Effects of heavy metals on benthic macroinvertebrate communities in high mountain streams

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Abstract – We characterized the responses of benthic macroinvertebrates to heavy metals released from mines in high mountain streams of the Gangqu River in the Shangrila Gorge, China. Benthic macro-invertebrates were collected with a kick-net at 32 sampling sites. In addition, 25 environmental variables including heavy metal concentrations were measured at each sampling site. Although the concentrations of heavy metals were not seriously high, their effects were reflected in the changes of community composition of benthic macroinvertebrates. Total abundance and species richness decreased with increasing heavy metal concentrations. Species richness of Plecoptera and Trichoptera, Margalef richness index, and percentage of scrapers in functional feeding groups were negatively correlated with heavy metal concentrations. A high variation was observed in the response of different taxa to heavy metals. Heavy metals mainly affected the sensitive taxa of Plecoptera, Ephemeroptera, and Trichoptera. However, some tolerant taxa were observed in Trichoptera (such as *Hydropsyche* sp.) and *Dugesia* sp. Cluster analysis and a non-metric multidimensional scaling analysis were applied to characterize specific macroinvertebrate taxa composition. The analyses revealed the effects of different environmental factors such as geographical, hydromorphological, physical, and chemical factors including heavy metals on the distribution of benthic macroinvertebrates in high mountain streams. Although the effects were compounded with different factors such as altitude, temperature, stream width, turbidity, and heavy metals, the influence of heavy metals on benthic macroinvertebrate communities was clearly identified (decreased species richness, changes in species composition). Even though the contamination of heavy metals was low in the sampling area, the consequences were clear, indicating that long-term exposure to heavy metals could seriously impact aquatic communities in high mountain streams.

Key words: Benthos / China / high mountain streams / mines / pollution / invertebrates

Introduction

Heavy metal pollution is of widespread concern for ecological management of streams and rivers. Drainage water from both active and abandoned mines may be the major source of heavy metal contamination in mountain streams. Most field studies of the effects of heavy metals on aquatic organisms, including benthic macroinvertebrates, have been conducted in mountain streams of Europe and the Americas, *e.g.*, in Sweden (Malmqvist and Hoffsten, 1999), Ireland (Gray and Delaney, 2008), Spain (Solà and Prat, 2006), Colorado (Clements, 1994), Idaho (Maret *et al.*, 2003), Virginia (Cherry *et al.*, 2001), and Bolivia (van Damme *et al.*, 2008). In China, the effects of heavy metal pollution are underestimated due to limited research in high mountain areas (Jiang *et al.*, 2008). There is, however, a pressing need for ecological risk assessments to protect and manage aquatic ecosystems in these areas.

Biomonitoring approaches have been used to assess the effects of contaminants on aquatic organisms since the early 1900s (Carpenter, 1924). Most of these researches have focused on responses of benthic macroinvertebrates (Carlisle and Clements, 2003). Benthic macroinvertebrate assemblages contain species with various sensitivities to contaminants and have been widely used to evaluate the ecological impacts of metal contamination in streams (Maret *et al.*, 2003). Metal contamination can reduce benthic macroinvertebrate species richness, as well as density, growth and production (Maret *et al.*, 2003; Gray and Delaney, 2008). Heavy metals can be accumulated

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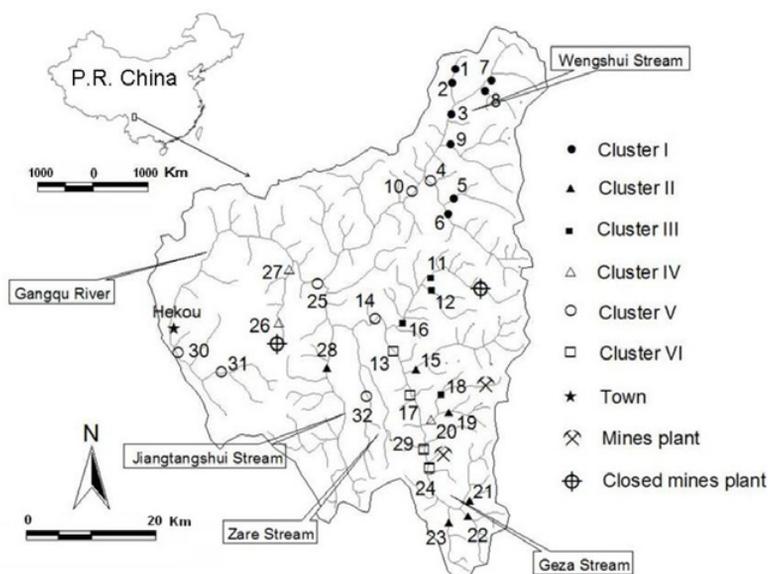*Corresponding author: parkys@khu.ac.kr

Fig. 1. Sampling sites in the Gangqu River of Shangrila Gorge. The different symbols indicate different clusters of the sampling sites based on the cluster analysis (see Fig. 3).

in the gut and tissues of individuals (Solà and Prat, 2006), which can affect predator-prey interactions in macro-invertebrate (Clements, 1999) and even fish communities (Freund and Petty, 2007) through the food web.

The effects of heavy metals on macroinvertebrates are highly variable among taxa (Kiffney and Clements, 1996). Heavy metals may also have an effect on ecosystem function. Clements et al. (2000) recorded a change in functional feeding groups (FFG) of benthic macroinverte-brates with lower abundances of scrapers, shredders, collectors, and predators at stations with medium- and high-concentrations of heavy metals, compared with reference stations. Predators may be the most sensitive species to heavy metals, especially Zn (Carlisle and Clements, 1999; Watanabe et al., 2008). Correspondingly, a shift in community composition from sensitive to tolerant taxa can occur when aquatic ecosystems are contaminated by heavy metals, thereby affecting the whole food web (Beltman et al., 1999). These general patterns are consistent across many regions in lowland areas (Freund and Petty, 2007). Benthic macroinvertebrates, however, were found to be more sensitive to heavy metals at higher elevations (Clements et al., 2000).

The generality of these responses in metal-polluted streams from high mountain areas has not been ex-tensively examined in China. In this study we aimed at characterizing the effects of low contaminations of heavy metals released from both active and abandoned mines on benthic macroinvertebrate communities. The study area is in the Gangqu River of the Shangrila Gorge at South-West of China. Human population density is low in this area, so the exploitation of minerals is the main human disturbance (Wu et al., 2007). The Shangrila Gorge has been defined as a national reserve park of China, and it is also located in the world natural heritage "Three Parallel Rivers", formed basically by three large rivers of Asia: the Yangtze, Mekong and Salween. This area is an epicentre of Chinese biodiversity, and also one of the richest temperate regions of the world in terms of biodiversity (UNESCO, 2003). We characterized the effects of low concentrations of heavy metals released from both active and abandoned mines on benthic macroinvertebrate communities in high altitude mountain streams of the Gangqu River system, an important upstream tributary of the Yangtze River.

Materials and methods

Study area

Sampling sites were located in the Gangqu River of the Shangrila Gorge (N28°07' ~ 28°36', E99°22' ~ 100°06') in China (Fig. 1). The Gangqu River is a tributary of the upper reaches of the Jingsha River which is one of main tributaries of the Yangtze River. The Gangqu River has a length of 98 km with catchment area of 2644 km^2. Its average discharge is 43.7 $m^3.s^{-1}$, with a maximum of 122 $m^3.s^{-1}$ and a minimum of 11.2 $m^3.s^{-1}$. It has an average gradient of 15% and a natural fall of more than 2000 m. There are two main tributaries of the Gangqu River: the Wengshui Stream located in the northern part of the watershed, and Geza Stream located in the southern region. The study area is highly canopied and undisturbed by human activity at high altitude areas (from 2150 to 3771 m).

Four mines (two active and two abandoned for Pb and Zn) were identified in the study area. The active mines are located at the watershed of the Geza Stream. The two abandoned mines – formerly active in Pb and Zn

mining – are at the tributaries of the Jiangtanghui and Geza streams, respectively (Fig. 1). Additionally, there were local reports of several abandoned mines in the northern part of downstream areas, although their precise numbers and locations were not presently determined because of limited information and difficulty to access the areas.

Sample collection

Benthic macroinvertebrates were collected at 32 sampling sites using a kick-net (1 m × 1 m, 500 μm mesh) in May 2005 before the monsoon season. Three replicates were taken at each sampling site with two samples in riffles and one sample in an area with relatively low velocity. Each sample was put into a separate one liter jar and fixed with 4% formalin. Considering the mines mainly located at the upstream area, 26 sites were sampled from the first-to-third order streams in the study area and the remaining six sites were from fourth and fifth order streams. Ten sampling sites were in the Wongshui Stream, 15 were in the Geza Stream, with the remainder located in the main portion of the stream and small tributaries (Fig. 1).

The organisms of each sample were preserved in 70% alcohol. In the lab, we rinsed the samples through a sieve (500 μm mesh). The retained invertebrates were sorted and identified generally to genus level. While Chironomidae and Hirudinea were identified to family level, the Oligochaeta, Nematoda and Hydracarina were not identified due to the relatively low numbers of individuals.

Environmental factors

At each sampling site, 25 physical, chemical, hydrological, and geographical factors were measured (Table 1). Dissolved oxygen (DO), water temperature, total dissolved solids (TDS), pH, nitrate (NO_3-N), calcium (Ca), chloride (Cl), fluoride (F), ammonia (NH_3-N), and turbidity were measured *in situ* with a water quality monitoring system (Horiba, W-23XD). Altitude was measured using a GPS receiver (Magellan 315). Current velocity was measured with an OTT® electromagnetic current meter. Stream width and water depth were measured with a ruler. The other environmental factors were measured in the lab. The concentrations of five heavy metals (Cu, Pb, Zn, Cd, and Ni) were determined by a standard atomic absorption spectrophotometric method (APHA, 1992) using a spectrophotometer (Hitachi, Z-8000). SiO_2 was measured using the molybdosilicate method (APHA, 1992). Total phosphorus (TP), total nitrogen (TN), chemical oxygen demand (COD_{Mn}), and phosphate (PO_4-P) were measured according to the Chinese Standard Methods (Huang, 1999), and nitrite (NO_2-N) and Chlorophyll-*a* (Chl-*a*) were determined by using UV spectrophotometric method according to the Standard Methods for Water and Wastewater Monitoring

Table 1. Characteristics of environmental factors measured at the sampling sites.

Environmental factors	Unit	Mean	SD	Range
Width	m	8.41	5.59	1.00–30.00
Depth	cm	26.30	12.17	4.33–60.00
Velocity	m.s^{-1}	0.79	0.28	0.32–1.79
Altitude	M	3144.34	392.54	2150–3771
Temperature	°C	9.05	3.33	4.55–18.99
pH		7.91	0.26	7.30–8.50
DO	mg.L^{-1}	11.72	1.54	8.38–14.16
Turbidity	NTU	22.83	29.05	3.10–120.00
TDS	mg.L^{-1}	90.31	30.95	40.0–160.00
Cl	mg.L^{-1}	1.33	1.16	0.07–4.67
F	mg.L^{-1}	0.11	0.15	0.004–0.623
Ca	mg.L^{-1}	71.79	127.80	4.48–621.00
SiO_2	mg.L^{-1}	4.95	0.96	3.62–8.15
Cd	μg.L^{-1}	0.35	0.13	0.12–0.61
Cu	μg.L^{-1}	5.87	10.29	1.10–57.70
Pb	μg.L^{-1}	12.05	14.80	3.60–89.10
Zn	μg.L^{-1}	13.12	23.69	0.00–97.30
Ni	μg.L^{-1}	3.25	0.89	1.90–5.20
PO_4-P	mg.L^{-1}	0.006	0.0064	0.0003–0.0202
TP	mg.L^{-1}	0.009	0.0050	0.0012–0.0214
NO3-N	mg.L^{-1}	0.299	0.0828	0.133–0.507
TN	mg.L^{-1}	1.05	0.17	0.678–1.358
NH_3-N	mg.L^{-1}	0.10	0.06	0.019–0.233
COD_{Mn}	mg.L^{-1}	1.19	0.54	0.60–2.85
Chl-*a*	mg.L^{-1}	45.71	44.24	2.73–174.71

and Analysis (Chinese Environmental Protection Chief Bureau, 2002).

Data analyses

We examined several commonly used metrics of community composition such as total abundance, total species richness, and species richness of selected taxa (Ephemeroptera, Plecoptera, and Trichoptera; EPT) based on the assumption that they are the most sensitive insect taxa to heavy metal pollution. Community indices such as Shannon diversity index (Shannon, 1948), Pielou's evenness (Pielou, 1966), and Margalef richness index (Clifford and Stephenson, 1975) were calculated at each sampling site. The following functional feeding groups (FFGs) were defined for each sampling sites: collector-filterers, collector-gatherers, predators, shredders, and scrapers (see Merritt and Cummins, 1996).

Differences of benthic macroinvertebrate communities in relation to environmental variables were analyzed using multivariate statistical analyses: hierarchical cluster analysis and non-metric multidimensional scaling (NMDS). Cluster analysis was used to classify the sampling sites based on the species abundance of macroinvertebrate communities using Ward's linkage method with Euclidean distance measure using PC-ORD software (McCune and Mefford, 1999). Differences among clusters were tested
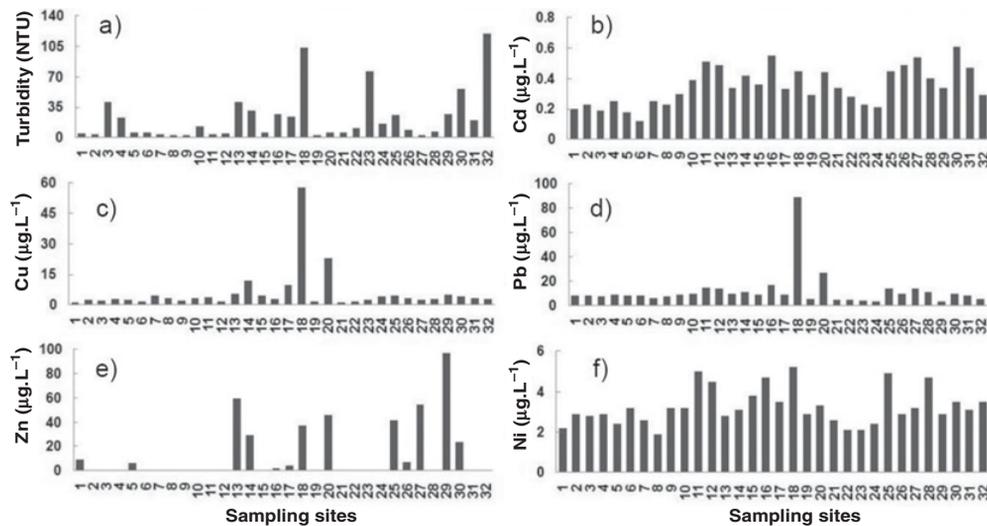
Fig. 2. Differences of environmental factors at each sampling site. a) Turbidity, b) DO, c) cadmium, d) copper, e) lead, f) zinc, g) nickel.

by the multi-response permutation procedure (MRPP), which is a non-parametric method for testing multivariate differences among pre-defined groups (Zimmerman *et al.*, 1985). The Sørensen coefficient was used as the distance measure in the MRPP. The significance of the null hypothesis that there was no difference among the groups was tested with a Monte Carlo randomization procedure with 1000 permutations using PC-ORD software (McCune and Mefford, 1999). The differences of environmental variables among different clusters were evaluated using Dunn's multiple comparison tests after Kruskal-Wallis (K-W) test through STATISTICA software (StatSoft, 2004). The community indices were also compared among different clusters by applying the K-W test.

The indicator value analysis (IndVal) (Dufrêne and Legendre, 1997) was used to define the indicator species for different clusters. The indicator value for each species in a group is the product of its relative abundance and its relative frequency ($\times 100$), and ranges from 0 (no indication) to 100 (perfect indication) (Peterson and Keister, 2003). A perfect indicator of a particular group should be faithful and exclusive to that group, never occurring in another group (McCune and Grace, 2002). The significance of the indicator value for each species was tested with a Monte Carlo randomization procedure with 1000 permutations. The indicator values analysis was carried out with the PC-ORD software (McCune and Mefford, 1999).

NMDS was used to characterize differences of benthic macroinvertebrate communities and relations with their environmental factors. The NMDS technique appears to be superior to other ordination techniques when applied to ecological data (Kenkel and Orloci, 1986; Bettinetti *et al.*, 2000; Kwon *et al.*, 2009; Paule *et al.*, 2009). NMDS was performed using PC-ORD (McCune and Mefford, 1999), based on the Bray-Curtis distance measure and 50 runs with a maximum of 100 iterations per run. A Monte Carlo test with 100 randomizations was used to determine the

probability that the observed stress value of the final solution would occur by chance alone (Laughlin and Abella, 2007). To assist interpretation of the changes in community profiles, Spearman correlation coefficients between dimension scores and the environmental variables were calculated and plotted in the NMDS ordination using PC-ORD (McCune and Mefford, 1999).

Prior to the multivariate statistical analyses, those rare species occurring at only one site were excluded, and species densities were $\log(x + 1)$ transformed to reduce variation.

Results

Environmental characteristics

The samples were collected from high mountain wade-able streams at an average elevation above sea level of 3144 m, and most of the sampling sites reflected the shallow riffle habitats with width and depth of 8.4 ± 5.6 m and 26 ± 12 cm, respectively (Table 1). The temperature was relatively low at most sampling sites ($9.0 \pm 3.3\,^{\circ}\text{C}$), whereas DO was relatively high ($11.7 \pm 1.5\,\text{mg.L}^{-1}$) (Table 1). TDS showed relatively high values in the Gangqu River ($90.3 \pm 31.0\,\text{mg.L}^{-1}$), whereas the values of chlorine, fluorine, calcium, and silicon dioxide were low. The lower concentrations of Chl-*a* and COD_{Mn} indicated lower nutrient levels in the Gangqu River.

The turbidity was relatively high with an average of 22.8 ± 29.0 NTU (Fig. 2a), showing the highest values at site 32 due to the erosion of the channel banks. The high turbidity at site 18 was mainly caused by mining activities at the upstream area. The concentrations of heavy metals at the sampling sites reflected the presence of mines at the upper reaches. The concentrations of Cu and Pb were significantly higher at sampling sites 18 and 20 located downstream of a mine (Figs. 1, 2c and 2d). The average

Table 2. Spearman rank correlation coefficients of the environmental variables and community indices. SR: species richness.

Variables	Turbidity	Cd	Cu	Pb	Zn	Ni
Total abundance	−0.27	−0.38*	−0.04	−0.58***	−0.42*	−0.29
Total SR	−0.57***	−0.05	−0.40*	−0.19	−0.35*	−0.19
Ephemeroptera SR	−0.14	0.20	−0.16	0.05	−0.17	0.22
Plecoptera SR	−0.67***	−0.31	−0.44*	−0.32	−0.28	−0.41*
Trichoptera SR	−0.43*	0.08	−0.39*	−0.07	−0.20	−0.19
Shannon diversity index	−0.47**	0.25	−0.34	0.21	−0.03	0.10
Evenness	−0.27	0.31	−0.25	0.28	0.11	0.15
Margalef richness index	−0.38*	0.21	−0.36*	0.12	−0.13	0.00
Collector-filter (%)	0.41*	−0.13	0.06	−0.33	0.02	−0.19
Collector-gather (%)	0.11	0.31	0.17	0.31	0.13	0.28
Predator (%)	−0.42*	0.03	−0.28	0.09	−0.06	−0.11
Scraper (%)	−0.27	0.04	−0.38*	0.11	−0.33	0.22
Shredder (%)	−0.21	0.19	0.03	0.10	0.17	0.24

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

concentration of Zn was 13.12 ± 23.69 µg.L^{-1} (range 0.00–97.30 µg.L^{-1}), showing the highest value at sampling site 29 (Figs. 1 and 2e). The average concentration of Ni was 3.25 ± 0.89 µg.L^{-1} (range 1.90–5.20 µg.L^{-1}), displaying the highest value at sampling site 18 (Fig. 2f). The concentration of Cd averaged 0.35 ± 0.13 µg.L^{-1} (range 0.61–0.12 µg.L^{-1}) with the highest value at sampling site 30 located at the lower reach of Hekou town, while the concentration was relatively low at the upper reach of Wengshui Stream (sampling sites 1–9) (Figs. 1 and 2b). Cd had relatively low concentration compared to other heavy metals. The high values of these heavy metals were likely due to the presence of active and/or formerly active mines upstream of the sampling sites (Fig. 1).

Benthic macroinvertebrates

A total of 86 taxa were identified in the sampling sites in the Gangqu River. Plecoptera, Ephemeroptera, Trichoptera (EPT), and Diptera comprised more than 89% of the total abundance. *Baetis* sp. and *Cinygmula* sp. (Ephemeroptera) were the most abundant taxa, representing 14.5% and 9.1% of the total abundance, respectively. Turbidity was negatively correlated with community indices ($r = -0.38$ to -0.57, $p < 0.05$) with the expection of total abundance and Pielou's evenness (both $r = -0.27$, $p > 0.05$) (Table 2). Among EPT, Plecoptera were the most strongly influenced by turbidity ($r = -0.67$, $p < 0.001$). However, correlations between heavy metals and relative abundance of FFGs were generally insignificant (Table 2).

Both total abundance and species richness were negatively correlated with heavy metals except Ni. Cu concentration was negatively correlated with the relative abundance of scrapers indicating that increased Cu concentration induced a decrease of scrapers (Table 2). Total abundance was not significantly influenced by Cu. Meanwhile, Cd and Pb reduced only total abundance

without decreasing species richness. Zn decreased both total abundance and species richness, whereas Ni did not have significant effects on community indices with the exception of Plecoptera species richness ($r = -0.41$, $p < 0.05$). The correlation analysis revealed the highest effects of Cu on species richness ($r = -0.40$, $p < 0.05$), whereas Pb strongly influenced total abundance ($r = -0.58$, $p < 0.001$).

Among the 86 taxa, 65 were selected based on an occurrence frequency exceeding 15%. Of these 65 taxa, 27 displayed a significant negative correlation with heavy metals (Table 3). Cd had a negative influence on the abundance of eight taxa including *Pericoma* sp. ($r = -0.56$, $p < 0.001$) in Diptera and *Peltoperlopsis* sp. ($r = -0.52$, $p < 0.01$) in Plecoptera. Cu was negatively correlated with 12 taxa including *Glossosoma* sp. ($r = -0.56$, $p < 0.001$) in Trichoptera. Pb had a negative influence on 10 taxa including *Simulium* sp. ($r = -0.62$, $p < 0.001$) in Diptera and *Apatania* sp. ($r = -0.58$, $p < 0.001$) in Trichoptera. Zn influenced negatively 14 taxa including *Cinygmula* sp. ($r = -0.61$, $p < 0.001$) in Ephemeroptera and *Tipula* sp. ($r = -0.56$, $p < 0.01$) in Diptera. Ni decreased the abundance of eight taxa including *Peltoperlopsis* sp. ($r = -0.64$, $p < 0.001$) and *Apatania* sp. ($r = -0.54$, $p < 0.01$). Some taxa were adversely affected by most of the tested heavy metals, while the majority of taxa were affected by only one or two heavy metals. For example, *Peltoperlopsis* sp. had a significant negative correlation with Cd, Cu, Pb, and Ni ($r = -0.44$–-0.64, $p < 0.05$), and *Pericoma* sp. in Diptera was significantly decreased with Cd, Cu, Zn, and Ni ($r = -0.42$–-0.56, $p < 0.05$). Conversely, seven species were positively correlated with the concentration of the heavy metals. Cd had a positive correlation coefficient with seven species including *Hydropsyche* sp. ($r = 0.62$, $p < 0.001$) in Trichoptera, and *Ephemera* sp. ($r = 0.53$, $p < 0.01$) in Ephemeroptera. Meanwhile 31 taxa did not display significant correlation coefficients with heavy metals ($r < 0.344$, $p > 0.05$).

Table 3. Spearman rank correlation coefficients between heavy metals and selected taxa, and indicator species in each cluster.

	Taxa	Occurrence frequency (%)	Correlation coefficients					Indicator species analysis	
			Cd	Cu	Pb	Zn	Ni	Indicator values	Indicating cluster
Plecoptera	*Stavsolus* sp.	53.1	− 0.30	− 0.49**	− 0.18	− 0.44*	− 0.16	39.8*	I
	Nemoura sp.	71.9	− 0.34	− 0.44*	− 0.26	− 0.42*	− 0.25	33.0**	I
	Suwallia sp.	93.8	− 0.39*	− 0.29	− 0.47**	− 0.38*	− 0.39*	25.0*	I
	Papacapnia sp.	53.6	0.37*	− 0.21	0.30	− 0.14	0.20	55.8*	VI
	Capnia sp.	62.5	− 0.23	0.12	− 0.37*	0.06	− 0.32	–	–
	Peltoperlopsis sp.	68.8	− 0.52**	− 0.50**	− 0.44*	− 0.19	− 0.64***	–	–
Ephemeroptera	*Baetis* sp.1	100.0	− 0.04	− 0.30	0.00	− 0.37*	0.13	31.0*	I
	Serratella sp.	75.0	0.15	− 0.13	− 0.22	− 0.29	− 0.02	34.4**	II
	Cinygmula sp.	93.8	− 0.08	− 0.44*	− 0.26	− 0.61***	− 0.04	26.3**	IV
	Ephemera sp.	57.5	0.53**	0.17	0.34	0.15	0.56***	27.1*	VI
	Epeorus sp.	96.9	− 0.44*	− 0.11	− 0.40*	− 0.33	− 0.28	–	–
	Rhithrogena sp.	53.1	− 0.13	− 0.21	− 0.01	− 0.45**	0.07	–	–
	Siphluriscus sp.	53.1	0.06	− 0.37*	0.10	− 0.35	0.08	–	–
Trichoptera	*Rhyacophila* sp.1	81.3	− 0.03	− 0.47**	− 0.04	− 0.43*	− 0.12	31.7*	II
	Sericostriata sp.	58.8	− 0.15	− 0.35	− 0.39*	− 0.40*	− 0.22	44.3*	II
	Brachycentrus sp.	68.8	− 0.09	0.35	− 0.34	0.17	− 0.27	43.4**	VI
	Apatania sp.	53.8	− 0.33	− 0.04	− 0.58***	− 0.02	− 0.54**	–	–
	Apsilochorema sp.	53.1	0.41*	0.37*	0.28	0.18	0.45**	–	–
	Ecclisomyia sp.	55.0	− 0.30	− 0.09	− 0.32	− 0.18	− 0.42*	–	–
	Glossosoma sp.	56.3	− 0.52**	− 0.56***	− 0.37*	− 0.32	− 0.34	–	–
	Himalopsyche sp.	59.4	− 0.10	− 0.51**	− 0.05	− 0.38*	− 0.10	–	–
	Hydropsyche sp.	57.5	0.62***	0.10	0.46**	0.14	0.37*	–	–
	Lepidostoma sp.	71.9	− 0.31	0.07	− 0.38*	− 0.05	− 0.37*	–	–
	Parapsyche sp.	58.8	− 0.10	− 0.38*	− 0.14	0.06	− 0.38*	–	–
	Polycentropus sp.	68.8	0.42*	0.03	0.39*	0.09	0.55**	–	–
	Tinodes sp.	57.5	0.41*	− 0.13	0.32	− 0.07	0.41*	–	–
Diptera	*Pericoma* sp.	59.4	− 0.56***	− 0.52**	− 0.31	− 0.42*	− 0.45*	40.0*	I
	Empididae sp.	81.3	− 0.37*	0.04	− 0.18	− 0.14	− 0.28	30.4*	I
	Tipula sp.	50.0	0.23	− 0.08	− 0.12	− 0.25	0.31	46.8**	IV
	Atherix sp.	100.0	− 0.53**	− 0.07	− 0.45**	− 0.25	− 0.44*	–	–
	Deuterophlebia sp.	65.0	− 0.28	− 0.13	− 0.18	− 0.36*	0.03	–	–
	Simulium sp.	81.3	− 0.50**	− 0.22	− 0.62***	− 0.34	− 0.33	–	–
Others	*Macronychus* sp.	90.6	0.16	− 0.13	− 0.18	− 0.25	− 0.06	31.2**	II
	Microcylloepus sp.	40.6	0.12	–	–	–	–	42.8*	II
	Gammarus sp.	37.5	− 0.01	–	–	–	–	58.0***	IV
	Dugesia sp.	59.4	0.44*	0.21	0.41*	0.28	0.41*	–	–
	Araneae sp.	65.6	− 0.27	− 0.31	− 0.26	− 0.51**	− 0.19	–	–

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Multivariate analyses

The dataset composed of 32 sampling sites with 65 selected taxa displaying more than 15% of occurrence frequency were used in the multivariate analyses. Thirty-two sampling sites were classified into six clusters (I–VI) based on the similarities of community composition (Fig. 3). MRPP revealed significant differences among the clusters (A = 0.137, $p < 0.001$). Overall, the classification reflected influences of the geographical location of sampling sites as well as heavy metals. Cluster I included sampling sites 1–9 (except site 4) located at

the upper reach of the Wengshui Stream, and cluster II included samples mainly from the upstream area of the Geza Stream (Figs. 1 and 3). Sampling sites in clusters III and IV were located at the lower reaches of mines, reflecting the difference of benthic macro-invertebrate communities compared with other sites. Cluster V included seven sampling sites from relatively downstream areas, and cluster VI included four sampling sites located at the main channel of the Geza Stream (Fig. 3).

Through the indicator species analysis, each cluster was characterized with different taxa (Table 3). The indicator
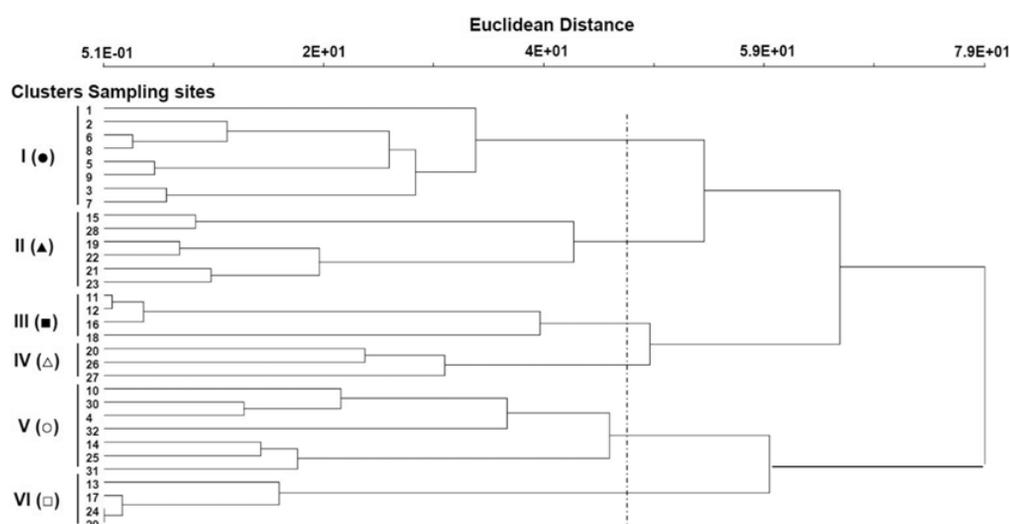
Fig. 3. Classification of the sampling sites with benthic macroinvertebrate communities based on a hierarchical cluster analysis using Ward's linkage method with Euclidean distance measure. Geographical distribution of sampling sites was indicated in Figure 1.
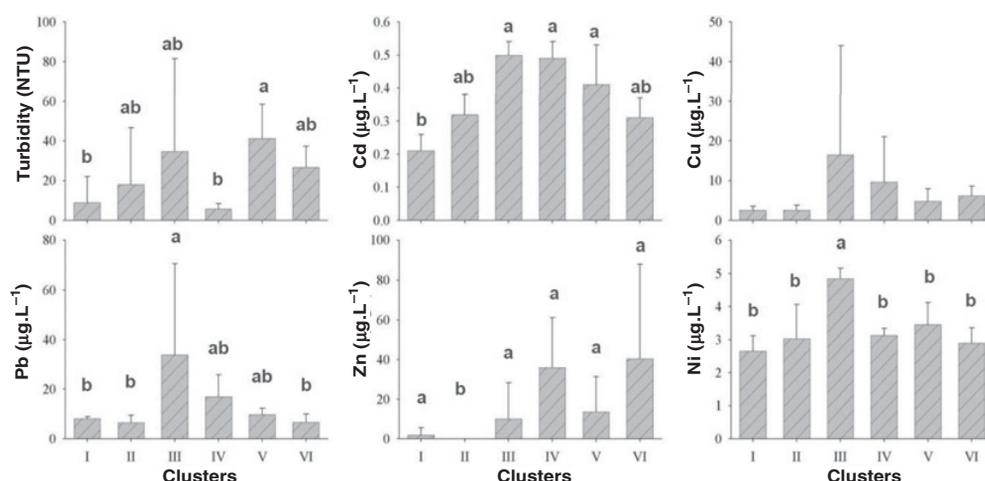

Fig. 4. Differences of heavy metals concentrations and turbidity level at different clusters. The error bars are standard deviation of each variable. The same letter on the error bars indicates no significant differences ($p < 0.05$) among clusters based on the non-parametric Dunn tests.

species were mainly Plecoptera, Ephemeroptera, and Trichoptera for clusters I, II, IV, and VI. However, the indicator species for clusters III and V were not identified. Six taxa including *Pericoma* sp. were identified as indicator species for cluster I, five taxa including *Sericostriata* sp. were indicator species for cluster II, three taxa including *Gammarus* sp. were identified as indicator species for cluster IV. Finally, three taxa including *Paracapnia* sp. characterized cluster VI (Table 3). Six taxa including *Pericoma* sp., *Stavsolus* sp., and *Sericostriata* sp. in clusters I and II were negatively correlated with contamination by heavy metals ($r = -0.39--0.56$, $p < 0.05$). In contrast, four taxa including *Paracapnia* sp. ($r = 0.37$, $p < 0.05$ with Cd) and *Ephemera* sp. ($r = 0.53$, $p < 0.01$ with Cd, and $r = 0.53$, $p < 0.001$ with Ni) in cluster VI were positively correlated with heavy metals (Table 3).

Turbidity was significantly higher in cluster V (41.34 NTU) than in clusters I and IV (5.70–8.85 NTU) (K-W test, $p < 0.05$), whereas other physical (*i.e.*, stream width and depth, altitude, velocity, and temperature) and chemical-environmental factors (DO) were not significantly different among clusters (Fig. 4). For the heavy metals, Cd had significantly higher values in clusters III, IV and V (0.41–0.50 µg.L^{-1}) compared with cluster I (0.21 µg.L^{-1}) (K-W test, $p < 0.001$). Pb was also significantly higher in cluster III (33.93 µg.L^{-1}) than in clusters I, II and VI (6.58–8.09 µg.L^{-1}) (K-W test, $p < 0.01$), and the Zn concentration was significantly lower in cluster II than in other clusters (K-W test, $p = 0.05$). Zn was not detected at sampling sites in cluster II, while it was the highest in cluster VI (40.35 µg.L^{-1}). Ni was significantly higher in cluster III (4.85 µg.L^{-1}) compared with
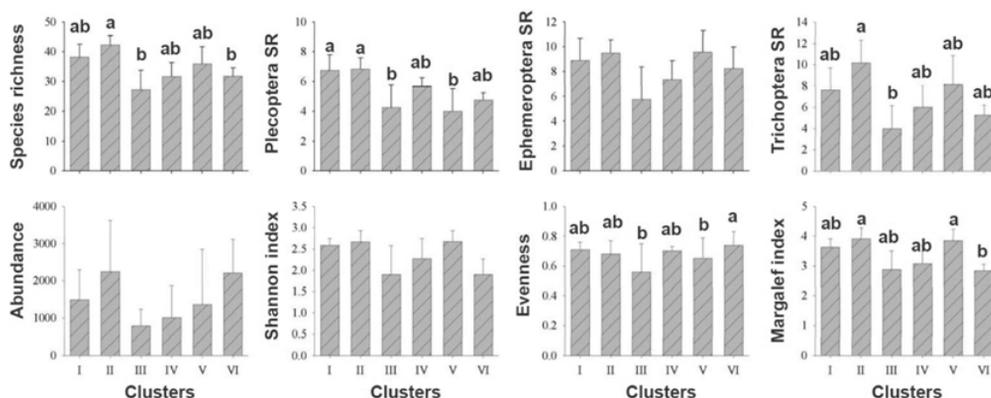
Fig. 5. Community indices at different clusters. The error bars are standard deviation of each variable. The same letter on the error bars indicates no significant differences ($p < 0.05$) among clusters based on the non-parametric Dunn tests.

other clusters (2.65–3.46 µg.L^{-1}) (K-W test, $p < 0.05$). Cu was not significantly different among different clusters, although it was relatively higher in cluster III (16.53 µg.L^{-1}) than in other clusters (2.48–9.60 µg.L^{-1}) (Fig. 4). The sampling sites contaminated with Cd, Cu, Pb, and Ni were mainly found in cluster III, whereas sampling sites influenced by Zn were in clusters IV and VI.

The species richness was significantly lower in clusters III (27 taxa) and VI (32 taxa) than in cluster II (42 taxa) (Fig. 5). Species richness of Plecoptera was significantly lower in clusters V and III (each with four species) compared with cluster I (seven species of Plecoptera), and species richness of Trichoptera was significantly lower in cluster III (four taxa) than in cluster II (10 taxa). Evenness was significantly higher in cluster VI (0.74) than in cluster III and V (0.65) (K-W test, $p = 0.044$), whereas Margalef's index was lower in cluster VI (2.83) compared with clusters II and V (3.85–3.91) (K-W test, $p < 0.01$). The species richness of Ephemeroptera, total abundance, and Shannon diversity index showed no significant differences among clusters (Fig. 5).

Two-dimensional NMDS ordination was obtained with 15.26 of final stress and 0.00 of final instability (Fig. 6). The axes explained significantly more variance than would be expected by chance, based on Monte Carlo permutation tests ($p = 0.01$). Clusters defined by cluster analysis (Fig. 3) were well-reflected in the NMDS ordination plots. The sampling sites of clusters I and II were mainly distributed in the upper middle part of the ordination map, and the sampling sites of cluster III were in the left part. In particular, sampling site 18 which suffered from the highest contamination of Pb and Cu (Fig. 2) was separated from other sites in the far left. The sampling sites of cluster IV were at the upper part of the map, the sampling sites of cluster V were in the lower middle part, and the sampling sites of cluster VI were mainly in the lower right area of the map.

The effects of environmental variables were characterized by calculating correlation coefficients between environmental factors and NMDS axis scores. The variables having significant correlation coefficients ($r < 0.344$, $p < 0.05$) are shown as arrows on the NMDS ordination

(Fig. 6a), where the arrow length indicates the magnitude of the correlation value and the arrow direction implies a correlation with each axis. Axis 1 was highly correlated with Pb ($r = -0.634$, $p < 0.001$) and Cu ($r = -0.527$, $p < 0.01$), thus showing the effects of heavy metals on benthic macroinvertebrate communities. Axis 2 was positively correlated with altitude thus showing the up-to downstream distribution of sites ($r = 0.609$, $p < 0.001$), but it was negatively correlated with width ($r = -0.612$, $p < 0.001$), turbidity ($r = -0.602$, $p < 0.001$), and temperature ($r = -0.499$, $p < 0.01$).

The clusters I and II displayed high relations with higher altitude and low values for turbidity, width, and temperature. In contrast, cluster V had higher turbidity, width, and temperature at lower altitude. Cluster III was explained by high values of heavy metals, indicating strong effects of heavy metal contamination on the species composition, especially at site 18 (Figs. 2 and 6a). The sampling site 26, located on the left part with high correlation with Pb and Cu, also reflected the effects of heavy metals. The sampling site 30 revealed high value of Cd. The cluster IV was in the upper part (high altitude), and the cluster V was at the middle part with high values for turbidity, stream width, and velocity.

Using the NMDS analysis, species could be ordinated on the biplot based on their contribution to the community ordination (Fig. 6b), reflecting their occurrence at different sites. The results were consistent with the IndVal analysis conducted to evaluate indicative species for each cluster. Most of the species negatively correlated with heavy metals were mainly distributed at the upper right part of the NMDS ordination (Fig. 6b). The indicator taxa of cluster I including *Pericoma* sp. and *Stavsolus* sp. were in the upper part of the map, whereas the indicator taxa of cluster II including *Sericostriata* sp. and *Rhyacophila* sp. were in the middle upper part. The indicator taxa of cluster VI including *Paracapnia* sp. and *Ephemera* sp. were in the right part of the map. The species sensitive to heavy metals such as *Peltoperlopsis* sp. (Table 3) were in the uppermost area. In general, all the sensitive species distributed at the reverse direction of the heavy metals contamination. In contrast, the tolerant species
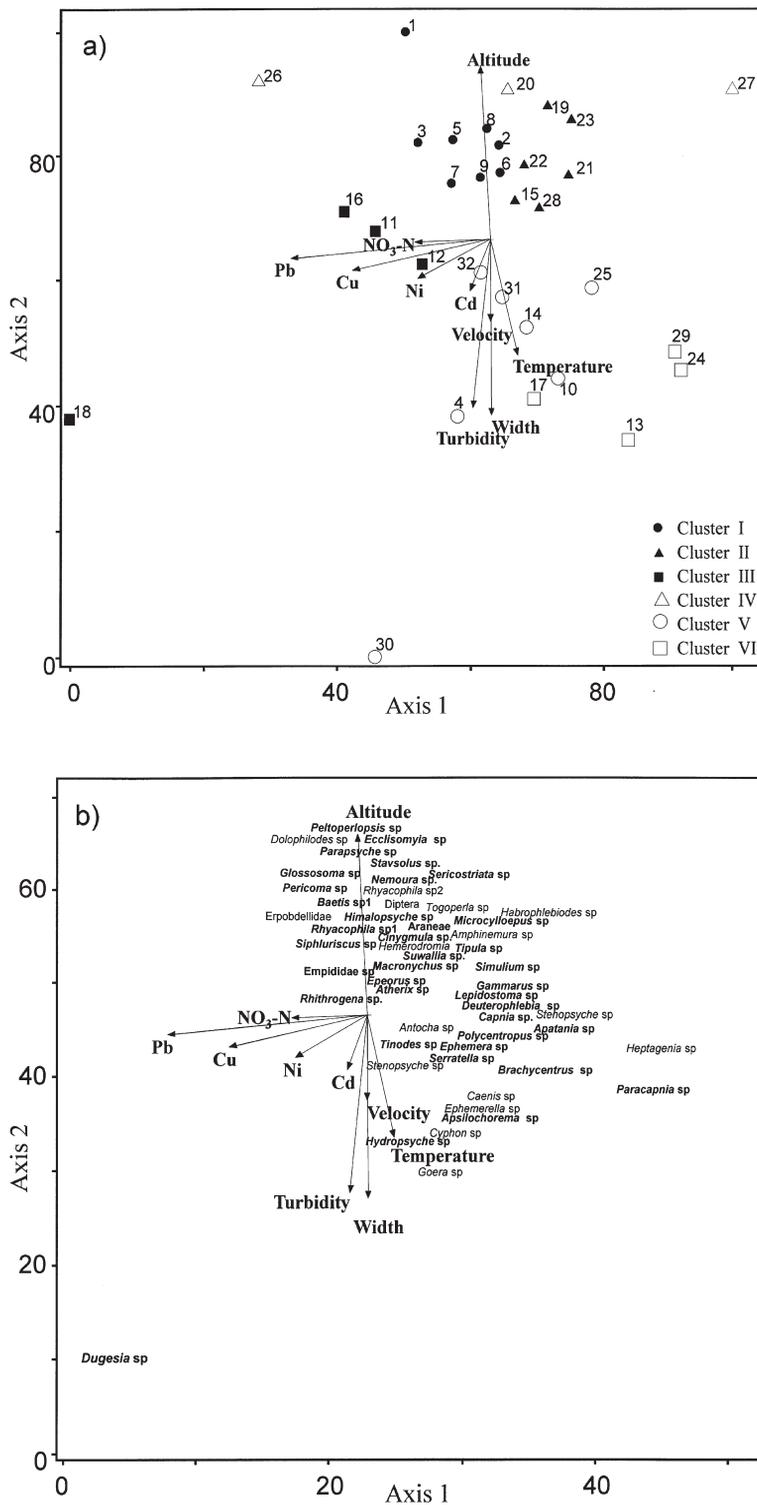
Fig. 6. NMDS ordination with benthic macroinvertebrate communities and environmental factors. a) Ordination of the sampling sites with significant environmental factors, and b) ordination of taxa with significant environmental factors. Correlation coefficients between ordination scores of each axis and environmental factors were calculated and visualized on the ordination plot. The sampling sites of different clusters defined in Figure 3 are indicated by different symbols. Indicator taxa were expressed as a bold type.

Paracapnia sp., *Hydopsyche* sp., *Ephemera* sp., *Polycentropus* sp., and *Apsilochorema* sp. were distributed in the middle and lower parts of the plot and positively correlated with Cd concentration ($r = 0.37$–0.62, $p < 0.05$), whereas

Dugesia sp., which was distributed at the lower left part of the plot, displayed positive correlation with Pb ($r = 0.41$, $p < 0.05$) (Table 3, Fig. 6b). The location of *Dugesia* sp. in the NMDS biplot matched the location of site 18

(cluster III), *Paracapnia* sp. matched heavy metal-contaminated sites 24 and 29 (cluster VI), and *Ephemera* sp., *Polycentropus* sp. and *Apsilochorema* sp. matched the location of sites 25, 14 and 31 (cluster V) (Fig. 6b).

Discussion

Low level contamination of heavy metals

The Gangqu River, in the high mountain area of Shangrila Gorge of China, has been impacted by heavy metals released from presently and formerly active mines. Presently, we observed an increase in Zn, Cu, and Pb concentrations ($> 20 \, \mu g.L^{-1}$) at sampling sites downstream of active and previously active mines, as compared with unpolluted areas that were not downstream of mines. According to the Chinese Environmental Quality Standards for Surface Water (Ministry of Environmental Protection, 2002), the concentrations of heavy metals measured in the sampling sites belonged to classes I and II representing freshwaters of headstreams or national reserves, and reserve area for resident drinking water, respectively. This indicated that contamination by heavy metals was not a serious problem in the study area. However, the deleterious effects of the contaminating heavy metals on macroinvertebrate communities in the Shangrila region watercourses resulted in reduced species richness and changes of the community compositions. The responses of these communities to heavy metals are both severe (Kiffney and Clements, 1996) and unrecoverable (van Damme *et al.*, 2008) in high mountain streams. Thus, it is conceivable that the low levels of heavy metals presently documented in some watercourses might profoundly affect the biological diversity and thus the ecological health of the affected streams.

Various responses of macroinvertebrates

In this study, the effects of heavy metals on macroinvertebrates were reflected in differences of community indices as well as community composition. Total abundance, species richness, and richness of Plecoptera and Trichoptera showed strong negative correlations with heavy metals (Table 2), reflecting the negative effects of heavy metals on stream ecosystems. In contrast, species richness of Ephemeroptera was not significantly decreased by heavy metals levels. However, heavy metal-related deleterious effects on Ephemeroptera have been reported (Specht *et al.*, 1984; Clements, 1994), mostly affected sensitive species (Clements *et al.*, 1988; Clements, 1994).

Heavy metal sensitivity of Ephemeroptera, Plecoptera, and Trichoptera species were apparent at the genus level. *Peltoperlopsis* sp. (Plecoptera) responded the most sensitively, especially with Ni. In Ephemeroptera, *Cinygmula* sp. was the most sensitive species, especially to Zn. *Baetis* sp. abundance decreased with Zn levels, in accordance with previous observations (Clements *et al.*, 1988). Contrarily, heavy metal tolerance of *Baetis* sp. has been reported (Beasley and Kneale, 2002; van Damme *et al.*, 2008). Even at low concentrations of Zn, *Rhithrogena hageni* showed significantly decreasing abundances, and was recorded as a sensitive species (Nelson and Roline, 1996). In Trichoptera, *Rhyacophila* sp. displayed negative responses to Cu and Zn. Similar results, showing decreased population density or absence in high altitude streams, were reported by Kiffney and Clements (1996). Considering the effects of different heavy metals, *Pericoma* and *Simulium sp.* in Diptera were sensitive to Cd and Pb, respectively.

Meanwhile, some taxa responded positively to increased concentrations of heavy metals. The abundance of *Paracapnia* sp. in Plecoptera increased significantly with increasing Cd concentration. In Trichoptera, the abundance of *Polycentropus* sp., *Tinodes* sp., *Hydropsyche* sp., and *Apsilochorema* sp. increased as a function of concentrations of heavy metals, especially Cd and Ni. The tolerance of Trichoptera, especially *Hydropsyche*, to heavy metals has been well reported (Clements and Kiffney, 1994; Clements *et al.*, 2000). *Dugesia* sp. in Turbellaria displayed positive correlations with concentrations of Cd, Pb, and Ni, although correlation coefficients were low. Correspondingly, *Dugesia* sp. was the second species in terms of density at site 18 (36 ind.m $^{-2}$) and 20 (12 ind.m $^{-2}$). Fennikoh *et al.* (1978) reported that *Dugesia* was not very sensitive to Cd. Therefore, our results contrasted with previous reports (Malard *et al.*, 1996). This may indicate that the concentrations of the heavy metals were lower than those critical concentrations that lead to decreases in abundance. These organisms are not only tolerant to heavy metal contamination but also to the conditions created by them (van Damme *et al.*, 2008). High numbers of tolerant species in contaminated areas may also be due to the absence of heavy metal sensitive predators (Weatherley *et al.*, 1980).

In the evaluation of community indices, only the Margalef richness index and percentage of scrapers in FFGs showed significantly negative correlations with Cu. These results are supported by bioassessments of contaminated sites that demonstrated strong correlations with heavy metals (Gray and Delaney, 2008; van Damme *et al.*, 2008). However, it should be noted that other studies did not report significant correlations between heavy metals and biological indices such as the Shannon diversity index and percentage composition of FFGs (Weigel *et al.*, 2002; Gray and Delaney, 2008). Thus, diversity indices such as the Shannon diversity index may not be suitable assessment tools due to their instability (Watanabe *et al.*, 2008). Moreover, the usefulness of FFGs could change for different streams due to the different trophic structures and sensitivities the corresponding species.

The clustering of macroinvertebrate communities based on species composition apparently reflected the natural characteristics of streams, and the effects of mines (Figs. 3 and 4). For instance, the sampling sites of clusters V and VI were located at the downstream area or main reach of the Gangqu River. Meanwhile, the sampling sites

of clusters III and IV were all located downstream of active or closed mines. According to the NMDS results, temperature, turbidity, stream width and velocity at low altitude were the main influential factors distinguishing the sampling sites in clusters V and VI from other sampling sites (Fig. 6a). Communities were characterized by relatively high altitude in clusters I, II, and IV. Altitude is a key factor to explain longitudinal changes in species composition, especially in mountain streams (Vannote *et al.*, 1980; Ward, 1994; Maiolini and Lencioni, 2001; Hollmann and Miserendino, 2008). The axis 1 of NMDS ordination showed the effects of heavy metals on benthic macroinvertebate communities (Fig. 6a), characterizing cluster III with high concentrations of Pb and Cu. Correspondingly, the species richness was low in cluster III compared with cluster II. Meanwhile, Trichoptera species in particular showed a significant decrease in cluster III (Fig. 5). The location of sensitive and tolerant species in the NMDS ordination was in accordance with the correlation analysis between the species and heavy metals (Table 3, Fig. 6b). For example, *Rhithrogena* sp. and *Siphluriscus* sp. were located mainly in the middle upper part in the NMDS biplot with lower concentration of heavy metals. However, *Dugesia* sp., *Paracapnia* sp. and *Ephemera* sp. were distributed in the lower middle part of the NMDS biplot showing extensive contamination of heavy metals (Fig. 6b). Meanwhile, the location of tolerant species on the biplot also matched the site locations of cluster III, V, and VI (Fig. 6a). The NMDS results thus coincided well with that of the cluster analysis.

Considering the differences of physical-chemical environmental factors including heavy metals among different clusters (Fig. 4), turbidity and heavy metals were significantly different among the different clusters. Turbidity correlated significantly with Cu and Pb, suggesting that high turbidity results from the erosion associated with mining activities. Therefore, the effects of high turbidity might be reflected in the heavy metal-related changes of benthic macroinveretebrate communities.

In conclusion, the effects of several environmental factors such as geographical, hydromorphological, physical, and chemical factors including heavy metals were revealed through both distributional and compositional patterns of benthic macroinvertebrates in high mountain streams. Although the effects were compounded with different factors such as altitude, temperature, stream width, turbidity, and heavy metals, the influence of heavy metals on benthic macroinvertebrate communities was clearly identified, with a decrease in species richness and changes in species composition. Even though contamination by heavy metals was low in the sampling area, the consequences upon benthic diversity were significant, indicating that the chronic effects of long-term exposure of aquatic communities to heavy metals could be serious in high mountain streams.

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