Internal elements of the millipede, *Chamberlinius hualienensis* Wang (Polydesmida: Paradoxosomatidae)

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Abstract

The concentrations of 54 elements of the millipede *Chamberlinius hualienensis* and the soil including leaf litters were examined using inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS). In all the millipedes, the Ca concentration derived from the calcium carbonate in the exoskeleton was the highest, at an average of 76 µg/mg-weight, and the other major elements were the following: Mg, K, Na, Fe, Al, Cu, Zn, Sr, Ba, Mn and Ti (>1 ng/mg-weight), whereas Ni, Cr, V, Ga, Mo, Co, Cd, etc. were at trace levels. Interestingly, the contents of Ca, Mg, K, Na, Zn, Sr, V, Co, Ag, and Sc in larvae were higher than in adult millipedes. Although the heavy metal contents, in the order of Cu>Pb>Cd, were similar to those of other invertebrates such as chilopoda, collembola, and carabidae, there was a high concentration of Cu in the millipedes. The other internal metals, As and Ag, were dependent on the soil concentrations in the habitat. Zn concentrations at sampling sites were different, but a constant level was maintained in the millipede. The results suggest that millipedes may provide information on the accumulation or regulation mechanism in the body for certain hazardous heavy metals. The C1 chondrite normalization pattern for lanthanoid series elements in both the millipede and soil indicated that the environmental habitats were well protected from pollution. The characteristics of internal elements and metal accumulation in the millipede in terms of relation to the habitat would be useful information for environmental pollution studies.

Key words: Millipede; *Chamberlinius hualienensis*; ICP-AES; ICP-MS; element

INTRODUCTION

Soil invertebrates have often been used as bioindicators for environmental pollution caused by heavy metals such as Cd, Cu, Pb, and Zn because their habitats are in close contact with contaminants in soils. The accumulation levels of heavy metals in earthworms are commonly used as risk assessments for environments or invertebrates eating animals (Roberts and Johnson, 1978; Ireland, 1979; Beyer et al., 1985; Heikens et al., 2001). Millipedes inhabit the same areas as other invertebrates and they are distributed widely around the world.

Millipedes discharge odorous secretions that cause headaches and unpleasant feelings for humans (Eisner et al., 1978; Mori et al., 1994;

Noguchi et al., 1997a). Recent studies demonstrated that the odorous compounds are different according to sex and habitat (Noguchi et al., 1997b; Taira and Arakaki, 2002; Taira et al., 2003). Such studies for active compounds in the millipede have elucidated much detailed information. However, knowledge regarding the essential elements or heavy metals is only provided for hazardous heavy metals like Cu, Cd, Pb, and Zn, and only for a few species of millipedes, and the amount of physiological data is yet insufficient (Beyer et al., 1985; Hunter et al., 1987; Gräff et al., 1997).

In Okinawa, *Chamberlinius hualienensis* Wang belongs to Polydesmida: Paradoxosomatidae migrates during the rainy season (from May to the end of June) and/or during the reproductive period (Higa et al., 1992). The millipede is found in damp

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areas and feeds on fallen or withered leaves in the soil. Its lifecycle is one year, and therefore slight changes in the environment over the short-term period are reflected in the body. In the present study, the internal elements of the millipede collected from non-polluted fields were examined by ICP-AES and ICP-MS.

MATERIALS AND METHODS

A mix standard (U, Be, Bi, Ce, Co, In, Pb, Mg, Ni) and other authentic elements were obtained from AccuStandard Inc. (CT, USA) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan). These were used to make calibration curves. Reagent grade water with a resistance of 18.2 M Ω made using a Milli-Q water purification system (Millipore Co., MA, USA) was used for all experimental preparations. Nitric acid, hydrogen peroxide and ethanol were of analytical reagent grade.

In the autumn of 2000, adults and 7th stage larvae of *C. hualienensis* were collected at Ginowan and Haeburu in Okinawa, Japan. Each insect was weighed and its sex was determined by the presence (male) or absence (female) of gonads. The millipedes were defecated for at least 1 d, and then individually preserved in 70% ethanol until measurement. The adult millipedes collected at Ginowan were 237 $\pm$ 45 mg (mean $\pm$ S.D., $n=3$). The Haeburu samples were 190 mg (mean, $n=2$) for adults and 75 $\pm$ 9 mg (mean $\pm$ S.D., $n=3$) for 7th stage larvae. Soil samples of 1 cm in depth from surface including leaf litters as the possible food source of the millipedes were collected at five points in each sampling area. The soil samples were mixed and air-dried. Pebbles were filtered out and the soil samples were then ground in an agate mortar.

Each millipede together with the ethanol solution was transferred into a Teflon beaker, and 5 ml of nitric acid (60%) was added. The mixture was covered with a watch glass and then carefully heated until the brown-colored gas was dissipated. After cooling the sample solution, the addition of 2 ml hydrogen peroxide (30%) completely decomposed the sample. The soil sample (500 mg) was broken down similarly to the millipede. Finally, all samples were adjusted to 50 ml with nitric acid (1%) for measurement using ICP-AES (Shimadzu ICPS-7000, Shimadzu) and ICP-MS (HP-4500 se-

Table 1. Analytical conditions of ICP-AES and ICP-MS

ICP-AES	
Plasma conditions	
RF frequency	40.68 MHz
RF power	1.0 kW
Coolant gas flow rate	8.0 l/min Ar
Auxiliary gas flow rate	0.6 l/min Ar
Carrier gas flow rate	0.6 l/min Ar
Observation height	8 mm
Nebulizer	Pyrex glass, coaxial type
Sample uptake rate	1.5 ml/min
ICP-MS	
Plasma conditions	
RF frequency	27.12 MHz
RF power	1.5 kW
Plasma gas flow rate	17 l/min Ar
Auxiliary gas flow rate	1.0 l/min Ar
Carrier gas flow rate	1.23 l/min Ar
Sampling conditions	
Sampling depth	8 mm
Sampling cone	Nickel
Skimmer cone	Nickel
Nebulizer	PEEK, Babington type
Sample uptake rate	0.4 ml/min
Data acquisition	
Data point	3 points/peak
Number of scan	100 times

ries, Hewlett Packard).

The concentrations of 49 elements in the millipede were determined using the ICP-MS with ^{72}Ge (10 ppb) and ^{103}Rh (10 ppb) as the internal standard. ICP-AES measurement with an internal standard Y (50 ppb) was applied to obtain accurate data for Ca, Mg, K, Na, and Fe because they are affected by interfering ions such as ArO^+ , ArOH^+ , and CO_2^+ . The detailed operational conditions of the equipment are summarized in Table 1. Each elemental concentration in the sample was determined by three times measurements.

RESULTS AND DISCUSSION

The concentrations of 54 elements in the millipedes and soil samples were examined using ICP-AES and ICP-MS (Table 2). In the millipedes, Ca (av. 76 $\mu\text{g}/\text{mg}$ -weight) occupied ca. 19% of the body weight, derived from the calcium carbonate in the exoskeleton (Hattori and Moriya, 1987). The other major elements having values larger than 1 ng/mg-weight in all specimens were the follow-

Table 2. Elemental contents in *Chamberlinius hualienensis* and soil

No ^a	Element	Wavelength (nm)	<i>m/z</i>	Millipede [ng/mg-weight]			Soil [ng/mg]	
				Ginowan	Haebaru		Ginowan	Haebaru
					Adult (<i>n</i> =3)	Adult (<i>n</i> =2) 7th stage larva (<i>n</i> =3)		
1	Ca	317.933		61,912	60,245	101,372	25,280	46,819
2	Mg	279.553		1,391	1,350	2,609	3,070	6,781
3	K	766.491		1,283	1,045	1,920	3,331	4,593
4	Na	589.592		504	462	819	1,272	1,001
5	Fe	259.940		120	305	252	24,186	16,852
6	Al		27	101	174	225	22,245	14,526
7	Cu		65	115	93	168	20	17
8	Zn		66	117	89	188	425	85
9	Sr		86	18	20	37	22	23
10	Ba		137	18	6.9	27	74	76
11	Mn		55	7.2	14	20	329	272
12	Ti		47	5.6	14	24	203	220
13	As		75	2.5	0.58	1.3	12	4.2
14	Pb		208	0.92	1.9	0.92	24	15
15	Ni		60	0.53	0.73	0.92	21	18
16	Cr		53	0.40	0.82	0.64	49	21
17	V		51	0.34	0.51	0.72	41	33
18	Ga		69	0.57	0.20	0.92	7.6	6.1
19	Li		7	0.10	0.34	0.41	25	23
20	Ce		140	0.16	0.29	0.23	39	17
21	Zr		90	0.16	0.19	0.23	6.6	6.1
22	Mo		95	0.13	0.22	0.16	0.64	0.69
23	Co		59	0.12	0.14	0.23	4.7	5.8
24	Y		89	0.10	0.14	0.12	28	6.1
25	La		139	0.089	0.15	0.14	20	7.8
26	Nd		146	0.082	0.15	0.12	20	8.2
27	Cd		111	0.079	0.072	0.11	0.89	0.35
28	Cs		133	0.062	0.051	0.036	2.2	1.9
29	Pd		105	0.043	0.053	0.068	0.39	0.14
30	Pr		141	0.022	0.033	0.028	4.7	1.9
31	Gd		157	0.017	0.029	0.022	4.3	1.6
32	Ag		107	0.049	0.073	0.36	0.062	0.33
33	Nb		93	0.0015	0.017	0.0018	0.20	0.12
34	Sn		118	<0.023 ^b	0.11	<0.013 ^b	0.40	0.055
35	Sm		147	<0.022 ^b	0.027	0.022	4.1	1.7
36	U		238	<0.0091 ^b	0.023	0.014	1.5	0.54
37	Sc		45	0.019	<0.010 ^b	0.044	7.0	4.7
38	Dy		163	<0.015 ^b	0.018	0.017	3.7	1.2
39	Er		166	<0.011 ^b	0.012	0.0085	2.0	0.55
40	Se		82	<0.18 ^b	<0.18 ^b	0.11	0.46	0.34
41	Eu		153	<0.0082 ^b	<0.0082 ^b	0.0051	0.95	0.36
42	Tl		205	<0.013 ^b	<0.013 ^b	<0.0078 ^b	0.21	0.091
43	Te		125	<0.0061 ^b	<0.0061 ^b	<0.0036 ^b	0.042	0.010
44	Yb		172	<0.011 ^b	<0.011 ^b	<0.0064 ^b	1.4	0.45
45	Be		9	<0.023 ^b	<0.023 ^b	<0.013 ^b	0.53	0.41
46	In		115	<0.011 ^b	<0.011 ^b	<0.0064 ^b	0.031	0.029
47	Tb		159	<0.0082 ^b	<0.0082 ^b	<0.0048 ^b	0.64	0.22

Table 2. (Continued)

No ^a	Element	Wavelength (nm)	<i>m/z</i>	Millipede [ng/mg-weight]			Soil [ng/mg]	
				Ginowan	Haebaru		Ginowan	Haebaru
					Adult (<i>n</i> =3)	Adult (<i>n</i> =2) 7th stage larva (<i>n</i> =3)		
48	Ho		165	<0.0088 ^b	<0.0088 ^b	<0.0051 ^b	0.74	0.21
49	Tm		169	<0.0098 ^b	<0.0098 ^b	<0.0058 ^b	0.25	0.075
50	Ta		181	<0.029 ^b	<0.029 ^b	<0.017 ^b	<0.013 ^b	<0.013 ^b
51	W		182	<0.11 ^b	<0.11 ^b	<0.063 ^b	<0.047 ^b	<0.047 ^b
52	Pt		195	<0.019 ^b	<0.019 ^b	<0.011 ^b	<0.0084 ^b	<0.0084 ^b
53	Au		197	<0.081 ^b	<0.081 ^b	<0.048 ^b	<0.035 ^b	<0.035 ^b
54	Bi		209	<0.024 ^b	<0.024 ^b	<0.014 ^b	0.16	0.17

^a Elements No. 1–5 were analyzed with ICP-AES and the others were obtained from ICP-MS.

^b Values indicate less than limit of quantitation.

ing: Mg, K, Na, Fe, Al, Cu, Zn, Sr, Ba, Mn, and Ti. On the other hand, 16 elements such as Ni, Cr, V, Ga, Mo, Co, and Cd were only present at trace levels (<1 ng/mg-weight), and the concentrations of Be, In, Tb, Ho, Tm, Ta, W, Pt, Au, and Bi were below the limit of quantitation. Generally, the major elements except for Al, Sr, Ba, Ti and the trace elements of Mo and Co are considered essential elements for almost all insects, but the physiological function of each metal is not yet understood (Bowen, 1979). Interestingly, the contents of Ca, Mg, K, Na, Zn, Sr, V, Co, Ag, and Sc in the larvae were higher than those in adult millipedes. Of these elements, Ca is a major element of the exoskeleton, thus other elements also may be required for developing the larvae with exfoliation.

Environmental pollution studies in the field have been widely conducted using terrestrial invertebrates as the biological indicator (Ireland, 1979; Beyer et al., 1985; Hunter et al., 1987; Van Straalen and Van Wensem, 1986; Rabitsch, 1995). The hazardous heavy metal concentrations in the millipedes were in the order of Cu>Pb>Cd, which is similar to other invertebrates such as chilopoda, collembola and carabidae (Rabitsch, 1995). In particular, Cu was found at a remarkably high concentration in the millipede; for example, the Cu content for dry-body weight was 7-, 3- and 32-fold higher than that for chilopoda, collembola and carabidae, respectively. A similar phenomenon was also observed in other species of millipede (Beyer et al., 1985; Rabitsch, 1995). Interestingly, the Cu

and Pb concentrations in the soil at both sampling sites were close, but the amount of Cu accumulated was more than 100-folds that of the Pb in the millipedes, and the internal concentration of Cu was 4–11 times higher than that of the soil. On the other hand, the concentrations of Cr, Ni, and As in the body were 1/100–1/10 of the soil concentrations. As concentration in the Ginowan soil sample was three times higher than that of the Haebaru soil sample, the difference in environmental habitat was well reflected in the millipedes of both sampling sites. A similar phenomenon was also seen in the case of Ag. The results suggest that the millipedes may provide information on the accumulation mechanism in the body for certain toxic metals. Such physiological features of the millipede would be a useful index environmental pollution.

The Zn concentration in the soil from Ginowan was approximately five times higher than that of the Haebaru sample, whereas all millipedes from both habitats had nearly similar concentrations. Zn is an essential element for various physiological functions related to metabolism. Heikens et al. (2001) reported that Zn is maintained at a constant level in invertebrates, independent of the soil concentration. The millipede also maintains Zn in a range of 77–205 ng/mg-weight as an essential element in the body.

Recently, lanthanoid series elements such as Ce, Pr, and Nb are used as raw materials for industrial products such as glass stains, permanent magnets and condensers, which then make their ways into

Fig. 1. C1 chondrite normalized lanthanoid series element patterns in the millipede and soil. Each point is expressed as an average of the values obtained from the millipedes (○) and soil (●) at Ginowan, or the millipedes (△) and soil (▲) at Haebaru.

the environment. It is expected that the mass release of such potent toxic elements may cause environmental destruction (Kawasaki et al., 1998). The lanthanoid series elements originate from meteorites and elements having even atomic numbers are more abundant than those of the adjoining odd atomic number by the Oddo-Harkins rule, which must be satisfied when the environment is well protected from pollution. The data are usually normalized by each lanthanoid series element in the C1 chondrite and are often applied for environmental assessment (Teranishi et al., 2003). As shown in Fig. 1, the C1 chondrite normalized lanthanoid series element patterns for both the millipede and soil sample were clearly expressed as decline lines accompanied by an increase in the atomic number. The results demonstrate that the environmental habitats were well protected from pollution.

In conclusion, it was determined for the first time that there are 54 elements in the millipede *Chamberlinius hualienensis*. Ca was the highest concentration, and other major elements included Mg, K, Na, Fe, Al, Cu, Zn, Sr, Ba, Mn, and Ti, whereas Ni, Cr, V, Ga, Mo, Co, Cd, etc. were found at trace levels (<1 ng/mg-weight). Internal As and Ag concentrations were dependent on the soil habitat. Zn concentration was maintained at a constant level as an essential element. The results suggest that the elemental contents of millipedes provide information on the accumulation and/or regulation mechanisms in the body for certain heavy metals, which would be useful information for environ-

mental pollution studies.

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