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Accumulation and Release of Heavy Metals (Cu, Zn, Cd and Pb) in the Mussel, *Mytilus galloprovincialis*; Reciprocal Transplantation Experiment

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Mussel, *Mytilus galloprovincialis,* was transplanted reciprocally between contaminated site (St. STP) and relatively less contaminated site (St. UB) in Onsan Bay, Korea in order to estimate heavy metal accumulation and release. Transplanted and indigenous mussels were collected 8 times over 108 days deployment at both sites and analyzed for Cd, Cu, Pb and Zn using ICP/MS. Cu and Zn concentration did not show any significant differences between transplanted and indigenous mussels throughout the experiment period, although dissolved Cu and Zn concentrations in seawater were significantly higher at contaminated site. Pb accumulated rapidly, while Cd did not show any accumulation in the mussels transplanted from St. UB to St. STP. These accumulation patterns might reflect the variation of dissolved metal concentration; dissolved Pb concentration was significantly higher in St. STP than St. UB, while dissolved Cd concentration was similar between both sites throughout this experiment. Release of Cd and Pb in the mussels transplanted from St. STP to St. UB was not significant during the transplantation period. The levels and variation of Pb and Cd concentrations in mussels transplanted from St. STP to St. UB were similar to those in the indigenous mussels at St. STP throughout the period. Therefore, Cd and Pb accumulated in indigenous mussels may indicate the integrated metals throughout their lives, not responding to the present status of seawater because the release of accumulated metals in mussels occurs very slowly when the seawater metal levels are lowered. On the other hand, since the transplanted mussels can respond immediately to the levels of metals in seawater, it may be suggested that the present status of heavy metals in seawater can be only acquired from the metal levels of mussels transplanted from clean environments.

Key words: Transplantation, Heavy Metals, Mussels, *Mytilus galloprovincialis*

INTRODUCTION

Bivalves including mussels have been widely used to monitor levels of heavy metal contamination in coastal seawater (Farrington *et al.*, 1983; Goldberg *et al.*, 1983; Gunther *et al.*, 1999; O'Connor, 2002). Heavy metals can be accumulated within tissues and shells of mussels through biological and/or non-biological processes. The mussels have merits as a biomonitor in the views of their considerable capacities of the heavy metal accumulation, time-integration, and direct assessment of bioavailable heavy metals.

Mussel as a biomonitor, however, has also several disadvantages: (1) biological factors such as the size, weight or age, and nourishment may affect to the concentrations of heavy metals in tissues (Phillips and Rainbow, 1994), (2) mussels cannot survive in heavily polluted coastal waters and (3) mussels with the same size (or age) may not exist everywhere. Although the size-normalized concentrations of heavy metals can be applied to the identification of pollution gradient, they still show inherent variability of organisms. In order to overcome above limitations, different method using mussels transplanted from a clean site rather than indigenous mussels can be used. Through the transplantation, we can employ statistically similar groups of organisms to all the monitoring sites, decide the exposure period and select sites freely for the identification of heavy metal contamination in seawater (Kramer, 1994). Therefore, transplantation of mussels from a clean environment to monitored sites may be a candidate to overcome the limitations

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caused by biological factors (Kramer, 1994; Phillips and Rainbow, 1994; Riget *et al.*, 1997).

Although the transplantation of mussels is conceptually reliable in using as a biomonitor, it is technically more complex than using indigenous mussels in a specific site. That is, mussels have to be collected in clean environments and be sorted by size. Furthermore, cages with mussels should be tightly bound in coastal area, which is practically a little difficult task, to endure for several weeks in harsh marine environments. Therefore, it is necessary to identify merits of transplantation relative to the method using indigenous mussels in detail. Accumulation and releases of heavy metals through transplantation have been assessed in several studies (Roesijadi *et al.*, 1984; Regoli and Orlando, 1994), while more detailed studies about merits or necessities of transplanted mussels as a biomonitor of heavy metals in seawater are needed through field research.

This study aims to estimate accumulation and release of heavy metals in mussels through reciprocal transplantation between clean and polluted sites, respectively. In addition, monitored results are compared between indigenous and transplanted mussels.

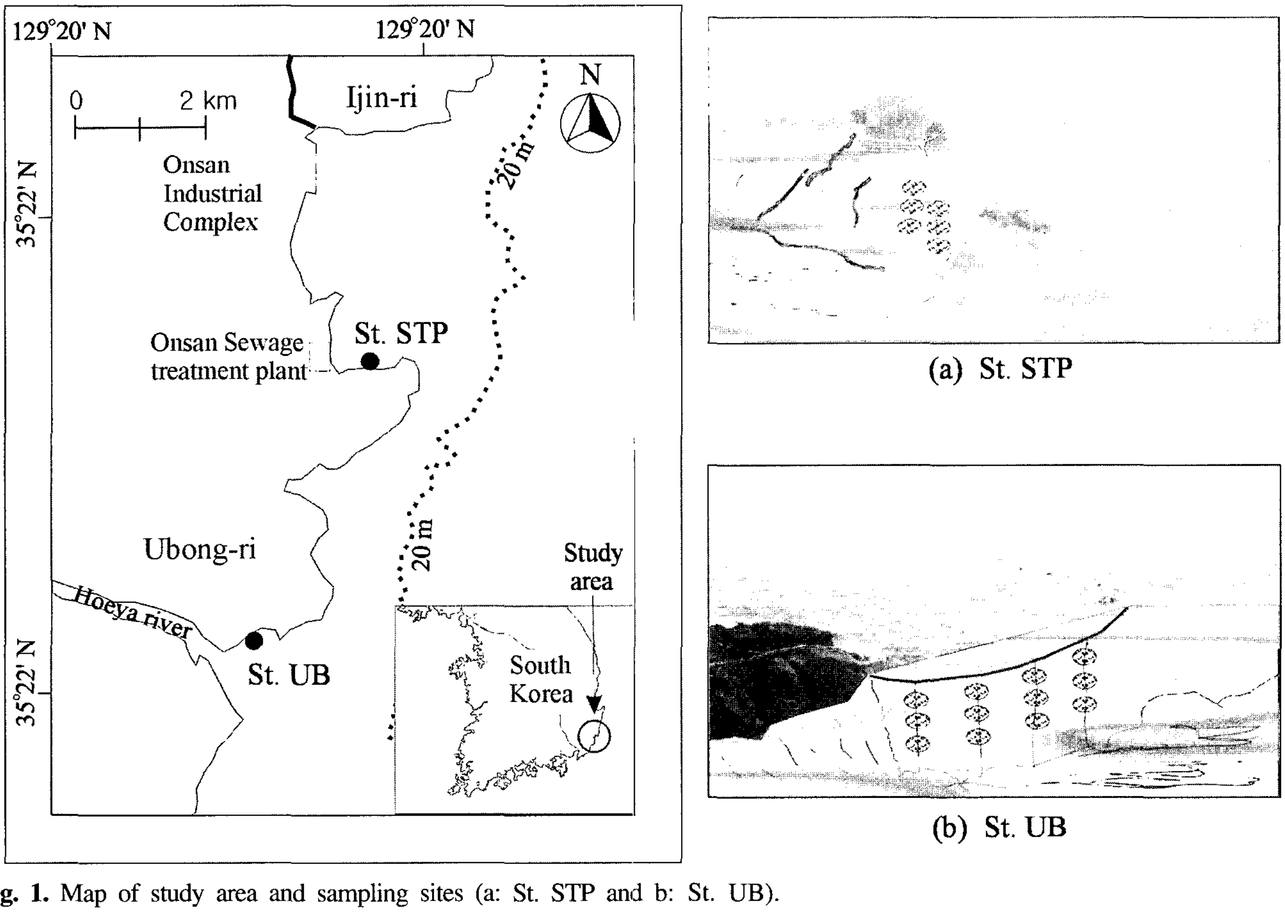
MATERIALS AND METHOD

Samples and analytical methods

The mussel, *Mytilus galloprovincialis*, may have been introduced since the World War and is found in all coastal waters of South Korea (Je *et al.*, 1990; Yoo, 1992; NFRDI, 1999; Min shell house, 2001). This species attaches itself to a rock or construction in the inter- or subtidal zone using a network of threads, named as byssi, which are secreted from a byssal gland in the foot, and feeds various organic matters suspended in seawater (NFRDI, 1999; Min shell house, 2001).

M. galloprovincialis was transplanted reciprocally from highly metal-contaminated site (St. STP) located near the Onsan sewage treatment plant to relatively less contaminated site (St. UB) located at 4 km south from St. STP, and *vice versa* (Fig. 1).

About 1600 mussels per each site were collected from St. STP and St. UB sites. The specimens were grouped into 3 size classes as 10–20 mm (class 1), 20–30 mm (class 2), and 30–40 mm (class 3). The mussels were maintained in a nylon mesh bag. Num-



(a) St. STP

(b) St. UB

Fig. 1. Map of study area and sampling sites (a: St. STP and b: St. UB).

Table 1. Experimental design for the reciprocal transplantation

Sampling date	Interval day	Cumulative day	Transplanted mussels		Indigenous mussels	
			St. STP	St. UB	St. STP	St. UB
14 Jan	0	0	O	O	O	O
22 Jan	8	8	O	O	-*	-
29 Jan	7	15	O	O	O	-
5 Feb	7	22	O	O	-	-
19 Feb	14	36	O	O	O	O
7 Mar	16	52	O	O	O	O
22 Mar	15	67	O	O	O	O
3 May	41	108	O	-	O	O

*: no sample

bers of the mussels in one nylon mesh bag were 20 (class 1), 30 (class 2), and 30 (class 3). The mooring lines were connected from a rock or other permanent structure to another rock. The mesh bags containing mussels attached to the mooring lines (Fig. 1a, 1b). The mussels were placed about 1 m water depth, and were collected periodically over 108 days (0, 8, 15, 22, 36, 52, 67, 108 days) from 14 January to 3 May 2002 by SCUBA diver. Indigenous mussels at St. STP and St. UB were also collected on the same date as transplanted mussels (Table 1).

Live specimens were kept in an aquarium containing the seawater collected from sampling sites for 24h to defecate the gut contents, and then were transported to the laboratory. During depuration, air was bubbled to supply oxygen to mussels. After depuration, soft tissues were dissected from about 10–20 individuals of each size class and pooled in three samples and stored at −20 °C, then freeze-dried for 2 days.

Dried mussel tissues were digested using 5 ml of concentrated HNO_3 in Teflon vessels on hot plates at 170 °C for 2 hours. After first digestion, solution was cooled down to room temperature, added with 1 ml of H_2O_2, and then heated at the same temperature again for overnight. Then, solution was evaporated on hot plates and added with 1% HNO_3 to appropriate volume. Heavy metals (Cd, Cu, Pb and Zn) in the aliquots were analyzed with Inductively Coupled Plasma Mass Spectrometer (X7 model, Thermo Elemental Ltd.). Recovery of analytical method was checked with a standard reference material of NIST oyster tissue (1566b) and was 104.1% for Cd, 93.4% for Cu, 97.2% for Pb and 96.1% for Zn. Reproducibility calculated from 8 SRM samples was over 90% for all heavy metals.

Seawater samples were also collected at the same date to sampling transplanted mussels using pre-cleaned HDPE bottle attached to a pole sampler. After the filtration of particulates using disposable vacuum filtration kit with 0.45 μm membrane filter paper in a calm area, filtered seawaters were transported to the laboratory and stored after the acidification with purified nitric acid to pH 1.5. All the used apparatus containing sampling bottles and filtration kits were precleaned with 10% (v/v) hydrochloric acid solution and then washed with Milli-Q water (18 MΩ). Dissolved metals were directly measured with High Resolution Inductively Coupled Plasma Mass Spectrometer (AXIOM MC model, ThermoElemental Ltd.) in Korea Basic Science Institute after 10 times dilution with Milli-Q water. Recoveries of Cu, Zn, Cd and Pb were checked with standard-spiked seawater and were 80~120% at 1 ng/ml level.

RESULTS AND DISCUSSION

Condition index

Integrated physiological status of a mussel can generally be estimated by determinations of the body condition index, which is defined as tissue weight or volume relative to shell weight or volume (Gosling, 1992). Many different methods exist for measuring the condition of mussel (Pridmore *et al.*, 1990). In this study, condition index was defined according to Lares *et al.* (2002) and presented as the following equation;

$$CI = \left(\frac{dry\ weight(g)_{tissue}}{L \times W \times H}\right)$$

where CI is a condition index and L, W, and H are shell length (mm), shell width (mm) and shell height (mm), respectively. Condition indices from mussels

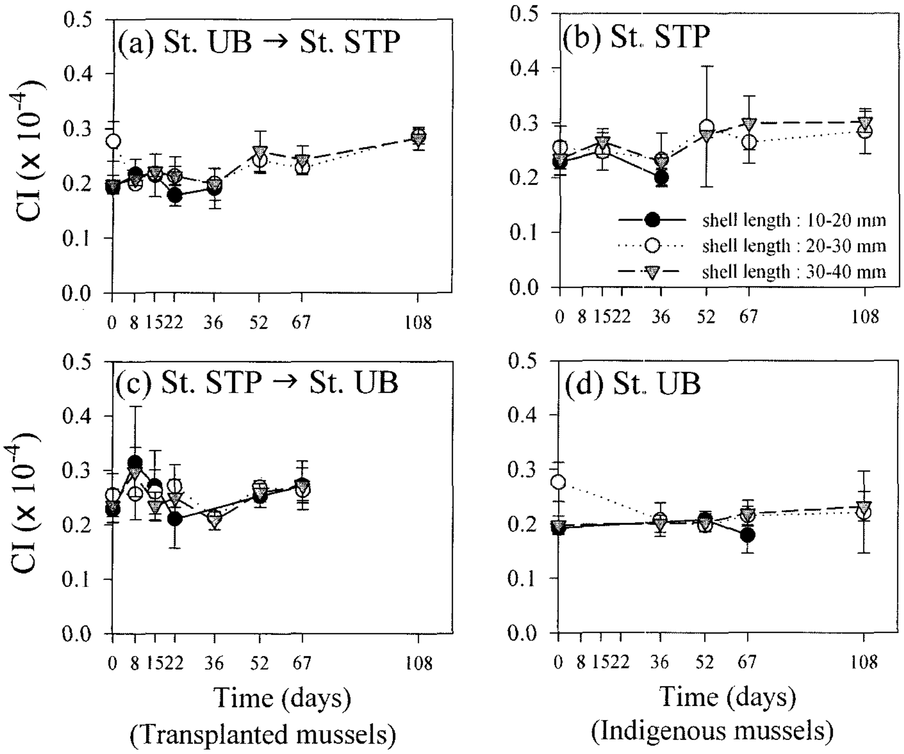


Fig. 2. Condition indices (C.I.) for transplanted and indigenous mussels during transplantation experiment at St. STP and St. UB.

are potentially very sensitive to pollution. However, condition indices are affected by a number of additional environmental stressors next to pollutants such as temperature, food supply and salinity that control the somatic growth and reproductive development (Gosling, 1992). Therefore, the physiological variation of mussel can be expected from the variation of the condition index. The variability of CI in this study was within about 20%, which indicated that there were few stressors enough to induce the change of tissue weight relative to shell volume during the experiment period (Fig. 2). Significant correlation between CI and heavy metal concentration in both transplanted and indigenous mussels were not shown: correlation coefficients in transplanted mussels (n=120) and indigenous mussel (n=40) are 0.46 and 0.41 for Cd, −0.22 and −0.16 for Cu, 0.38 and 0.42 for Pb, and −0.15 and −0.12 for Zn, respectively. Therefore, according to CI values, it seemed that heavy metal concentrations in mussel were not largely affected by physiological factors such as somatic growth and reproductive development.

Dissolved metals in seawater

Dissolved metal concentrations in seawater at each

Table 2. Dissolved metal concentrations(μg/l) in seawaters collected at St. STP and St. UB.

Site	Metal	Sampling date												mean	stdev	Other study*
		Jan						Feb		Mar		May				
		12	14	15	16	22	29	5	19	7	22	3	24			
St. STP	Cd	0.06	0.04	0.10	-	0.05	0.04	0.05	0.03	0.04	0.08	0.05	0.01	0.05	0.02	0.47
	Cu	1.50	1.53	2.02	-	0.95	0.53	0.85	0.81	0.67	0.63	0.65	0.73	0.99	0.48	1.93
	Pb	0.89	1.50	1.12	-	0.53	0.26	0.40	0.31	0.30	0.52	0.39	0.28	0.59	0.41	1.08
	Zn	4.92	6.74	8.15	-	4.42	2.71	2.96	2.23	2.63	3.06	7.02	1.72	4.23	2.19	9.14
St. UB	Cd	0.02	0.06	0.05	0.04	0.05	0.04	0.01	0.05	0.05	0.04	0.04	0.01	0.04	0.01	0.06
	Cu	0.31	0.32	0.34	0.27	0.44	0.43	2.02	0.37	0.53	0.53	0.56	0.92	0.59	0.48	0.43
	Pb	0.29	0.14	0.10	0.16	0.19	0.10	0.40	0.96	0.15	0.15	0.12	0.18	0.24	0.24	0.07
	Zn	1.15	0.95	1.14	0.98	1.72	1.39	5.57	1.34	2.16	2.16	1.22	2.50	1.86	1.28	1.04

*Other study: previous data in stations close to sites of this study (Kang *et al.*, 1999)

Table 3. Heavy metal concentrations (mg/g dry weight) in mussels (*Mytilus galloprovincialis*) collected at St. STP and St. UB on day 0

Site	Size class	Cd	Cu	Pb	Zn	n*
		(mean ± stdev)	(mean ± stdev)	(mean ± stdev)	(mean ± stdev)	
St. STP	10-20 mm	7.5 ± 0.71	12.0 ± 1.08	23.0 ± 1.66	59.1 ± 4.21	3(4-6)**
	20-30 mm	10.5 ± 1.92	12.2 ± 2.37	25.0 ± 6.89	65.5 ± 8.89	3(4-6)
	30-40 mm	12.7 ± 1.80	10.6 ± 1.48	20.8 ± 4.28	69.4 ± 14.2	3(4-5)
	Total	10.2 ± 2.61	11.6 ± 0.87	22.9 ± 2.10	54.7 ± 5.20	
St. UB	10-20 mm	2.7 ± 0.02	14.6 ± 0.32	3.5 ± 0.65	65.9 ± 4.85	3(4-6)
	20-30 mm	2.9 ± 0.41	14.1 ± 1.24	2.8 ± 0.49	59.6 ± 10.4	3(4-6)
	30-40 mm	4.1 ± 0.55	15.3 ± 1.07	3.3 ± 0.57	75.7 ± 15.0	3(4-5)
	Total	3.2 ± 0.76	14.7 ± 0.60	3.2 ± 0.36	67.1 ± 8.11	

*n: number of samples
**(): number of individual

site are shown in Table 2 during the experiment. For Cd, two sites showed very similar concentrations (t-test, $p<0.05$). Concentrations of dissolved Cu, Pb, and Zn at St. STP were 5–6 times higher than those at St. UB during the first 8 days (22 Jan) and then, dissolved Cu concentrations became little different between two sites while dissolved Pb and Zn concentrations at St. STP decreased to the levels of about 1.5 times higher than those at St. UB.

Dissolved metal levels of this study are within the range of the previously reported concentrations except for Cd at St. STP. Mean Cd concentration in St. STP was about 10 times lower than previous one (Kang *et al.*, 1999). The reason why Cd concentration decreased so much was unidentified while it may be related to the decrease of pollution intensity.

Heavy metals in initial mussels at the starting point of transplantation experiment

Before the deployment of transplanted mussels, mean metal concentrations in mussels at St. STP and St. UB are shown in Table 3. The levels of Cd and Pb in mussels collected at St. STP were 3 times and 7 times higher than those at St. UB, respectively, and these levels are comparable to those of high concentration reported previously in the same location as well as in the metal-polluted areas of the world (O'Connor, 1996; Szefer *et al.*, 1997; Kang *et al.*, 1999; Kim, 2000). This fact may indicate that the pollution of Cd and Pb was severe in this area.

The concentrations of Cu and Zn in mussels were similar between sites. In addition, the levels of Cu and Zn in mussels were similar to the previous data in this area (Kang *et al.*, 1999; Kim, 2000).

Heavy metals in mussels from the reciprocal transplantation

Cadmium: Cadmium was not accumulated in mussels transplanted from St. UB to St. STP (Fig. 3a) throughout the experiment period but, in transplanted mussels from St. STP to St. UB, Cd concentration in mussels increased during the first 15 days from 10.5 μg/g to 15 μg/g for 20–30 mm size class and was maintained thereafter (Fig. 3c). In indigenous mussels at St. STP, there were 1.7 times increase of Cd concentrations during the 52 days from 10.5 μg/g to 17 μg/g for 20–30 mm size class and then decreased to 12 μg/g in the last sample (108 day) (Fig. 3b). There was no significant variation of Cd concentrations in indigenous mussels at St. UB during the experiment period (Fig. 3d). That is, Cd was not accumulated in mussels collected at St. UB but was accumulated in mussels collected at St. STP regardless of transplanted site.

Since the concentration of Cd in initial mussels collected at St. UB was 3 times lower than that at St. STP, cadmium accumulation in transplanted mussels from St. UB to St. STP was expected but not shown. This fact indicates that there was decrease of either Cd input or bioavailable Cd species at St. STP because Cd concentration in mussels depends on Cd level in aquatic environment (Odžak *et al.*, 1994). Actually, dissolved Cd concentration throughout the experiment period during 4 months at both sites was very low and similar between sites. However, Cd in mussels collected at St. STP including transplanted and indigenous mussels increased even though dissolved Cd concentration was very low. Therefore, it may need that there were other Cd accumulation mechanisms in polluted mussels regardless of dissolved Cd level.

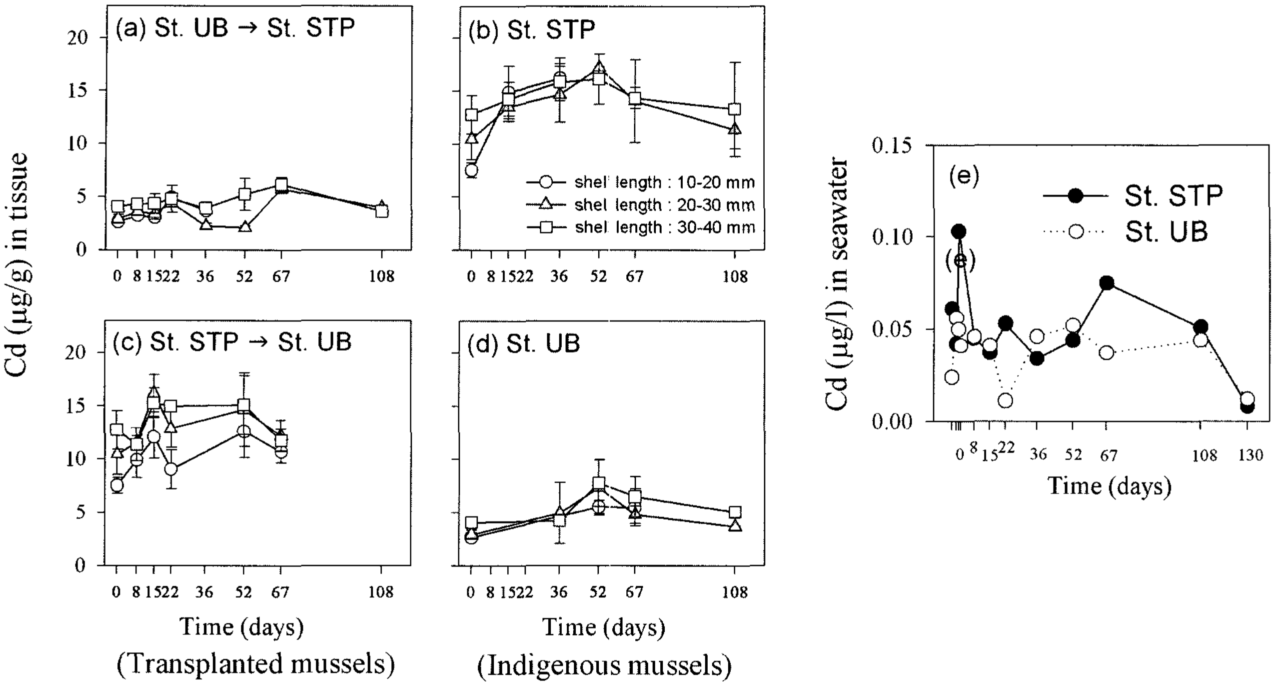


Fig. 3. Cadmium concentrations in the mussels (μg/g dry wt.) and seawater (μg/l) during transplantation experiment.

The release of Cd in mussels seemed to be very slow. The elimination of Cd in indigenous mussels at St. STP and in transplanted mussels from St. STP to St. UB was expected because dissolved Cd concentration in both sites were similar and Cd concentration in the initial mussels of St. STP was 3 times higher than those from St. UB. However, actual release in those mussels occurred only after 52 days of deployment. This observation agrees with that of Luten *et al.*(1986) in *Mytilus edulis* and may result from the fact that a biological half-life of Cd was 96–190 days (Borchardt, 1983). There were no differences in three size classes with respect to the accumulation and release behavior of Cd in mussels.

Therefore, Cd accumulated in indigenous mussel may reflect the integrated ones through their lives, but does not show the present status of seawater because the release of Cd in mussels occurs very slowly. Cd in transplanted mussel from clean site rather than indigenous mussel, however, shows the present status of seawater. In case of the reduction of pollution intensity in a specific area, it may be difficult for Cd in indigenous mussels to reflect the environmental Cd level anymore. And only transplanted mussels from clean site can respond to the environmental change.

Copper: The Cu concentration in both transplanted and indigenous mussels varied over time in similar pattern; that is, slight increase at the early period (initial 15 days), consecutive decrease from 15 days to 36 days and increase again at 67 days (Fig. 4). Although dissolved Cu concentration was higher in St. STP than in St. UB at the earlier experimental periods, mussel Cu concentration was higher in St. UB (14.7 μg/g) than in St. STP (11.6 μg/g) (Table 3). This uncoupling between mussel and environment was also shown in transplanted mussels from St. STP to St. UB, which showed initial increase of Cu even though mussels had lived in contaminated area exposed to clean seawater. Therefore, slight differences of Cu concentration in initial mussels used in transplantation experiment made the evaluation of the accumulation and release of Cu through reciprocal transplantation difficult.

Lead: Lead concentrations in transplanted mussels from St. UB to St. STP gradually increased from 2.8 μg/g to 14 μg/g (size class 2) during experiment period (Fig. 5a). Lead concentrations in transplanted mussels from St. STP to St. UB gradually decreased from 25.0 μg/g to 17 μg/g (size class 2) after 67 days deployment (Fig. 5c). In indigenous mussels at both sites, there was little variation of Pb concentration over time (Fig. 5b and 5d). The increase of Pb in mussels over time was resulted from higher concentrations of dissolved Pb in St. STP than those in St. UB during the experiment, which could be found in other study (Martinčić *et al.*,1992) that showed the significant correlation between body Pb concentrations and Pb amount in the surrounding water.

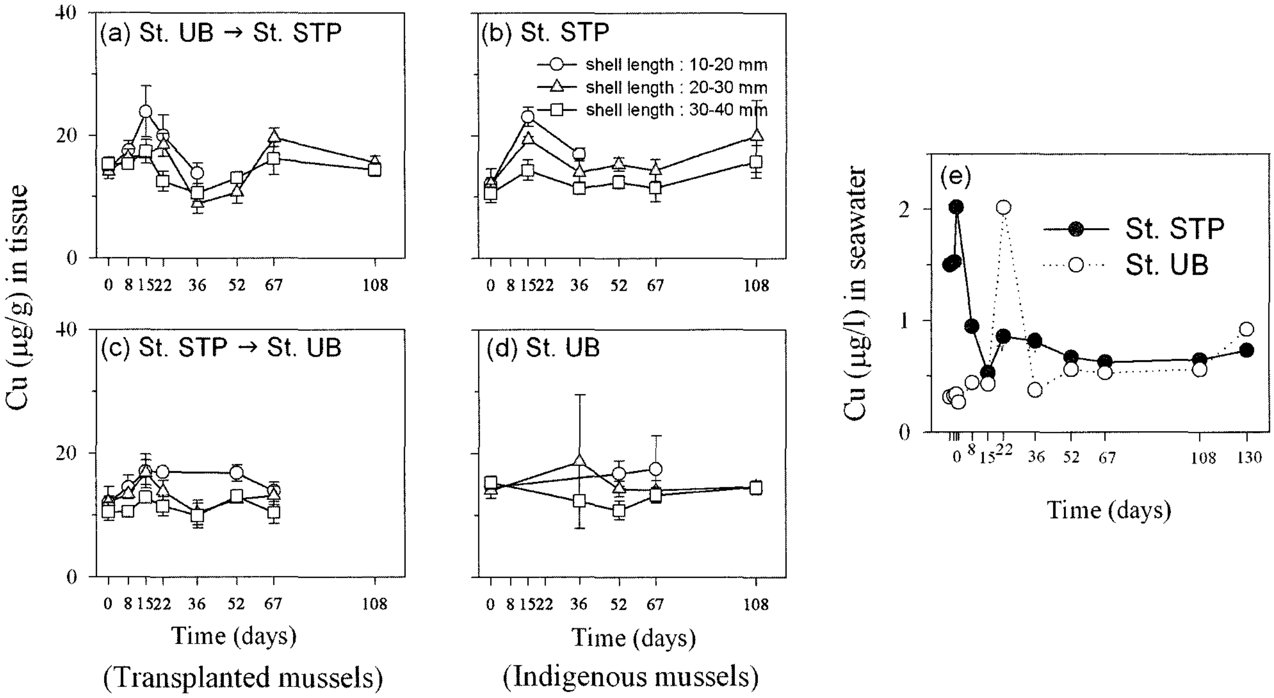


Fig. 4. Copper concentrations in the mussels (μg/g dry wt.) and seawater (μg/l) during transplantation experiment.

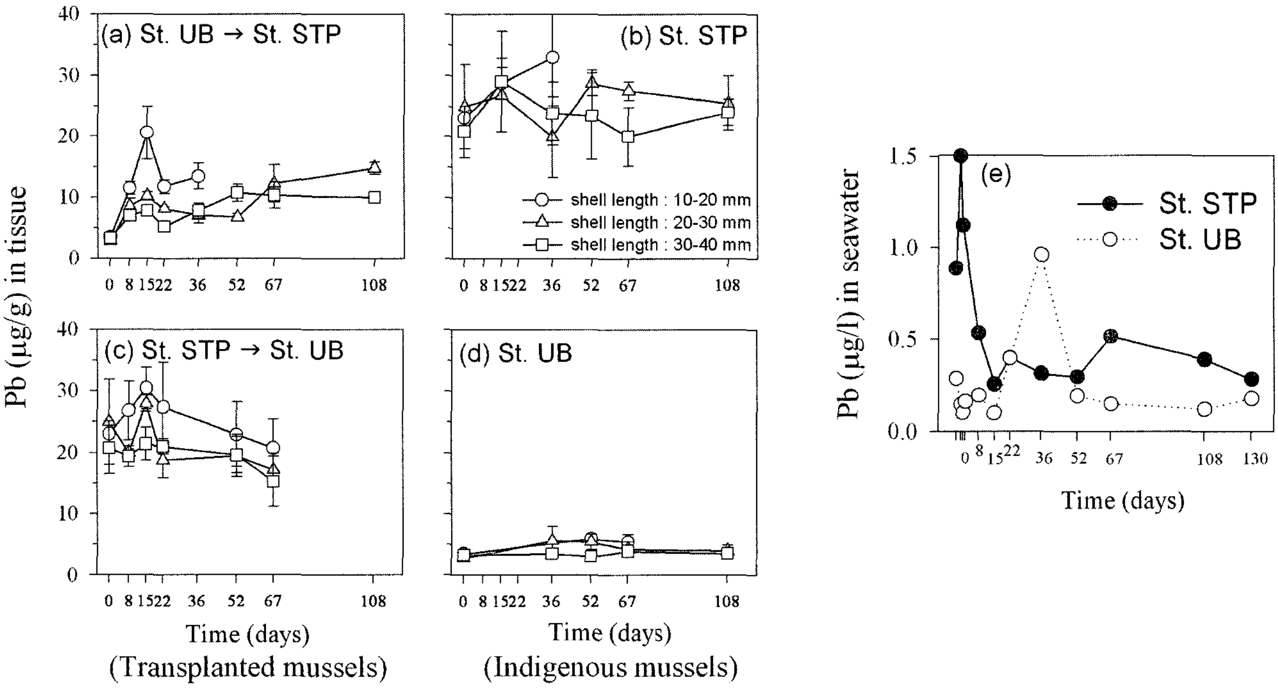


Fig. 5. Lead concentrations in the mussels (μg/g dry wt.) and seawater (μg/l) during transplantation experiment.

The greatest accumulation of Pb in transplanted mussels at the early period after deployment could be found in size class 1 from 3.5 μg/g to 20 μg/g although the rapid accumulation of Pb was also found in other size classes at the early period after deployment. The significant increase of Pb concentrations in younger aged mussels was observed in other studies (Odžak *et al.*, 1994; Phillips and Rainbow, 1994). After the rapid increase of Pb concentrations, there were sudden drops of Pb in mussels of all the size classes at 22days after deployment, which may reflect the rapid decrease of dissolved Pb concentration in St. STP

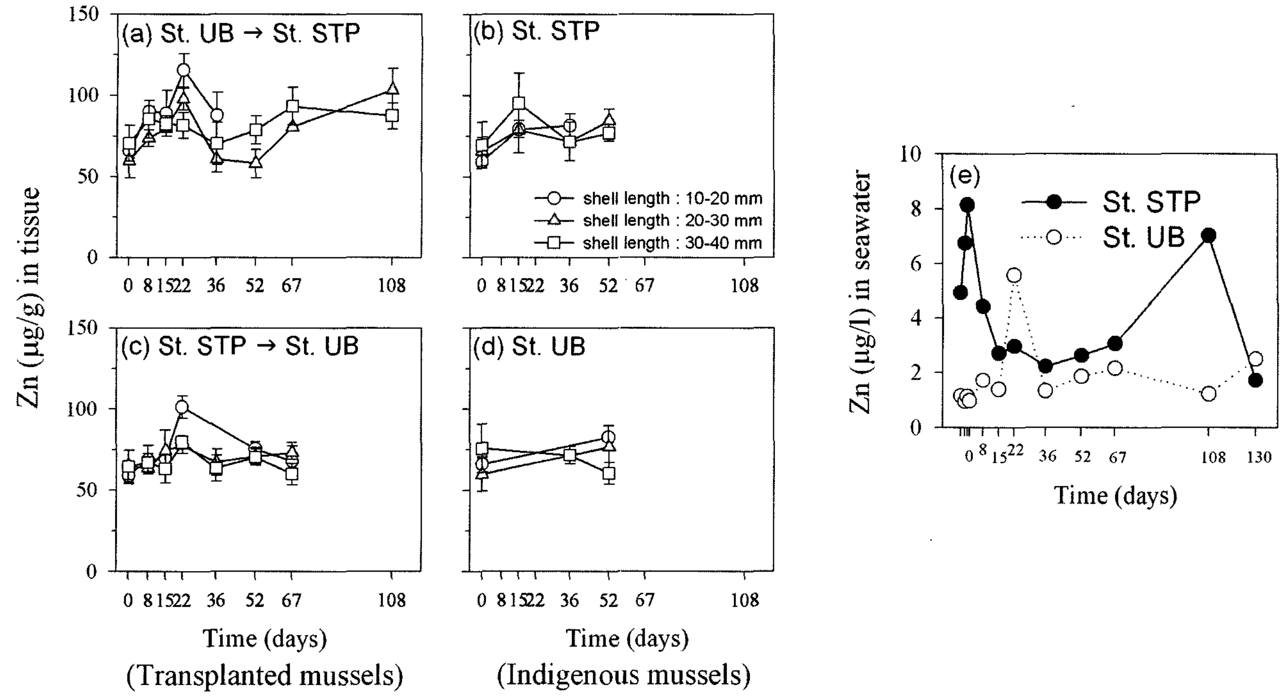


Fig. 6. Zinc concentrations in the mussels (μg/g dry wt.) and seawater (μg/l) during transplantation experiment.

(Fig. 5e). Since the indigenous mussels in St. STP didn't respond, however, to the decrease of dissolved Pb concentration, the rapid release of accumulated Pb from mussels would not be expected. As the physiological variation, indicated as condition index, cannot describe the significant decrease of Pb in mussel, it may be necessary to speculate over the mechanism describing different release pattern between transplanted and indigenous mussels such as the difference of newly accumulated Pb and aged Pb in transplanted and indigenous mussels, respectively.

The release of accumulated Pb in mussels seemed to occur very slowly (Fig. 5c). After 52 days deployment, the decrease of Pb concentrations in transplanted mussel from St. STP to St. UB was observed. This slow elimination of Pb from mussel is consistent with other results showing the long depuration half-life of Pb (Regoli and Orlando, 1994; Boisson *et al.*, 1998). Regoli and Orlando (1994) found that the loss of lead in the digestive gland of *M. galloprovincialis* was approximately 15% after 5 months depuration and suggested that some long-term storage components within tissue for Pb had been made. Riget *et al.* (1997) found that after two to three years deployment in a clean environment, the release of lead was stopped, and the mussels seemed to be able to depurate only about half of the originally taken up Pb.

From above observation, it may be concluded that Pb accumulated in mussels depended on seawater Pb level, and mussel has shown long biological half-life release for accumulated Pb. Therefore, like Cd, when the input of Pb decreased at a Pb polluted area in the past, indigenous mussel cannot reflect the present status of Pb in seawater while transplanted mussels from a clean environment can only do it.

Zinc: The initial concentration of Zn in mussels of St. STP and St. UB was comparable such as 64.7 μg/g and 67.1 μg/g, respectively, even though dissolved Zn concentration in seawater was higher in St. STP than in St. UB (Fig. 6). The variation of Zn concentration over time in transplanted mussels seemed to be similar in both sites; that is, early increase, consecutive decrease from 22 days to 52 days and then increases again. Furthermore, temporal variability of Zn concentration in both transplanted and indigenous mussels was just similar to that of Cu. This decoupling between mussels and environment for Cu and Zn might be explained in that there are a certain regulatory capacity in mussels or similar bioavailability of Cu and Zn in both sites (Regoli and Orlando., 1994; Riget *et al.*, 1997).

Consideration for Mussel Watch for monitoring heavy metals in Korean coastal waters

In this study, accumulation of heavy metals in soft tissue of *Mytilus galloprovincialis* was shown in only Pb (Fig. 5a). Cd and Cu accumulation of transplanted mussel from St. UB to St. STP were not observed due to the similarity of Cd and Cu levels in seawater at both sites (Fig. 3a; Fig. 4a). Although there was difference of Zn concentrations in seawater at both sites, Zn accumulation of transplanted mussel from St. UB to St. STP was not shown (Fig. 6a). Release of heavy metals in mussel with respect to difference of heavy metal levels in seawater at both sites was observed in Cd and Pb (Fig. 3c; Fig. 5c), but their release occurred very slowly.

These results confirm that mussels may exhibit different accumulation and release patterns depending on individual metal (Boisson *et al.*, 1998; Martinčić *et al.*, 1992; Regoli and Orlando, 1994). The fact that Zn body concentration does not relate with Zn concentrations in the surrounding waters will limit the utility of mussels as biomonitor for Zn (Martinčić *et al.*, 1992; Regoli and Orlando, 1994; Riget *et al.*, 1997). Release of Cd and Pb in *M. galloprovincialis* over the experiment time (108 days) was found to be slow processes. Due to the slow elimination of Cd and Pb, the concentrations of these heavy metals from indigenous mussels can reflect past contamination that is not present any longer. On the other hand, using transplanted mussels, it is possible to measure the actual presence of these heavy metals. If Cd and Pb inputs into coastal areas are reduced by administrative regulations in metal-polluted areas such as industrial complex or harbour, transplanted mussels rather than indigenous mussels are to be useful as a biomonitor. Therefore, it may be suggested that transplanted mussels rather than indigenous mussel are more useful for monitoring heavy metals of coastal seawaters.

CONCLUSIONS

This study is a first attempt to estimate heavy metal accumulation and release by mussels through field reciprocal transplantation in Korean coastal water. Our experiments show that Cd and Pb accumulated in *Mytilus galloprovincialis* are slowly depurated when the mussels are transplanted from contaminated site to relatively less contaminated site. In addition, accumulation of metals in transplanted mussels responded to the present levels of Pb and Cd in seawaters, while metals accumulated in indigenous mussels indicated the past gradients of metal levels. Therefore, in order to investigate the present status of metal pollution in seawaters, mussels transplanted from a clean area rather than indigenous mussels should be assessed although the practical performance of transplantation experiment is more complicate.

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