent of the water was exchanged daily.

2.2. Mercury analysis in mussel tissue and water

The total Hg concentration was determined in mussel tissues using a protocol modified from the methods of U.S. EPA (1997), Odžak (2000), and Gašpić (2006). Lyophilized and homogenized tissue (0.1–0.2 g) was mixed with 5 ml of conc. HNO_3 and 0.040 g of V_2O_5 in Teflon digestion vessels and left for 1 h. After heating for 5-10 min, 3 ml of $K_2Cr_2O_7$ was added and diluted to 40 ml. with Mili-Q water. Total Hg concentrations were determined by Cold Vapor Atomic Adsorption Spectrophotometry (CVAAS). For Hg analysis in water, the protocol was modified from the method of Qu´emerais and Corssa (1997). Total mercury in water was reduced to elemental mercury by an addition of stannous chloride ($SnCl_2$), vaporized by argon bubbling, transported by an argon current to a gold trap, and detected by Cold Vapor Atomic Fluorescent Spectrophotometry (CVAFS) at 253.7 nm.

2.3. Experimental set up

2.3.1. Laboratory study

The experiment was conducted in five 800-L tanks and each tank contained 200 mussels. Mercuric chloride ($HgCl_2$) was applied to each tank making up Hg concentrations of 0.0, 0.5, 1.0, 2.5, and 5.0 nmol/L (0.0, 0.1, 0.2, 0.5 and 1.0 µg/L), respectively. Water quality was monitored every 2 days for ammonia, nitrite, and nitrate using the methods described by Strickland and Parsons (1972). Ten percent of the water was exchanged every day, and $HgCl_2$ was applied with the new water to maintain the original concentration in every tank. Ten mussels from each tank were collected weekly. Shell length and the weight of each individual specimen were measured. Tissues from collected samples were kept at -20˚C for Hg analysis.

2.3.2. Field study

Mussels were transplanted to 3 stations at petroleum production platforms (station A, B, and C) and maintained for 90 days (Fig. 1). Mussels (15-20 specimens) were put into a net bag and each net bag was attached to a rope at 5, 20 and 40 m below the surface level. The end of the rope was weighed with heavy concrete to maintain position. Seven ropes were placed at each platform. Mussel sampling was performed monthly in the period of 3 months. At each sampling time, one rope was detached and mussels (10 specimens) were collected from each net bag. Average shell length of the collected mussels was determined. Tissue samples were stored in sealed plastic bags at -20˚C for Hg analyses. At the same time as mussel collection, plankton sampling was performed using a cylindrical net with 20 micron mesh size, 50 cm in diameter, and 150 cm long. Plankton was sampled vertically in a water column of 40 m depth and preserved in 3% neutral formaldehyde.

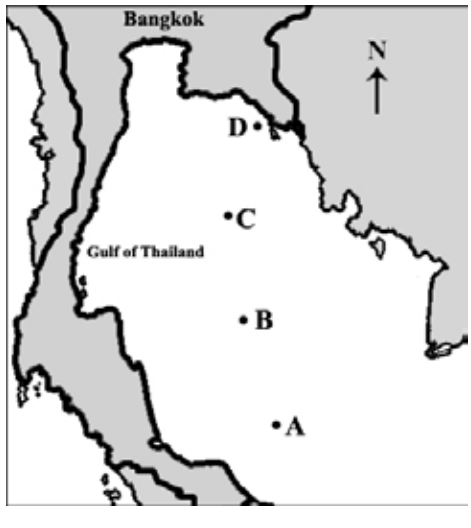

Figure 1. Experiment sites located in the Gulf of Thailand. Station A, B and C are the field study locations at petroleum production platforms. Station D is the reference site.

2.4. Statistical Analysis

Variables of each experiment were typically analyzed using the statistical package in SPSS Version 15 for Windows. The difference of each variable among groups of treatment was tested for normality and variance homogeneity using Shapiro-Wilk and Levene's test. Significant differences among groups of treatments were examined using Duncan's test at $p<0.05$.

3. Results and Discussion

3.1. Survival and growth rates of transplanted mussels

One hundred and eighty mussels were initially transplanted to each station. At the end of the experiment 87, 101, 111, and 180 mussels were survived. The survival rates of mussels from stations A, B, C, and D after 3 months of transplantation were 48.33, 56.11, 61.67, and 100 %, respectively. The survival rates of transplanted mussels at station A, B, and C decreased rapidly within the first month and then decreased slightly, while no mortality of mussels was detected at station D during 3 months of experiment. No significant differences were detected between the survival rates of mussels reared at different water depths. There was no significant difference between survival rates of the mussels from stations A, B and C, but the survival rates from these stations were significantly lower than that from station D ($p< 0.05$) (Fig. 2).

The average shell lengths of mussels collected from station A, B, and C after 3 months of transplantation were 5.72, 6.56, and 6.23 cm, respectively, which was significantly lower than that of station D (7.65 cm). There were no significant differences of shell length between different depths and between transplantation times (Fig. 3).

3.2. Water quality in laboratory study

The average levels of ammonia, nitrite, and nitrate measured from the mussel rearing water were 54.38±43.75, 5.22±0.65, and 190.81±1.29 μmol/L (0.84±0.70, 0.24±0.03, and 11.83±0.08 mg/L) respectively. These results (Fig. 6) indicate that the amounts of nitrogenous waste in all experiment tanks did not reach the toxic level.

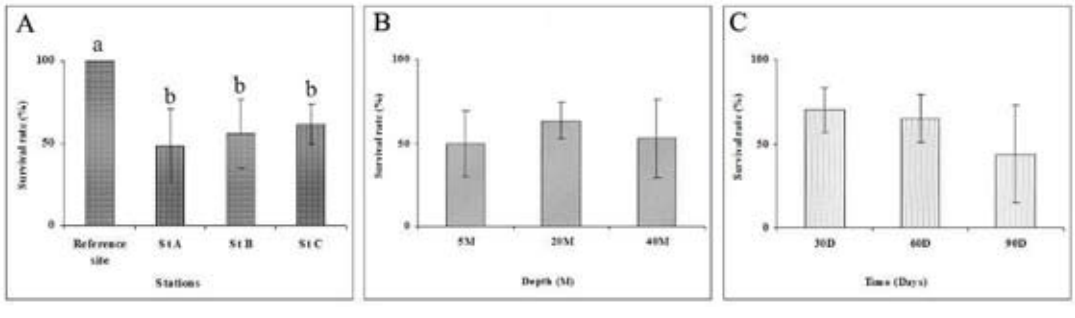

Figure 2. Survival rates of transplanted mussels from different stations (A) and different depths (B) after 3 months of transplantation (C). The values are shown as mean±SD (n=60). Significant differences between groups of experiment sites are indicated by letter a and b ($p < 0.05$). The same letter indicates no significant difference ($p\geq0.05$).

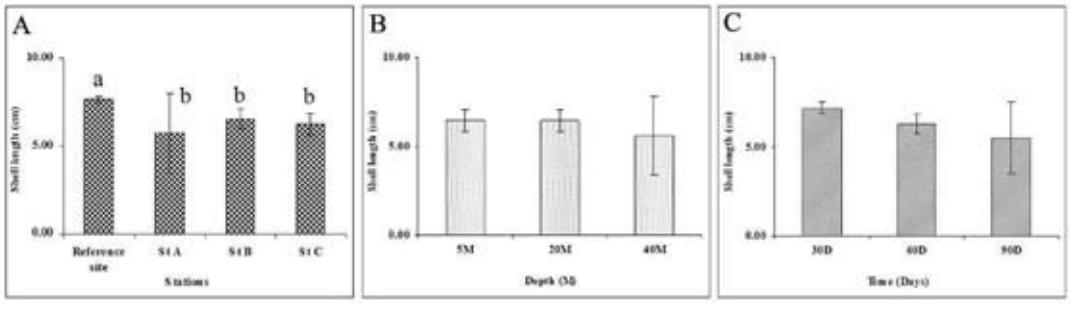

Figure 3. Shell lengths of transplanted mussels from different petroleum production platforms (A) at different depths (B) after 3 months of transplantation (C). The values are shown as means ± SD ($n=30$). Significant differences between groups of experiment sites, depths, and experiment times are indicated by letter a and b ($p< 0.05$). The same letter or no letter indicates no significant difference ($p\geq0.05$).

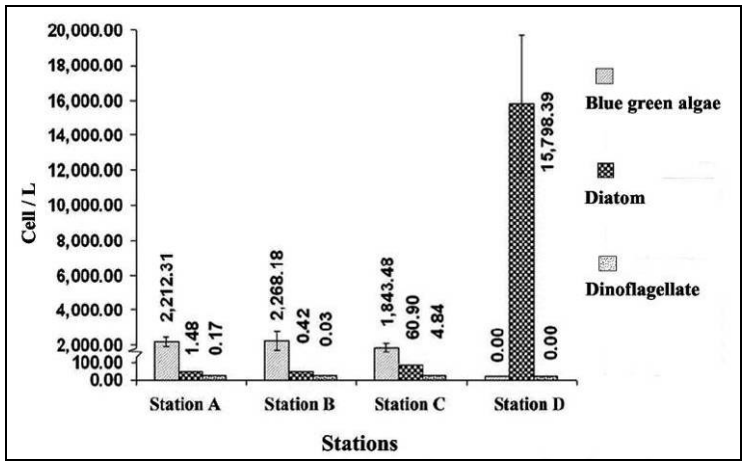

Figure 4. Plankton compositions from Petroleum Production Platforms (Station A, B, C) and reference site (station D). The values are shown as means ± SD (*n*=3).

3.3. Plankton composition

Our study revealed no significant differences of number and group of planktons within offshore stations (station A, B, and C) whereas the density of planktons, especially diatom, at the reference site (station D) was much higher than that of stations A, B and C ($p < 0.005$) where blue green algae was the dominant group. The survival and growth rates of mussels from stations A, B, and C were much lower than that from reference site presumably due to less abundance of food.

3.4. Determination of mercury concentration

3.4.1. Mercury concentration in water

During 8 weeks of $HgCl_2$ treatment, Hg concentration in the experiment water from each treatment was not significantly different. The average level of Hg detected in water of tanks 1 to 5 were 0.01±0.01, 0.03±0.01, 0.05±0.04, 0.09±0.06 and 0.20±0.19 nmol/L, respectively (Fig. 5). This reveals that Hg levels remain in the water at 0.049, 0.054, 0.036, and 0.040 %, respectively, after 24 h of application. This result coincides with most experiments, since inorganic Hg has been known to be exchanged quickly in aquatic environment and mostly transformed into organic Hg by living organisms (Sanchez *et al*., 1998; Kannan *et al*., 1998). Hg tends to be absorbed by surrounding organic materials quickly and remains in the water with a certain amount, regardless how much Hg was initially applied to the water, indicating that the amount of Hg measured from the water within a certain time can not be efficiently used as indicator for determining the actual amount of Hg previously released into water.

In the field study, Hg concentrations in the water around the platforms were monitored every 3 or 4 years by the Department of Mineral Fuel, Ministry of Energy. The standard value for the offshore seawater

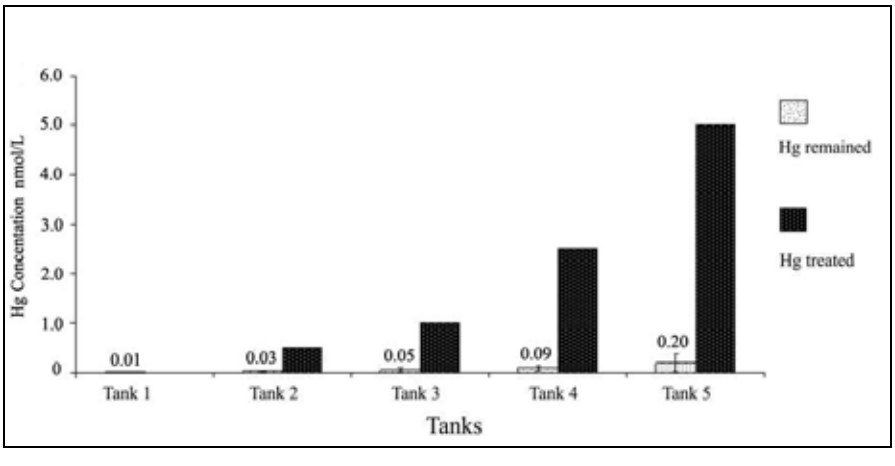

Figure 5. Hg concentrations in the mussel rearing water in tank 1 (0), tank 2 (0.5 nmol/L), tank 3 (1.0 nmol/L), tank 4 (2.5 nmol/L), tank 5 (5.0 nmol/L).

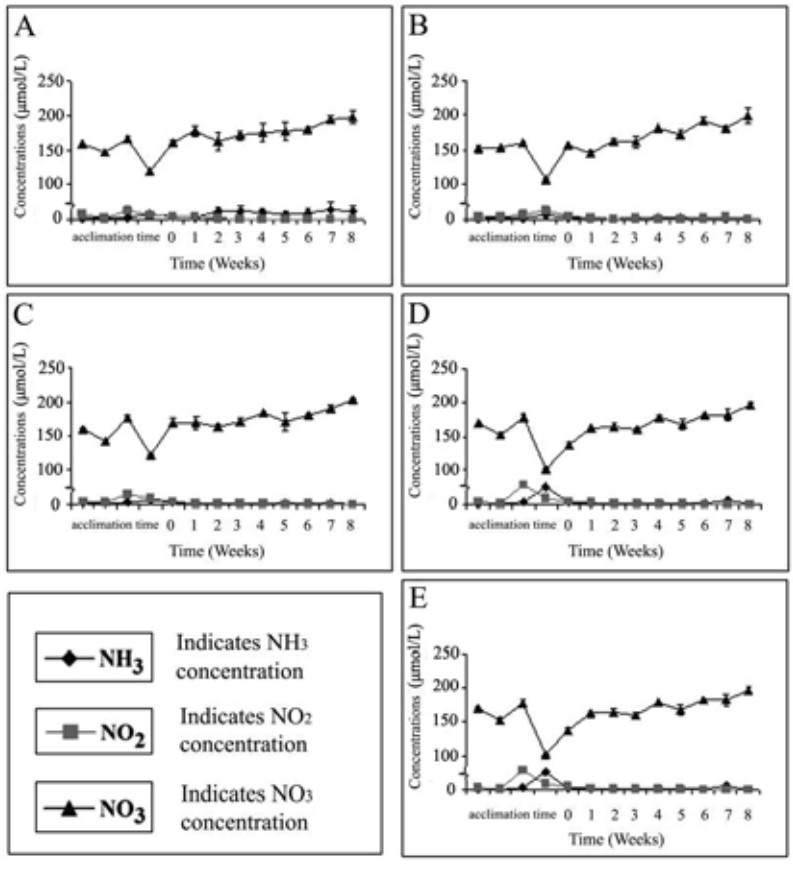

Figure 6. Ammonia, nitrite, and nitrate concentrations of mussel rearing water from the experiment tanks. A, B, C, D, and E indicate the results of tank 1 to 5, respectively. The values are shown as means ± SD (*n=3*).

regulation is less than 0.10 µg/L or 0.5 nmol/L (PCD, 1997), while the average Hg concentrations of water detected at Stations A, B, C, and D (reference site) were 0.023, 0.010, 0.002, 0.002 nmol/L, respectively. This indicates that Hg concentration of water at every target site was lower than the standard limit values of the offshore seawater regulation.

3.4.2. Mercury concentration in mussel tissue

In the laboratory experiments, Hg concentrations in all remained at the same level, except in tanks 3 and 4 where it appeared to be higher [Fig. 7(A)]. After 8 weeks, the differences between treatments were statistically significant [Fig. 7(B)]. Hg levels increased in correspondence to the increasing levels of Hg that were applied to the mussels. The average level of Hg in the mussel before treatment was 0.01±0.01 µg/g while the level from the highest Hg treatment for 8 weeks was 0.14±0.08 µg/g. The Hg level was thus approximately 10 times higher than initially.

The increasing level of Hg in mussel tissue in this study coincides with the increasing level of Hg applied to the test tank, and the tissue Hg levels were thousand folds higher than that of water. This is in agreement

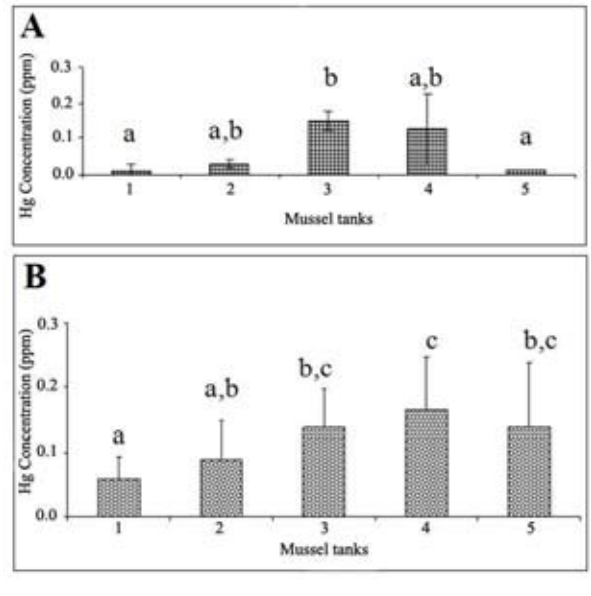

Figure 7. Hg concentrations in mussel tissue. The results of Hg level in mussel tissue at week 1(A) and 8 weeks (B). The values are shown as mean±SD (*n=3*). Significant differences of Hg concentrations in mussels between experiment tanks are indicated by letter a, b, and c ($p<0.05$). The same letter indicates no significant difference ($p\geq0.05$).

with previous studies (Fowler *et al*., 1978; Phillips and Buhler, 1978; Thompson *et al*., 1990; Yamada *et al*., 2003).

In field study, Hg levels detected in mussels from all stations ranged from less than 0.0100 to 0.1725 μg/g. Hg concentrations in mussel tissues from all stations remained at the same levels during the first month and became significantly higher after 2 months. After 3 months, Hg levels were slightly decreased but still remained in the same level as detected in the second month [Fig. 8(B)]. At the reference site, Hg levels in mussels were not significantly different during 3 months of experiment [Fig. 8(A)].

Significant differences of Hg levels between mussels rearing in different depths were obvious after 3 months of transplantation. It showed that the Hg levels of transplanted mussels increased corresponding to the increasing depths of the water [Fig. 9(C)] ($p<0.05$).

When the levels of Hg in mussels from each station were compared according to time, the results showed that Hg concentrations remained at the same levels among transplantation sites within the first month [Fig. 10(A)]. After 2 months, all sites at petroleum production platforms (A, B, and C) tended to be higher than the reference site (D) [Fig. 10(B)]. After 3 months of transplantation, the difference between Hg of mussels from difference locations was significant ($p<0.05$) where average Hg level of mussels from station B was lower than that from station A and C [Fig. 10(C)].

The assimilation efficiency of Hg from food of Mussel, *Mytilus edulis*, was reported to be 1-9 % for inorganic Hg and 30-87% for MeHg (Gagnon and Fisher, 1997). In this study, the maximum accumulation of Hg (48.89 %) was found in mussels exposed to the lowest level of Hg (0.50 nmol/L or 0.1 μg/L) (Table 3). The levels of accumulation seemed to be reduced when the level of Hg applied to the mussels was increased. It was quite interesting to note that very small amounts of Hg were detected from the control treatment (both tissue and water) where Hg was not deliberately applied to the tank (Table 3). The amount of Hg detected in control mussel tissues and water were

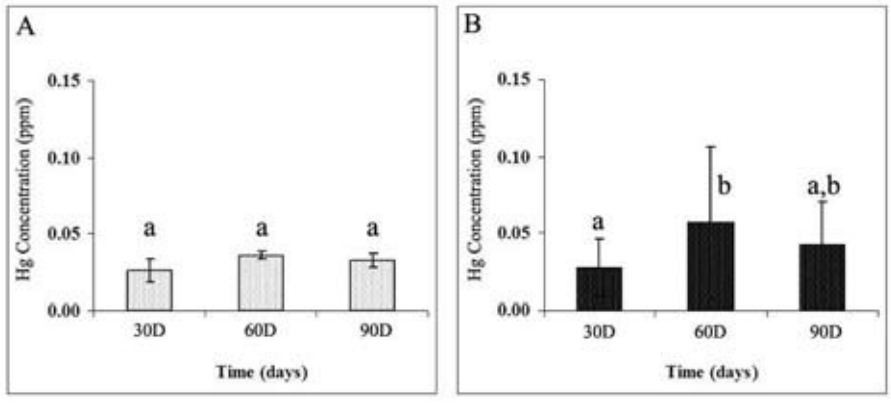

Figure 8. Hg concentrations of transplanted mussels at petroleum production platforms (B) and reference site (A) after 3 months of transplantation. The values are shown as mean±SD ($n=3$). Significant differences of Hg concentrations in mussels at different experiment times are indicated by letter a, and b ($p<0.05$). The same letter indicates no significant difference ($p\geq0.05$).

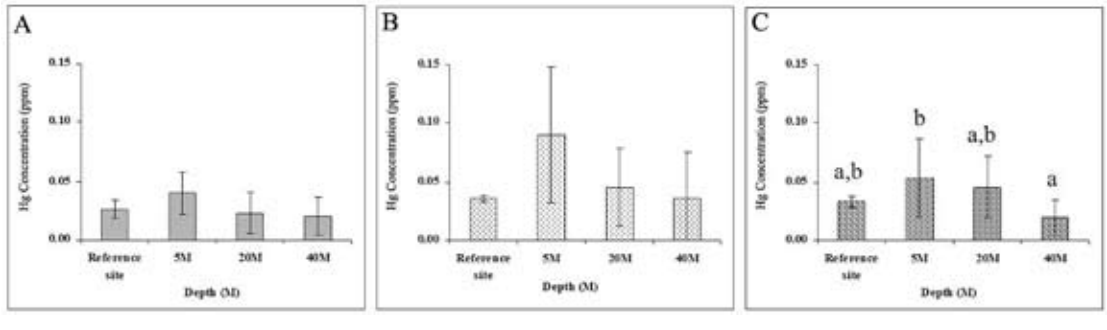

Figure 9. Hg concentrations of transplanted mussels at petroleum production platforms at different water depths after 30 (A), 60 (B), and 90 (C) days of transplantation. The values are shown as mean±SD ($n=3$). Significant differences of Hg concentrations in mussels at different depths are indicated by letter a, and b ($p<0.05$). The same letter or no letter indicates no significant difference ($p\geq0.05$).

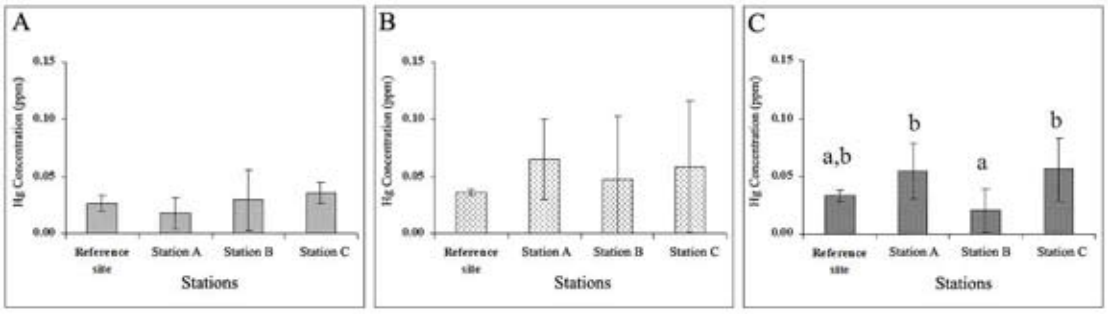

Figure 10. Hg concentrations of transplanted mussels at different stations during 3 months of transplantation (A) 30, (B) 60, and (C) 90 days of transplantation. The values are shown as mean±SD *(n=3)*. Significant differences of Hg concentrations in mussels at different stations are indicated by letter a, and b ($p<0.05$). The same letter or no letter indicates no significant difference ($p\geq0.05$).

in close to those detected from the reference site in the field study, indicating that the Hg detected in control samples was presumably the background concentration of Hg normally found in mussels from natural habitat (Gagnon and Fisher, 1997).

4. Conclusion

The use of active an bioindicator for determining Hg contamination in marine environment surrounding areas of petroleum platform in the Gulf of Thailand was investigated, focusing on the feasibility and validity of using green mussel, *P.viridis*, as transplanted species for monitoring the change of Hg contamination in target areas. Inorganic Hg at sub-lethal levels (between 0.5 to 5.0 nmol/L or 0.1 to 1.0 µg/L) can significantly increase Hg concentrations in mussel tissues up to more than 10 times within 8 weeks. Also, Hg can be taken up into living organisms almost completely within 24 h, and Hg can accumulate in the mussel tissue at a concentration of more than 1,000 higher than the Hg level in the surrounding water. This indicates that the response of Hg levels in tissues of exposed *P.viridis* can be used as an indicator for Hg contamination at very low levels. The results of growth and survival rates of the experiment mussels from laboratory and in field studies are relatively normal when compared to the mussels rearing in their natural habitat, and the transplanted mussels at petroleum production platforms showed no sign of any physical anomalies. This demonstrates that transplanted mussels maintained in un-natural habitat can be used as bioindicators at locations such as petroleum production platforms where food is much less abundant, for up to 3 months without significant physical change.

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Table 3. Summary for mass balance of Hg in mussel water tank after 8 weeks of experiment (Laboratory study)

Mussel tank	Hg concentrations exposed to mussel (nmol/L)/(µg/L)	Hg in water tank (%)								Remark
		Hg treated		Hg accumulate in mussel tissue		Hg remain in water		Hg loss		
		µg	%	µg	%	µg	%	µg	%	
Tank 1	0	0	-	75.31	-	15.41	-	-	-	Control
Tank 2	0.5/0.1	304.00	100	148.64	48.89	27.60	9.08	127.77	42.03	
Tank 3	1.0/0.2	608.00	100	167.59	27.56	60.81	10.00	379.60	62.43	
Tank 4	2.5/0.5	1,520.00	100	220.71	14.52	108.22	7.12	1,191.07	78.36	
Tank 5	5.0/1.0	3,040.00	100	137.10	4.50	224.74	7.40	2,678.16	88.09	

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