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Cu, Zn, Fe AND Mn CONCENTRATIONS OF SOME GASTROPODS IN THE WAKAGAWA ESTUARY TIDAL FLAT

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ABSTRACT: The Wakagawa estuary tidal flat was heavily polluted by industrial wastewater in the past. Since then, organic pollution has decreased due to inflow regulations, etc., and it is now an important wetland with rich biota. However, it is expected that metallic elements remain in the tidal flat and affect benthic organisms. Therefore, some benthic animals, mainly sediment feeders, which are expected to be strongly affected by the sediment, were collected in 2016. Then the concentrations of some metallic elements in their bodies were measured. In this paper, copper, zinc, iron, and manganese concentrations in the soft bodies of six benthic gastropod species are reported. It was suggested that soil zinc concentration was not uniform and some areas were high in this tidal flat, especially in places where water flow is obstructed topographically and mud tends to accumulate. Some degree of partial accumulation in the sediment within this tidal flat was suggested for copper, iron and manganese. Exclusive or partial detritus feeders showed higher metal concentrations than microalgae feeders. Among gastropods, three species of genus *Batillaria* (*B. attramentaria*, *B. multiformis* and *B. zonalis*) for copper; *Batillaria multiformis*, *B. zonalis* and *Pirenella nipponica* for zinc; *Batillaria zonalis* and *Pirenella nipponica* for iron and manganese, are considered useful as an indicator species.

Keywords: Snails, Batillaria, Pirenella, Clithon, Heavy metals

1. INTRODUCTION

The Wakagawa estuary is located in the northwestern part of the Kii Peninsula. The tidal flat area is about 35 hectares, and it is thought that more than 300 species of benthos and fish inhabit there. It has been selected as one of the "500 Important Wetlands in Japan" by the Ministry of the Environment (Ministry of the Environment Nature Conservation Bureau 2001) [1]. However, from around the early 1900s, the number of dye, leather, and chemical factories increased, especially along the Wakagawa River. Rivers and tidal flats were polluted by the factory and domestic wastewater, and it became a major social problem, such as the death of farmed seaweed. Since then, various efforts have been made to reduce pollution, such as wastewater control and restriction of inflow into estuaries [2].

Although organic pollutants are decomposed and reduced, it is expected that the factory effluent at that time contained various metals, and most of them may remain on the tidal flats in some way, affecting benthic organisms. However, before the establishment of the environmental standards for the discharge of factory wastewater, there was no application system for establishing a factory, and it is not possible to know the content of factory wastewater from documents. There are many studies on heavy metal contamination around the coasts of the Kii Peninsula and along the catchment of Kinokawa River using seaweed, aquatic insects, aquatic plants, bryophytes, and freshwater crabs [3-8]. Various investigations about the Wakagawa estuary have been conducted for a long time. However, until recent years, most of them have been related to biota [9,10] and nutrients such as nitrogen [11,12]. There seems to be no investigation regarding metal contamination. In general, the properties of tidal flat sediments have discontinuous distribution characteristics and sometimes have completely different properties at a distance of several meters. The representativeness of the value of one point is very low compared to water quality [13]. Therefore, it is conceivable that benthic animals that roam the tidal flats and feed on detritus could be useful as environmental bio-monitors. In this study, the concentrations of some metals were measured for some benthos, mainly detritus feeders, which are thought to be strongly affected by sediment. An attempt was made to estimate the current state of metal pollution in this tidal flat that could affect organisms and to find candidate indicator species, which could be useful for future tidal flat environmental research. If it becomes clear that metal pollution remains in tidal flats for many years and continues to affect benthic organisms, it will have important implications for considering environmental issues and policies. In this paper, concentrations of copper, zinc, iron and manganese in six species of gastropods are reported.

2. RESEARCH SIGNIFICANCE

Concerning the environmental pollution of tidal flats, attention is often paid to organic pollution. Investigation of metal contamination of the sediments is desired. However, in general, the properties of tidal flats differ depending on the microtopography, and the properties of bottom sediment show a discontinuous distribution, and the representativeness of one point of sediment is low [13]. Therefore, it is conceivable that environmental pollution assessment using indicator organisms is important. This research provides one of the basic findings for establishing the method. It also contributes to showing the continuity of metal pollution and its effects in tidal flats.

3. STUDY AREA

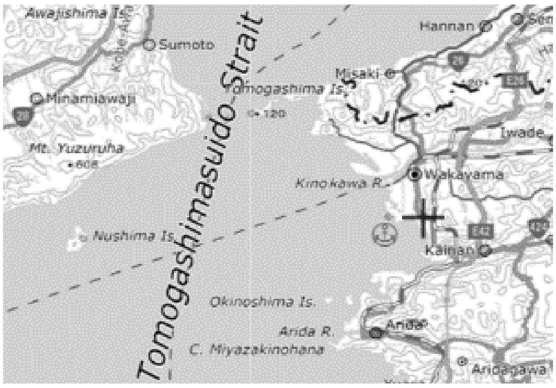

Fig. 1 Study area
(Map from Geospatial Information Authority of Japan)

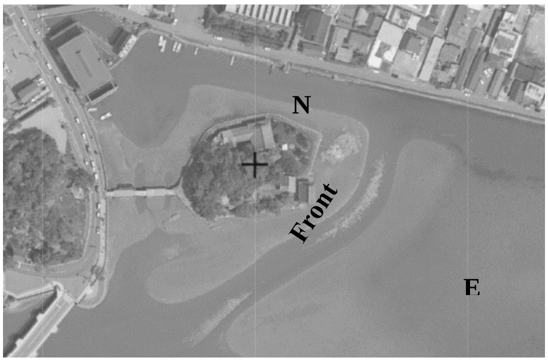

Fig. 2 Sampling site
(Geospatial Information Authority of Japan map Vector)

Wakagawa estuary is located in the northern part of Wakayama Prefecture. Fig. 1 shows the location on a map, and Fig. 2 shows the sampling points in this tidal flat. Fig. 2 is a part of the photo that seems to have been taken at low tide. At high tide, all sampling points sink into the sea, leaving small islets with a plus mark and bridges. Around the small islet the area near the islet is sandy, and the mud increases as it gets closer to the water's edge. Among them, Site Front is relatively sandy, and Site N, which is sandwiched between the islet and land, is relatively muddy and the sandy area is narrow. Site E, which is closer to the sea across the channel from Site Front, is sandy. Sampling was mainly performed at Site Front and was also performed at Site N, E as a reference.

4. SPECIES INVESTIGATED

Table 1 Species investigated and collection date in 2016

Familly	Species	Site	Month	n	feeding	Red data
Batillariidae	*Batillaria attramentaria*	Front	Mar.	1	detritus	
			May	3		
		E	Apr.	4		
	Batillaria multiformis	Front	Jan.	1	detritus	NT
			Mar.	4		
			May	3		
			Sep.	6		
		N	Jan.	1		
			Mar.	6		
		E	Apr.	3		
	Batillaria zonalis	Front	Mar.	5	detritus	VU
			May	2	&suspension	
			Sep.	6		
		N	Jan.	1		
			Mar.	4		
		E	Apr.	4		
		S	Mar.	2		
Potamididae	*Pirenella nipponica*	Front	Mar.	4	detritus	NT
			May	4		
			Sep.	6		
		N	Mar.	7		
		E	Apr.	4		
		S	Mar.	3		
Neritidae	*Clithon oualaniensis*	Front	May	4	microalgae	NT
			Sep.	4	&detritus	
		E	Apr.	3		
	Nerita japonica	Front	May	1	microalgae	
			Sep.	5		

All sampling was done in 2016. Red Data Rank: Ministry of the Environment. VU: vulnerable, NT: near threatened

All sampling was done in 2016, mainly in spring and September. Details are shown in Table 1.

5. METHOD

The collected snails have washed off the mud and fasted in 50% artificial seawater for about a week to expel the contents of the digestive tract. Then, the whole snails were ultrasonically cleaned, and then the soft bodies were carefully taken out and washed with Millipore water. After sufficiently drying them in a dryer at 60 ° C, they were weighed and dissolved with concentrated nitric acid. After filtering with the 0.45μm membrane filter, the concentrations of metal elements were measured using ICP-OES (AMETEK Inc. SPECTRO ARCOS). For some samples, the previous model Seiko Instruments Inc. SPS1700HVR was used.

6. RESULTS AND DISCUSSION

6.1 Copper concentration

Figure 3 shows the copper concentration in the soft body of snails. Between spring (March to May) and autumn (September), there was no significant difference in the mean values for all sites except for *Pirenella nipponica* (t-test, significance level 5%).

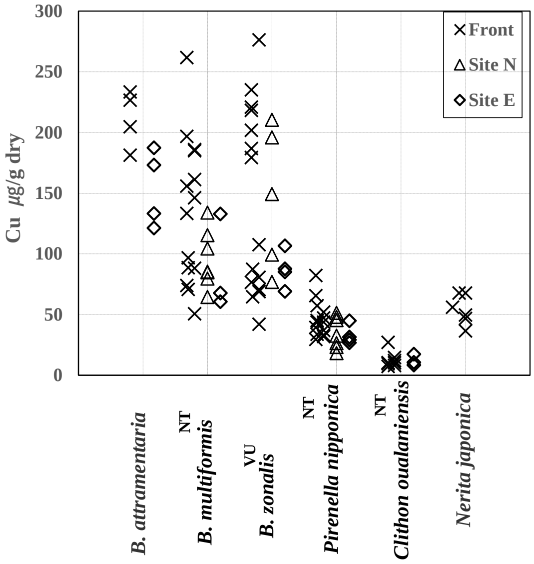

Fig.3 Cu concentration in the soft body of snails.

As for *Pirenella nipponica*, the mean value was 59.7μg/g in March-May and 40.9μg/g in September, the difference was only 18.8μg/g. Table 2 shows the mean, standard deviation and median values of copper concentration when totaled for all sampling periods for each species. Except for *Batillaria attramentaria*, there was no significant difference between sites (ANOVA, significance level 5%).

Three species of the genus *Batillaria* showed large value variation and higher concentration distribution than other species. *Pirenella nipponica*, which is also a detritus-feeder, showed similar concentration distribution to *Nerita japonica*, which is a microalgae feeder. *Clithon oualaniensis*, a microalgae and detritus feeder, showed very low concentration distribution.

Since the standard deviation varied greatly depending on the species, simple comparison using representative values was difficult, but both the mean and median values for genus *Batillaria* were two to four times higher than those of *Pirenella nipponica* and *Nerita japonica*, and approximately 10 times those of *Clithon oualaniensis*.

Table 2 Cu concentration (μg/g-dry) in the soft body of snails when totaled for all sampling periods.

Species	Site	Mean	SD	Median	n
Batillaria attramentaria	Total	182.7	40.2	184.4	8
	Front	211.6	23.6	215.7	4
	Site E	153.8	31.5	153.2	4
Batillaria multiformis	Total	117.7	52.8	100.6	24
	Front	135.4	60.1	139.8	14
	Site E	87.1	39.8	67.8	3
	Site N	95.5	23.8	85.4	7
Batillaria zonalis	Total	133.2	68.8	103.0	24
	Front	141.1	77.6	107.6	15
	Site E	87.2	15.4	86.5	4
	Site N	146.4	58.3	149.2	5
Pirenella nipponica	Total	45.7	20.3	43.8	28
	Front	53.1	21.7	45.2	17
	Site E	33.1	8.1	30.4	4
	Site N	35.1	13.2	32.6	7
Clithon oualaniensis	Total	12.3	5.7	10.4	11
	Front	12.4	6.4	10.3	8
	Site E	12.2	4.7	10.6	3
Nerita japonica	Front	54.1	12.3	52.9	6

Fukuda et al. [14] evaluated metal contamination in Banzu tidal flat (Chiba Prefecture) and Nakatsu tidal flat (Oita Prefecture) using *Batillaria cumingii* (Currently, the scientific name has been changed to *B. attramentaria*. Hereafter referred to as *attramentaria* in this paper.). From their graph, the mean value of copper concentration of *Batillaria attramentaria* can be read as about 300 μg/g in Banzu tidal flat and about 100 μg/g in Higashihama of Nakatsu tidal flat. They removed the stiff leg muscles and used only the visceral parts. Although it is necessary to be careful when comparing the values, the *Batillaria attramentaria* in this study were intermediate between those of Bansu Tidal Flat and Higashihama. The mean and median values for *B. multiformis* and *B. zonalis* were slightly lower than for *B. attramentaria* but the concentration distributions of all three species overlapped. There was large variation in the values for all three species.

The copper concentration of Gastropod *Assiminea brevicula* in the mangrove belt of Bangladesh is 3.66 ± SD 0.89 μg/g [15], which is low compared to any species in this study. In bivalves, *Cyclina sinensis*, filter-feeders in the Shiogawa tidal flat in Aichi Prefecture, the median value was 8.64-31.4 μg/g [16]. It is almost the same as *Clithon oualaniensis* and *Pirenella nipponica* in this study, or slightly lower. All three species of genus *Batillaria* in this study have concentrations more than three times higher than these. These results suggest that there is some partial copper contamination in the Wakagawa estuary tidal flat.

6.2 Zinc Concentration

Figure 4 shows the zinc concentration in the soft body of snails. There was no significant difference in mean values between spring (March to May) and September (t-test, significance level 5%).

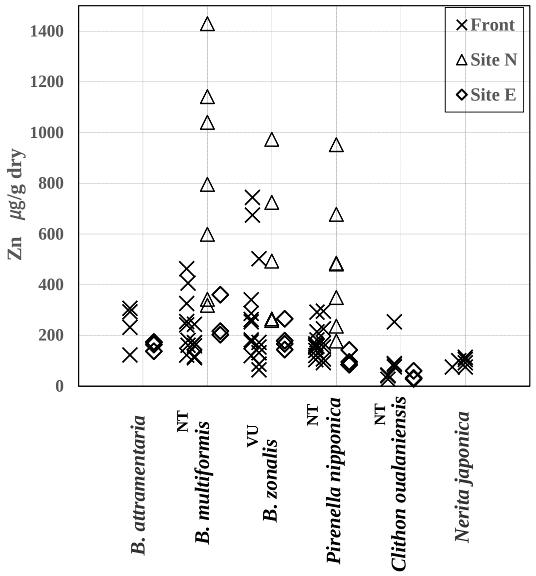

Fig.4 Zn concentration in the soft body of snails.

Table 3 shows the mean, standard deviation and median values of zinc concentration at each site, when totaled for all sampling periods.

From the graph of Fukuda et al. [14], the mean values of zinc concentration of *Batillaria attramentaria* in Banzu tidal flat and Nakatsu tidal flat Higashihama are read to be about 200 and 100 μg/g, respectively. For *Batillaria attramentaria* in this study, the mean value was 238.9μg/g at Front, 162.3μg/g at Site E, and the total average of both sites was 200.6 μg/g, which was about the same as the concentration of Banzu tidal flat. At Site E, except for *Batillaria multiformis*, the average value tended to be low, but the concentration distribution completely overlapped with that of other Sites.

Batillaria multiformis and *B. zonalis* showed high concentration distribution and had some extremely high values, especially at Site N. In *Pirenella nipponica*, some very high values were observed at Site N. These three species are mainly detritus feeder (*B. zonalis* also feed on suspensions), and red data species. In *Batillaria multiformis*, the mean and median values at Site N were 809.7 and 795.7 μg/g, respectively, and they were about four times higher than that of *B. attramentaria* in Banzu tidal flat [14], and about eight times higher than the mean value of Higashihama in Nakatsu tidal flat [14].

Table 3 Zn concentration (μg/g-dry) in the soft body of snails when totaled for all sampling periods.

Species	Site	Mean	SD	Median	n
Batillaria attramentaria	Total	200.6	69.3	173.7	8
	Front	238.9	83.7	262.7	4
	Site E	162.3	17.0	168.6	4
Batillaria multiformis	Total	399.2	354.3	249.5	24
	Front	223.8	108.2	176.4	14
	Site E	260.2	87.5	217.0	3
	Site N	809.7	418.6	795.7	7
Batillaria zonalis	Total	258.4	188.2	179.3	24
	Front	276.9	207.8	182.4	15
	Site E	189.1	53.1	173.2	4
	Site N	543.1	307.7	493.2	5
Pirenella nipponica	Total	238.1	197.0	163.6	28
	Front	170.4	58.3	157.8	17
	Site E	102.2	27.7	90.4	4
	Site N	480.2	267.6	481.9	7
Clithon oualaniensis	Total	71.0	64.5	44.4	11
	Front	82.3	72.9	60.2	8
	Site E	40.7	17.5	32.7	3
Nerita japonica	Front	93.7	15.7	96.2	6

The zinc concentration in *Assiminea brevicula* from Bangladesh is 110 ± 8.82 μg/g [15]. *Cyclina sinensis* in the Shiogawa tidal flat has a median value of 137-155 μg/g [16]. Both Mean and median of *B. zonalis* at Site N (543.1 and 493.2) were more than three times higher than these.

It is strongly suggested that the Wakagawa tidal flat has hotspot zinc contamination in the bottom sediments.

At Site N, small islets tend to block water flow, mud accumulates easily. The wide area of this Site is muddy. During periods of severe environmental pollution, just below the surface of the sediment were anaerobic and had a bad odor like hydrogen sulfide. Even at high tide, the interstitial water in the mud did not seem to be sufficiently exchanged with the seawater outside. It is presumed that some of the metal contaminants still remain in the sediment.

6.3 Iron Concentration

Figure 5 shows the iron concentration in the soft body of snails. There was one extremely high value (2537.4 μg/g) in *Pirenella nipponica*, but it was excluded here for ease of comparison. Table 4 shows the mean, standard deviation and median values of iron concentration when totaled for all sampling periods for each species.

Batillaria zonalis and *Pirenella nipponica* showed high concentration distribution with large variations in values. Both mean and median values were highest for *Pirenella nipponica* (538.1 and

412.7), followed by *Batillaria zonalis* (357.7 and 252.7), and both species have very large standard deviations (449.2 and 315.6). The iron concentration of both species were higher than that of *Assiminea brevicula* from Bangladesh, which is 235 ± 10.11 μg/g [15]. It is suggested that there is partial iron contamination within the tidal flats.

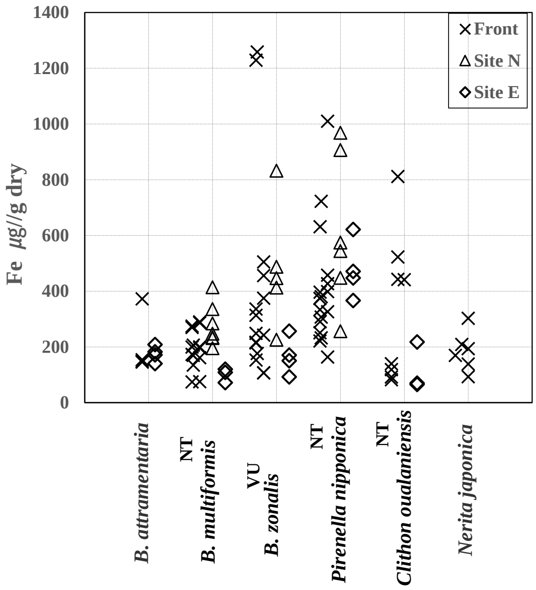

Fig.5 Fe concentration (μg/g-dry) in the soft body of snails.

Table 4 Fe concentration (μg/g-dry) in the soft body of snails, when totaled for all sampling periods.

Species	Site	Mean	SD	Median	n
Batillaria attramentaria	Total	190.5	76.9	162.9	8
	Front	205.3	111.5	151.4	4
	Site E	175.7	28.3	177.1	4
Batillaria multiformis	Total	206.6	86.2	199.7	24
	Front	193.8	70.3	198.2	14
	Site E	100.3	25.0	108.8	3
	Site N	277.6	75.1	247.5	7
Batillaria zonalis	Total	375.7	315.6	252.7	24
	Front	396.0	363.3	248.6	15
	Site E	167.7	68.0	160.8	4
	Site N	481.3	220.5	447.2	5
Pirenella nipponica	Total	538.1	449.2	412.7	28
	Front	407.3	209.6	374.1	17
	Site E	476.9	106.4	459.5	4
	Site N	890.8	767.7	574.6	7
Clithon oualaniensis	Total	273.2	246.4	140.0	11
	Front	331.4	265.3	290.6	8
	Site E	117.9	86.6	70.1	3
Nerita japonica	Front	184.6	71.1	181.8	6

As for mean values, these species were followed by *Clithon oualaniensis*, *Batillaria multiformis*, *Batillaria attramentaria*, and *Nerita japonica* in descending order. However, as for median values, they were followed by *Batillaria multiformis*, *Nerita japonica*, *Batillaria attramentaria*, and *Clithon oualaniensis* in descending order. For third place and below, the rankings differ significantly between the mean and the median. It seems that interspecific comparisons of iron accumulation in snails are more suitable using concentration distributions than using representative values such as mean and median values.

In *Clithon oualaniensis*, a microalgae and detritus feeder, four individuals showed very high values. These were collected in September. Except for these, the concentration distribution was low. Iron is an essential element for the growth of phytoplankton and microalgae, and iron uptake into microalgae may vary seasonally. Investigations such as seasonal changes in snails and microalgae are needed to discuss the cause of the high values.

There was no significant difference in mean value between spring (March to May) and September (t-test, significance level 5%) for the four detritus feeder species.

6.4 Manganese Concentration

Figure 6 shows the manganese concentration in the soft body of snails. There was no significant difference in mean values between spring (March to May) and September (t-test, significance level 5%).

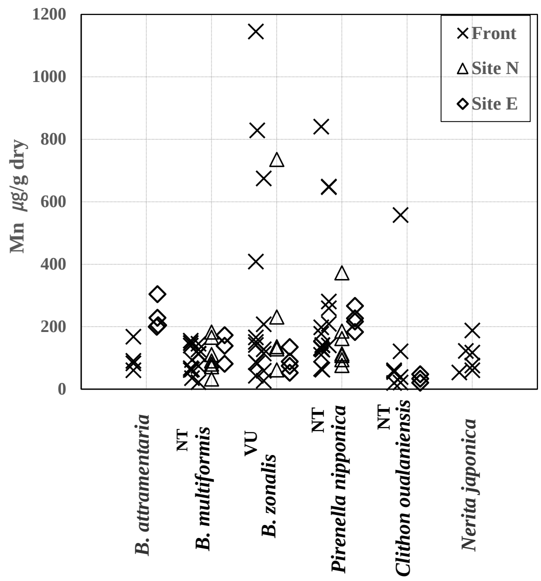

Fig.6 Mn concentration in the soft body of snails.

In *Batillaria zonalis*, the values varied greatly, and some individuals showed extremely high concentrations. In *Pirenella nipponica*, a slightly higher concentration distribution and some higher values were observed. It is suggested that there may be partial manganese contamination within the tidal flat. Except for *Batillaria attramentaria*, there was no significant difference between sites (ANOVA, significance level 5%).

Table 5 Mn concentration (μg/g-dry) in the soft body of snails, when totaled for all sampling periods.

Species	Site	Mean	SD	Median	n
Batillaria attramentaria	Total	167.2	84.1	183.7	8
	Front	100.2	46.7	86.3	4
	Site E	234.3	48.4	216.4	4
Batillaria multiformis	Total	103.4	46.1	101.7	24
	Front	96.9	43.6	102.6	14
	Site E	130.9	46.5	138.7	3
	Site N	104.7	53.4	90.3	7
Batillaria zonalis	Total	246.0	294.8	131.7	24
	Front	284.1	335.2	141.5	15
	Site E	87.5	34.7	81.5	4
	Site N	258.5	273.5	135.7	5
Pirenella nipponica	Total	225.3	188.0	169.6	28
	Front	253.3	229.8	141.2	17
	Site E	223.0	34.5	221.5	4
	Site N	158.6	101.8	113.0	7
Clithon oualaniensis	Total	93.9	156.4	47.4	11
	Front	116.4	181.0	56.6	8
	Site E	33.7	13.5	33.2	3
Nerita japonica	Front	102.6	50.5	96.0	6

Table 5 shows the mean, standard deviation and median values of manganese concentration when totaled for all sampling periods for each species. *Batillaria zonalis* had the highest mean value (246.0 μg/g), followed by *Pirenella nipponica* (225.30 μg/g) and *Batillaria attramentaria* (167.2 μg/g) in descending order. However, regarding the median value, *Batillaria attramentaria* was the highest (183.7 μg /g), followed by *Pirenella nipponica* (169.6μg/g) and *Batillaria zonalis* (131.7μg/g) in descending order.

The ranking was different between the mean and the median. It seems that interspecific comparisons of manganese accumulation in snails are more suitable using concentration distributions than using representative values. In *Batillaria multiformis*, the variation in values was small, showing a low concentration distribution, and both the mean and median values were low. It cannot be explained by feeding habits or species affinity.

On the other hand, for *Clithon oualaniensis*, there was one extremely high value (557.4μg/g), so the standard deviation (156.4) was larger than the average (93.9μg/g). Excluding this extremely high value (557.4) as an outlier yields an average of 47.5 with a standard deviation of 30.2. This outlier was 11.7 times higher than the mean of 47.5. As for *Clithon oualaniensis*, it is presumed that most of the populations in this tidal flat show low manganese concentrations.

6.5 Discussion About Value Variability

For each of the four elements, there were species with small variations in body concentration values and species with large variations. Variation was not uniform. There was a large difference between the concentrations exhibited by most of the population and some outlier concentrations. These outliers, non-uniformity of variation, and concentration distribution patterns were thought to have important implications. It seems more reasonable to think that there is a partial difference in the bottom sediment rather than a mere error or ordinary physiological individual difference.

In general, the sediment properties of tidal flats show a discontinuous distribution [13]. Although not shown in the results because the sediment was not investigated in this study, on September 11, 2022, when the sediment pH was measured at 14 locations at Site Front, pH values were 3.5 to 6.5. The values differed greatly depending on the measurement points. From these, it is expected that the bottom sediment is not uniform even in this tidal flat. And it is presumed that the large variations in metal concentrations in the snails represent the heterogeneity of the metal concentrations in the sediment. In order to clarify this, a fine-grained and detailed sediment survey is desired. In the survey, sufficient attention and ingenuity will be required for the sampling method of sediments.

6.6 Suggestions for Indicator Species in Gastropods

Based on the above results and considerations, the proposed index species for the six gastropod species are summarized in Table 6. Although the sediment was not investigated in this study, exclusive or partial detritus feeders seemed to be more effective as an indicator species than microalgae feeders from the concentration distribution and the degree of variation in values.

For all four elements, *Batillaria zonalis* is considered suitable as an indicator species. They feed on sediment-feeding like other Bitillariidae species, but it was reported in 2004 [17] to also feed in suspension [18]. Although the feeding rate is not specified, the filtration rate is said to be lower than that of bivalves in the same tidal flat [17]. This species is ranked as Vulnerable in Red Data and is the most endangered species in the survey. *Pirenella nipponica*, a near-threatened species, is considered suitable as an indicator species for zinc, iron and manganese. Possibly, red data species might be more sensitive to metal contamination.

Table 6 Species considered to be effective as indicator species

Species	RD	Feeding	Cu	Zn	Fe	Mn
Batillaria attramentaria		detritus	○	△		
Batillaria multiformis	NT	detritus	○	○		
Batillaria zonalis	VU	detritus& suspension	○	○	○	○
Pirenella nipponica	NT	detritus		○	○	○
Clithon oualaniensis	NT	microalgae &detritus			△	
Nerita japonica		microalgae				

It is known that since iron and manganese have similar chemical properties, they often behave together in the environment, and there are many cases where iron and manganese coexist in groundwater [19]. Iron-manganese oxides are also widely present in suspended-sedimented particles in seawater [20]. It makes sense that iron and manganese share the same species that are considered useful as indicators.

7. CONCLUSIONS

Regarding copper, *Batillaria attramentaria*, *B. multiformis*, which are detritus feeder, and *B. zonalis*, which are detritus and suspension feeder, showed higher concentrations and large variations in copper (121.4-233.4, 50.7-261.8 and 42.1-276.3 μg/g-dry) than other species. The mean values were 182.7, 117.7 and 133.2μg/g, respectively. Among gastropods, these three species of genus *Batillaria* are considered to be useful as indicator species for copper.

Regarding zinc, *Batillaria multiformis*, *B. zonalis*, and *Pirenella nipponica* showed a higher concentration distribution than microalgal feeders. They showed very large variability (112.2-1429.7, 64.1-973.4 and 85.1-952.3 mg/g), with several individuals showing extremely high concentrations. The mean values of these species were 399.2, 258.4 and 238.1 μg/g, respectively, which were much higher than those of *Batillaria attramentaria* in the literature [14] (about 200 at Banzu, about 100 at Higashihama). Even within the same species, the pattern of concentration distribution differed greatly depending on the site, it was presumed that it reflected the difference in zinc concentration in the environment. They are considered useful as indicator species for zinc.

Regarding iron, *Batillaria zonalis* and *Pirenella nipponica* showed a higher distribution of iron concentration than *Nerita japonica*, an algae feeder, and a very large variation of iron (92.5-1258.4 and 163.8-2537.4μg/g). Among gastropods, these two species are considered useful as indicator species for iron.

Regarding manganese, in *Batillaria zonalis*, the values varied greatly (26.8-1145.4μg/g), and some individuals showed extremely high concentrations. *Pirenella nipponica* showed a rather high concentration distribution and wide variation (62.3-840.7μg/g). Among gastropods, these two species are considered to be useful as indicators of manganese.

Extremely high concentrations of zinc were observed, especially in the inner part of the tidal flat, sandwiched between small islands and land. Here, the water flow tends to be blocked by islets, mud accumulates easily, and it has been anaerobic in the past. Wakagawa estuary tidal flat was severely polluted by industrial wastewater in the past. It is suggested that there is zinc contamination in this estuary at the time and that it remained as a hotspot in areas of poor water exchange within the tidal flats.

As for copper, iron and manganese, it is suggested that there is partial contamination in this tidal flat.

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