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CU AND ZN CONCENTRATIONS OF NATURAL OYSTERS IN OSAKA BAY, JAPAN

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ABSTRACT: Cu and Zn concentrations found in oysters in Osaka Bay were 0.09 to 7.8 ‰ and 1.7 to 37 ‰. Particularly high Cu concentration was found in oysters at fishing ports as well as a yacht harbor and leisure boat wharf. High Zn concentration oysters were also found at fishing ports, yacht harbor, wharf and near food factories. Anti-fouling paint, found in large quantity at the small fishing port, contained Cu_2O as a biocide for biofouling and Zn concentrations of anti-fouling paint were also high. This anti-fouling paint was suspected to be the main Cu and Zn contaminant for oysters. Among all oyster parts for natural and cultivated oysters, Cu and Zn concentrations were highest in the oyster gills. Cu and Zn concentrations in the mantle and hepatic gland were almost the same value while adductor muscle values were low. As Cu and Zn concentrations in cultivated oysters were very low, less than 0.1 and 1.1 ‰, natural oysters are considered as effective for getting Zn and Cu in Osaka Bay.

Keywords: Oysters, Environmental Index, Heavy-metal concentrations, Anti-fouling paint

1. INTRODUCTION

The absorption and accumulation of heavy metals in seawater by aquatic organisms were reported by Ikuta [1]. Concentration and distribution of toxic and essential metals in biological samples collected in Okayama prefecture were reported by Morita and Ogata [2]. Metal contamination of seawater was evaluated by seaweed [3] and in particular, the metal concentration of seaweed along the Kii Peninsula was measured and high Cu, Fe and Zn concentrations were found around metal mines [4]. Use of seaweed is useful for sea contamination. The use of bivalves as possible sentinel marine organisms to monitor metal pollution in coastal water was reported by Takayanagi and Sakami [5].

"*Ostrea gigas*," a very popular coastal oyster, lives in the same location for several years, thus has high potential as a heavy metals index. The relation of Pb, Cd and Cr between sea water, sea sediment and oyster (*Crassostrea virginica*) was clarified and influence of metal contamination caused by the runoff of household and industrial discharge on oyster was evaluated in Mexico [6]. Then, this study is to determine the potential use of oysters as an environmental index for heavy metal concentrations in Osaka Bay. Heavy metal concentrations in oysters were measured over a wide area of Osaka Bay under various conditions. The heavy metals measured were Cu, Zn, Fe, Mn, Pb, and As. The source of these heavy metals was an anthropogenic factor.

2. OYSTER SAMPLING

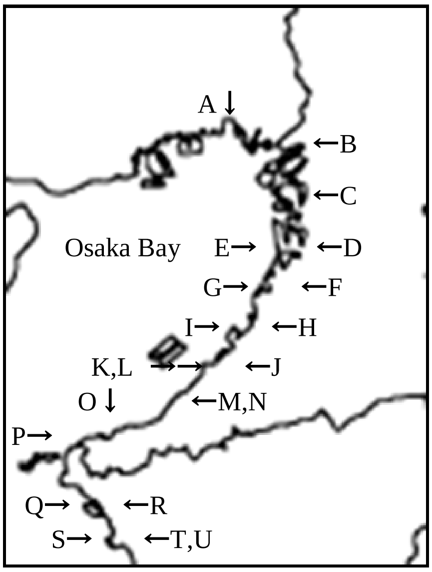

Fig.1 21 sampling points covered the entire east side of Osaka Bay. Oysters were sampled in 2014 August.

Fig.1 and Table 1 show 21 sampling points covered the entire east side of Osaka Bay and site environment. Oysters were sampled in 2014 August from Nishinomiya City in Hyogo Prefecture to Kainan City in Wakayama Prefecture. Sampling area covered almost the entire east side of Osaka Bay. A number of sampling points was 21. Most oysters were attached to concrete walls at the fishing port. Rolling oysters were found at the beach and the slope of the wharf. Attached oysters were stripped carefully from rocks or concrete walls by spatula and hammer. Table 1 shows details about sampling points for address, habitat and state. Sampled oysters were 60 to 70 mm in length.

Sampling point E “Takaishi City” was a small fishing port and a small beach. This point was heavily polluted, and some oysters had green tissues. Therefore, oysters here were sampled intensively from 2014 March to 2016 March.

Table1 Details about sampling points

	Address	Habitat	State
A	Nishinomiya City	Artificial wharf	attached
B	Osaka City north	Mouth of river	attached fixed
C	Osaka City south	Artificial wharf	attached fixed
D	Sakai City	Mouth of river	attached
E	Takaishi City	The artificial slope of the fishing port	rolling
F	Izumiohtsu City	The artificial slope of the yacht harbor	rolling
G	Kishiwada City	Mouth of river	attached
H	Kaizuka City	Mouth of river	attached
I	Izumisano City	Mouth of river	attached
J	Izumisano City	Artificial wharf	attached
K	Sennan City	Mouth of river	attached
L	Sennan City	Natural beach	rolling
M	Hannan City	Artificial wharf	attached
N	Hannan City	Artificial wharf	attached
O	Misaki Town	Natural rock	attached
P	Wakayama City	Artificial wharf	attached
Q	Wakayama City	Artificial slope of fishing port	rolling
R	Wakayama City	Mouth of river	attached
S	Wakayama City	Artificial wharf	attached
T	Kainan City	Natural rock	attached
U	Kainan City	Artificial leisure boat wharf	attached

Note: The artificial slope was made of gentle gradient concrete for ship maintenance. Oysters and stones were rolled by waves on the slope. The artificial wharf was made of a vertical concrete wall to protect the port against waves. Oysters were directly attached to the concrete wall keeping them out of waves.

3. METHOD

Shells of sampled oysters were opened carefully with a stainless-steel knife. The soft tissues of the oysters were cut into small pieces and put into pre-weighted laboratory dishes. The soft tissues were dried at 60 degrees C for a week. Each dried sample was ground manually into fine powder form by mortar and a pestle. Each dried ground sample was stored separately in airtight polypropylene bags.

0.020g of the dried ground sample was mixed with 15.0 mL of concentric nitric acid in a glass vessel and then kept for a week at room temperature. After ground samples were dissolved by nitric acid completely, the solution was filtered with a 0.45μm filter and then dry weight concentrations of heavy metal were measured by Inductively Coupled Plasma (ICP).

4. RESULT

4.1 Dry Weight Heavy Metal Concentrations of Oysters around Osaka Bay

Table 2 shows dry weight heavy metal concentrations for 21 sampling points. Seawater in Osaka Bay is polluted by metropolitan sewage, however, Mn, Pb and As concentrations in oysters at the inner area of Osaka Bay were not high relative to other heavy metal concentrations. Mn concentrations were under 0.001 to 0.13 ‰. Pb concentrations were under 0.001 to 0.04 ‰. As concentrations were 0.018 to 0.081 ‰. On the other hand, Fe concentrations were 0.21 to 2.1 ‰. Cu concentrations were 0.09 to 7.8 ‰. Zn concentrations were 1.7 to 37 ‰. Then, concentrations of Cu, Zn, and Fe widely changed with sampling location and oysters are thought to be suitable for use as an environmental index of Cu, Zn, and Fe.

Fig.2 shows Cu concentrations in oysters measured at 21 sampling points. At sampling points E, F, Q, and U, Cu concentration was high and the color of their oyster tissues was green. Sampling points E, F, and Q were artificial slopes. Sampling point E and Q were fishing ports. Sampling point F was a yacht harbor. Sampling point U was a leisure boat wharf. High Cu concentration oysters were found around the ports. Therefore, Cu concentrations in oysters displayed a high potential as an environmental index.

Fig.3 shows Zn concentrations in oysters measured at 21 sampling points. At sampling points, F, G, I, and Q, particularly high Zn concentrations of over 10 ‰ were found although the minimum Zn concentration was 1.7 ‰. Sampling point F (yacht harbor) and point Q (fishing port) showed very high concentrations of both Cu and Zn. Sampling point I was located near many food factories.

Table2 Dry weight heavy metal concentrations of oysters

	Mn	Pb	As	Fe	Cu	Zn
A	0.046	0.004	0.057	0.379	0.402	6.199
B	0.051	0.006	0.052	0.718	0.347	6.506
C	0.086	0.000	0.073	0.411	0.340	7.264
D	0.108	0.009	0.049	0.567	0.094	3.268
E	0.036	0.002	0.029	0.353	3.211	5.010
F	0.057	0.000	0.081	1.393	7.870	13.07
G	0.064	0.010	0.061	1.137	0.379	12.63
H	0.089	0.010	0.072	2.092	0.642	8.019
I	0.057	0.022	0.062	1.014	1.112	37.23
J	0.083	0.040	0.064	0.266	0.228	1.725
K	0.036	0.003	0.077	0.898	0.576	8.655
L	n.a.	n.a.	0.018	0.229	0.458	6.090
M	0.134	0.017	0.064	1.208	0.375	5.717
N	0.025	0.010	0.072	0.230	0.372	5.609
O	0.001	0.002	0.020	0.209	0.240	3.045
P	0.014	0.002	0.063	0.675	0.512	3.780
Q	0.049	0.013	0.030	1.522	4.488	12.35
R	0.063	0.006	0.065	0.429	0.553	4.159
S	0.049	0.007	0.047	0.633	0.840	6.666
T	0.034	n.a.	0.049	0.280	0.440	5.857
U	0.054	n.a.	0.060	0.475	2.727	5.867

(‰ [drywt])

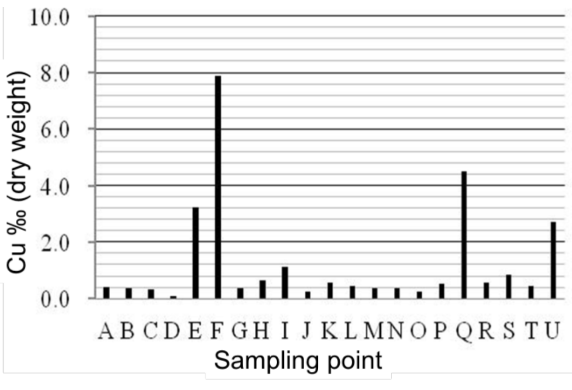

Fig.2 Cu concentrations in oysters measured at 21 sampling points

Fig.4 shows Fe concentrations in oysters measured at 21 sampling points. Some differences were found in concentrations of Fe between sampling points. At sampling points F, G, H, I, M, and Q, Fe concentrations showed very high values over 1.0 ‰. Sampling point H was located near wire rope production factories.

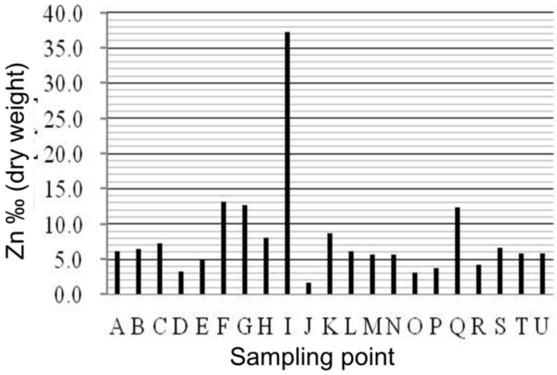

Fig.3 Zn Concentrations measured at 21 sampling points.

Concentrations of Cu, Zn, and Fe in oysters changed with the characteristic situation of each sampling point. At sampling point E, two different characters of oyster were found. Some oysters had green tissues, while others had normal color tissues. When the color of oyster tissues was green, their Cu concentration was high. Then, sampling point E was thought suitable for research into the potential of oyster in detail.

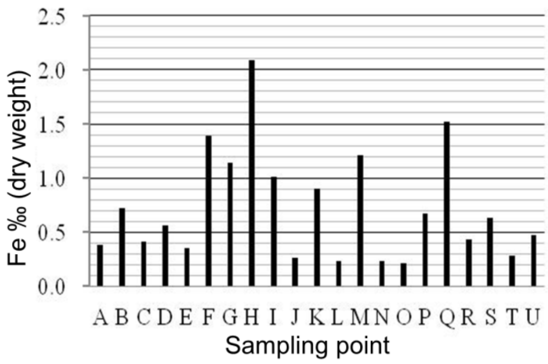

Fig.4 Fe Concentrations in oysters measured at 21 sampling points

4.2 Concentrations of Cu, Zn, and Fe in oysters at Sampling Point E1 to E5

Fig.5 shows sampling point E1, E2, E3 and E5. Sampling point E was the small fishing port and small beach. The distance from sampling point E1 to E5 was about 200 m. Oysters at sampling point E1 and E2 had green tissues. They were called “Green oyster”. But oysters at sampling points E3, E4, and E5 had normal color tissues.

At sampling point E1 and E2, oysters were picked from the artificial slopes. At sampling point, E3, E4 and E5, oysters were picked on a natural beach. Oysters were found under the low tide level.

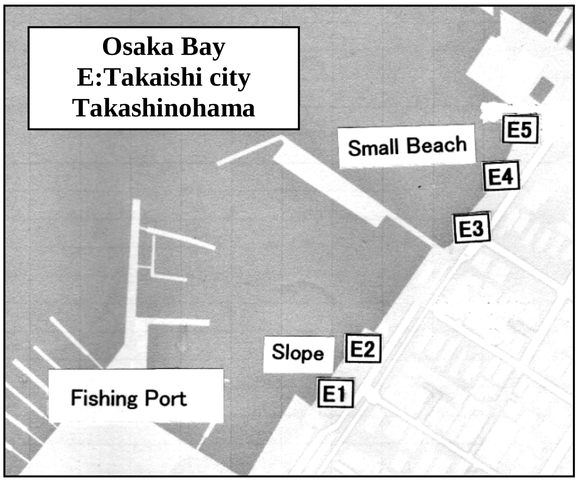

Fig.5 Sampling point E1, E2, E3 and E5

4.2.1 Concentration of heavy metal in seawater from sampling point E1 to E5

Concentrations of Cu, Zn, and Fe in seawater at sampling points E1 to E5 were measured by ICP. Metal concentrations of seawater at each point were very low although metal concentrations of oysters were over several ‰. Seawater Cu and Fe concentrations were under the detection limit of 0.001 ppm. However, seawater Zn concentrations were 0.032 ppm at sampling point E1 and 0.043 ppm at sampling point E3.

4.2.2 Concentration of heavy metals in the sludge at sampling points E1 and E3

Fig.6 shows heavy metal concentrations in the sludge at sampling point E1 and E3. Dried sludge heavy metal concentrations were measured using the same method as oysters. The sludge was black and reeked of hydrogen sulfide. The concentration of Cu in sludge MD1 at sampling point E1 was 2.0 ‰, but the concentration of Cu in sludge MD2 at sampling point E3 was 0.1 ‰.

On the artificial slope at the sampling point, E1, many small pieces of red paint were found and red paint metal concentration was subsequently measured. Small pieces of red paint were steeped in nitric acid and kept for a week at room temperature. After one week, the small pieces remained, however, the color of the solution changed to orange. After the solution was filtered using a 0.45μm filter, the color of the solution turned green.

Fig.7 shows heavy metal concentrations of red paint dissolved with nitric acid. Heavy metal concentrations in the dissolved solution were 58.6 ppm for Cu, 59.7 ppm for Zn, 0.6 ppm for Fe, 0.3 ppm for Mn, 0.1 ppm for Pb, and n.a. for Cd as shown in Fig.7. Cu and Zn concentrations had very high values relative to other heavy metals. The red paint flakes were anti-fouling paint which had peeled off from the bottoms of boats. The main metal ingredients of anti-fouling paint were thought to be Cu_2O and ZnO [7].

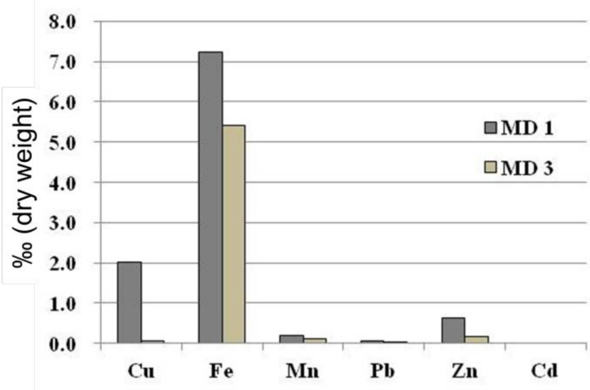

Fig.6 Heavy metal concentrations in the sludge at sampling point E1 (MD1), and E3 (MD3)

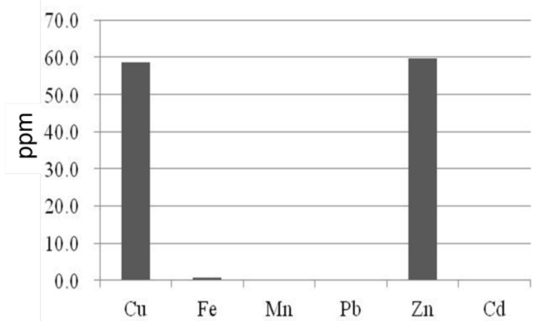

Fig.7 Heavy metal concentrations of red paint dissolved with nitric acid.

4.2.3 Concentration of heavy metals in oysters at sampling points from E1 to E5

Oysters on the artificial slope were found under the low tide level as shown in Fig.8. Oysters were rolled by waves with stones on the slope as shown in Fig.9. Many small pieces of red paint were found on the slope. The width of the beach was less than 100 m. Oysters on the natural beach were found under the low tidal level. Very high concentrations of Cu in the oyster, 3.0 ‰, were found at sampling point E1, whereas very low concentrations, 0.5 ‰, were found at sampling points E3 and E5 as shown in Fig.10. These results coincided with sludge Cu concentrations. Therefore, Cu concentrations of oysters were thought to be influenced by differences between the artificial slope and small beach.

High concentrations of Zn, 4 to 7 ‰, were found at all sampling points. Sampling point E1 was contaminated with Cu and Zn from sludge and red paint pieces so the Cu concentration in oyster was very high at sampling point E1. However, Zn concentrations in oyster were high overall and Zn concentrations of oysters sampled at the next small beach, E3 and E5 were also high as shown in Fig.11. Zn concentrations of oysters were not thought to be influenced by differences between the artificial slope and small beach.

Fig.8 Sampling points E1 and E2 (artificial slopes at the fishing port)

Fig.9 Sampling points E3 and E5 (small natural beach)

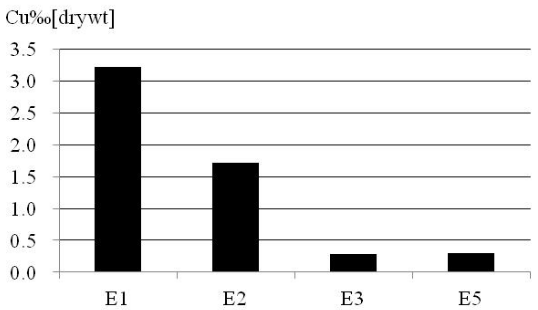

Fig.10 Concentrations of Cu in oysters at sampling points from E1 to E5

4.2.4 Seasonal change for Cu and Zn concentration in oysters at sampling point E1

Figs.12 and 13 show Cu and Zn seasonal change in an oyster at sampling point E1. At sampling point, E1, Cu concentration of oyster changed seasonally and a maximum value of about 10 ‰ was recorded in January as shown in Fig.12. Concentrations of Zn were 4 ‰ to 6 ‰ and the difference between seasonal changes was smaller than those of Cu concentrations, however, the Zn seasonal change pattern was similar to Cu as shown in Fig.13.

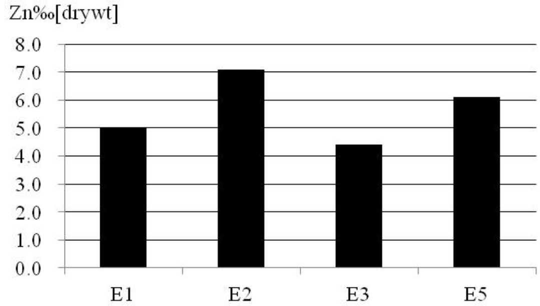

Fig.11 Concentrations of Zn in oysters at sampling points from E1 to E5

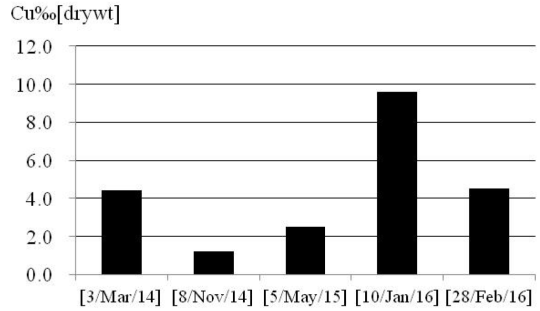

Fig.12 Seasonal change for Cu concentration of oysters at sampling point E1

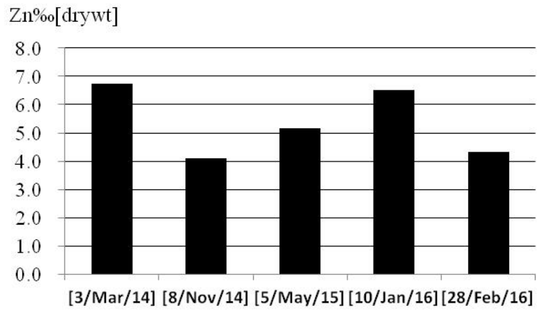

Fig.13 Seasonal change for Zn concentration in oysters at sampling point E1

4-3 Concentrations of Cu and Zn in various Oyster Parts

To check concentrations of Cu and Zn in each oyster part, oysters were sampled at sampling points E1 and E3. Oysters at sampling point E1 which had green color tissues were called “green oyster” as shown in Fig.14. Oysters at sampling point E3 which had normal color tissues were called “normal oyster” as shown in Fig.15.

Oysters at sampling point E1 which had green color tissues were called “green oyster” as shown in Fig.14. Oysters at sampling point E3 which had normal color tissues were called “normal oyster” as shown in Fig.15.

Fig.14 “Green oyster” at sampling point E1
1: Mantle 4: Hepatic gland [8]

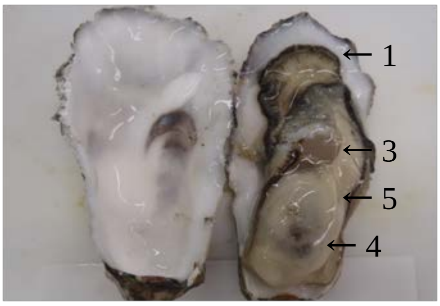

Fig.15 “Normal Oyster” at sampling point E3
1: Mantle 3: Adductor muscle
4: Hepatic gland 5: Other internal organs [8]

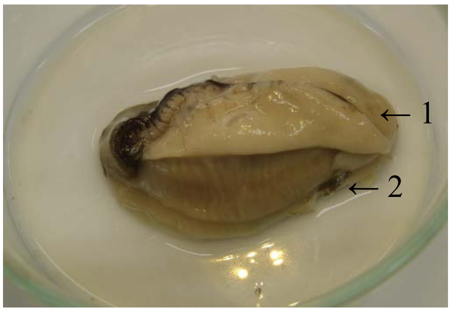

Fig.16 Rolled up the mantle and exposed gill
1: Mantle 2: Gill

After sampling and opening the shell, the rolled-up mantle was cut as shown in Fig. 16. Then, the exposed gill and the adductor muscle were cut. Following this, the brownish hepatic gland was cut, and each tissue of oyster was removed separately. Small stainless-steel scissors used for trimming eyebrows were useful for cutting the oyster tissues. The dissection of the oyster’s tissues was performed in this way.

Fig.17 shows Cu concentration in various oyster parts for sampling point E1 and E3. At sampling point, E1, Cu concentrations in the mantle, gill, hepatic gland and the others were over 4 ‰. In particular, Cu concentration in the gill had the highest value at 6.4 ‰. However, Cu concentrations in the adductor muscle were less than 1 ‰. At sampling point E3, Cu concentrations in each oyster part showed very low relative to E1. At sampling point E3, the highest value of Cu concentration was 0.37 ‰ in the gill as shown in Fig.17. Cu concentration in the mantle and hepatic gland were almost identical values and those of the adductor muscle were low. Cu concentrations of each oyster parts were analyzed at Oman Sea [9] and those of tissue were higher than those of shell although Pb concentrations of tissue were lower than those of shell.

The Cu concentrations of various oysters sampled on the artificial slope were higher than those at the small beach. Therefore, Cu concentrations of oysters were thought to be influenced by differences between the artificial slope and small beach.

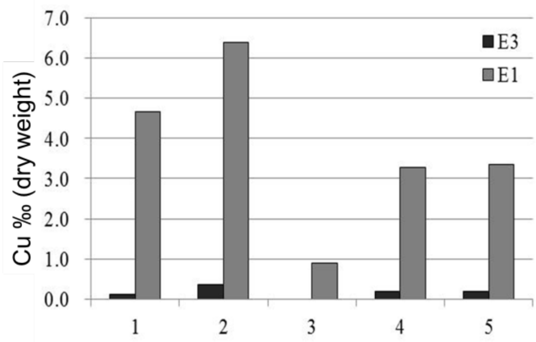

Fig.17 Concentrations of Cu in various oyster parts
1: Mantle, 2: Gill, 3: Adductor muscle,
4: Hepatic gland, 5: Other internal organs

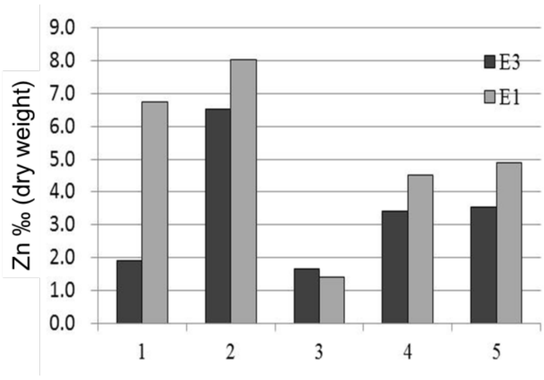

Fig.18 Concentration of Zn in oyster parts
1: Mantle, 2: Gill, 3: Adductor muscle,
4: Hepatic gland, 5: Other internal organs

Fig.18 shows Zn concentration in oyster parts. No difference in Zn concentrations between sampling points E1 and E3 was found, and Zn concentrations in each oyster part were virtually the same value except for the mantle. The differences between the artificial slope and natural beach did not relate with Zn concentration of oyster.

8.0 ‰ Zn concentrations, the highest found, were detected in oyster gill at sampling point E1, and 6.5 ‰ at sampling point E3. Zn concentrations of the mantle were 6.8 ‰ at sampling point E1, and 1.9 ‰ at sampling point E3. Zn concentrations in the hepatic gland and others were also high values ranging from 3.5 to 4.8 ‰. However, Zn concentrations in the hepatic muscle were low. Zn concentrations of each oyster part were almost the same at both the artificial slope and small beach. Zn concentrations of oysters were therefore not thought to be influenced by the difference between artificial slope and small beach.

Heavy metal concentrations of each scallop (*patinopecten yessoensis*) parts purchased at Tokyo wholesale market [10] and each bivalves at Mikawa Gulf, Aichi prefecture [11] were measured and Cu and Zn concentration of muscle was low and gill, mantle and hepatic gland were high as well as this study.

4-4 Concentrations of Cu and Zn in relocated oysters

Oysters were sampled from 10th January 2016 to 28th February 2016 at sampling point E1 and E3 to check for changes of Cu and Zn concentrations after the oysters had been relocated.

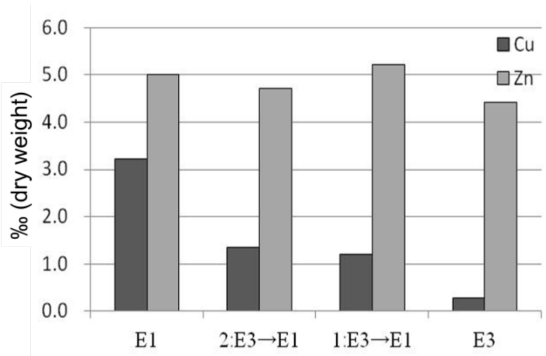

Fig.19 Concentrations of Cu and Zn in relocated oysters.

The oyster relocation was done in the following way; “1:E3→E1” indicates that oysters were sampled 10th January 2016 at sampling point E3 and then were released at sampling point E1 as shown in Fig.19. Sampling point E3 was an area of normal color oysters, and sampling point E1 was an area of green oysters. The replaced oysters have been sampled again at sampling point E1, 23rd January 2016. Therefore, the “living time” at sampling point E1 was 13 days.

“2:E3 → E1” indicates that oysters were sampled 11th February 2016 at sampling point E3 and then were released to sampling point E1. The relocated oysters were sampled again at sampling point E1, 28th February 2016. Therefore, the “living time” at sampling point E1 was 17 days.

Fig.19 shows the relation between Cu and Zn concentrations of the oysters and living time at sampling point E1. Cu concentration increased with living time 0.3, 1.2, 1.7 and over 3.0 ‰ for living time from 0, 13, 17 days to long term (birth to sampling). But Zn concentrations in oysters did not change with location and living time at sampling point E1 and values were uniform. Therefore, sampling point E1 increased Cu concentration in the oysters but did not increase Zn concentration.

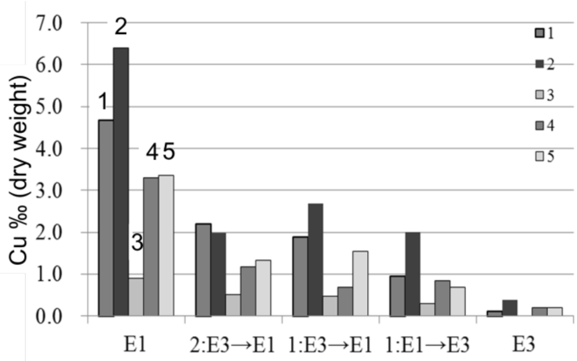

Fig.20 Concentration of Cu in various oyster parts for relocated oysters
1: Mantle, 2: Gill, 3: Adductor muscle,
4: Hepatic gland , 5: Other internal organs

Next, the relation between Cu concentration of each oyster part and “living time” at sampling point E1 and E3 was studied as shown in Fig.20. “1:E3→E1” and “2:E3→E1” in Fig.19 and Fig.20 was the same condition. “1:E1→E3” in Fig.20 showed that oysters were sampled 10th January 2016 at sampling point E1 and then were released to sampling point E3. The relocated oysters have been sampled again at sampling point E3, 23rd January 2016. Therefore, the “living time” at sampling point E3 was 13 days.

Cu concentrations of all parts of the oyster at sampling point E1 were higher than those at sampling point E3 and, Cu concentration of the gill at sampling point E1 and E3 were over 6 ‰ and 0.5 ‰. However, Cu concentration of each part of oysters originally sampled at sampling point E1 decreased with living time at E3 and Cu concentration in each oyster part originally sampled at sampling point E3 increased with living time of E1. Therefore Cu concentration of each oyster part changed in a short time of fewer than two weeks.

4-5 Comparison of cultivated oysters and natural oysters in Osaka Bay

Oyster "Cult. 1," cultivated in the Murotsu area in Hyogo Prefecture, was sold for raw eating. Oyster "Cult. 2," cultivated in Hiroshima Prefecture, required heating before consumption. The Cu and Zn concentrations in cultivated oysters were very low as shown in Fig.21 and 22. The highest concentration value of Cu in "Cult. 1" was 0.079 ‰ in the gill and the highest concentration value of Zn in "Cult. 1" was 0.61 ‰ in the gill. The highest value of Cu was 0.10 ‰ in the gill of "Cult. 2" and the highest value of Zn was 1.2 ‰ in the gill of "Cult. 2". "Cult. 1" was kept in clear water and intensively washed with sterilized water before being shipped. Both values for the cultivated oyster were very low and the difference between "Cult. 1" and "Cult. 2" was also very low.

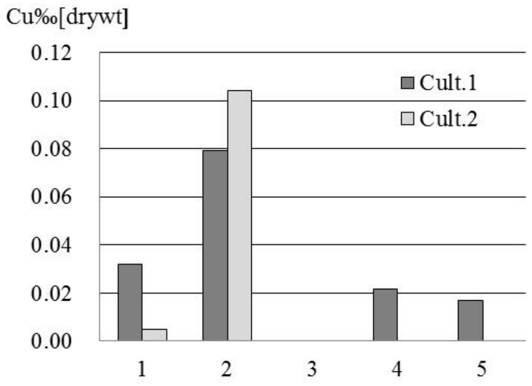

Fig.21 Concentrations of Cu in cultivated oyster
1: Mantle, 2: Gill, 3: Adductor muscle,
4: Hepatic gland, 5: Other internal organs

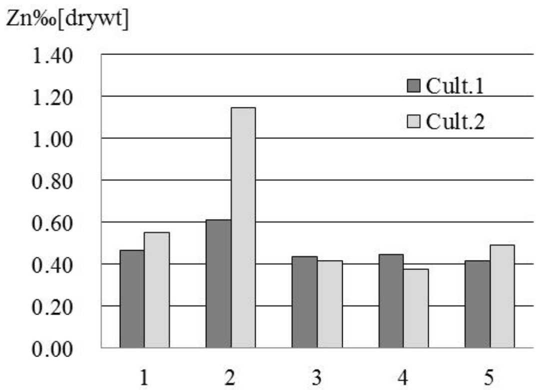

Fig.22 Concentrations of Zn in cultivated oyster
1: Mantle, 2: Gill, 3: Adductor muscle,
4: Hepatic gland, 5: Other internal organs

Cu and Zn concentrations in the cultivated oysters were lower than those of natural oysters in Osaka Bay. Zn concentration of the cultivated oyster was extremely low, less than 1.1 ‰. In contrast, Zn concentration in the natural oyster was extremely high, 1.7 to 37 ‰. Cu concentrations in the cultivated oysters were less than 0.1 ‰ and the natural oysters were 0.09 to 7.9 ‰ respectively.

For both natural and cultivated oysters, Cu and Zn concentration in the gill was the highest among all oyster parts. Cu and Zn concentration of the mantle and hepatic gland were almost identical values and those of adductor muscle were low.

4-6 Influence of Anti-fouling Paint on Concentration of Cu in Oysters in Osaka Bay

Oysters with high Cu concentrations were found at the fishing ports, yacht harbor, leisure boat wharf and particularly on the slope of the artificial slope where many red small pieces of anti-fouling paint were also found. The Cu highest sampling season was January.

Winter is maintenance season for boats. In winter, many red small pieces were found on the slope. The red small pieces were flakes of anti-fouling paint coming from the bottoms of boats. Anti-fouling paint included Cu and Zn as a biocide for biofouling. Therefore, the concentration of Cu and Zn in seawater at the ports of Osaka was thought to be influenced by anti-fouling paint.

5. CONCLUSION

Mn, Pb and As concentrations in oysters in the inner area of Osaka Bay were very low, under 0.001 to 0.13 ‰, under 0.001 to 0.04 ‰ and 0.018 to 0.081 ‰. In contrast, Fe concentrations were 0.21 to 2.1 ‰, Cu concentrations were 0.09 to 7.8 ‰ and Zn concentrations were 1.7 to 37 ‰. In particular, oysters with high Cu concentration were found at the fishing ports, yacht harbor and leisure boat wharf. Oysters with high Zn concentration were found both at the fishing ports, yacht harbor, leisure boat wharf and near food factories. Oysters with high Fe concentration were found near the wire rope maker's factories.

At the small fishing port, much anti-fouling paint, which contained Cu_2O as a biocide for biofouling, was found. Oysters with high Cu concentration were sampled and Cu concentration in oysters was the highest in winter. Since winter is maintenance season for boats anti-fouling paint was thought to be the main Cu contaminant for oysters.

Zn concentration was also high at the small fishing port because Zn concentration of anti-fouling paint was also high as well as Cu. However, Zn concentration was high near many food factories. Therefore, the source of Zn in oysters was anti-fouling paint and food factories

Cu and Zn concentration in the gill was the highest among all oyster parts for natural and cultivated oysters. Cu and Zn concentration of mantle and hepatic gland were almost the same values and those of the adductor muscle were low.

Zn and Cu are important required heavy metals. Cultivated oysters are also a very important food to get Zn and Cu [12]. However, Zn and Cu concentrations in cultivated oysters were very low, less than 1.1 and 0.1 ‰, therefore, the natural oysters in Osaka Bay are effective for getting Zn and Cu.

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