Variation in metal concentrations in the brown alga *Undaria pinnatifida* in Osaka Bay, Japan

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SUMMARY

In order to evaluate the usefulness of a biomonitoring system using seaweeds for assessing the geographic distribution of metal ions in coastal seawaters, the metal concentrations in the sporophytes of an annual kelp, *Undaria pinnatifida* (Harvey) Suringar, were collected at 15 localities in Osaka Bay, Japan and compared. About 160 cm² of the blade was cut out from the central part of clean sporophytes, rinsed in filtered seawater using an ultrasonic cleaning bath, and freeze-dried. After digestion with 12% HNO₃ in a microwave apparatus, metal concentrations in the samples were determined by inductively coupled plasma mass spectrometry. The concentrations (dry weight basis) of most examined elements were in the parts per million range; Cr, 0.48–3.18; Ni, 0.77–5.94; Cu, 3.20–43.8; Zn, 11.3–86.8; Pb, 0.14–3.53. Comparisons of metal compositions of the *U. pinnatifida* samples from the northeastern area of the bay, which has a large urban population and highly developed industries inland, showed high concentrations of Cd, Pb and Cu compared with the samples from the southwestern area of the bay where the population and industries are much smaller. This suggests that *U. pinnatifida* metal loads can be used as a marker to track the geographic distributions of the metal concentrations in coastal seawaters, reflecting inland human activities such as shipbuilding and repairing in port areas, and can be used as a useful biomonitoring system of coastal environments for long-term trend.

Key words: biomonitoring, coastal seawater, metal pollution, Osaka Bay, *Undaria pinnatifida*.

INTRODUCTION

Metal concentrations in seawater, sediments, and marine organisms in coastal areas have been studied in order to assess coastal pollution (Haritonidis & Malea 1999; Sanchiz et al. 2001; Tabudravu et al. 2002; Beiras et al. 2003). In the case of seawater, the routine determination of metal concentrations is rather difficult because of the very low concentrations of toxic elements and the interferences of high concentrations of matrix elements such as Na and Mg. In addition, the complicated current system and the considerable influence of metal elements in the bottom sediments prevent us from simulating the degree of metal pollution by occasional sampling and analyzing of seawater samples, especially in the shallow enclosed sea such as Osaka Bay. In order to overcome these problems, we need some samples that preserve long-term information on the seawater pollution by metal elements.

Some marine organisms are known to accumulate various metal elements from the ambient seawater or sediment through biological processes. For example, the coastal phytoplankter *Thalassiosira pseudonana* Hasle et Heimdal has a system to concentrate Zn and Cd (Sunda & Huntsman 1998), and the seagrass *Cymodocea nodosa* (Ucria) Asch accumulates metals from the ambient sediments (Martín-Guirao et al. 2005). Similarly, the mussel *Dreissena polymorpha* Pallas accumulate various metals, and is used as a bioindicator of metal concentrations in coastal areas (Berloots et al. 2005).

However, if the animals take up and concentrate the metals through their food, the metal concentrations in the animal tissues may be considerably influenced by the trophic level of the food organisms (Wang & Rainbow 2000), because the metal concentrations per food volume/weight become higher at the higher trophic levels by repeated bioconcentration. In contrast, the primary producers such as algae and sea grasses, which take up metals directly from the ambient seawater or sediments, are not influenced by such bioconcentration mechanisms (Fortin & Campbell 2001; Campbell et al.)

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Variation in metal concentrations in the brown alga

Channel. Five large rivers flow into the northeastern Channel, and to the Pacific Ocean by the Kitan to the western part of the Seto Inland Sea by the Akashi easternmost part of the Seto Island Sea. It is connected As shown in Figure 1, Osaka Bay is located in the Study area

MATERIALS AND METHODS

area, where the bay is rather stagnant. The coastal region of the northeastern area has a population of about 16,000,000 and has highly developed industries inland, and consequently the concentrations of nutrient salts in the bay are high (Nakatuji 1998). In contrast, Awaji Island, on the western side of the bay, has a population of only 160,000 and is basically an agricultural and fishery area. Due to the strong tidal current along Awaji Island through the Akashi and Kitan Channels, the water in the southwestern area of the bay is regularly exchanged with the open-ocean water of the Pacific. Reflecting the remarkably different environmental conditions, the total nitrogen (TN) and total phosphate (TP) of the northeastern area of the bay is 0.6–1.0 mg/L and 0.04–0.1 mg/L, respectively whereas they are less than 0.3 mg/L and 0.03 mg/L, respectively, in the southwestern area (Osaka Prefecture 2000).

Bottom sediments of the northeastern side of the bay are reported to contain relatively high concentrations of metal elements (Kitano et al. 1981; Gohda & Yamazaki 1982; Sue et al. 1983; Hoshika & Shiozawa 1986; Nagaoka et al. 2004; Yamada et al. 2006) and this is thought to be a result of industrial discharge, especially during the period of rapid industrial development from the 1950s to the 1970s. The concentration of Hg, Cd and Cu are higher in the sediment samples from the northeastern side (Nagaoka et al. 2004).

Comparison of the metal concentrations in the blade and sporophyll

The sporophyte of *U. pinnatifida* (Phaeophyceae, Laminariales) is composed of three major parts: lobed blade with conspicuous midrib, stipe, and holdfast. The sporophyll, the reproductive part of the sporophyte

2002), and thereafter are considered to reflect the metal concentrations in the ambient seawater more directly. Among the primary producers, phytoplankton and seaweeds (macroalgae) take up metals from the ambient seawater, in contrast to sea grasses (marine phanerogams), which can take up metals from the sediments via roots. Therefore, we considered that it would be useful if we could establish a monitoring system of coastal metal concentrations using algae to compliment the systems using benthic animals (e.g. mussels). Among the algae, phytoplanktons (mostly unicellular algae) have very short life-spans, it is very difficult to collect sufficient quantities of specimens for analyses, and it is often difficult to identify the species. In contrast, individual macroalgae have longer life-spans than phytoplankton (months to years), in general they are easier to identify, and it is easier to collect sufficient quantities of specimens for measurements.

We selected the kelp species *Undaria pinnatifida* (Laminariales, Phaeophyceae) as the model species because of the following features: (i) *U. pinnatifida* is an annual species, but its sporophyte (macroscopic generation) has a relatively long life span (5–6 months); (ii) the taxonomic identification of the species is easy; (iii) the maintenance of unialgal cultures (gametophytes) is easy, and the sporophytes can be easily generated from gametophytes and grown as laboratory cultures using artificial seawater; (iv) the species is widely distributed in the temperate regions of Far Eastern Asia, and is commonly found even in highly eutrophicated, enclosed seawater areas. Furthermore, by unintentional introductions *U. pinnatifida* is now widely distributed in Europe, southeastern Australia, New Zealand, southern South America and some areas of Pacific North America; and (v) the sporophyte of the species is relatively large, so that a part of one individual is sufficient for the determination of metal concentrations using inductively coupled plasma mass spectrometry (ICP-MS).

In this study, we intend to discuss the differences of metal concentrations in different parts of the thallus of *U. pinnatifida* and the differences of metal concentrations among *U. pinnatifida* blades collected from 15 localities along Osaka Bay, in order to assess the usefulness of metal composition of *U. pinnatifida* as a bio-indicator of seawater pollution in inland seas.

MATERIALS AND METHODS

Study area

As shown in Figure 1, Osaka Bay is located in the easternmost part of the Seto Island Sea. It is connected to the western part of the Seto Inland Sea by the Akashi Channel, and to the Pacific Ocean by the Kitan Channel. Five large rivers flow into the northeastern

Fig. 1. Topographic map of Osaka Bay and sampling sites.
forming sori of unilocular sporangia, is formed on both sides of the lower part of the stipe.

In order to assess the differences in metal concentrations in different parts of the thallus, we compared the blade and the sporophyll. For this experiment, we used *U. pinnatifida* sporophytes collected from Amagasaki, Hyogo Prefecture on March 5 2005. The tissues of the blade and sporophyll were cut out from the sporophytes, and then rinsed in filtered seawater using an ultrasonic cleaning bath for 30 min, three times, in order to remove debris and epiphytic organisms from the thallus. These samples were freeze-dried and used for the analyses.

Metal concentrations of *Undaria pinnatifida* collected from various sites of Osaka Bay

We collected *U. pinnatifida* sporophytes from 15 sites along the bay on March 5, 6 and 7 2005. Approximately 160 cm² of the lower central part of the blade beside the midrib was cut out, rinsed as described above, and dried.

Approximately 0.5 g of pulverized sample was weighed and digested with 8 mL of 12% HNO₃ using a laboratory microwave system (Microwave Labstation mlm 1200 mega, Milestone Inc., Kawasaki, Japan). After decomposition, 8 mL of ultra-pure water was added to the vessel and this was then heated at 110°C in an oven for 1 h. The sample was diluted with ultra-pure water to about 24 mL and analyzed for Mg, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Sn, Hg, and Pb by an ICP-MS (HP4500, Yokogawa Analytical Systems, Tokyo, Japan). Each element was calibrated with mixed standard solutions prepared from commercial 1000 p.p.m. solutions. Analytical results are expressed in percentage, p.p.m. or p.p.b. of dried sample weight (% dwt, p.p.m. dwt and p.p.b. dwt, respectively).

RESULTS

Comparison of the metal concentrations between blade and sporophyll

Figure 2 shows the comparison of the metal concentrations between the blade and sporophyll (*n* = 3) in *U. pinnatifida*. Both blade and sporophyll showed remarkably high Mg concentrations, being % dwt. The Cu and Zn concentrations were relatively high both in the blade and sporophyll compared to other metals, and Cd, and Sn were relatively low. Most metal elements, except for Mg, Cd and Hg, showed higher concentrations in the blade than in the sporophyll, although the values of some metals overlapped in the error range.

Metal concentrations of *Undaria pinnatifida* specimens collected from various sites in Osaka Bay

Averages and standard deviations of metal concentrations of 15 *U. pinnatifida* samples along Osaka Bay are shown in Table 1. Concentrations of most elements (Cr, Mn, Ni, Cu, Zn, As, Se and Pb) were in the p.p.m. range, whereas Mg was as high as 10–30%. The concentrations of Co, Cd and Sn were as low as 50–600, 10–550 and 100–1200 p.p.b., respectively.

Relatively constant Mg and As contents in *U. pinnatifida* are observed irrespective of sampling locality in the bay (Fig. 3a,b). On the other hand, Zn and Ni concentrations were variable according to the sampling locality (Fig. 3c,d); samples from the Honshu side showed higher Zn and Ni concentrations (30–90 p.p.m. and 1–6 p.p.m., respectively) compared with those from the Awaji Island coast (10–30 p.p.m. and c. 1 p.p.m., respectively). Figure 4 shows the distributions of Cu, Pb and Cd concentrations in *U. pinnatifida* along the bay. The Cu concentration in the Amagasaki sample (43.8 parts per million (p.p.m.)) was the highest among the all samples (3–16 p.p.m.). *U. pinnatifida* from Kobe Port showed extremely high concentrations of Pb (3.53 p.p.m.) and Cd (1257 parts per billion (p.p.b.)).
Table 1. Effect of sampling location on the variability of metal concentrations in *Undaria pinnatifida* from Osaka Bay in view of analysis of variance ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Mg</th>
<th>Cr</th>
<th>Mn</th>
<th>Co</th>
<th>Ni</th>
<th>Cu</th>
<th>Zn</th>
<th>As</th>
<th>Se</th>
<th>Cd</th>
<th>Sn</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>**</td>
<td>46.61**</td>
<td>23.53</td>
<td>49.00**</td>
<td>43.01**</td>
<td>52.44**</td>
<td>27.29*</td>
<td>59.37**</td>
<td>42.75**</td>
<td>32.82**</td>
<td>42.09**</td>
<td>39.18**</td>
<td>53.47**</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01.

Fig. 3. Comparisons of Mg (a), As (b), Zn (c) and Ni (d) concentrations in *Undaria pinnatifida* (*n = 4*) along Osaka Bay. For names of the sampling localities, refer to Figure 1 and Table 2. The bar on the data box shows standard errors.
DISCUSSION

Comparison of the metal concentrations between the blade and sporophyll

Comparison of the metal concentrations between the blade and sporophyll revealed that concentrations of most metals, except for Mg, Cd and Hg, were higher in the blade than in the sporophyll. This uneven distribution may be explained by the following mechanism. Absorption of elemental ions into the algal cells first occurs in the blade when the division and enlargement of the cells occurs, and the elements are considered to be secondarily transferred to the sporophyll by active transport via inner hyphae in kelp species (Pfister 1992; Wu & Meng 1997; Kumura et al. 2006). Therefore, the lower metal concentrations in the sporophyll might be explained by the difference of transfer ten-
dency of the metals through the transport system. Considering these results, as well as the characteristic growth mode of the laminariaceae species wherein the lower part of the blade is the youngest tissue in the thallus, and therefore normally has fewer epiphytes on the surface, we decided to use the lower part of the blade in the later analyses.

The coefficients of variation for each metal calculated for 15 *U. pinnatifida* samples along Osaka Bay ranged from 2 to 161%. None of the analyzed metals were within a statistically acceptable limit of variability (30–35%). In algal samples, however, the coefficients of variation often exceed 50% even among the specimens collected in the same locality (cf. Zbikowski et al. 2006). Therefore, the differences in metal concentrations found among 15 *U. pinnatifida* collected in Osaka Bay were tested by ANOVA (Table 1). As the result, the differences of the metal concentrations of *U. pinnatifida* specimens were concluded to be statistically meaningful except for Cr, whose P-value was larger than 0.1.

Comparison of the metal concentrations between the *Undaria* tissues from Osaka Bay and Venice, Italy

The metal concentrations in *U. pinnatifida* collected from Venice, Italy in the Mediterranean Sea (Caliceti et al. 2002) are shown in Table 2. The metal concentrations of the Venice *U. pinnatifida* were mostly in the range of those in the Osaka Bay samples; Cr, 0.5 for Venice (0.48–3.2 for the Osaka Bay); Ni, 1.2 (0.77–5.9); Cu, 2.0 (3.2–44); Zn, 97 (11–87); Cd, 100 (0.047–1.3); Pb, 2.2 (0.14–3.53) in p.p.m. dwt.

Specimens of *U. pinnatifida* examined by Caliceti et al. (2002) were collected from two localities, one close to the parking area of Tronchetto and characterized by heavy maritime traffic, and the other located in the northeast of Venice close to a highly populated area where household activities and maritime traffic are the main pollution sources. Although the metal concentrations in the seawater surrounding these two sampling localities in Venice were not studied by Calicetti et al. (2002), Scarponi et al. (1998) reported the metal concentrations in the seawater near the two localities: Cd, 0.03 p.p.b.; Pb, 0.45 p.p.b.; Cu 0.5 p.p.b. On the other hand, the Cd concentration of seawater in the innermost part of Osaka Bay (Nishinomiya area) was reported to be less than 1.0 p.p.b. (Hyogo Prefecture 2003). *U. pinnatifida* collected at Nishinomiya showed high Cd concentrations around 500 p.p.b. In contrast, the *U. pinnatifida* from Venice concentrated Cd up to 100 p.p.b. from the ambient seawater containing 0.03 p.p.b. Cd. These facts suggest that *U. pinnatifida* effectively concentrates Cd in the ambient seawater through biological processes.

**Table 2.** The averages (±SD) of metal concentrations (‰, p.p.m and p.p.b. dry weight) in *Undaria pinnatifida* along Osaka Bay

<table>
<thead>
<tr>
<th>Locality</th>
<th>Mg (dry weight/‰)</th>
<th>Zn (p.p.m.)</th>
<th>Cu (dry weight/‰)</th>
<th>Pb (dry weight/p.p.m.)</th>
<th>Cd (dry weight/p.p.b.)</th>
<th>Ni (dry weight/p.p.m.)</th>
<th>Cr (dry weight/p.p.m.)</th>
<th>Fe (dry weight/p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Yokkaichi)</td>
<td>15 ± 0.81</td>
<td>11 ± 0.47</td>
<td>17 ± 1.35</td>
<td>2.4 ± 0.64</td>
<td>2.3 ± 0.64</td>
<td>2.7 ± 0.26</td>
<td>1.0 ± 0.53</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>B (Atsumi)</td>
<td>16 ± 0.22</td>
<td>15 ± 0.73</td>
<td>9.6 ± 0.25</td>
<td>1.7 ± 0.53</td>
<td>1.4 ± 0.13</td>
<td>1.3 ± 0.29</td>
<td>1.1 ± 0.53</td>
<td>2.7 ± 0.73</td>
</tr>
<tr>
<td>C (Wakayama)</td>
<td>17 ± 0.15</td>
<td>16 ± 0.59</td>
<td>2.5 ± 0.34</td>
<td>2.1 ± 0.34</td>
<td>2.0 ± 0.12</td>
<td>2.3 ± 0.36</td>
<td>2.1 ± 0.73</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>D (Buzunashiki)</td>
<td>18 ± 0.36</td>
<td>17 ± 0.92</td>
<td>3.8 ± 0.73</td>
<td>2.0 ± 0.53</td>
<td>1.7 ± 0.34</td>
<td>1.0 ± 0.36</td>
<td>1.2 ± 0.53</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>E (Wakayama)</td>
<td>19 ± 0.3</td>
<td>18 ± 0.97</td>
<td>3.5 ± 0.71</td>
<td>2.1 ± 0.53</td>
<td>2.0 ± 0.12</td>
<td>2.3 ± 0.36</td>
<td>2.1 ± 0.73</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>F (Nishinomiya)</td>
<td>20 ± 0.36</td>
<td>19 ± 0.92</td>
<td>3.8 ± 0.73</td>
<td>2.0 ± 0.53</td>
<td>1.7 ± 0.34</td>
<td>1.0 ± 0.36</td>
<td>1.2 ± 0.53</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>G (Kobe Port)</td>
<td>21 ± 0.3</td>
<td>20 ± 0.97</td>
<td>3.5 ± 0.71</td>
<td>2.1 ± 0.53</td>
<td>2.0 ± 0.12</td>
<td>2.3 ± 0.36</td>
<td>2.1 ± 0.73</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>H (Nakayama)</td>
<td>22 ± 0.3</td>
<td>21 ± 0.97</td>
<td>3.5 ± 0.71</td>
<td>2.1 ± 0.53</td>
<td>2.0 ± 0.12</td>
<td>2.3 ± 0.36</td>
<td>2.1 ± 0.73</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>I (Iwaya)</td>
<td>23 ± 0.3</td>
<td>22 ± 0.97</td>
<td>3.5 ± 0.71</td>
<td>2.1 ± 0.53</td>
<td>2.0 ± 0.12</td>
<td>2.3 ± 0.36</td>
<td>2.1 ± 0.73</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>J (Jizohama)</td>
<td>24 ± 0.3</td>
<td>23 ± 0.97</td>
<td>3.5 ± 0.71</td>
<td>2.1 ± 0.53</td>
<td>2.0 ± 0.12</td>
<td>2.3 ± 0.36</td>
<td>2.1 ± 0.73</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>K (Izumisano)</td>
<td>25 ± 0.3</td>
<td>24 ± 0.97</td>
<td>3.5 ± 0.71</td>
<td>2.1 ± 0.53</td>
<td>2.0 ± 0.12</td>
<td>2.3 ± 0.36</td>
<td>2.1 ± 0.73</td>
<td>4.2 ± 1.4</td>
</tr>
</tbody>
</table>

†Averages and standard deviations are quoted from Calicetti et al. (2002), p.p.m., parts per million; p.p.b., parts per billion.
Although the Pb concentration of seawater in the Nishinomiya area (2.0 p.p.b.) was higher than that in Venice (0.45 p.p.b.), the Pb content of *U. pinnatifida* from the Nishinomiya area (0.3 p.p.m.) was apparently lower than that from Venice (2.2 p.p.m.). The relatively high Pb content in Venice *U. pinnatifida* was comparable to that in the Kobe Port sample (3.53 p.p.m.). Both Venice and Kobe Port are active harbors. Heavy oils, the fuel of cargo ships, contain many metal elements with various concentrations. For example, Dou et al. (1998) reported that heavy oil originating in northeastern China contains 0.46–1.63 p.p.m. Pb. Therefore, local contamination from cargo ships in Venice and Kobe Port may be the source of the high Pb content in *U. pinnatifida*.

Cu is also concentrated by *U. pinnatifida*. Samples from Nishinomiya contained 10 p.p.m. Cu and those from Venice 2 p.p.m. (Caliceti et al. 2002), whereas Cu concentrations in the ambient seawater were 1 p.p.b. for Nishinomiya (Hyogo Prefecture 2003) and 0.5 p.p.b. for Venice (Scarponi et al. 1998), suggesting that *U. pinnatifida* concentrates Cu about 5000-fold. This high enrichment factor suggests that *U. pinnatifida* can be a useful bio-indicator of coastal seawater pollution.

### Comparison of the metal concentrations between *U. pinnatifida* and sediments in Osaka Bay

Metal element concentrations in seaweeds are reported to have positive relationships with those in the sediments near the habitat of the seaweeds (e.g. Haritonidis & Malea 1999; Sanchiz et al. 2001; Tabudravu et al. 2002). In Osaka Bay, the bottom sediments of the northeastern area contain relatively high concentrations of Ni, Cd and Cu (Kitano et al. 1981; Gohda & Yamazaki 1982; Sue et al. 1983; Hoshika & Shiozawa 1986; Nagaoka et al. 2004). Nagaoka et al. (2004) reported high Ni concentrations of 35–55 μg/g in the sediments from northeastern and eastern Osaka Bay and relatively low concentrations of 10–40 μg/g for the sediments off Awaji Island. As shown in Figure 3d, Ni concentrations of *U. pinnatifida* in the northeastern and eastern areas ranged from 1.35 to 5.94 p.p.m., and they were considerably higher than those on the Awaji Island side (0.77–1.48 p.p.m.). Ni contents in *Mytilus galloprovincialis* Lamarrck also showed a similar trend, higher in the northeastern and eastern areas than on the Awaji Island side (Eguchi et al. 2004). The same tendency was seen in Cd and Ni. However, the significant statistical correlations among the Ni, Cd and Cu concentrations between sediments and *U. pinnatifida* were not found (r = 0.236, 0.252 and 0.148, respectively).

Morrow (2003) has pointed out that coating materials containing Cd are used in shipbuilding and repairing processes because of their high resistance to seawater. The highest Cd content was observed in *U. pinnatifida* collected at the Port of Kobe, where a number of shipbuilding and repairing industries are located in the port and its vicinity. Therefore, it can be suggested that the high Cd concentrations in *U. pinnatifida* at Kobe Port may be due to anthropogenic contamination related to shipbuilding.

As shown in Figure 4a, the concentration of Pb was remarkably high in the specimen collected at the Port of Kobe, a major industrial port, and also relatively high in the localities in the southeast area, most of which are fishing ports. In the port area, in addition to the materials related to shipbuilding and repairing, Pb is a component of ships' fuels (Cochran et al. 1998; Hoven et al. 1999; Stukas et al. 1999; Saad et al. 2003) and storage batteries.

Similarly, various heavy metals are components of the paints used on ships (e.g. Zn, Sn and Cu; Stærgård et al. 2001) and for cathodic protection (e.g. Zn, Cd, Sn, Ni, Cr, Cu and Pb; Strekalov & Panchenko 2001) and storage batteries (Pb, Cd, Cr and Ni; Corbus et al. 1993). Therefore, it is likely that these metals are dissolved into the seawater as various ions, especially in port areas, and become accumulated in algal biomass.

In conclusion, in order to establish a biomonitoring system for assessing coastal seawater pollution, we investigated the metal concentrations in *U. pinnatifida* specimens in Osaka Bay. Our results indicate that *U. pinnatifida* concentrates metal elements from the ambient seawater for long-term trends and should be considered as a bio-indicator of metal contaminations for Cu, Ni, Cd and Pb due to human activities such as shipbuilding in the port area. In order to make *U. pinnatifida* a more quantitative bio-indicator of metal pollution, it will be necessary to estimate the enrichment factor of metals incorporated into *U. pinnatifida* from seawater. For this, correlated determinations of metals in both seawater and *U. pinnatifida* samples are needed, together with incubation experiments using seawater spiked with metal elements.

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