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Localization of Heavy Metals in the Viscera and the Muscular Tissues of *Haliotis discus* exposed to Selected Metal Concentration Gradients

Kunio Ikuta*[1]

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Black abalones were exposed to cadmium, chromium, copper, lead and manganese concentration gradients of 0 (not artificially added), 5, 10, 15, 20 and 25 $\mu g/l$ for 120 days, and subsequently the metal concentrations in the visceral and the muscular parts were determined at the interval according to the elapsed days.

Extremely high distribution rates in the viscera of the control groups were obtained for cadmium with *ca.* 93–96%, which was followed by the somewhat low values, *ca.* 77–93, for lead, *ca.* 76–80 for manganese and *ca.* 50%'s for chromium and copper.

The metals were divided into three groups by the differences in the values of distribution rates and induced Localization Indices (*i*-LI) of the two compartments. (1) *Cadmium*: the rates of this metal in the viscera of the exposure groups decreased, and consequently those of the muscular tissues increased; and the *i*-LI values for the viscera of the control were similar to those of the exposure groups, while the values for the muscular tissues weakened about two times in the exposure groups in negative direction. (2) *Lead and Copper*: the rates of the two metals in the viscera increased gradually in the exposure groups according to the concentration gradients, so that those of the muscular tissues decreased; and the *i*-LI values increased slightly in the viscera, and steadily in the muscular tissues in the negative direction. (3) *Chromium and Manganese*: there were no differences in the values of distribution rates and *i*-LI of the two metals in the two compartments among the control and the exposure groups regardless of the concentration gradients.

Extremely higher levels of heavy metals and their radioisotopes can be accumulated in the visceral tissues of gastropod mollusks than in their muscular tissues under normal environmental conditions. These facts for gastropod species have been documented in the several articles[1–10] on abalones *Haliotis discus*[1,2,10], ivory shells *Babylonia japonica*,[1] perry whelks *Volutharpa ampullacea perryi*,[6–9] which were collected from normal marine environments or reared under laboratory conditions. In these studies, except for a few instances, the specimens were separated into several parts of soft bodies, and the distributions of the metals and the radioisotopes were discussed in relation to the biological or experimental stages, such as the pre- and post-spawning for black abalones[4,5] or the accumulation and elimination phases for perry whelks.[6,7] Ikuta[10] revealed that in top shells exposed to several concentration levels of cadmium, the metal concentrations increased exponentially in the viscera and linearly in the muscular tissues with increased exposure times, and the concentrations of this metal in the two compartments could reach extremely high levels if the exposure times were lengthened. Further, there was a clear difference in concentration; higher in the viscera than in the muscular tissues. Moreover, he documented that the localization indices of cadmium in the muscular tissues decreased in exposure groups (5–20 $\mu g/l$ cadmium) compared to the control.

In this paper, the behaviours of heavy metals in soft bodies of a gastropod *Haliotis discus* are discussed from the viewpoint of trends of distributions and localizations of cadmium, chromium, copper, lead and manganese in the viscera and the muscular tissues of black abalones exposed to some metal concentration gradients.

Materials and Methods

Experimental specimens, black abalones, *Haliotis discus* reared for a year and half with the brown alga *Eisenia bicyclis* were received from the

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[1] Laboratory of Aquatic Environment and Ecology, Department of Fisheries, Faculty of Agriculture, Miyazaki University, Miyazaki 889-21, Japan (生田國雄: 宮崎大学農学部).

Table 1. Weights (g) and weight percentage (%) of the visceral and the muscular parts of specimens

Experimental group ($\mu g/l$)	Weight (g)		Weight percentage (%)	
	Viscera	Muscle	Viscera	Muscle
0	1.31±0.15	2.01±0.25	40.39±1.84	59.61±1.84
5	1.26±0.17	1.93±0.24	39.44±1.54	60.56±1.54
10	1.22±0.20	1.82±0.21	40.11±3.53	59.89±3.53
15	1.29±0.13	2.00±0.21	39.19±1.31	60.81±1.31
20	1.25±0.13	1.93±0.18	39.20±1.83	60.80±1.83
25	1.18±0.15	1.81±0.17	39.35±0.86	60.65±0.86

Note: These data are obtained from exposure experiments of the five metals.

Seed Production Section, Chiba Prefectural Fisheries Experimental Station. Averages and standard deviations of shell length and whole, soft part, visceral and muscular weights of specimens (N=90) used for experiment were 3.72±0.32 (cm) and 6.03±1.50 (g), 3.72±0.95 (g), 1.29±0.34 (g) and 1.96±0.61 (g), respectively. There were no significant differences in size and weight of specimens among the experimental groups of the five metals mentioned below. Weights and weight percentages of the viscera and the muscular tissues were shown in Table 1, being separated into experimental groups according to concentration gradients, with averages and standard deviations.

The standard solutions of the five metals were prepared according to Testing Methods for Industrial Waste Water (K 0102–1971).[12] The original concentrations for cadmium, chromium, copper, lead and manganese were 1000, 500, 1000, 1000 and 100 ppm, respectively. The stock solutions of metals for experiments were diluted with deionized water to 10 ppm, and they were added in rearing seawaters respectively, being diluted with seawater before use. The seawater contained initial levels of 0.05 for cadmium, 2.9 for copper and 2.8 $\mu g/l$ for manganese and traces for chromium and lead, which were determined after the extraction with dithizone-chloroform for the former three and iron-coprecipitation for the latter two.

The exposures to cadmium, chromium, copper, lead and manganese were set up at the following concentration gradients: 0 (control group, containing the initial levels of the metals), 5, 10, 15, 20 and 25 $\mu g/l$. These concentration gradients were below criteria for public health in the world governmental bodies[13] except cadmium and manganese. Cadmium is regulated at 10 $\mu g/l$ for drinking water and 50 $\mu g/l$ for waste water discharge while manganese is not regulated.

The duration of exposure was 120 days, during which three specimens each were removed at 0, 15, 30, 60, 90 and 120 days to determine the metal concentrations in the viscera, including digestive diverticula, stomach, digestive tract, gonad, kidney, heart, *etc.* and the muscular tissues including foot, columellar muscle, snout, buccal mass and free edge of mantle.

Fifteen individuals of specimens were initially held in rearing seawaters of 15 litres which were renewed at the regular interval of 24 h throughout the experiment, and volumes of seawater were decreased in proportion to numbers of specimens in aquaria to maintain ratio of biomass to water volume.

During the exposure, abalones were fed with thalli of *Undaria pinnatifida* with metal levels of 1.14 for cadmium, 1.65 for copper, 1.50 for chromium, 6.17 $\mu g/g$ for manganese and trace for lead on a dry weight basis. The thalli of 2 g were laid in each aquaria every evening.

The conditions for rearing the abalones were water temperature of 20.0±0.33°C, specific gravity of 24.11±0.11 at 15°C, dissolved oxygen of *ca.* 4.80 ml/l (87% of the saturation value), which were preferable to physiological activities of the abalones.

Determinations of the metals in the two compartments and the food stuffs were made by atomic absorption spectrophotometry after wet-digestion with a mixture of perchloric and nitric acids, and also for the metals in the seawater.

Distribution rates, in percentage, of the metals in the viscera or the muscular tissues were calculated from ratios of absolute amounts in the viscera or the muscular tissues to sums of absolute amounts of the metals contained in the two compartments. Even if a metal concentration in the viscera is equal to that in the muscular tissue, absolute amounts in the two compartments can differ depending on their weight differences.

Hence, in order to eliminate the influence of weights on absolute amounts of metals and to demonstrate variations in localization trends in the two compartments caused possibly by exposures to metals, the induced Localization Index obtained by subtracting ratios of the viscera (or the muscular tissues) weight percentages in whole soft bodies to absolute amount percent ages of metals in the viscera (or muscular tissues) from their reciprocals, was introduced in this paper. This calculating method for *i*-LI value and its availability to consider localization trends had already been explained in detail.[2,3] Namely, it has been very useful to compare localization of a metal in different tissues with various concentrations, since the index is not affected by the concentration levels of the metal and the unit indications (*e.g.* different units for stable- and radio-elements)[4,10] by virtue of the nature of calculation method of the index. In this paper, the indices of the controls were compared with those of the exposure groups to the metals, and their variation trends of the muscular tissues after exposure were noted as a biological indicator in relation to marine environmental pollution of heavy metals.

Table 2. Metal concentrations (μg/g wet wt) in the visceral and the muscular parts of black abalones exposed to concentration gradients of different metals

Metals & Concns (μg/l)	Days and Parts											
	0		15		30		60		90		120	
	VP	MP	VP	MP	VP	MP	VP	MP	VP	MP	VP	MP
(1) *Cadmium*												
0	3.34	0.05	3.94	0.05	4.04	0.01	2.62	0.18	3.28	0.07	3.33	0.09
5			5.16	0.26	7.84	0.36	8.80	0.46	11.4	0.65	13.1	0.64
10			8.69	0.36	10.3	0.51	15.2	0.83	20.1	1.16	28.5	1.36
15			7.73	0.35	16.4	0.73	20.3	0.90	30.3	1.32	35.7	1.59
20			9.51	0.50	18.8	0.81	25.7	1.11	45.9	1.92	43.8	1.78
25			11.1	0.55	18.9	0.91	25.0	1.38	49.9	2.01	53.3	2.90
(2) *Lead*												
0	4.77	1.28	4.92	1.10	6.19	0.62	4.15	0.71	5.73	1.18	4.79	1.08
5			8.89	0.98	9.27	0.93	9.17	0.98	12.3	1.29	16.1	1.48
10			11.1	1.02	13.0	1.31	21.7	1.44	25.7	1.85	44.6	2.15
15			12.4	1.26	25.3	1.28	28.3	1.56	40.0	2.15	54.5	2.81
20			18.4	1.44	30.5	1.40	36.1	1.85	79.5	2.64	76.2	2.87
25			24.0	1.42	31.4	1.61	42.0	2.06	91.2	3.37	87.2	4.49
(3) *Copper*												
0	22.2	15.5	13.7	13.6	20.8	17.4	21.3	17.5	23.8	15.5	13.7	14.5
5			18.3	9.03	27.2	17.4	26.0	18.6	19.5	15.8	21.2	22.3
10			18.8	8.09	28.1	18.7	25.9	18.6	31.4	25.4	26.3	21.0
15			26.5	12.6	31.8	18.9	32.0	22.8	35.9	21.1	47.4	31.4
20			31.7	14.9	48.0	25.0	41.8	24.0	54.6	36.8	52.5	33.2
25			35.4	16.9	41.0	23.4	51.1	29.7	68.0	32.4	65.0	41.6
(4) *Manganese*												
0	1.70	0.37	1.53	0.33	1.63	0.42	1.97	0.31	1.81	0.38	1.58	0.33
5			2.47	0.46	2.30	0.36	1.93	0.39	1.95	0.34	1.35	0.30
10			2.19	0.44	2.38	0.33	1.85	0.38	1.95	0.39	1.91	0.34
15			1.91	0.48	2.16	0.38	2.09	0.37	1.82	0.39	2.50	0.36
20			2.20	0.39	2.75	0.42	1.98	0.34	2.70	0.36	1.66	0.32
25			2.40	0.43	2.24	0.41	2.21	0.44	2.17	0.47	2.00	0.50
(5) *Chromium*												
0	0.36	0.24	0.38	0.22	0.35	0.30	0.67	0.35	0.46	0.25	0.34	0.22
5			0.67	0.32	0.70	0.36	0.87	0.56	0.79	0.46	0.70	0.66
10			0.97	0.29	0.98	0.64	1.09	0.98	1.21	0.99	1.36	1.20
15			0.72	0.52	1.22	0.89	1.47	0.84	1.48	1.06	2.27	1.93
20			1.17	0.47	1.55	0.81	1.29	0.84	2.03	1.27	2.48	1.45
25			1.36	0.63	1.42	1.04	2.29	1.20	2.61	1.79	3.17	2.49

Note: Visceral and muscular parts are abbreviated to VP and MP, respectively.

Results and Discussion

Accumulation of the Five Metals in the Two Compartments

Except for manganese the other four metals were taken up steadily in the two compartments according to concentration gradients with the exception of control groups as shown in Table 2. The general equations for accumulations of the four metals were able to be calculated as follows: $\log Y = \log A + B \log X$, for the viscera and $Y = a + bX$, for the muscular tissues, in which Y is metal concentration in the two compartments on the exposure day (X). These accumulation patterns were similar to those of top shells *Batillus cornutus*[10] examined with cadmium previously. Regression coefficients for the regressed formulae were in the range of $p<0.05$ to $p<0.001$ of significance except a few instances. The formulae calculated for the four metals are left out, since the description for accumulation patterns deviate from the subject of relevant problem. From the pattern of accumulation curves, it was suggested that accumulation amounts in the two compartments did not reach the maxima for 120-days-exposure. Especially, the muscular tissues seemed to accumulate much more of the metals when the

Table 3. Distribution rates (%) and *i*-LI values of the visceral and the muscular parts for cadmium, lead, chromium, copper and manganese, respectively

Metals & Concns (*μg/l*)	DR-VP[*1] (%)	DR-MP[*2] (%)	*i*-LI-VP[*3]	*i*-LI-MP[*4]
(1) *Cadmium*				
0	95.97±2.55	4.03±2.55	1.96±0.14	−18.71±0.12
5	92.80±1.59	7.20±0.43	1.96±0.13	−8.29±0.49
10	93.01±1.59	6.99±1.59	1.92±0.23	−8.62±1.27
15	93.55±0.43	6.45±0.43	1.99±0.11	−9.33±0.41
20	93.60±0.76	6.40±0.76	1.99±0.13	−9.47±1.01
25	92.97±0.67	7.03±0.67	1.96±0.10	−8.57±0.99
(2) *Lead*				
0	76.86±5.79	23.14±5.79	1.38±0.22	−2.37±1.01
5	86.53±0.70	13.47±0.70	1.73±0.10	−4.27±0.23
10	89.87±2.87	10.13±2.87	1.79±0.23	−6.13±1.92
15	91.19±3.03	8.81±3.03	1.89±0.09	−7.21±1.75
20	92.98±2.27	7.02±2.27	1.95±0.15	−9.20±2.66
25	92.95±1.17	7.05±1.17	1.93±0.06	−8.66±1.53
(3) *Copper*				
0	43.99±4.95	56.01±4.95	0.16±0.25	−0.13±0.19
5	47.69±7.67	52.31±7.67	0.36±0.29	−0.31±0.28
10	50.28±7.39	49.72±7.39	0.43±0.26	−0.38±0.27
15	51.80±3.91	48.20±3.91	0.56±0.16	−0.47±0.16
20	53.21±4.39	46.79±4.39	0.61±0.14	−0.53±0.17
25	54.49±2.64	45.51±2.64	0.66±0.13	−0.58±0.14
(4) *Manganese*				
0	75.54±3.42	24.46±3.42	1.34±0.12	−2.07±0.43
5	77.73±2.28	22.27±2.28	1.46±0.12	−2.36±0.30
10	78.41±4.54	21.59±4.54	1.43±0.14	−2.48±0.57
15	77.20±3.81	22.80±3.81	1.46±0.15	−2.34±0.48
20	79.74±1.50	20.26±1.50	1.54±0.15	−2.68±0.31
25	76.16±2.24	23.85±2.24	1.41±0.10	−2.16±0.28
(5) *Chromium*				
0	50.93±5.31	49.07±5.31	0.46±0.18	−0.40±01.9
5	51.64±7.13	48.36±7.13	0.53±0.28	−0.47±0.27
10	50.38±10.94	49.62±10.94	0.43±0.44	−0.43±0.54
14	47.57±4.42	52.43±4.42	0.38±0.15	−0.30±0.14
20	54.07±5.81	45.93±5.81	0.64±0.18	−0.58±0.24
25	51.07±5.59	48.93±5.59	0.51±0.23	−0.44±0.24

Note: Abbreviations asterisked and numbered are as follows: [*1] (distribution rate of visceral part); [*2] (distribution rate of muscle part); [*3] (*i*-LI of visceral part) and [*4] (*i*-LI of muscle part).

exposure time was lengthened.

Variations of Distribution Rates of Metals in the Two Compartments

Distribution rates given in Table 3 are summarized as averages and standard deviations of the respective metals and their concentration group; since there were no clear trends (such as ascending or descending) in variations of the rates in the two compartments according to exposure days, irrespective of the concentrations gradients.

For cadmium (Table 3-(1)), the distribution rates of the two compartments for the control differed from those of the exposure groups with the opposite relationship. In the exposure groups, the rate decreased in the viscera and contrarily increased in the muscular tissues.

On the contrary, the relationship for lead was reversed, *i.e.* the rates of the viscera increased gradually according to the increase in the concentration gradient, and those of the muscular tissues decreased in a similar trend (Table 3-(2)). The difference between rates of the control and the experimental group of 25 $\mu g/l$ reached *ca.* 16%, which was the highest among such differences for the metals. For copper in the two compartments (Table 3-(3)), the increase and decrease trends of the rates were exactly similar to those of lead. The rates for the viscera were clearly less for cadmium and lead, and consequently, the rates for the muscular tissues were higher.

Distribution rates of manganese in the control were nearly equal to those of the exposure groups for the two compartments; the rates for the viscera were *ca.* 3.5 times higher than those of the muscular tissues (Table 3-(4)). For chromium (Table 3-(5)), there was no difference between the rates for the viscera and the muscular tissues of the control and the exposure groups, since the metal was distributed equally in the two compartments.

Variations of Induced Localization Indices before and after Exposure

Cadmium: In the muscular tissues, the values (*ca.* −19 on average) of *i*-LI were extremely low in the control with the normal concentration level of cadmium as shown in Table 3-(1), and in the exposure groups (from 5 to 25 $\mu g/l$) the values showed invariably almost no difference among the groups. Namely, the *i*-LI values ranged from *ca.* −8 to *ca.* −9 on averages for the exposure groups and weakened about two times (in the negative direction) that of the control. On the other hand, in the viscera, there was no difference between *i*-LI values of the control and the exposure groups. It could be explained that the clear differences in *i*-LI values of muscular tissues between the control and the exposure groups is resulted from differences in distributions of the levels of the metal in the muscular tissues are extremely low compared to those of viscera, and concentration ratios of metal levels after exposure to initial levels are clearly higher in the muscular tissues than in the viscera.

Lead and Copper: the *i*-LI values of lead in viscera increased gradually according to the concentration gradients as shown in Table 3-(2). On the other hand, the values of the muscular tissues decreased with the increase in concentration gradients, being limited in the negative direction. These correlations, especially for *i*-LI values of the muscular tissues, were reversed when compared with the distribution and localization of cadmium as mentioned above. In the two compartments, the increase and decrease trends of *i*-LI's (Table 3-(3)) for copper were exactly similar to those of lead (Table 3-(2)), but the absolute values of *i*-LI were clearly less than those of lead.

Manganese and Chromium: the *i*-LI values of the two metals in the controls were almost similar to those of the exposure groups irrespective of the concentration gradients (Table 3-(4 & 5)). This differed from the distribution patterns for the three metals mentioned above.

Comparison of the Results Obtained Here with Those of Batillus Cornutus and Other Considerations

The results obtained in this study for cadmium was similar to that of *B. cornutus.*[10] The similarity found in the two species, *H. discus* and *B. cornutus* results from the similarity of accumulation pattern; The metal was taken up linearly in the muscular tissues and exponentially in the viscera with exposures of three or four months, and also uptake ratio of levels after exposure to initial level were definitely higher in the muscular tissues than in the viscera. The values of distribution rate and *i*-LI for lead and copper increased in the viscera of the exposure groups, while those for the muscular tissues decreased. From these results, the behaviours of lead and copper in the abalone differed greatly from those of cadmium in the two species. It will be pointed out that the difference between cadmium, and lead and copper originated from the diversities of distribution modes and affinities of the metal in the two compartments. For these three metals, it can be inferred that transportation patterns of the metals in soft bodies under conditions that enhanced the heavy metal conmetal to the two compartments. Namely, initial centrations differ greatly from those under conditions with normal concentration levels of metals. The distribution patterns of manganese and chromium were distinguished from those of cadmium, lead and copper, with the respective proper rates. For both the control and exposure groups, the values of the rates and *i*-LI for manganese and chromium were similar.

Utility of i-LI *Value in the Muscular Tissue for Heavy Metal Pollution*

Bivalve and gastropod mollusks, in general, have been used as biological indicators of heavy metal pollutions for the past decade. In any case, heavy metal concentrations in soft bodies of mollusks have been proposed as indicative of heavy metal pollution in sea environments.

From the results obtained in this study, the variations of *i*-LI values in the muscular tissues of black abalones before and after the exposures to the heavy metals suggest that the *i*-LI values for cadmium, lead and copper may be applied as indicators of the metal pollution in sea environments and is more useful than comparisons of the concentration levels of the metals in the muscular tissues. In the further study$^{11)}$ relevant to the use of *i*-LI value as an indicator to monitor cadmium pollution in sea environments, it will be discussed that the *i*-LI values of cadmium in the muscular tissues of gastropod mollusks, four species (two herbivores and two carnivores), are used as an indicator for detecting the contamination of living body under the increased levels of the metal concentrations in the environments.

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