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Distribution and Association of Trace Metals in Soft Tissue and Byssus of *Mytilus edulis* from the East Coast of Kyushu Island, Japan

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Abstract. Concentrations of Cd, Pb, Zn, Cu, Ag, Cr, Co Ni, Mn, and Fe in soft tissues and byssi of blue mussel (*Mytilus edulis*) from three sites along the east coast of Kyushu Island, Japan, were determined by AAS method. Large inter-regional differences in metal concentrations in both soft tissues and byssi (Cu, Cd, and Pb and Cu, Pb, Co, Ni, Mn, and Fe, respectively) were recorded. Highly significant correlations ($p < 0.01$) were observed between tissue and byssal concentrations of Pb, Cu, Zn, and Mn. The tissue concentrations of Cu, Cd, and Pb were two orders of magnitude greater in *Mytilus edulis* from expected to be the most contaminated locations compared to those from a nonindustrialized area. Intercomparison of the present study data with those published previously indicates that the soft tissue and especially byssus are useful in detecting some areas of some metallic contaminants. The high concentrations of Cd, and especially Pb and Cu, in Saganoseki mussels and moderately elevated concentrations of these metals in Akamizu mussels may be attributed to the anthropogenic emissions from a metallic refinery and an artificial fiber factory, respectively. It is evident that, compared to the soft tissue, the increase of Cu levels relative to Zn levels in the byssi of *M. edulis* are eight times higher, with a slope b (Cu/Zn) of 7.5 for byssus and 0.93 for soft tissue. This suggests that byssus, as compared to soft tissue, is a more sensitive bioindicator for Cu. From the data obtained, the soft tissue and especially byssi of *M. edulis* appear to be good bioindicators for identification of coastal areas exposed to metallic contaminants.

Much attention has been paid to the ability of marine organisms, especially molluscs, to accumulate metallic toxicants from the marine environment. Marine organisms, compared to bottom sediments, exhibit a greater spatial sensitivity and a greater ability to concentrate some metals.

In previous investigations, emphasis has been placed on the potential of molluscs for signaling contamination of aquatic environments by heavy metals. Some organisms like *Mytilus* spp. (Fowler and Oregioni 1976; Phillips 1980; Koide *et al.* 1982; Ritz *et al.* 1982; Goldberg *et al.* 1978, 1983; Szefer and Szefer 1985, 1990, 1991; Ikuta 1986a, 1986b, 1988; Szefer 1991; Broman *et al.* 1991; Lauenstein and Dolvin 1992; Fabris *et al.* 1994) and other zoobenthos (McGreer 1982; Bryan *et al.* 1983; Bryan *et al.* 1985; Savari *et al.* 1991; Anderlini 1992; Fowler *et al.* 1993) are potential bioindicators for monitoring metallic contamination of marine ecosystems. Extensive critical reviews concerning concentration, distribution and/or bioaccumulation of heavy metals in marine mussels, with special reference to their comparative evaluation as quantitative indicators of metallic pollutants, have been published (Phillips 1980; Bryan 1984; Cossa 1988; Fowler 1990, Bryan and Langston 1992).

However, byssus secreted from byssal glands in the foot has not been extensively analyzed for metals. Thus, the number of available articles on the distribution of trace elements in byssi of the bivalves from regions other than the Japanese coast (Ikuta 1986a, 1986b) is very scant (Coombs and Keller 1981).

The present study was designed to examine availability of byssus of *M. edulis* as a bioindicator for monitoring metallic contamination on the basis of metal concentrations in soft tissues and byssi, using the mussels collected from Japanese southern region of the Pacific. This survey of the spatial distribution of contaminants was designed carefully with appropriate selection of sampling sites close to known or suspected sources of contaminants (Saganoseki and Akamizu) including reference location (Urashiro).

Materials and Methods

About 1,000 specimens of a blue mussel (*Mytilus edulis*) were collected from rocky shores in inter-tidal zones around the sea areas of Urashiro, Akamizu, and Saganoseki along the east coast of Kyushu, Japan, during low water of spring tide in April 1994 (Figure 1). The sampling sites were selected according to expected different degree of their exposure to metal sources. A metallic refinery (Saganoseki Refinery of Nipponkougyo Industry) is located in Saganoseki while Akamizu is situated near a fiber factory (Asahikasei Industry in Nobeoka). Urashiro is far from metallic pollution sources.

Living specimens were transported immediately to the laboratory and subsequently kept in seawater from the sampling sites in an aquarium for 24 h to defecate the contents in alimentary canals. The specimens were sorted with respect to their sizes and were separated

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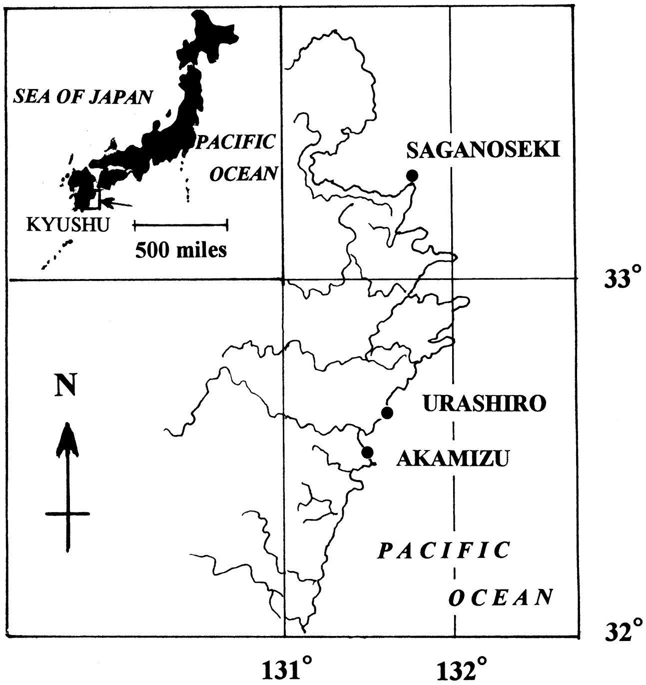

Fig. 1. Location of the sampling stations

into soft part, shell valves, and byssal tuft, and each were weighed. It should be mentioned that criterion used to set separate size class was differentiation relative to the shell length within each individual group collected at each sampling site. Wet soft tissue and shell isolated from each specimen were separately weighed after separation of byssal threads. Five analytical groups were prepared, in which soft tissue of 20 individuals were pooled for each group to obtain means of the samples.

Byssus was cleaned of shell fragments, debris, seaweeds, etc., which commonly adhere to it. Pooled samples were prepared as well as the treatment of soft tissues and, after rinsing with seawater, were preserved in plastic bags with ice blocks in a freezer.

All pooled samples were weighed, dried at 105°C to a constant weight, and homogenized in a porcelain mortar. Three to five replicate subsamples of each were then prepared. The biometric data are listed in Table 1.

After weighing, dried materials were digested in an Automatic Microwave Digestion System (MLS 1200) using concentrated HNO_3 Suprapur® "Merck" and triple distilled water (obtained in apparatus Destamat® "Heareus Quarzglas"). Cadmium, Cu, Zn, Pb, Ag, Cr, Co, Ni, Mn, and Fe concentrations were determined by AAS method (Philips PU 9100 and VIDEO 11 E Atomic Absorption Spectrophotometers) using deuterium-background correction. The quality of the method used was checked and confirmed in a separate comparative study of metals in a standard reference material, *i.e.*, Fish Flesh MA-B-3/TM (IAEA, Monaco). The agreement between the analytical results for the reference material and their certified values were satisfactory; *i.e.*, the recovery and the standard deviation were $>87\%$ and $<10\%$, respectively. To check for contamination, blanks were analyzed using this procedure after every five samples. Both the correlation matrix and linear regression analysis of the chemical data were then calculated using GRAPHER Version 1.09 (Golden Software, Inc.) program.

Results and Discussion

Concentrations of Metals

Inter-regional variations of both the tissue and byssal concentrations of metals in successive size classes of the mussel are illustrated in Figures 2, 3. The mean values of tissue and byssal concentrations obtained in the present study are compared with those from other sites of the eastern coast of Kyushu Island (Table 2).

Figure 2 shows that the tissue concentrations of Cu and Pb in different size classes of *M. edulis* from Saganoseki were up to two orders of magnitude greater than those from Urashiro. The concentration of Cd was also greater, by a factor of 20, in Saganoseki tissues. As for Zn and Mn, such regional-dependent variations were considerably smaller, *i.e.*, Saganoseki mussels contained 3–4 times more of these two elements than did mussels from Urashiro. Akamizu mussels were also characterized by higher concentrations of Cu and Pb as compared to those from Urashiro. These levels were, however, lower by an order of magnitude than those observed for mussels from Saganoseki.

The inter-regional distribution of Cu, Pb, Mn and Fe in byssi was similar to that in soft tissues, but byssal concentrations were higher (by an order of magnitude for Cu, 1.5–3 times for Pb, 2–6 times for Fe), especially for Mn (10–50 times) as compared with those of soft tissues (Figures 2, 3). It should be emphasized that concentrations of Cd were lower in byssi than in soft tissues, in particular in the samples collected from Saganoseki (10–60 times lower). The byssal concentrations of Cu, Pb, and Mn obtained for Saganoseki mussels in the present study are evidently greater as compared with those reported for the same species from the Ythan, Scotland (Coombs and Keller 1981). By contrast, the concentration of Zn was greater in byssus from the latter region.

It is evident that Saganoseki *M. edulis* contained in soft tissue and byssus much more of the elements studied, except Zn, as compared with specimens inhabiting not only the Urashiro and Akamizu regions but also other areas of Japan. For example, this concerns Cu (739 ± 119 and 2480 ± 338 µg g^{-1}), Pb (182 ± 17.8 and 182 ± 18.7 µg g^{-1}), Cd (18.4 ± 1.73 and 4.39 ± 1.14 µg g^{-1}), Cr (9.99 ± 0.57 and 30.7 ± 6.82 µg g^{-1}), Ni (16.0 ± 2.07 and 100 ± 45.0 µg g^{-1}) and Fe (1460 ± 190 and 5580 ± 3340 µg g^{-1}) (Table 2; Ikuta 1986a).

The tissue concentrations of Pb, Ag, Cd and Mn are mostly comparable with maximum values reported for *M. edulis* from different aquatic regions. For Ag in tissue, the concentrations determined here were lower as compared to a maximum tissue value (16.9 µg g^{1} dry wt.) recorded in Whitehaven harbor, England (Bryan *et al.* 1985). Based on the results obtained in the present study and earlier published data for other geographical regions (Hung *et al.* 1981; Bryan *et al.* 1985; Cossa 1988; Anderlini 1992) including numerous studies have been published in the United States, *e.g.*, both NOAA's and EPA's mussel watch programs (NOAA 1989; Lauenstein and Dolvin 1992; Lauenstein *et al.* 1990), it can be concluded that Saganoseki area is one of the most polluted areas reported to date.

Interelemental Relationships

Significant correlation coefficients between metal concentrations in soft tissue and between metals in byssus as well as between metals in soft tissue vs metals in byssus are presented in Tables 3 and 4, respectively. Figures 4 and 5 illustrate the relationships between concentrations of some metals in the soft tissue (Cd–Cu, Cd–Mn, Cd–Pb, Cu–Pb, Zn–Pb, Zn–Cu) and byssi (Cu–Pb, Pb–Mn, Pb–Co, Pb–Ni, Pb–Ag, Cu–Zn). The

Table 1. Biometric data (mean and standard deviation corresponding to one specimen) and water content for various size classes of *Mytilus edulis*

Size class	N[a]	Shell length [mm]	Wet weight of whole specimen [g]	Wet weight of soft tissue [g]	Water content [%]
Urashiro					
ML	5 (20)	48.8 ± 0.59	13.4 ± 0.62	2.07 ± 0.15	83.3 ± 0.79
M	5 (20)	43.2 ± 0.34	8.68 ± 0.20	1.45 ± 0.02	92.6 ± 0.52
S	5 (20)	31.6 ± 0.70	3.35 ± 0.22	0.58 ± 0.03	83.2 ± 3.66
Akamizu					
L	5 (20)	68.6 ± 1.50	31.1 ± 2.72	4.47 ± 0.48	96.1 ± 0.3
ML	5 (20)	58.3 ± 1.00	21.7 ± 0.29	0.61 ± 0.10	83.5 ± 0.6
M	5 (20)	46.9 ± 1.03	12.3 ± 0.76	0.39 ± 0.10	83.3 ± 0.77
S	5 (20)	28.6 ± 0.93	2.6 ± 0.16	0.38 ± 0.02	82.3 ± 0.31
Saganoseki					
L	1 (12)	56.2 ± 5.62	18.0 ± 4.79	2.2 ± 0.87	90.1
M	5 (20)	35.7 ± 1.19	4.47 ± 0.54	0.4 ± 0.05	84.4 ± 0.45
S	5 (20)	25.5 ± 0.51	1.78 ± 0.09	0.14 ± 0.02	84.6 ± 0.95

[a] Number of pooled samples and number of specimens per sample in parentheses

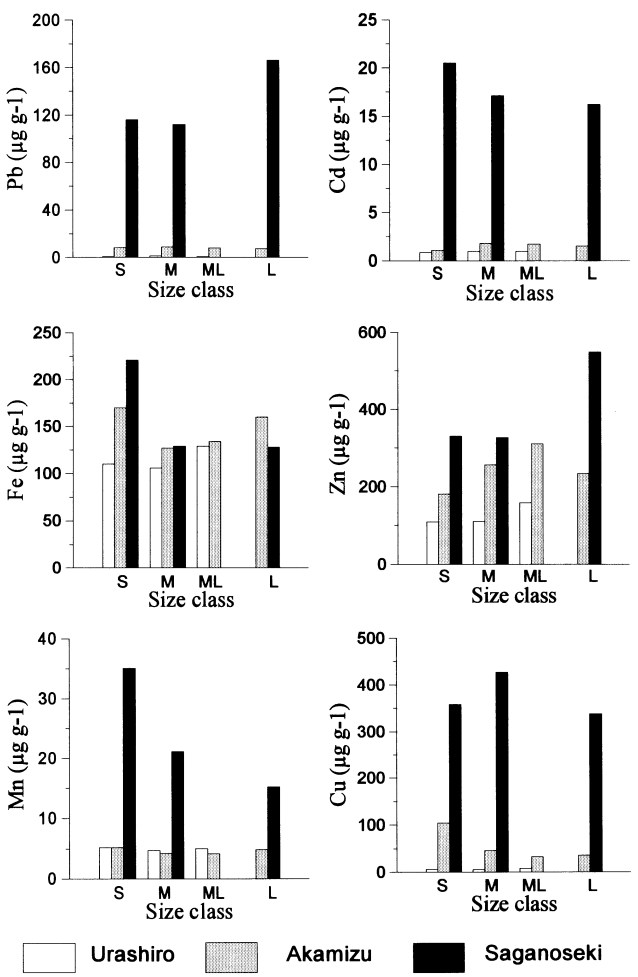

Fig. 2. Inter-regional distribution of selected metals in the soft tissue of successive size classes of *M. edulis*

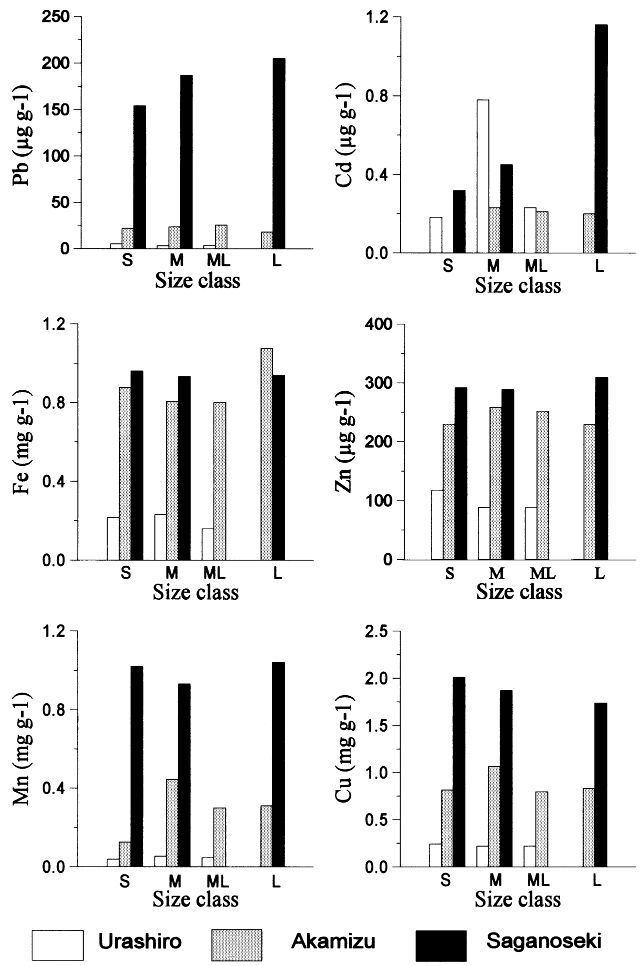

Fig. 3. Inter-regional distribution of selected metals in the byssus of successive size classes of *M. edulis*

relationships between metals in soft tissue vs metals in byssus are shown in Figure ;6.

Significant positive correlations ($p < 0.01$ or $p < 0.05$) in the soft tissues of *M. edulis* from the three sites were found for pairs Pb–Mn, Pb–Cu, Pb–Cd, Pb–Zn, Cu–Mn, Cd–Mn, and Cd–Cu. (Table 3). There is a positive correlation ($p < 0.01$ or $p < 0.05$) between the concentrations of metals in byssus for the assemblages of Cu–Pb–Zn–Cr–Co–Ni–Mn, Ag–Pb–Cu–Co–Ni–Mn

Table 2. Concentrations of selected metals (µg/g dry wt.) in the soft tissue (ST) and byssal threads (B) of *Mytilus edulis* from different locations on the east coat of Kyushu Island

Location		Pb	Cd	Cu	Zn	Ag	Cr	Co	Ni	Mn	Fe	Literature
Urashiro	ST	0.73 ± 0.4	0.92 ± 0.08	6.5 ± 0.92	127 ± 21.2	<0.37	0.67 ± 0.44	0.46 ± 0.19	<0.2	4.98 ± 0.28	115 ± 13.9	present
	B	3.96 ± 0.77	0.4 ± 0.26	22.9 ± 0.96	98.4 ± 13.1	<1.36	0.85 ± 0.02	1.26 ± 0.46	5.71 ± 0.95	46.2 ± 4.89	203 ± 29.1	paper
Akamizu	ST	8.08 ± 0.79	1.52 ± 0.23	51.8 ± 22.4	244 ± 46.0	1.1 ± 0.73	0.92 ± 0.26	0.56 ± 0.25	0.2	4.63 ± 0.48	149 ± 19.0	present
	B	22.2 ± 2.2	0.16 ± 0.08	876 ± 94.4	243 ± 13.0	1.44 ± 0.26	2.24 ± 0.79	3.13 ± 0.56	5.56 ± 0.19	341 ± 144	891 ± 94.4	paper
Saganoseki	ST	122 ± 17	18.4 ± 1.73	385 ± 36.3	360 ± 54.1	5.36 ± 4.1	0.87 ± 0.24	0.83 ± 0.4	<0.2	26.3 ± 7.57	169 ± 45.2	present
	B	182 ± 18.7	0.64 ± 0.34	1870 ± 91.1	297 ± 8.67	2.87 ± 0.55	5.93 ± 1.88	12.0 ± 0.96	22.4 ± 0.75	997 ± 44	943 ± 11.1	paper
Saganoseki	ST	58.2 ± 22.4	10.5 ± 1.95	739 ± 119	245 ± 38.6	—	9.99 ± 0.57	—	16.0 ± 2.07	28.4 ± 2.59	537 ± 162	Ikuta
	B	152 ± 58.6	ND[a]	2480 ± 338	230 ± 10.9	—	30.7 ± 6.82	—	100 ± 45.5	229 ± 97.3	5580 ± 3340	[1986a]
Baba	ST	5.31 ± 0.84	0.6 ± 0.08	5.06 ± 0.51	118 ± 14.0	—	1.9 ± 0.25	—	1.44 ± 0.23	7.21 ± 0.75	180 ± 37.8	Ikuta
	B	ND	ND	86.2 ± 12.3	168 ± 25.7	—	ND	—	31.9 ± 6.7	1190 ± 279	1680 ± 96.2	[1986a]
Beppu	ST	ND	1.2 ± 0.23	3.76 ± 0.3	233 ± 25.6	—	ND	—	1.37 ± 0.52	45.0 ± 9.00	161 ± 0.5	Ikuta
	B	ND	1.05 ± 0.32	18.8 ± 3.35	204 ± 29.7	—	ND	—	9.3 ± 3.65	893 ± 235	1180 ± 272	[1986a]
Hizi	ST	0.84 ± 0.05	0.92 ± 0.03	5.14 ± 0.34	—	—	—	—	—	20.4 ± 4.5	—	Ikuta
	B	43.8 ± 19.3	1.9 ± 0.73	52.9 ± 6.15	—	—	—	—	—	672 ± 102	—	[1986a]
Kamiura	ST	ND	0.84 ± 0.27	5.67 ± 0.19	156 ± 13.8	—	0.14 ± 0.1	—	0.35 ± 0.05	6.54 ± 0.24	133 ± 15.9	Ikuta
	B	ND	ND	21.0 ± 0.76	191 ± 10.6	—	2.79 ± 2.04	—	14.8 ± 0.38	61.8 ± 12.5	606 ± 82.6	[1986a]

[a] ND Not detected

Table 3. Significant correlation between selected metals in the soft tissue and between metals in byssus of *Mytilus edulis* from the east coast of Kyushu Island at $p < 0.01$ and $p < 0.05$. Biometric data are also taken into account: whole weight (WW), tissue weight (TW) and shell length (SL)

	Soft tissue	Byssus
(SL)	(+)WW^{a}, (+)TW^{a}	–
Pb	(−)TW^{b}	–
Mn	(+)Pb^{a}, (−)SL^{a}, (−)WW^{b}, (+) TW^{a}	(+)Pb^{a}
Cu	(−)SL^{b}, (−)WW^{b}, (−)TW^{a}, (+)Pb^{a}, (+)Mn^{a}	(+)Pb^{a}, (+)Mn^{a}
Cd	(+)Pb^{a}, (+)Mn^{a}, (+)Cu^{a}, (−)SL^{b}, (−)WW^{b}, (−)TW^{b}	–
Fe	(+)Mn^{a}, (+)Cu^{b}, (+)Cd^{b}	(+)Cu^{b}
Zn	(+)Pb^{a}, (+)Mn^{b}, (+)Cu^{a}, (+)Cd^{a}	(+)Pb^{b}, (+)Mn^{b}, (+)Cu^{a}, (+)Fe^{a}
Ag	(+)Cu^{b}	(+)Pb^{b}, (+)Mn^{a}, (+)Cu^{a}, (+)Zn^{b}
Co	–	(+)Pb^{b}, (+)Mn^{a}, (+)Cu^{a}, (+)Zn^{a}, (+)Ag^{a}
Ni	–	(+)Pb^{a}, (+)Mn^{a}, (+)Cu^{a}, (+)Cd^{b}, (+)Ag^{a}, (+)Co^{a}
Cr	(−)Ag^{b}, (+)Co^{b}, (+)SL^{a}, (+)WW^{a}, (+)TW^{a}	(+)Pb^{a}, (+)Mn^{a}, (+)Cu^{a}, (+)Zn^{b}, (+)Co^{a}, (+)Ni^{a}

^{a} $p < 0.01$
^{b} $p < 0.05$
(+) = positive correlation
(−) = negative correlation

Table 4. Significant correlation between metals in byssus vs metals in soft tissue of *Mytilus edulis*

Byssus	Soft tissue
Pb	(+)Pb^{a}, (+)Mn^{a}, (+)Cu^{a}, (+)Cd^{a}, (+)Zn^{a}
Mn	(+)Pb^{a}, (+)Mn^{a}, (+)Cu^{a}, (+)Cd^{a}, (+)Fe^{b}, (+)Zn^{a}
Cu	(+)Pb^{a}, (+)Mn^{a}, (+)Cu^{a}, (+)Cd^{a}, (+)Fe^{a}, (+)Zn^{a}
Cd	(+)Pb^{b}
Fe	–
Zn	(+)Pb^{b}, (+)Cu^{b}, (+)Cd^{b}, (+)Fe^{b}, (+)Zn^{a}

^{a} $p < 0.01$
^{b} $p < 0.05$
(+) = positive correlation
(−) = negative correlation

(Table 4). From the clustering of points in Figure 4 and 5, it is seen that such associations of metals are connected with inter-regional differentiation of their concentrations. It is evident that, compared to the soft tissue, the increase of Cu relative to Zu in the byssi of *M. edulis* is eight times higher, with a slope b (Cu/Zn) of 7.5 for byssus and 0.93 for soft tissue. Bearing in mind that all the regions studied in principle were not contaminated by Zn (comparable concentrations in all the organisms analyzed), Zn in such a comparison may be chosen as a reference element. Hence, a significantly higher slope constant Cu/Zn for the byssus than that for the soft tissue may reflect a extremely higher bioaccumulation of Cu relative to Zn in the former material. This suggests that byssus, as compared to soft tissue, is a more sensitive bioindicator for Cu. Byssal Pb, Mn, and Cu correlated significantly ($p < 0.01$) with tissue Pb, Mn, Cu, Cd, and Zn. Moreover, there is a significant relationship between the concentrations of byssal Cu and tissue Fe as well as byssal and tissue Zn.

Figure 6 reveals a strong correlation between the concentrations of some byssal and tissue metals. It is concluded from the relationships byssal Zn–tissue Zn and byssal Cu–tissue Cu that distribution of Zn in the both parts of *M. edulis* is similar in contrast to that of Cu. There is extremely greater discrimination in favour of Cu versus Zn in the byssus. This observation let us to conclude that, relative to soft tissues, the byssi accumulated Cu to the greatest extent, and Zn (and Cd) to a lesser extent.

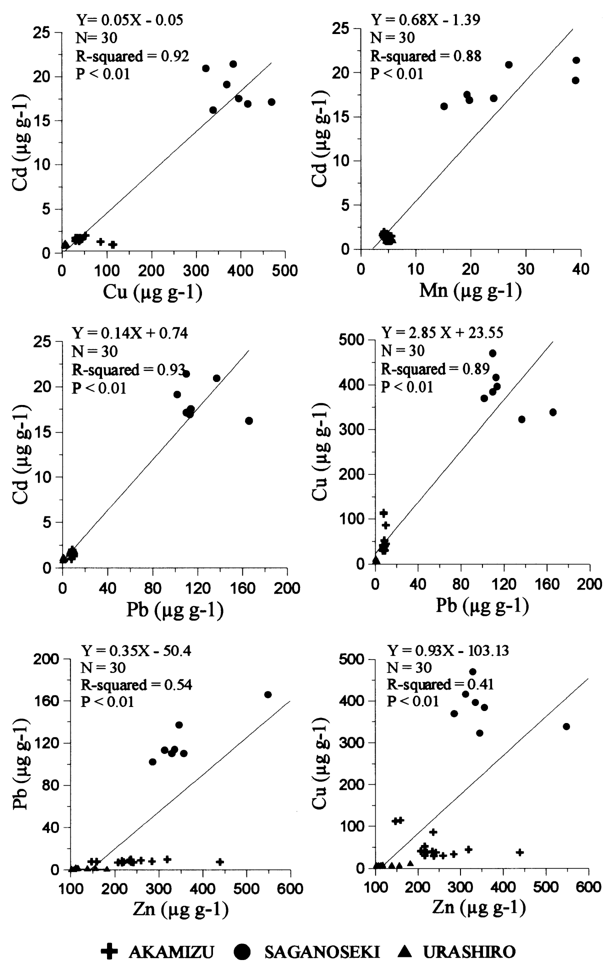

Fig. 4. Relationships between selected metals in the soft tissue of *M. edulis* reflecting significant ($p < 0.01$) inter-regional differentiation of their concentrations

Conclusion

The data obtained in the present study and those reported by Ikuta (1986a, 1986b) show that the soft tissues and byssi of *M. edulis* are good biomonitors for identification of coastal areas

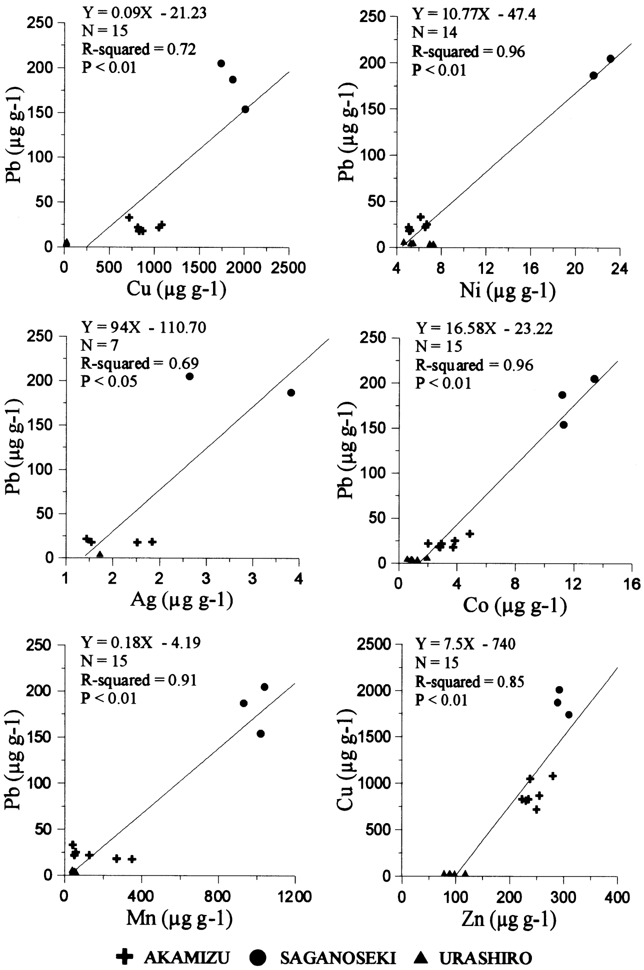

Fig. 5. Relationships between selected metals in the byssus of *M. edulis* reflecting significant ($p < 0.01$ and $p < 0.05$) inter-regional differentiation of their concentrations

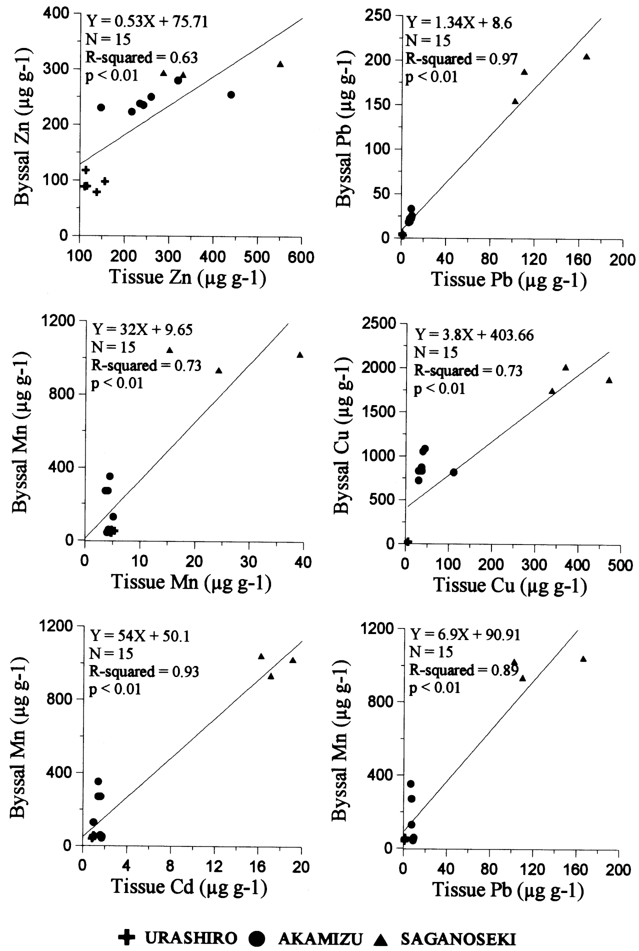

Fig. 6. Relationship between selected metals in byssus vs metals in soft tissue of *M. edulis*

exposed to the influx of some metallic toxicants. In contrast to Cd, byssus showed significantly higher levels of Cu, Co, Cr, Ni, Fe, and Mn. It may therefore be a more sensitive indicator of these elements as compared with soft tissue of the mussels. Intercomparison of our data with those published previously (Phillips 1980; Coombs and Keller 1981; Bryan *et al.* 1985; Ikuta 1986a, 1986b; Szefer and Szefer, 1991) indicates that the soft tissue and especially the byssi is useful in detecting areas of expected metallic pollution. The high concentrations of Cd, and especially Pb and Cu, in Saganoseki mussels and moderately elevated concentrations of these elements in Akamizu mussels may be attributed to the anthropogenic emissions from a metallic refinery and an artificial fiber factory. It is noteworthy that concentrations of Cu, Ni, Cr, and Fe in both the soft tissue and byssi from Saganoseki are some of the highest recorded values amongst presently available data for *M. edulis.* It seems that, significantly higher slope constant Cu/Zn for the byssus than that for the soft tissue may reflect a extremely higher bioaccumulation of Cu relative to Zn in the former material.

It should be emphasized that *M. edulis* is a very effective but not an exclusive bioaccumulator inhabiting the east coast of Kyushu Island. Soft tissue of *Crassostrea gigas* also exhibited a great spatial sensitivity and ability to concentrate some metals (Cu, Pb, Cd, Ag, and Fe) in the study area (Szefer *et al.* 1987). Bottom substrata for the mussels analyzed cannot be considered as a sink for heavy metals because of its rocky structure.

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