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Antimony and Heavy Metals Accumulation in Some Macroinvertebrates in the Yesilirmak River (N Turkey) near the Sb-mining Area

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All aquatic invertebrates accumulate trace metals in their bodies whether or not these metals are essential to metabolism. Antimony is a trace element with quite low content in the earth's crust about 0.2 ppm. Antimony is considered geochemically immobile (Ainsworth et al. 1991). Nevertheless, mobility and the biological role of Sb, its behavior and transfer into food chain, are not well known (Baroni et al. 2000). Total Sb concentrations in natural waters have been reported to be in the range of 0.01–1.1 mg/L (US EPA 1996). Acutely toxic concentrations of Sb are in the range of 22–36 mg/L fish (Lin and Hwang 1998), and 9–20 mg/L for daphnids (Anderson 2000), although the toxicity database is small. All these concentrations are above the typical range of concentrations in mine effluents. Therefore, Sb is unlikely to contribute appreciably to effluent acute toxicity. The aquatic toxicity database for Sb and the knowledge of species effluents from Sb mine are limited.

Antimony accumulation is not often studied for aquatic invertebrate and macrophytes (Sanders and Cope 1968; Murphy et al. 2002). A decline in biodiversity of macroinvertebrate communities has generally related to metal pollution (Hill et al. 1997; Mori et al. 1999) but Sb is not often studied in contrast to Zn, Cu and Cd (Mori et al. 1999). Metals such as Fe, Cu, Zn, Cd and Pb are known to be especially high in association with mining activities (Axtmann et al. 1997; Pestana et al. 1997; Quinn et al. 2003). Aquatic invertebrates are often excellent bioindicators of trace element pollution in aquatic ecosystems (Cain et al. 1992). They take up trace elements directly from water and sediments and diet and serve as a tropic link to higher food chain organisms such as fish and aquatic birds (Wayland and Crosley 2006). This work presents total Sb, Cd, Pb, Zn and Cu accumulation in water, sediments, some macroinvertebrate from active antimony mining area. We were interested in comparing heavy metal levels between mine impacted and non impacted sites. Also, this study addresses the impact of Sb-mining on biological components of macroinvertebrate of this part of Yesilirmak River.

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Materials and Methods

The total basin of the Yesilirmak River is 2352.8 km^2, and 519 km length. The study area is located in the Turhal district, which is part of Tokat city (Northern Turkey). By the time, Sb production exceeded the Turkish domestic consumption. However, this mining area produced a significant proportion of the Sb, which is exporting to other countries. Sampling sites were classified as reference sites is located before the mining company (reference site = station 1) and mine-affected site is located 500 m down the mining company (station 2) (Fig. 1). Macroinvertebrate samples were collected monthly from June 2004 to November 2004.

Sediment samples were dried for 3 h at 110°C and ground to pass through 200 µm mesh sieves. After homogenization, 100 mg amount of sediment sample was weighed and transferred to a 100 mL beaker. Ten milliliter of aqua regia was added. The sample was heated to 95°C. After the evaluation of NO_2 fumes had ceased, the mixture was evaporated almost get dry on a sand-bath and was

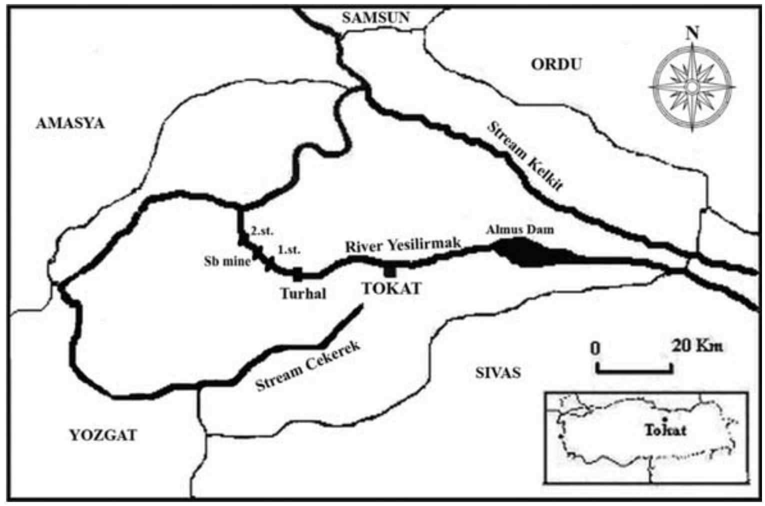

Fig. 1 Location of Sb mining area and sampling sites, *st* station

cooled. After cooling, 10 mL of aqua regia was added to the sample and mixed. The mixture was again evaporated till get dry then 10 mL of distilled water was added to the residue. The suspension was filtered through a blue band filter paper (Whatman, 41) and the insoluble part was washed with distilled water. The final solution was diluted to 10 mL with distilled water (Soylak et al. 1999). The same procedure was applied to the blank solution. The final solution was analyzed for the determination of total antimony by graphite furnace atomic absorption spectrophotometry (AAS, Perkin Elmer Analyst 700).

Analyses of the water samples were performed monthly. Water samples were filtered through a 0.45 μm Milipore membrane and then acidified to pH ≤ 2 using high purity HNO_3 (Merck, Munich, Germany) immediately after sampling. Then, the samples were kept in the refrigerator at 4°C until analysis (Ballinger 1979). All chemicals obtained from commercial sources were of super pure grade unless otherwise stated. All aqueous solutions were prepared using doubly distilled water. Glassware and plasticware were cleaned by soaking in dilute HNO_3 (1 + 9) before rinsing with distilled water prior to use. Standard stock solutions (1,000 mg/L) of the working elements were prepared by dissolution of their salts (Merck, Darmstadt, Germany). The working solutions were prepared by dilution.

Macroinvertebrate; *Asellus aquaticus*, *Hydropsyche pellucidula*, *Leucorrhinia dubia*, and *Gammarus pulex* were weighted (1.0 g) and dissolved with 8 mL of the mixture of acids HNO_3–$HClO_4$–H_2O_2 (2:1:1). The samples were heated at 170°C for 3 h, cooled down, and then 2 mL of H_2SO_4 and 8 mL of acid mixture were added. These solutions were centrifuged at 2,500 rpm for 5 min. The solutions were filled up to 25 mL with 1 M HNO_3 (Divrikli et al. 2003). The antimony level was determined based on dry weight of samples. Also, the blank digest was carried out in the same way. The total antimony concentration in the final solution was determined by graphite furnace atomic absorption spectrophotometry. Quality assurance measures included blanks, replicate analyses, and matrix spikes. Recoveries from matrix spikes ranged about 90–112%. Repeated analyses did not reveal differences greater than 10%.

The minimum and maximum values of the analytical results are as given below: Antimony 0.015–0.5 μg/L, cadmium 0.02–0.96 μg/L, lead 0.93–28.6 μg/L, zinc 5.36–37.4 mg/L and copper 10.93–56.25 μg/L. The recommended values for natural waters in Turkish Standards (1988) were as given below: Antimony 1.0 μg/L, cadmium 3–10 μg/L, lead 10–50 μg/L, zinc 200–2,000 mg/L and copper 20–200 μg/L. The concentration levels (μg/L) of some trace metal ions in the water samples investigated were below the detection limit of AAS analysis. The detection limit of Sb, Cd, Pb, Zn and Cu were found to be 0.6 μg/L, 4.10^{-3} μg/L, 0.25 μg/L, 0.6 mg/L and 0.1 μg/L, respectively.

Experimental data were analyzed using One-way ANOVA and Mann-Whitney test and any significant difference was determined at a 0.05 probability level using Minitab 13.2 statistical software.

Results

At the two stations total 79 taxa were found. The reference site had the richest taxa than the mine-affected site. The high total Sb values and some heavy metals caused changes on composition of the macroinvertebrate population in the Yesilirmak River. The taxonomic diversity decreased from 79 (reference site) down to 36 (mine-affected site), because

Table 1 Density (Organizm m^{-2}) of some macroinvertebrates in reference site and Sb mine-affected sites

Species	Reference site	Sb mine-affected site
Asellus aquaticus	10	125
Chironomus thumni	78	374
Gammarus pulex	154	35
Leucorrhinia dubia	9	37
Hydropsyche pellucidula	21	67

of impact of the mine. The maximal abundance of some macroinvertebrates in reference and mine-affected sampling sites is presented in Table 1.

Heavy metals concentration had an impact on macroinvertebrate of the population. There were significant interaction effects between site type (One-way ANOVA, $p < 0.05$) and macroinvertebrate taxon on total Sb, Cd, Pb, Zn and Cu levels (One-way ANOVA, $p < 0.05$). Concentrations of total Sb (One-way ANOVA, $p < 0.05$), Cd (One-way ANOVA, $p < 0.05$), Zn (One-way ANOVA, $p < 0.05$), Cd (One-way ANOVA, $p < 0.05$) and Cu (One-way ANOVA, $p < 0.05$) were significantly higher in mine-affected water samples than in samples from reference site (Table 2). We examined the simple effects of site types on accumulation of these heavy metals, which are separately for each macroinvertebrate taxon. Sb concentrations in macroinvertebrates were different significantly among the taxa (Mann-Whitney test, $p < 0.05$), but were not different significantly among the months (One-way ANOVA, $p > 0.05$).

The simple effects of each macroinvertebrate taxon on concentration of heavy metals examined separately for each site type. Total Sb concentrations differed significantly among macroinvertebrate taxa at both sites (One-way ANOVA, $p < 0.05$) while total Sb not detected at the reference site. Total Sb levels in *Asellus aquaticus*, and *Hydropsyche pellucidula* were significantly greater than in *Leucorrhinia dubia* and *Gammarus pulex* at the Sb mine-affected site (Mann–Whitney test, $p < 0.05$). Pb levels in *A. aquaticus*, *L. dubia* and *H. pellucidula* were greater significantly than in *G. pulex* at the mine-affected site (Mann–Whitney test, $p < 0.05$). Zn levels in *A. aquaticus* and *G. pulex* were greater significantly than in *L. dubia* and *H. pellucidula* at reference site (Mann-Whitney test, $p < 0.05$). Cu level in *A. aquaticus* was greater significantly than in *L. dubia*, *H. pellucidula* and *G. pulex* at reference site (Mann–Whitney test, $p < 0.05$). Also, Cd levels were greater significantly at reference site than at Sb mine-affected site (One-way ANOVA, $p > 0.05$) while at reference site Cd levels were in *A. aquaticus*, *L. dubia* and *H. pellucidula* were greater significantly than in *G. pulex* (Mann–Whitney test, $p < 0.05$). Total Sb, Pb, Zn and Cu levels of sediments were higher significantly than waters except Cd (One-way ANOVA, $p > 0.05$).

Discussion

Total Sb and Pb concentrations in macroinvertebrates were elevated at Sb mine-affected site compared to reference site. However, Cd, Zn and Cu concentrations in macroinvertebrates were high at reference site compared to Sb mine-affected site. Concentrations of total Sb, Cd, Zn, Pb and Cu differed from macroinvertebrate taxa. Significant differences between Sb mine-affected site and reference site were found in *A. aquaticus*, *L. dubia* and *H. pellucidula* for Sb and Pb. Generally, *A. aquaticus* had the highest median concentrations, which is followed by *H. pelluci-*

Table 2 Average concentrations of heavy metals in water (μg L^{-1}) and macroinvertebrates and sediment (μg g^{-1} dry weight) from active Sb mine-impacted and reference sites near the Yesilirmak River, in the Turhal district

Sample type	S	Sb	Cd	Pb	Zn	Cu
Asellus aquaticus	S1	ND	0.0702 ± 0.076	3.428 ± 0.19	258.6 ± 4.79	126.38 ± 2.45
	S2	3.262 ± 0.09	0.0530 ± 0.022	19.62 ± 0.98	19.8 ± 0.078	81.74 ± 1.89
Hydropsyche pellucidula	S1	ND	0.0564 ± 0.065	3.82 6 ± 0.082	128.2 ± 3.56	44.725 ± 1.54
	S2	1.995 ± 0.034	0.052 ± 0.001	21.54 ± 0.19	123.4 ± 2.81	28.82 ± 1.12
Leucorrhinia dubia	S1	ND	0.0442 ± 0.001	2.425 ± 0.074	144.0 ± 2.83	23.94 ± 1.21
	S2	0.694 ± 0.001	0.0395 ± 0.001	16.953 ± 0.1	132.6 ± 2.39	17.56 ± 0.96
Gammarus pulex	S1	ND	0.0074 ± 0.001	0.848 ± 0.001	285.6 ± 4.92	29.34 ± 1.19
	S2	0.43 ± 0.001	0.002 ± 0.001	1.180 ± 0.009	131.1 ± 2.17	11.6 ± 1.02
Sedimen	S1	ND	0.066 ± 0.092	19.063 ± 1.06	37.4 ± 1.39	56.25 ± 1.89
	S2	0.5 ± 0.002	0.0924 ± 0.037	28.613 ± 1.18	32.22 ± 1.22	31.52 ± 1.20
Water	S1	ND	0.025 ± 0.022	0.093 ± 0.023	5.369 ± 0.16	11.89 ± 0.41
	S2	0.015 ± 0.00	0.967 ± 0.21	1.087 ± 0.09	6.238 ± 0.25	10.93 ± 0.49

ND Not deceted, *S* Stations

dula, *L. dubia* and *G. pulex*. Relatively high levels of Se, As, Pb, Zn and Cu in herbivorous or detritivorous mayflies and lower levels of these elements in predatory stoneflies have been noted in other studies (Kiffney and Clements 1993; Saiki et al. 2001). Resistance to metalloid pollution and the decrease in inter specific competition, due to the low variety of benthic macroinvertebrate in polluted zones, could explain the proliferation of the species (Mori et al. 1999). Indeed, composition of macroinvertebrate and abundance of some macroinvertebrate reveal differences compared to the Sb mine-affected site and reference sites (Table 1).

A number of reasons have been offered to explain why some types of macroinvertebrates contain greater concentrations of heavy metals than others. Food is potentially an important vector of metal uptake to aquatic organisms (Luoma 1983). However, establishing the relative importance of food versus water as routes of metal exposure has proven to be difficult. In an attempt to develop a model for determining the importance of biotic uptake in causing toxicity, Dallinger et al. (1987) proposed a model that—-food chain effect—this model hypothesises that metal transfer through aquatic food chains can be high enough to be harmful to fish under certain ecological situations. Furthermore, metal contamination in aquatic ecosystems is more often reflected by high metal levels in sediments, macrophytes and benthic animals than by elevated concentrations in water (Prosi 1981).

Metal availability also differs among functional feeding groups (Kiffney and Clements 1993). Benthic invertebrates that feed primarily on periphyton (i.e., algae, bacteria, fine detritus and sediment accumulating on rock surfaces) bioaccumulated significantly more metals (Cd, Cu, Zn) than predaceous invertebrates (Kiffney and Clements 1993). Filter feeders, collector-gatherers, shredders, and grazers tend to accumulate higher levels of certain trace elements than omnivores and predators (Kiffney and Clements 1993; Besser et al. 2001). Therefore, the complete dominance and heavy metals levels of grazer *Asellus* in the Sb mine-affected site can very probably be attributed to the feeding behaviour.

Body size strongly influences levels of trace elements in aquatic insects with smaller-bodied insects having greater concentrations than larger-bodied ones (Cain et al. 1992; Kiffney and Clements 1993; Mason et al. 2000; Besser et al. 2001). The inverse relationship between body size and trace element concentration is believed to be related to the greater surface area/volume ratios in smaller bodied insects (Hare 1992; Mason et al., 2000). Regarding the macroinvertebrate analyzed in this study, *A. aquaticus* is smaller than the *H. pellucidula*, which is, in turn, smaller than *L. dubia* and *G. pulex*. Thus, for the most part, our results are consistent with observations of other studies, and show that smaller-bodied macroinvertebrate have greater concentrations of heavy metals than larger-bodied ones. Accumulation of heavy metals availability also differs between two Crustacean species. This is because, *Gammarus* is more sensitive to organic pollution than *Asellus* and the relative abundance of the two taxa has been proposed as a pollution index (MacNeil et al. 2002).

Cd, Cu and Zn concentrations were higher in references site than Sb mine-affected site. This is might due to the competition between H^+ and metal ions for binding sites on inorganic and organic ligands. Because of the relationship between pH and concentration of free metal ions, it has been assumed that metals are more likely to be toxic to biota in acidic than in neutral waters (Campbell and Stokes 1985). However, a research suggests that a decrease in pH might result in a decreased biological response (e.g., toxicity) for some metals (Cd, Cu, and Zn). Evidence to support the notion by Hare and Tessier (1996) that H^+ ions compete with metal ions for binding sites was provided.

Total Sb, and Pb were elevated at Sb mine-impacted sites compared to reference sites in macroinvertebrate. A concern arising from such a result is whether consumers of these aquatic macroinvertebrates are placed at increased risk of toxic effects in Sb mine-impacted streams.

Natural diets consisting of metal-contaminated insects are often toxic at lower concentrations than artificially spiked commercial diets (Farag et al. 1999), possibly because of additive or synergistic effects of multiple metals in the natural diet, the relatively poor nutritional quality of natural diets in metal-contaminated streams, or the organic binding of metals in natural foods (Farag et al. 1999; Saiki et al. 2001).

The concentration levels measured in this study are not very higher than compared to other studies (Mori et al. 1999; Baroni et al. 2000). However, the present study has demonstrated that total Sb and some heavy metals (Pb, Cu, Zn and Cd) contamination has a strong impact on the macroinvertebrates community. In addition, the vulnerability of the individual species clearly depends on the place in the food web and on feeding behavior. Collectively, these results suggest that aquatic dietary total Sb and other heavy metals are unlikely to affect macroinvertebrate population in Sb mine-affected running waters. Overall, this study stated that elevated concentrations of total Sb, Cd, Pb, Zn and Cu in at least one or two macroinvertebrate taxa in Sb mine-affected site. Total Sb and Pb concentrations were high enough to be of potential concern for *Asellus* and *Hydropsyche* whereas Cd, Cu and Zn concentrations remained below levels at Sb mine-affected site.

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