BioMetals (2005) 18:651–658
DOI 10.1007/s10534-005-4575-z

Distribution of elements in the millipede, *Oxidus gracilis* C. L. Koch (Polydesmida: Paradoxosomatidae) and the relation to environmental habitats

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Received 31 May 2005; accepted 24 October 2005

Key words: millipede, *Oxidus gracilis*, element, ICP-AES, ICP-MS

Abstract

The concentrations of 55 elements in the millipede, *Oxidus gracilis*, soil and plant in the habitat were examined using inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS). In all the millipedes, Ca concentration derived from calcium carbonate in the exoskeleton was the highest at average 94 μg/mg-weight. The other major elements were the following: Mg, K, Na, Zn, Fe, Al, Cu, Sr, Ba, Mn and Ti (>1 ng/mg-body weight), whereas Se, Mo, Ag, Cd, Co, Li and Ce etc. were in trace levels. Interestingly, the various 15 elements such as Ca, Na, Zn, Al, Ba, Ga, Ag, Cd, Co and Y in environmental habitats were well reflected in the body of the millipede. Although the heavy metal contents, in the order of Cu > Pb > Cd, were similar to those of other invertebrates, Cu in the millipede was remarkably high concentration. Zn was maintained in a range of 72–394 ng/mg-weight as essential element in the body and no difference was found in the sexes. The C1 chondrite normalization pattern for lanthanoid series elements in the millipede, soil and plant indicated that the environmental habitats were well protected from pollution. These characteristics of internal elements and metal accumulation in the millipede or relation to their habitats would be useful information for the environmental pollution studies.

Introduction

Soil invertebrates have often been used as bioindicator for environmental pollution caused by heavy metals such as Cd, Cu, Pb and Zn, because they inhabit in close contact with contaminants in soils. It has been reported that metal accumulations in many species were in line with site contamination (Roberts & Johnson 1978; Hunter *et al.* 1987; Rabitsch 1995; Heikens *et al.* 2001). Millipedes inhabit the same areas with other invertebrates and they distribute widely around the world.

Millipede discharges smell secretions, which cause headaches and unpleasant feelings for humans (Eisner *et al.* 1978; Mori *et al.* 1994; Noguchi *et al.* 1997a). Recent studies demonstrated that the smell compounds were different in the sexes and habitats (Noguchi *et al.* 1997b; Taira & Arakaki 2002; Taira *et al.* 2003). Such studies for active compounds in the millipede have been elucidated in detail. However, knowledge regarding the essential elements or heavy metals is only provided for hazardous heavy metals of Cu, Cd, Pb and Zn in a few species of millipedes, and the amount of physiological data is yet insufficient

(Beyer *et al.* 1985; Hunter *et al.* 1987; Rabitsch 1995; Gräff *et al.* 1997). On the other hand, the various potent hazardous metals are used in raw materials of industrial products with technical development, which are accumulating in environment. Therefore the establishment of fundamental data for the various metal accumulations or elemental distribution in organisms is important point to consider the environment.

The millipede, *Oxidus gracilis*, distributes widely over the world and is usually found in damp area with other invertebrates and migrates during the rainy season in Okinawa (from May to the end of June) and/or their reproductive period. Its life cycle is one year, and therefore slight changes in the environment over the short term period would be reflected in the body of the millipede.

The present study established the various 55 elemental distributions in the millipede and its environmental habitat.

Materials and methods

Adult millipedes *O. gracilis* were collected from different habitats around the middle (Ginowan) and the southern areas (Haebaru) in Okinawa Island, Japan. Each individual was weighed and its sex was determined by the presence (male) or absence (female) of gonads. The millipedes were defecated for at least one day and then the individually preserved in 70% ethanol until measurements. The millipedes collected at Ginowan were 40 ± 5 mg (mean $\pm$ SD, $n = 5$) for male and 66 ± 8 mg (mean $\pm$ SD, $n = 5$) for female. The Haebaru samples were 44 ± 2 mg (mean $\pm$ SD, $n = 5$) for male and 54 ± 5 mg (mean $\pm$ SD, $n = 5$) for female. Soil of ca. 1 cm in depth and plant (withered leaf) collected at five points in each area were air-dried and were ground in an agate mortar. Finally each sample was mixed and measured as a typical sample in the habitats.

The authentic elements including a mix standard (U, Be, Bi, Ce, Co, In, Pb, Mg, Ni) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and AccuStandard Inc. (CT, USA). Reagent grade water with a resistance of 18.2 MΩ made by Milli-Q water purification system (Millipore Co., MA, USA) was used for all experimental preparations. All chemicals were of analytical reagent grade. Each millipede together with 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 up until the brown-colored gas was disappeared. After cooling the sample solution, the addition of 2 ml hydrogen peroxide (30%) completely decomposed the sample. Each 500 mg of the soil or plant sample was digested similarly to the millipede.

Samples were adjusted to 50 ml with nitric acid (1%) for measurement using ICP-AES (Shimadzu ICPS-7000, Shimadzu) and ICP-MS (HP-4500 series, Hewlett Packard). The 50 elements in the sample were determined by the ICP-MS with ^{103}Rh (10 ppb) as an internal standard. To obtain accurate data, ICP-AES measurement with an internal standard Y (50 ppb) was applied for the high concentrations of Na, Mg, K, Ca, and Fe affected by the interfering ions such as ArO^+ and $ArOH^+$. The parameters of the equipments were summarized in Table 1.

Each elemental concentration in the sample was determined in triplicate measurements. The statistical analysis was carried out with non-parametric Mann–Whitney *U*-test and *p*-value less than 0.05 expressed as a significant difference.

Results and discussion

The concentrations of 55 elements in the millipede, soil and plant in the habitats were examined using ICP-AES and ICP-MS (Tables 2 and 3).

In the millipedes, Ca (average 94 μg/mg-weight) occupied ca. 24% of the body weight, derived from calcium carbonate in the exoskeleton (Hattori & Moriya 1987). The other major elements having value larger than 1 ng/mg-weight in all individuals were the following: Mg, K, Na, Zn, Fe, Al, Cu, Sr, Ba, Mn and Ti. On the other hand, 16 elements such as Se, Mo, Ag, Cd, Co, Li and Ce were present at trace levels (<1 ng/mg-weight), and the concentrations of Au, Bi, Be, Pt, In, Ta, Lu, Nb were below the limit of quantitations. Generally, the major elements except for Al, Sr, Ba, Ti and the trace elements of Mo and Co are considered as essential elements for almost all insects, but the physiological functions of each metal is not yet clear (Bowen 1979). Interestingly, the contents of 20 elements in the body showed significant

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
Internal standard element	Y (wavelength: 371.029 nm)
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
Internal standard element	Rh (mass number: 103)

difference between the habitats and the concentrations of 15 elements such as Ca, Na, Zn, Al, Ba, Ga, Ag, Cd, Co and Y seem to be influenced by their environment.

Okinawa Island soils are classified in three major groups of Kunigami-mahji, Shimajiri-mahji and Jahgaru (Onaga & Yoshinaga 1988). In the sampling areas, Ginowan of Shimajiri-mahji was covered with a dark-red colored soil and Haebaru of Jahgaru areas were a gray colored soil. A few metals, Ca, Mg, K, Na in the soil eluent at both areas was measured but not sufficient to discuss from a view point of the environmental habitats (Tokashiki & Shimo 1983). This study examined the various elements in soil and established elemental distribution as shown in Table 3.

The difference of elemental distribution between both sites was clearly demonstrated. The 24 elements such as Cr, Zn, As, Cd, La, Ce, U in Ginowan soil were contained 2 times higher concentrations than in Haebaru, whereas major elements of Ca, Mg, K and Na were high concentration in Haebaru. The pH of Jahgaru in Haebaru was at 7.8, which was slightly alkaline soil compared to that of Shimajiri-mahji (pH 7.4) in Ginowan. The alkaline pH of the soil may be affected by amounts of Ca, Mg, K and Na. Interestingly, the elements of Ca, Na, Zn, Al, Ba, Ga, Ag, Cd, Co and Y in soil at both sites were well reflected in the body of millipede. The order of metal contents in plant the following Ca, Zn, Ag, Cd, Co and Y was also correspondent to that of millipede. The results demonstrated internal metals of millipede would be affected by environmental habitat.

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 & van Wensem 1986; Rabitsch 1995). The hazardous heavy metal concentrations in the millipedes were in the order of Cu > Pb > Cd, which was similar to the 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 3-, 4- and 13-fold higher than that for chilopoda, collembola and carabidae, respectively. A similar phenomenon was also observed in the other species of millipede (Beyer *et al.* 1985; Rabitsch 1995). Interestingly, Cu and Pb concentrations in the soil at both sampling sites were in close, but the amount of Cu accumulated was more than 200-fold that of the Pb in the millipedes. The internal content of Cu was 3–8 times higher than that of the soil, whereas Pb was only concentrated 1/1000-1/20 of the soil concentration. Hemocyanin which has a function as oxygen transporter in millipedes is large respiratory cooper protein (Jaenicke *et al.* 1999). A large amount of Cu may be required to construct of the significant metalloprotein in tissue. Taken together, the results suggested that the millipede may provide information on accumulation or regulation mechanisms in the body for certain toxic metals.

Zn contents in the soil and plant from Ginowan were high levels of three or eight times compared to those of the Haebaru samples. The differences in the environmental habitat were well reflected in the millipede. Zn is an essential element with various physiological functions related to metabolism. Heikens *et al.* (2001) reported that Zn was

Table 2. Internal distribution of elements in *O. gracilis*.

Element[a]	Wavelength (nm)	m/z	Ginowan [Average ± SD (ng/mg-body weight)]			Haebaru [Average ± SD (ng/mg-body weight)]		
			Male ($n=5$)	Female ($n=5$)	Total average ($n=10$)	Male ($n=5$)	Female ($n=5$)	Total average ($n=10$)
**Ca	317.933		94700 ± 5770	74071 ± 4420	84385 ± 11903	106497 ± 12617	99271 ± 8225	102884 ± 10739
Mg	279.553		5045 ± 326	3733 ± 123	4389 ± 730	5096 ± 647	4736 ± 467	4916 ± 565
K	766.491		2741 ± 444	2358 ± 316	2550 ± 416	2517 ± 265	2590 ± 165	2554 ± 211
*Na	589.592		312 ± 158	174 ± 31	243 ± 129	313 ± 44	387 ± 138	350 ± 104
**Zn		66	255 ± 68	281 ± 95	268 ± 79	114 ± 52	173 ± 66	143 ± 64
Fe	259.940		247 ± 82	136 ± 43	192 ± 85	226 ± 98	237 ± 68	231 ± 80
*Al		27	228 ± 15	121 ± 42	174 ± 64	87 ± 45	117 ± 43	102 ± 45
Cu		65	141 ± 38	106 ± 21	124 ± 34	155 ± 22	137 ± 35	146 ± 29
**Sr		86	23 ± 4.2	18 ± 2.7	21 ± 4.2	38 ± 7.4	34 ± 3.6	36 ± 5.9
**Ba		137	38 ± 15	25 ± 4	31 ± 13	14 ± 2	14 ± 2	14 ± 1.8
**Mn		55	8.5 ± 2.5	7.7 ± 1.5	8.1 ± 2.0	20 ± 8.1	38 ± 10.0	29 ± 13
Ti		47	12 ± 6.3	12 ± 3.0	12 ± 4.7	11 ± 3.2	11 ± 2.5	11 ± 2.7
Zr		90	4.5 ± 3.3	2.5 ± 1.4	3.5 ± 2.6	1.5 ± 0.4	1.8 ± 0.8	1.6 ± 0.57
*As		75	2.7 ± 3.0	0.6 ± 0.0	1.7 ± 2.3	1.8 ± 0.9	1.6 ± 0.4	1.7 ± 0.70
Ni		60	1.0 ± 0.67	0.57 ± 0.36	0.81 ± 0.56	0.55 ± 0.17	0.75 ± 0.13	0.65 ± 0.18
**Ga		69	1.3 ± 0.54	0.86 ± 0.13	1.1 ± 0.45	0.37 ± 0.08	0.34 ± 0.05	0.36 ± 0.065
Pb		208	0.65 ± 0.50	0.54 ± 0.57	0.59 ± 0.51	0.77 ± 0.28	0.85 ± 0.16	0.81 ± 0.22
Sn		118	0.84 ± 1.9	0.92 ± 1.4	0.88 ± 1.6	0.33 ± 0.45	0.088 ± 0.20	0.21 ± 0.35
Se		82	0.58 ± 0.20	0.45 ± 0.08	0.52 ± 0.16	0.44 ± 0.14	0.41 ± 0.07	0.42 ± 0.11
V		51	0.56 ± 0.43	0.33 ± 0.10	0.44 ± 0.32	0.41 ± 0.20	0.42 ± 0.13	0.42 ± 0.16
Cr		53	0.56 ± 0.47	0.27 ± 0.09	0.42 ± 0.35	0.28 ± 0.14	0.45 ± 0.18	0.37 ± 0.17
*Mo		95	0.20 ± 0.25	0.23 ± 0.11	0.22 ± 0.18	0.49 ± 0.32	0.47 ± 0.18	0.48 ± 0.24
*Ag		107	0.23 ± 0.09	0.18 ± 0.17	0.20 ± 0.13	0.43 ± 0.21	0.30 ± 0.23	0.36 ± 0.22
**Cd		111	0.32 ± 0.18	0.36 ± 0.20	0.34 ± 0.18	0.13 ± 0.023	0.10 ± 0.016	0.12 ± 0.024
**Co		59	0.14 ± 0.047	0.14 ± 0.024	0.14 ± 0.035	0.24 ± 0.064	0.33 ± 0.123	0.28 ± 0.10
**Li		7	0.11 ± 0.067	0.063 ± 0.046	0.087 ± 0.060	0.25 ± 0.091	0.30 ± 0.088	0.27 ± 0.089

Ce	140	0.21 ± 0.11	0.17 ± 0.13	0.19 ± 0.11	0.14 ± 0.068	0.20 ± 0.079	0.17 ± 0.076
La	139	0.13 ± 0.053	0.10 ± 0.065	0.12 ± 0.058	0.08 ± 0.035	0.10 ± 0.035	0.087 ± 0.035
*Y	89	0.15 ± 0.074	0.097 ± 0.030	0.13 ± 0.061	0.071 ± 0.036	0.075 ± 0.019	0.073 ± 0.027
Nd	146	0.13 ± 0.057	0.10 ± 0.065	0.11 ± 0.059	0.073 ± 0.030	0.095 ± 0.039	0.084 ± 0.035
**Pd	105	0.034 ± 0.044	0.014 ± 0.0071	0.024 ± 0.032	0.088 ± 0.027	0.071 ± 0.012	0.080 ± 0.022
*Cs	133	0.030 ± 0.0084	0.018 ± 0.0078	0.024 ± 0.010	0.046 ± 0.031	0.039 ± 0.0085	0.043 ± 0.022
Pr	141	0.028 ± 0.012	0.023 ± 0.015	0.025 ± 0.013	0.017 ± 0.006	0.024 ± 0.0089	0.020 ± 0.0082
Tl	205	0.022 ± 0.012	0.027 ± 0.015	0.024 ± 0.013	0.022 ± 0.004	0.019 ± 0.0032	0.021 ± 0.0036
Gd	157	0.023 ± 0.012	0.016 ± 0.0094	0.019 ± 0.011	0.016 ± 0.007	0.020 ± 0.0071	0.018 ± 0.0068
Sm	147	0.022 ± 0.013	0.019 ± 0.011	0.020 ± 0.012	0.013 ± 0.008	0.019 ± 0.0065	0.016 ± 0.0074
Dy	163	0.017 ± 0.011	0.011 ± 0.0050	0.014 ± 0.0084	0.008 ± 0.006	0.013 ± 0.0060	0.010 ± 0.0065
Er	166	0.010 ± 0.0061	0.0072 ± 0.0016	0.0087 ± 0.0045	0.0066 ± 0.0038	0.0076 ± 0.0017	0.0071 ± 0.0028
Eu	153	0.0054 ± 0.0037	0.0050 ± 0.0019	0.0052 ± 0.0028	0.0041 ± 0.0024	0.0043 ± 0.0013	0.0042 ± 0.0018
Yb	172	0.0037 ± 0.0035	0.0019 ± 0.0014	0.0028 ± 0.0027	0.0026 ± 0.0027	0.0042 ± 0.0012	0.0034 ± 0.0021
Tb	159	0.0018 ± 0.0016	0.0023 ± 0.0016	0.0020 ± 0.0015	0.0023 ± 0.0012	0.0027 ± 0.0011	0.0025 ± 0.0011
Ho	165	0.0021 ± 0.0020	< 0.0014	0.0017 ± 0.0015	0.0023 ± 0.0011	0.0030 ± 0.00063	0.0026 ± 0.00093
**W	182	< 0.092[b]	< 0.092[b]	< 0.092[b]	0.26 ± 0.16	0.12 ± 0.10	0.19 ± 0.15
**U	238	< 0.0078[b]	< 0.0078[b]	< 0.0078[b]	0.020 ± 0.0066	0.027 ± 0.0085	0.023 ± 0.0081
**Te	125	< 0.0052[b]	< 0.0052[b]	< 0.0052[b]	0.0062 ± 0.0053	0.0060 ± 0.0043	0.0061 ± 0.0046
Sc	45	< 0.0090[b]	< 0.0090[b]	< 0.0090[b]	< 0.0090[b]	< 0.0090[b]	< 0.0090[b]
Tm	169	< 0.0013[b]	< 0.0013[b]	< 0.0013[b]	< 0.0013[b]	< 0.0013[b]	< 0.0013[b]
Au	197	< 0.069[b]	< 0.069[b]	< 0.069[b]	< 0.069[b]	< 0.069[b]	< 0.069[b]
Bi	209	< 0.021[b]	< 0.021[b]	< 0.021[b]	< 0.021[b]	< 0.021[b]	< 0.021[b]
Be	9	< 0.019[b]	< 0.019[b]	< 0.019[b]	< 0.019[b]	< 0.019[b]	< 0.019[b]
Pt	195	< 0.016[b]	< 0.016[b]	< 0.016[b]	< 0.016[b]	< 0.016[b]	< 0.016[b]
In	115	< 0.0094[b]	< 0.0094[b]	< 0.0094[b]	< 0.0094[b]	< 0.0094[b]	< 0.0094[b]
Ta	181	< 0.0025[b]	< 0.0025[b]	< 0.0025[b]	< 0.0025[b]	< 0.0025[b]	< 0.0025[b]
Lu	175	< 0.0012[b]	< 0.0012[b]	< 0.0012[b]	< 0.0012[b]	< 0.0012[b]	< 0.0012[b]
Nb	93	< 0.00061[b]	< 0.00061[b]	< 0.00061[b]	< 0.00061[b]	< 0.00061[b]	< 0.00061[b]

Significant difference for comparing both sampling sites: *$p < 0.05$, **$p < 0.01$. [a]Elements of Ca, Mg, K, Na and Fe were analyzed with ICP-AES and the others were obtained from ICP-MS. [b]Values indicate less than limit of quantitation.

Table 3. Distribution of elements in soil and plant in habitats.

Element[a]	Wavelength (nm)	m/z	Ginowan		Haebaru	
			Soil (ng/mg)	Plant (ng/mg)	Soil (ng/mg)	Plant (ng/mg)
Ca	317.933		24619	25940	63865	29772
Mg	279.553		3192	2948	10285	3278
K	766.491		2621	4041	5275	3911
Na	589.592		153	2391	787	1215
Zn		66	307	542	99	71
Fe	259.940		47522	850	32680	1025
Al		27	43533	957	27966	1087
Cu		65	29	9.8	23	11
Sr		86	38	5.9	36	11
Ba		137	136	12	125	28
Mn		55	567	92	485	59
Ti		47	383	23	406	33
Zr		90	13	0.38	12	0.40
As		75	23	0.60	7.7	0.72
Ni		60	42	1.2	34	1.8
Ga		69	14	0.63	11	1.1
Pb		208	45	2.4	23	6.1
Sn		118	0.81	< 0.010[b]	0.11	< 0.010[b]
Se		82	0.88	0.050	0.56	0.12
V		51	80	2.4	63	3.0
Cr		53	96	1.9	40	2.3
Mo		95	0.52	0.76	0.31	1.1
Ag		107	0.10	0.024	0.62	0.029
Cd		111	1.2	0.59	0.43	0.27
Co		59	9.2	0.24	11.2	0.44
Li		7	49	1.1	45	2.0
Ce		140	76	1.4	33	1.2
La		139	39	0.78	15	0.60
Y		89	54	1.2	12	0.52
Nd		146	40	0.79	16	0.57
Pd		105	0.74	0.034	0.24	0.041
Cs		133	4.2	0.17	3.5	0.20
Pr		141	9.3	0.18	3.7	0.14
Tl		205	0.40	0.020	0.17	0.015
Gd		157	8.5	0.17	3.0	0.10
Sm		147	8.1	0.16	3.2	0.11
Dy		163	7.3	0.14	2.3	0.074
Er		166	3.9	0.079	1.1	0.034
Eu		153	1.9	0.035	0.70	0.033
Yb		172	2.8	0.056	0.87	0.025
Tb		159	1.3	0.024	0.42	0.015
Ho		165	1.4	0.028	0.41	0.014
W		182	< 0.047[b]	< 0.047[b]	< 0.047[b]	< 0.047[b]
U		238	2.9	0.065	1.0	0.043
Te		125	0.083	< 0.0027[b]	0.020	< 0.0027[b]
Sc		45	14	0.30	9.0	0.30
Tm		169	0.49	0.0094	0.15	0.0047
Au		197	< 0.035[b]	< 0.035[b]	< 0.035[b]	< 0.035[b]
Bi		209	0.30	0.025	0.31	0.028

Table 3. Continued.

Element[a]	Wavelength (nm)	m/z	Ginowan		Haebaru	
			Soil (ng/mg)	Plant (ng/mg)	Soil (ng/mg)	Plant (ng/mg)
Be		9	1.0	0.02	0.79	0.03
Pt		195	< 0.0084[b]	< 0.0084[b]	< 0.0084[b]	< 0.0084[b]
In		115	0.060	< 0.0048[b]	0.052	0.006
Ta		181	< 0.013[b]	< 0.013[b]	< 0.013[b]	< 0.013[b]
Lu		175	0.41	0.0074	0.14	0.0036
Nb		93	0.36	0.041	0.19	0.049

[a]Elements of Ca, Mg, K, Na and Fe were analyzed with ICP-AES and the others were obtained from ICP-MS.[b]Values indicate less than limit of quantitation.

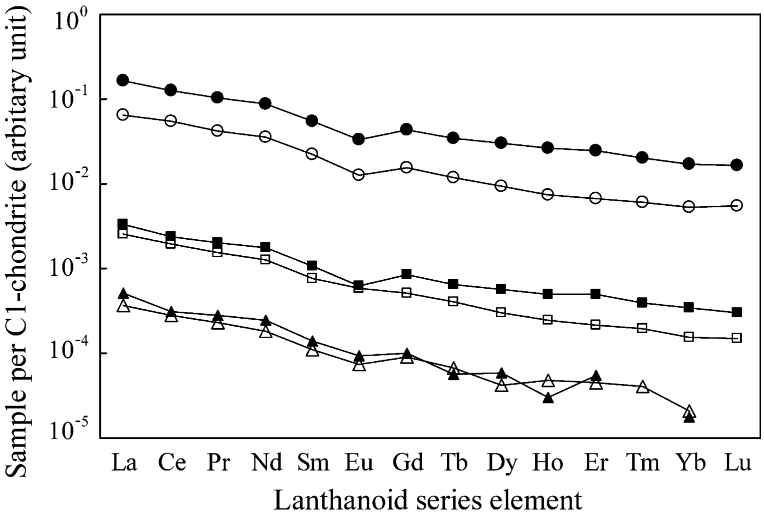

Figure 1. C1 chondrite normalized lanthanoid series element patterns in the millipede, soil and plant. Each point is expressed as an average of the values obtained from the millipedes (▲), soil (●) and plant (withered leaf) (■) at Ginowan, or the millipedes (△), soil (○) and plant (withered leaf) (□)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

maintained at a constant level in invertebrates. The millipede also maintained Zn in a range of 72–394 ng/mg-weight as an essential element in the body and no differences were found in the sexes.

Recently, the lanthanoid series elements such as Ce, Pr and Nd are used as raw materials for industrial products such as glass stains, permanent magnets and condensers, which spread into environment. It is supposed that the mass release of such potent toxic elements may cause environmental destruction in near future (Kawasaki *et al.* 1998). The lanthanoid series elements originate in meteorite and elements having even atomic numbers are more abundant than those of the adjoining odd atomic numbers 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 Figure 1, the C1 chondrite normalized lanthanoid series elements patterns for the millipede, soil and plant samples were clearly expressed as declined lines accompanied by an increase in the atomic number. The results demonstrated that the environmental habitats were well protected from pollution.

In conclusion, it was determined for the first time that there are 55 elements in the millipede *O. gracilis*. Ca was the highest concentration and other major elements were the following: Mg, K, Na, Zn, Fe, Al, Cu, Sr, Ba, Mn and Ti, whereas Se, Mo, Ag, Cd, Co, Li and Ce etc. were in trace levels (< 1 ng/mg-body weight). Interestingly, the various 15 elements such as Ca, Na, Zn, Al, Ba, Ga, Ag, Cd, Co and Y in environmental habitats were well reflected in the millipede. The present study suggested that the elemental contents of millipedes provide information on the accumulation and/or regulation mechanisms in the body for certain metals, which would be useful information for the environmental pollution studies.

Acknowledgements

We would like to thank S. Sakiyama their (Okinawa Prefectural Enterprise Bureau) and Y. Higa (Okinawa Millipede Research Laboratory) for their warm cooperation throughout this study. We are also grateful to Dr. A. Tokuyama and Mr. M. M. Kombo of University of the Ryukyus for their helpful discussions on this paper.

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