Characteristics of Concentration and Composition of Heavy Metals in Seawater, Sediment and Macroalgae, *Ulva* sp., in Yatsu Tidal Flat in Tokyo Bay, Japan

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Abstract

Macroalgae, *Ulva* sp., seawater and sediments in Yatsu tidal flat, Japan were examined to clarify the characteristic distributions of heavy metals and their interactions within these compartments of the ecosystem. The heavy metal concentrations were higher in fall than summer for Fe, Cu and Zn, but were constant with respect to season for Pb and Cd. The distribution of heavy metals in *Ulva* sp. harvested from Yatsu tidal flat was close to in *Ulva* sp. grown in other parts of the world with the following decreasing order of concentration of $\text{Fe} \gg \text{Zn} > \text{Cu} \geq \text{Pb} \gg \text{Cd}$, but differed from that of other. *Ulva* spp. was considered to have an inherent characteristic ratio of heavy metals. In the sediments, heavy metal content was higher in the muddy area of the tidal flat than sandy area, while the compositions were similar for the two areas. The difference in heavy metal content would be attributed to the difference in organic matter and sediment particle size. The major heavy metal in the suspended particulate matter (SPM) in seawater was determined to be Fe followed by Zn, Pb, Cu and Cd, which exhibited a similar composition as those in the sediments. SPM was considered to be derived by tidal shear forces. Absorption of heavy metals into *Ulva* sp. did not depend on the seawater concentrations, but on the absorbability inherent in *Ulva* sp. In comparisons of Concentration Factors for heavy metals in *Ulva* sp., essential elements as Fe, Cu and Zn were preferentially absorbed compared to non-essential metals as Pb or Cd.

Key words: bioaccumulation, Concentration Factor, inherent composition, selective absorption, *Ulva* sp.

INTRODUCTION

Due to their toxicity, heavy metals are important for evaluating the pollution of

marine environments. Numerous studies on heavy metal contamination in macroalgae have been carried out in recent years^{1, 2)}. It has been reported that macroalgae could be

an indicator of the degree of heavy metal contamination in costal areas since the macroalgae exhibit a large capability for accumulating heavy metals^{3, 4)}. Heavy metal contamination in urban areas is principally caused by human activities such as industrialization, urbanization and mining. Heavy metals absorbed in organisms are biologically concentrated in higher animals or plants through the food chain.

The contamination of heavy metals in macroalga in Tokyo Bay has not been examined, though large amounts of green macroalga *Ulva* sp. were observed recently in the costal areas of Tokyo Bay, which is surrounded by the Tokyo and Yokohama metropolitan areas. Several tidal flats in the area have been artificially developed. One of these, the Yatsu tidal flat located in an innermost area in Tokyo Bay has an area of 40 ha and was used as one of the tidal flats in this study. Excess growth of *Ulva* sp. was observed at Yatsu tidal flat, as well as the other tidal flat or shallow sea areas in Tokyo Bay. In 1993, the Yatsu tidal flat was registered by the International Union for Conservation of Nature and Natural Resources (IUCN) under the "Convention on Wetlands (Ramsar, Iran, 1971)" with the purpose of preserving migratory areas since it is used as a feeding grounds and resting area by many migrant species. Yatsu tidal flat is valuable not only for wildlife, but also for residents of cities, as a remnant of nature in an urban area. When *Ulva* sp., which is a primary producer in the tidal flat ecosystem, becomes polluted by heavy metals, detrimental effects on higher-level predators can be anticipated.

In this study, the concentrations and relative composition of heavy metals Fe, Pb, Cu, Zn and Cd in *Ulva* sp. harvested from the Yatsu tidal flat were observed to clarify the influence of heavy metals on the tidal flat ecosystem. The characteristics of heavy metal content in macroalgae were compared with values found in the literature. The relationship among heavy metal contents of seawater, sediments and *Ulva* sp. were also discussed.

SITE DESCRIPTION

Yatsu tidal flat shown in Fig.1 is located in the Tokyo Inner Bay, Japan, at 35°40' N and 140°0' E. The tidal flat was once part of an extensive foreshore-type tidal flat on the coastline of Tokyo Bay. In 1971, it was modified to an artificial lagoon-type tidal flat surrounded by a concrete wall as part of the reclamation works for industrial and residential lands, and as a result, the flow of seawater between the tidal flat and Tokyo Bay became restricted, with exchange being possible only through the Yatsu and Takase channels. Yatsu tidal flat was separated in two parts, main part and south-west part by the freeway construction. Those two parts were connected by four water conduits setting under the freeway. Inflow loads of domestic wastewater to the tidal flat were gradually decreased every year by the development of sewage disposal systems. The tidal flat had become eutrophicated by large amounts of wastewater flowing in from the drainage basin, but has improved recently. The salinity of the tidal flat water also gradually increased to the same levels as the seawater as inflows of domestic fresh water containing abundant organic matter was reduced. The sandy area consisting of sand and larger particles increased and the mud area consisting of small particles decreased in the tidal flat; the amounts of earth and sand, as well as organic materials supplied from the surrounding land area, were decreased by changing the sewage line, resulting in breaking the dynamic balance of the particles in the sediment of the tidal flat by flowing out the small particles to Tokyo Bay⁵⁾. The expansion of the sandy area is a transition in the environmental conditions that permits the growth of *Ulva* sp. (Chlorophyta) in tidal flats. The Yatsu tidal flat became suitable for the growth of macroalga *Ulva* sp. when the sandy sediment area sank below the level of the seawater. As most of Yatsu tidal flat was a horizontal flat, the sediment was shown up leaving gut area at ebb tide and sunk down completely under the level of seawater at high tide. The sediment conditions were not uniform over the tidal flat; sand and sand-mud layers are

Fig. 1 Geographical features of Yatsu tidal flat and sampling points

dominant in the east region while the mud layer is dominant in the west region. *Ulva* sp. was observed in whole area in the sand and sand-mud areas, but not in the mud area. *Ulva* sp. grew, withered and piled up within the tidal flat, as they could not move out to Tokyo Bay due to the isolation of the tidal flat. The enclosed condition of the tidal flat has led to severe, detrimental effects on the ecosystem in the tidal flat; for instance, macrobenthos, which are the main food source for migratory birds were suffocated by the piles of *Ulva* sp.⁵⁾.

MATERIALS AND METHODS

Field sampling

Macroalgae Macroalga *Ulva* sp. samples were collected at Sts. 1 through 3 located in the east area of the Yatsu tidal flat in July and October 2002 (Fig. 1). Collected samples were rinsed with on-site seawater to

remove particulate matter and benthic organisms adhering to the surface of the algae. The samples were placed in vinyl bags and transported to the laboratory.

Seawater Assuming that heavy metal concentrations in seawater do not exhibit seasonal variation, seawater was sampled 6 times from March to May 2003 at St. A of a connecting point of Yatsu tidal flat and Yatsu channel (Fig. 1). The surface water was collected using a water sampler. The seawater samples were poured into polystyrene bottles and transported to the laboratory.

Sediment Sediment samples were collected at 5 locations of Sts. 1 through 5 in October 2002. A difference in heavy metals content between Sts. 1 through 3 where macroalgae were observed and Sts. 4 and 5 where macroalgae were absent were expected because the sediment properties at Sts. 1 through 5, and particularly Sts. 1 through 3 in the *Ulva* sp. area, might be influenced by the withered and piled macroalga on October. Surface sediments within a 2 cm depth were collected using a shovel and ruler. The sediment samples were put into plastic containers after removing debris such as macrobenthos and shells and transported to the laboratory.

Sample pretreatment and analysis of heavy metals content

Macroalgae The collected macroalgae were rinsed with tap water and dried in ambient conditions for 48 hours followed by drying in an oven at 110 °C for 24 hours until a constant weight was attained. Dried samples of 6 g each were digested in a mixture of concentrated nitric acid and concentrated hydrochloric acid on a hot plate. The digested solution was diluted by de-ionized water and the concentrations of Fe, Pb, Cu, Zn and Cd were determined on an Atomic Absorption Spectrophotometer (AAS; HITACHI Atomic Absorption Spectrophotometer 180-30).

Seawater Heavy metal concentrations were determined on filtered and unfiltered

seawater. Filtered seawater was obtained by passing seawater through membrane filters (pore size 0.45 μm ; ADOVANTEC). Nitric acid (5 vol%) was added to both types of samples and boiled to digest the organic substances in the sample. For Fe analysis, ammonium acetate, hydroxylammonium chloride and ammonia solutions were added to the seawater sample to precipitate the Fe. The precipitate was redissolved in 50% HCl solution after filter separation. The concentration of Fe was determined by Spectrophotometer (SHIMADZU Double-Beam Spectrophotometer UV-210A) using 1, 10-phenanthroline hydrochloride as a coloring reagent. For analysis of Cu, Pb, Zn and Cd, 1% N, N-diethyldithiocarbamate trihydrate (DDTC) was added to the seawater sample to form a metal complex with DDTC followed by extraction with butyl acetate twice. The organic solvent was evaporated by heating the extract on a hotplate. The trace organic substances remained in the flask were digested in a mixture of concentrated nitric acid and concentrated hydrochloric acid by heating on a hotplate. The remaining heavy metals were diluted with aqueous 0.1 N HNO_3 . Concentrations of Pb, Cu, Zn and Cd were determined by AAS, as in the analysis of the macroalga.

Sediment The collected sediments were dried in ambient conditions for a week followed by drying in an oven at 110 $^{\circ}\text{C}$ for 24 hours until a constant weight was attained. Dried samples (4 g each) were digested in concentrated nitric acid with heating on a hot plate to completely remove organic substances. The extract containing heavy metals was passed through the membrane filter, and the filtered extract was diluted with de-ionized water. The concentrations of Fe Pb, Cu, Zn and Cd were also determined by the AAS.

RESULTS AND DISCUSSION

Heavy metal content in *Ulva* sp. The heavy metal content in *Ulva* sp. harvested in the Yatsu tidal flat is shown in Table 1. Based on the average concentrations, the heavy metals in *Ulva* sp. in decreasing order of concentration were $\text{Fe} \gg \text{Zn} > \text{Pb} \geq \text{Cu} \gg \text{Cd}$

in July, and $\text{Fe} \gg \text{Zn} > \text{Cu} > \text{Pb} \gg \text{Cd}$ in October. It is generally known that there are seasonal variations in heavy metal bioaccumulation in the macroalgae; the extent of bioaccumulation was less remarkable in the maximum growing period of summer than in the growth period of fall or winter⁶. This seasonal variation of the uptake of heavy metals into macroalgae is linked with metabolic processes⁷. A similar result was observed for *Ulva* sp. in the Yatsu tidal flat as relatively lower concentrations of heavy metals were observed in July than in October for some heavy metals. The concentrations of Fe, Cu and Zn of *Ulva* sp. in October were more than 2 times the levels observed in July. On the contrary, seasonal variations could not be observed for Pb or Cd, even though a small decline was observed for Pb. The results indicated that the extent of bioaccumulation of Fe, Cu and Zn in *Ulva* sp. followed the general understanding of seasonal variations in organisms but those of Pb and Cd did not.

Heavy metal content of *Ulva* sp. found in the literature^{4, 8-11}) are presented in Table 2. Some differences in the heavy metal concentrations were present; Pb was lower in *Ulva* sp. from Greece and Turkey and Cd was lower in *Ulva* sp. of Turkey. The average values for *U. rigida* in Table 2 were calculated in which Fe concentration were tabulated and Pb concentrations of more than 1.0 $\mu\text{g/g}$ dry wt. Based on average literature concentrations, heavy metals were found in decreasing order of $\text{Fe} > \text{Zn} > \text{Cu}$. $\text{Pb} > \text{Cd}$. The rank of heavy metal concentrations of *Ulva* spp. were the same between Yatsu tidal flat and the data in the literature without some exception despite differences in growing season and region. The Fe and Pb content of *Ulva* sp. in Yatsu tidal flat were about 10 and 3 times as much, respectively, as the average value published in the literature. Very low content levels of Fe were reported in the data at Thermaikos Gulf⁴) (Greece) and Black Sea⁹) (Turkey). Both study area, Thermaikos and Black Sea, are effected by inflow loads of domestic and industrial wastewater from drainage basin area^{4, 8, 9}) as same as Tokyo Bay. Both of Gulf and Sea are always sunk down under seawater surface, while Yatsu

tidal flat is suffered from dryness at ebb tide. Therefore, *Ulva* spp. blooming in both Thermaikos and Black Sea cannot contact atmosphere directly. In Tokyo Bay area, the metal content, especially Fe, in descent particulates are observed high levels as a result in steel industrial activity. Fe in descent particulates at neighborhood of Yatsu tidal flat were monitored/reported as 1068 kg/km²/year at 1998, 852 kg/km²/year at 1999, 636 kg/km²/year at 2000, 924 kg/km²/year at 2001 and 948 kg/km²/year at 2002¹²⁾, while at Mediterranean Sea bordered Greece and Italy were estimated 32-140 kg/km²/year¹³⁾ and 5-27 kg/km²/year¹⁴⁾. There are possible that those descent particulates stick on the surface of *Ulva* sp., and act as the content rising of Fe and other metals. *Ulva* sp.

corrected from Venice lagoon (Italy) where is drained at ebb tide^{11, 15)} as same as Yatsu tidal flat shows higher content than both Thermaikos and Black Sea in Fe and other heavy metals. It is therefore considered that the trend of heavy metals in *Ulva* spp. at drained tidal zone is increased. Fe was the major component of *Ulva* sp. in Yatsu tidal flat (99%) and for *Ulva* spp. in the literature (95%). The Zn, Cu, Pb and Cd concentrations were one or two orders of magnitude lower than Fe for both in the experiments and in the literature. The heavy metal composition of other species of seaweed differed from *Ulva* spp.; for instance, the composition of *Grateloupia* in the order Florideophyceae was 52.9% Fe, 0.538% Pb, 1.97% Cu, 44.5% Zn, and 0.0359% Cd¹¹⁾ and that of *Ascophyllum nodosum* in the order Phaeophyceae was 80.9% Fe, 0.0757% Pb, 1.99% Cu, 16.8% Zn, and 0.218% Cd¹⁰⁾. Though the composition of heavy metals for *Grateloupia* and for *Ascophyllum* is also similar to that of *Ulva* spp., the relative composition differed from *Ulva* spp. The Fe composition was 50-80% for *Grateloupia* and *Ascophyllum* while it was more than 95% for *Ulva* spp. The Zn composition in *Grateloupia* and *Ascophyllum* was 17-45% and was higher than that of *Ulva* spp. (less than 4%). The heavy metal ratios in *Ulva* spp., therefore, were similar within *Ulva* spp., but were distinct from the other seaweeds.

Table 1 Heavy metals in *Ulva* sp. grown in Yatsu tidal flat
μg/g dry wt.

St. No.	Fe	Pb	Cu	Zn	Cd
1	8160	22.7	18.2	31.6	0.460
2	8350	21.0	18.9	32.7	0.452
3	7280	18.9	17.1	31.7	0.456
July Ave.	7930	20.9	18.1	32.0	0.456
(Composition (%))	(99.1)	(0.261)	(0.226)	(0.400)	(0.00570)
1	12000	15.4	29.5	78.8	0.489
1	13400	19.0	33.4	87.0	0.565
2	14300	18.7	45.1	102	0.483
3	23000	22.2	45.5	105	0.614
October Ave.	15600	18.8	38.4	93.2	0.538
(Composition (%))	(99.0)	(0.119)	(0.244)	(0.592)	(0.00342)

Table 2 Reference data of heavy metals in *Ulva* spp.

Species	Geographic area/season	Fe	Pb	Cu	Zn	Cd	Reference
<i>U. lactuca</i>	Thermaikos, N.Krini (Greece)	—	0.02	7.40	43.70	0.42	8
<i>U. lactuca</i>	Thermaikos, Kalochori (Greece)	—	0.02	11.10	88.00	0.76	8
<i>U. lactuca</i>	Pagastikos, Pefka (Greece)	—	2.80	8.30	49.30	0.24	8
<i>U. lactuca</i>	Pagastikos, Lehonía (Greece)	—	0.02	9.00	79.90	0.54	8
<i>U. lactuca</i>	Crete, Heraklion (Greece)	—	0.02	7.00	16.40	0.42	8
<i>U. lactuca</i>	Crete, Chania (Greece)	—	0.02	14.50	56.30	1.10	8
<i>U. lactuca</i>	Black Sea (Turkey)/autumn	778	<0.1	13.80	21.20	<0.02	9
<i>U. lactuca</i>	Black Sea (Turkey)/autumn	550	<0.1	3.87	9.60	<0.02	9
<i>U. rigida</i>	Black Sea (Turkey)/autumn	235	1.30	2.53	3.90	0.10	9
<i>U. lactuca</i>	St. Lawrence River (Canada)/summer	2486	1.64	19.20	33.3	0.22	10
<i>U. rigida</i>	Thermaikos (Greece)/all year	97.20	14.70	2.20	57.30	1.00	4
<i>U. rigida</i>	Venice Lagoon (Italy)/spring and autumn	1033	7.30	13.00	64.00	0.20	11
Ave.		962.8	6.235	9.23	39.63	0.38	
(Composition (%))		(94.6)	(0.612)	(0.907)	(3.89)	(0.0373)	

Heavy metals concentrations in seawater

Concentrations of heavy metals in seawater of the Yatsu tidal flat are shown in Table 3. The metals in decreasing order of concentration were $\text{Fe} \gg \text{Zn} > \text{Cu}$. $\text{Pb} \gg \text{Cd}$ for both unfiltered and filtered seawater. The Fe content was decreased by more than 95% while that of Pb, Cu, Zn or Cd was decreased only by 5-10% by passing through the filter. The composition of Fe also decreased from 95.2% of the metals for unfiltered seawater to 58.2% for the filtered seawater. Fe concentration at Yatsu tidal flat was Higher levels than the concentrations at Tokyo Bay measured by Akagi and Haraguchi (1984). In general, at the shallow sea area such as tidal flat and/or coastal zone, the sediment is remarkably resuspended by sea waves and tidal current compared with relatively deep-sea area. Therefore, it is considered that Fe and other metals concentrations are increased result in resuspension of sediment into seawater. However, the concentrations of the other measured heavy metals, Pb, Cu, Zn and Cd, were similar levels to Tokyo Bay reported by Akagi and Haraguchi (1984) and Hirao *et al.* (1983). Consequently, it is able to gather that the sediment in Yatsu tidal flat

compose much Fe compared with other heavy metals. The concentrations of Pb and Cd were lower levels than ambient environmental quality standard in Japan ($\text{Pb} < 0.01\text{mg/l}$, $\text{Cd} < 0.01\text{mg/l}$). Moreover, concentrations of Zn, which was added as “the environmental standard for preservation of aquatic creatures” at 2003, were generally lower level compared with the standard ($< 0.01\text{mg/l}$). It is accordingly considered that the heavy metal pollutions in Yatsu tidal flat are lower levels.

The insoluble heavy metal content, most of which would be contained in the suspended particulate matter (SPM) fraction of seawater, were calculated by subtracting the metal content in filtered seawater from that in unfiltered seawater as expressed in concentration in the seawater in Table 3. The insoluble heavy metal content was $370 \mu\text{g/l}$ for Fe, 2.4 for Pb, 0.57 for Cu, 6.2 for Zn and 0.016 for Cd. By relative composition, the samples were 97.6% Fe, 0.641% Pb, 0.151% Cu, 1.64% Zn and 0.00414% Cd as shown in Fig.2. Based on these results, Fe was found to be the major component in SPM compared to the other heavy metals of Pb, Cu, Zn and Cd.

Table 3 Heavy metals in seawater

		$\mu\text{g/l}$				
		Fe	Pb	Cu	Zn	Cd
Unfiltered	March	—	6.7	3.4	28	—
	April	—	3.2	1.4	5.2	0.093
	April	—	4.5	1.5	6.8	0.069
	April	—	8.7	1.9	7.1	0.093
	May	380	5.1	2.7	8.6	0.12
	May	380	—	—	—	—
	May	390	—	—	—	—
	Ave.	380	5.6	2.2	11	0.094
	(Composition (%))	(95.2)	(1.40)	(0.542)	(2.79)	(0.0232)
	March	—	2.2	1.9	5.0	0.23
Filtered	April	—	3.2	0.37	3.0	0.046
	April	—	4.0	1.9	6.0	0.044
	April	—	3.0	2.4	6.8	0.015
	April	—	0.21	1.2	3.6	0.015
	May	17	6.6	1.8	5.9	0.11
	May	11	—	—	—	—
	Ave.	14	3.2	1.6	5.0	0.078
	(Composition (%))	(58.2)	(13.5)	(6.78)	(21.2)	(0.328)
SPM	Ave.	370	2.4	0.57	6.2	0.016
	(Composition (%))	(97.6)	(0.641)	(0.151)	(1.64)	(0.00414)

Fig. 2 Heavy metal composition in the sediment and insoluble heavy metals (ISHM) in seawater

Heavy metal content in sediment

Sediment heavy metal content in October in the Yatsu tidal flat are shown in Table 4. The metals in decreasing order of concentration were $\text{Fe} \gg \text{Zn} > \text{Cu}$. $\text{Pb} \gg \text{Cd}$ for both areas; sand and mud areas. It is generally recognized that heavy metals are bound more in the fraction due to high surface area and the presence of organic complex¹⁸⁻²²⁾. It is also widely shown that heavy metals are adsorbed to specific surface functional groups as carboxyl, carbonyl, hydroxyl and amino groups^{23, 24)}. Yu reported that the heavy metal concentrations decreased with increasing sand composition in the surface of the sediment²⁵⁾. As shown in Table 4, similar results were obtained in the Yatsu tidal flat; heavy metal content was higher in the muddy area of Sts. 4 and 5 than in the sandy area of Sts. 1 through 3. The levels of ignition loss (IL) at sandy sediment area were lower than the levels at muddy sediment area in Yatsu tidal flat as shown in Table 5. in which IL is one of indicators for

Table 5 Ignition Loss (IL) in sediments corrected form both sandy and muddy area

Sampling points	Sediment condition			
	sandy		muddy	
	St.1	St.3	St.4	St.5
Ignition Loss (%)	4.06	3.48	8.56	6.41

organic matter composed in sediment; particles in the muddy area contain much amount of organic matter compared to in the sandy area. It is well known the organic matter, *i.e.* humic acids, provides surface functional groups for metal adsorption from the aqueous system²⁶⁾. Difference in heavy metals content between the muddy and sandy areas is supposed to be due to difference in content of organic matter. On the other hand, Lian and Wong recently (2003) concluded that the heavy metals can be retained in sediment not only by surface adsorption but also by precipitation²⁷⁾. In Yatsu tidal flat, seawater is flown from Tokyo

Table 4 Heavy metals in sediments

St. No.	Fe	Pb	Cu	Zn	Cd
1	538000	394	222	923	7.19
2 (sandy area)	518000	218	234	993	6.79
3	546000	242	236	998	6.06
1-3 Ave.	534000	285	231	972	6.68
(Composition (%))	(99.7)	(0.0532)	(0.0431)	(0.181)	(0.00125)
4	723000	425	796	2310	12.5
5 (muddy area)	703000	459	957	2800	14.0
4, 5 Ave.	713000	442	877	2560	13.2
(Composition (%))	(99.5)	(0.0616)	(0.122)	(0.357)	(0.00185)

Bay at high tide and flown out to the bay at ebb tide with tidal cycle of the Bay by the two channels. It is able to be considered that water quality, e.g. salinity, pH, SPM concentration, in the tidal flat is similar characteristics at either sandy or muddy area. Accordingly, it should be ignored the third retained factor i.e. precipitation, because seawater in the tidal flat is similar quality in all part of Yatsu tidal flat. Influence of organic sedimentation derived from *Ulva* sp. was infinitesimal by the reason of low IL levels on sandy area covered with *Ulva* sp.. In addition, the influence of *Ulva* sp. on the content of heavy metals in sediment was also negligible as shown in Table 4, i.e. the amount of heavy metals in *Ulva* sp. is much smaller than the difference in concentration of the heavy metals in the sediments.

The heavy metal compositions of the sediments are depicted in Fig.2 and are compared to the insoluble heavy metal (ISHM) composition in seawater. The heavy metal compositions of Zn, Cu, Pb and Cd, excluding Fe, are also depicted in Fig.2. When the insoluble heavy metal composition was assumed to be identical with that of SPM, the composition of SPM was similar to that of the sediments, not only for Fe as an abundant heavy metal, but also for the other four elements of Zn, Cu, Pb and Cd. Considering the fact that there is no material supply from the land area of Yatsu tidal flat, the SPM observed in the seawater would be coming from the sediment released in the tidal flat by tidal shear force.

Bioaccumulation of Heavy Metals In general, it was well recognized that bio-absorption of heavy metals into algae occurs by direct metal absorption from seawater^{28, 29)}. Concentration Factor (CF) defined by Eq.(1) was employed to represent bio-absorption of heavy metals by *Ulva* sp. from seawater.

$$CF = \frac{C_{Ulva}}{C_{sw}} \quad (1)$$

where C_{Ulva} and C_{sw} are the heavy metal concentrations in *Ulva* sp. ($\mu\text{g/g}$) and in the filtered seawater (mg/l), respectively. The average values calculated for July and

October samplings of all stations were used for C_{Ulva} and C_{sw} . The resulting CFs were 575000 for Fe, 6510 for Pb, 11200 for Cu, 6350 for Zn and 5850 for Cd in July, and 1130000 for Fe, 5860 for Pb, 23900 for Cu, 18500 for Zn and 6910 for Cd in October as shown in Fig. 3. There were no major differences in CF ratios for Pb, Zn and Cd in July, which is the maximum growing season of *Ulva* sp. The content of Fe, Cu or Zn in October was two or three times higher than that in July. Assuming that the absorbability of each heavy metal species to *Ulva* sp. was similar, the heavy metal content in *Ulva* sp. must be proportional to the heavy metal concentration in seawater. Although the Fe concentration of filtered seawater was only a little greater than that of Pb, Cu, Zn and Cd, the CF of Fe was extraordinarily higher than any other metal found in *Ulva* sp., as shown in Fig. 3, indicating that *Ulva* sp. absorbed Fe with particularly high selectivity in its growing process. The CF of Cu for *Ulva* sp. was higher than that of Pb or Zn both in July and October despite the Cu concentration in filtered seawater being less than Pb or Zn. The CF of Cd was close to that of Pb or Zn in July, though the Cd concentration was more than one order of magnitude lower than that of Pb or Zn. The results indicated that Cd was also selectively absorbed to *Ulva* sp. The difference in absorption capacities of heavy metals, therefore, depends on the difference in elemental absorbabilities rather than the differences in concentration.

Fig. 3 Concentration factors of heavy metals for *Ulva* sp.

From another point of view, Fe, Cu and Zn are essential elements for the growth of algae, while Pb and Cd are toxic elements without any biological function³⁰⁾. Based on the CFs observed in October, Fe, Cu and Zn were relatively easily absorbed compared to Pb and Cd. Consequently, it was considered that *Ulva* sp. would efficiently absorb essential elements for the formation of cells. The increases in CFs for Pb and Cd were limited from July to October, while the increase in CFs were observed for Fe, Cu and Zn. Comparing the CFs of Cu with Zn, the increase in Zn from July to October was more pronounced than that of Cu. Cu is known to be a toxic element in a large numbers of organisms but is essential in suitable amounts. In general, marine algae has the capability to eliminate the influence of Cu when it exists in excess amounts in the cell, by methods such as exclusion out of the cell, decrease in absorption ability, detoxification in cell or conversion to non-toxic stable compounds. Most macroalgae generate an organic ligand that can form the complex with Cu ions outside the cell to control the Cu concentration inside the cell³¹⁾. The smaller increase in CF of Cu than Zn from July to October could be attributed to the capability of controlling Cu concentration in *Ulva* sp.

CONCLUSIONS

By examining the heavy metal concentrations of *Ulva* sp., the seawater and the sediments in Yatsu tidal flat and their relationships, the following conclusions were deduced.

1. In *Ulva* sp., the heavy metal concentrations of Fe, Cu and Zn increased from July to October, but remained unchanged for Pb and Cd. Hence, the increases in Fe, Cu and Zn contents of *Ulva* sp. obeyed general seasonal variations, though Pb and Cd contents did not show seasonal variations.
2. Comparing the heavy metal concentrations of *Ulva* spp. in Yatsu tidal flat with those of other regions, a similar metal composition was obtained within the species, despite differences in season and region, but differences arose in other species. Therefore, *Ulva* spp. were

considered to have an inherent heavy metal composition.

3. The heavy metal concentrations of sediments in the Yatsu tidal flat were higher in the muddy area than in the sandy area. Difference in heavy metals concentration between the muddy and sandy areas is proposed to be caused by difference in concentration of organic matter. The influence of *Ulva* sp. on the heavy metals content in the sediments could be negligible because the content of heavy metals in the sandy area were much smaller than those in the muddy area.
4. When the insoluble heavy metal composition was assumed to be identical with that of SPM, the composition in the SPM was similar to that of the sediments, not only for Fe as a major heavy metal component, but for the other minor elements of Zn, Cu, Pb and Cd. The SPM in Yatsu tidal flat seawater would be come from the stirred up sediment because no material was supplied from the land area.
5. Considering CFs of heavy metals in *Ulva* sp., the difference in absorption capacities of heavy metals would depend on the differences in elemental absorbabilities, rather than the differences in heavy metal concentrations in seawater; *Ulva* sp. efficiently absorbs essential elements of Fe, Cu or Zn for the formation of cells and selectively absorbs the toxic elements that lack biological function, such as Pb and Cd.

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