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Research Paper

www.publish.csiro.au/journals/env K. Telford et al., *Environ. Chem.* **2009**, *6*, 133–143. doi:10.1071/EN08097

Bioaccumulation of antimony and arsenic in a highly contaminated stream adjacent to the Hillgrove Mine, NSW, Australia

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Environmental context. Concern over the presence of antimony (Sb) in the environment because of chemical similarities with arsenic (As) has prompted a need to better understand its environmental behaviour and risks. The present study investigates the bioaccumulation and uptake of antimony in a highly contaminated stream near the Hillgrove antimony–gold mine in NSW, Australia, and reports high Sb (and As) concentrations in many components of the ecosystem consisting of three trophic levels, but limited uptake into aboveground parts of riparian vegetation. The data suggest that Sb can transfer into upper trophic levels of a creek ecosystem, but that direct exposure of creek fauna to creek sediment and soil, water and aquatic autotrophs are more important metalloid uptake routes than exposure via riparian vegetation.

Abstract. Bioaccumulation and uptake of antimony (Sb) were investigated in a highly contaminated stream, Bakers Creek, running adjacent to mining and processing of Sb–As ores at Hillgrove Mine, NSW, Australia. Comparisons with arsenic (As) were included owing to its co-occurrence at high concentrations. Mean metalloid creek rhizome sediment concentrations were $777 \pm 115\,\mu g\,g^{-1}$ Sb and $60 \pm 6\,\mu g\,g^{-1}$ As, with water concentrations at $381 \pm 23\,\mu g\,L^{-1}$ Sb and $46 \pm 2\,\mu g\,L^{-1}$ As. Antimony and As were significantly elevated in aquatic autotrophs (96–$212\,\mu g\,g^{-1}$ Sb and 32–$245\,\mu g\,g^{-1}$ As) but Sb had a lower uptake efficiency. Both metalloids were elevated in all macroinvertebrates sampled (94–$316\,\mu g\,g^{-1}$ Sb and 1.8–$62\,\mu g\,g^{-1}$ As) except Sb in gastropods. Metalloids were detected in upper trophic levels although biomagnification was not evident. Metalloid transfer to riparian vegetation leaves from roots and rhizome soil was low but rhizome soil to leaf As concentration ratios were up to 2–3 times greater than Sb concentration ratios. Direct exposure to the rhizosphere sediments and soils, water ingestion and consumption of aquatic autotrophs appear to be the major routes of Sb and As uptake for the fauna of Bakers Creek.

Additional keywords: ecosystem, food web, uptake.

Introduction

Antimony (Sb) is one of the less common natural elements, with an estimated crustal abundance of 0.2–$0.3\,\mu g\,g^{-1}$.[1–4] In contrast to neighbouring subgroup 15 metalloid arsenic (As), the biogeochemistry of Sb has received less attention and consequently there is limited understanding of its behaviour as an environmental contaminant.[5] Antimony has four oxidation states in nature, (−3, 0, +3, +5), with Sb^{III} and Sb^{V} predominating. Similar to As, toxicity of Sb species decreases in the order inorganic Sb^{III} > inorganic Sb^{V} > methylated Sb compounds.[5–10] Both metalloids are clastogenic in the trivalent state and have carcinogenic potential.[6] Antimony toxicity to organisms is not well understood and is often assumed to be comparable with As, which may not necessarily be true. Antimony has no known biological function[11–15] and consequently, the occurrence of elevated Sb concentrations in natural media may prove to be of environmental concern in view of the chemical similarities with As.

Typical background concentrations of Sb are $<1\,\mu g\,L^{-1}$ in stream water, $0.2\,\mu g\,L^{-1}$ in ocean water, ~0.5–$1\,\mu g\,g^{-1}$ in soil and $0.2\,\mu g\,g^{-1}$ in rocks.[3,12,16] Antimony enrichment in the environment occurs both naturally and anthropogenically due primarily to mineralization, mining processing, smelting and industrial activities.[3] Although global production is only $140\,000\,t\,year^{-1}$ from ores and as a by-product of smelting of other metallic ores,[3] Sb has a wide range of industrial uses including being used in metal alloys, glass, plastics, fire retardants, semiconductors, batteries, brake linings, paints, pigments and enamels.[3,17–20] It has also been used therapeutically as the primary treatment of the intracellular parasite *Leishmania donovani* and in the treatment of HIV.[21] Increasing use of Sb and its compounds has resulted in a need over recent years for improved

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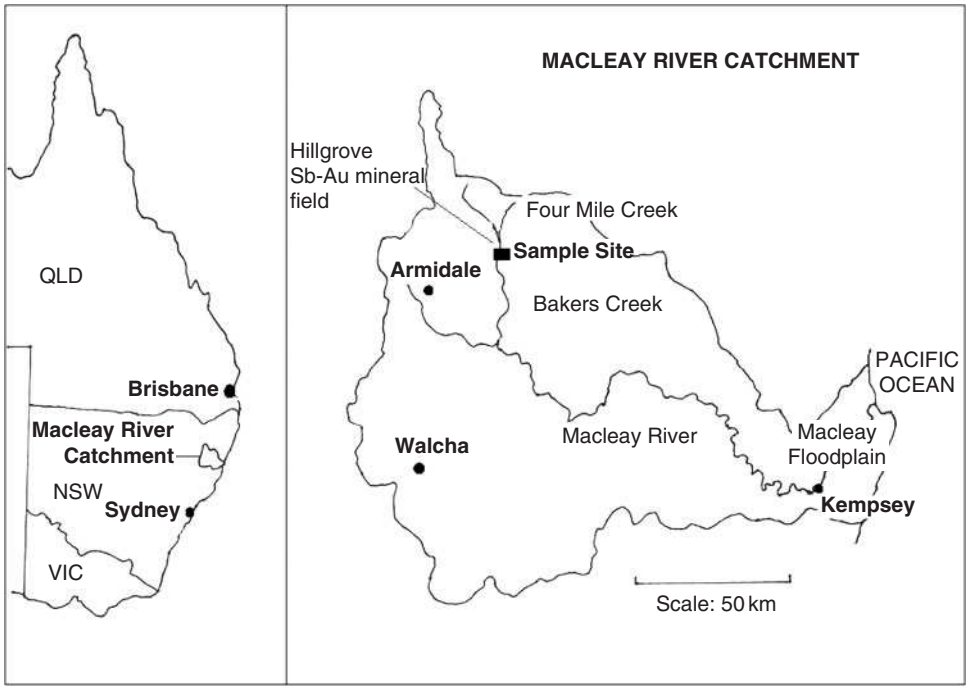

Fig. 1. Location of Macleay River Catchment and Bakers Creek.

understanding of its bioaccumulation and the risks associated with its presence in environmental systems.

The objective of the present study was to investigate the bioaccumulation and uptake of Sb with comparisons with As in a highly contaminated stream, Bakers Creek. Bakers Creek traverses the Hillgrove mining field (centred on 30°35′S, 151°54′E), New South Wales (NSW), Australia, where deep underground mining has exploited vein and breccia-hosted ores producing significant quantities of Sb, Au and W from 1878 to the present. Arsenic was integral to the study because of its similar geochemical behaviour, and because it is present as a major contaminant in Bakers Creek, occurring at high concentrations in the ore.[22] Its co-occurrence provided useful comparisons with Sb behaviour.

Experimental

Study site

Bakers Creek is a major tributary of the Macleay River in north-eastern NSW, Australia. The Macleay catchment is ~11 450 km^2 and the river drains into the Pacific Ocean (Fig. 1). Bakers Creek within and downstream of the Hillgrove mine flows in a gorge 450–500 m deep and provides a high-energy fluvial environment, with riffle and pool zones. Its flow regime is highly variable, depending on rainfall in the catchment. The region experiences a temperate, subhumid climate with an average annual rainfall of 790 mm, and an average summer daily maximum temperature of 26°C and an average winter daily minimum temperature of 1°C.[23]

In the Hillgrove region, Bakers Creek has a bedrock substrate of metasedimentary rocks (shale, siltstone, greywacke originally) and intrusive igneous rocks (mostly monzogranite, with smaller amounts of granodiorite, diorite and lamprophyre). It has been estimated that ~7 Mt of mine waste material has entered the creek[24] from early mining and processing operations (pre-1970s mainly). Although sulphide minerals (e.g. pyrite, arsenopyrite, stibnite) are common in the mine waste, there is no acid mine drainage owing to the buffering action of carbonate minerals.[25] Small volumes of highly contaminated water also enter Bakers Creek from old mine workings and other seepages (up to 54.7 mg L^{-1} Sb and 7.2 mg L^{-1} As),[25] but the main contributor to stream water contamination is by reaction between water and the highly contaminated stream bed material.[24] Earlier studies have shown that Sb and As are bioavailable in the Bakers Creek fluvial environment, with uptake into riparian vegetation, aquatic algae[24,26] and macroinvertebrates reported.[27] The contamination plume extends 300 km downstream in the Macleay River to the coastal floodplain and the Pacific Ocean.[24,28]

The sampling site was ~300 m downstream of the confluence of Bakers Creek and Four Mile Creek within the mine lease area. At the site, the stream forms a large pool up to 1 m deep, bound by riffles up to 50 cm deep, with bedrock outcrop on one side and a vegetated point bar on the other. Stream sediments range from boulders to silt, with the point bar being colonised by species typical of the riparian zone of the region such as *Paspalum distichum*, *Lomandra* spp. and *Persicaria* spp. Earlier sampling of water in the vicinity of the site has demonstrated wide ranges in pH (6.76–9.12), electrical conductivity (230–690 μS cm^{-2}) and concentrations of Sb (125–1800 μg L^{-1}) and As (15–488 μg L^{-1}).[26,29]

Sample collection and preservation

Plants

Samples were collected at the study site in November 2006. Five species of riparian vegetation (*Persicaria* spp., *Pennisetum clandestinum*, *Lomandra* spp., *Juncus krussii* and *Paspalum distichum*), three species of aquatic plants (an unidentified emergent, milfoil (*Myriophyllum*) and floating green macroalgae) and

Table 1. Mean antimony and arsenic concentrations (±s.e.) (dry weight) measured in aquatic flora and fauna sampled from Bakers Creek

Taxa	Order	Common name	Number of samples	Antimony ($\mu g\,g^{-1}$)	Arsenic ($\mu g\,g^{-1}$)
Carnivores					
Insecta	Odonata	Dragonfly	1	146	12
Insecta	Coleoptera	Water beetle	1	302	57
Philypnodon sp.		Flat-head gudgeon	10	0.3 ± 0.3	1.6 ± 0.4
Omnivores					
		Tadpole	2	174 ± 10	62 ± 2
Insecta	Trichoptera	Caddisfly	7	277 ± 89	35 ± 9
Detritivores					
Oligochaeta		Worm	1	316	47
Gastropod		Snail	1	<0.03[A]	1.8
Herbivores					
Insecta	Ephemeroptera	Mayfly	1	193	50
Insecta	Coleoptera (*Psephenidae*)	Water pennies	1	94	42
Autotrophs					
		Macroalgae	5	96 ± 13	56 ± 20
Myriophyllum	Leaves	Milfoil	5	212 ± 30	32 ± 8
	Roots			533 ± 229	577 ± 197
Unknown emergent	Leaves		5	139 ± 10	245 ± 63
	Roots		5	348 ± 80	565 ± 66
Other					
Seston[B]			2	0.24 ± 0.01	0.33 ± 0.06
Source					
Water[C]			4	381 ± 23	46 ± 2
Rhizome sediments		Milfoil	5	958 ± 152	71 ± 10
		Emergent macrophyte	5	596 ± 141	50 ± 6
		Mean	5	777 ± 115	60 ± 6

[A]Below detection limit. Detection limit for Sb = 0.03 $\mu g\,g^{-1}$ and As = 0.1 $\mu g\,g^{-1}$.
[B]μg in suspended sediment L^{-1} of water filtered.
[C]Water concentrations in $\mu g\,L^{-1}$.

their associated rhizosphere soil or sediment were collected. All were common to the area and represented a range of different riparian and aquatic vegetation species. Five randomly selected samples of each species were collected and, for the rooted plants, leaves, roots and soil and sediment were separated for analysis. All samples were transported on ice and frozen at −10°C before analysis.

Animals

For each species, at least five replicates were collected. Because of the low mass of some species, some of the replicates were pooled for analyses. Macroinvertebrates were collected using the Australian Capital Territory Australian River Assessment System (AUSRIVAS ACT) kick net method.[30] Five replicate macroinvertebrate samples were collected from ~1-m^2 sections of the creek using a rectangular-framed hand-held dip net, with a 350 mm-wide opening and a 250-μm mesh. In total, seven species of macroinvertebrate were collected (Table 1). In addition, one unidentified species of tadpole (*Anuran*) was collected from the shallow waters on both sides of the creek and one fish species, *Philypnodon* sp. (flat-head gudgeon), also using the dip net (10 samples). All organisms collected were depurated and were preserved in 70% ethanol (AR).

Three water samples (1 L) were collected from ~30 cm below the water surface. Each sample was filtered through a 0.45-μm glass fibre filter (PALL, Ann Arbor, MI, USA) using a hand pump. Seston was collected on the filter paper for analysis. Samples were transported on ice and frozen at −10°C before analysis.

Sample preparation

Water samples were thawed, homogenised using a vortex mixer, centrifuged at 5943*g* at 10°C (4000 rpm) for 5 min using an Eppendorf 5804 centrifuge (Eppendorf, Hamburg, Germany) and acidified to 1% v/v with nitric acid (Aristar, BDH, Poole, UK). Filter papers with seston were oven-dried at 100°C for 6 h.

Rocks, large gravel particles and some organic matter were removed from the rhizosphere soil and sediment samples. The samples were then oven-dried at 40°C for 72 h, homogenised using an agate mortar and pestle, and then passed through a 250-μm nylon mesh twice.

Plant samples were rinsed repeatedly with deionised water to remove foreign material. In addition, root samples were sonicated in high-purity deionised water (18 MΩ cm, Sartorius AG, Goettingen, Germany). Fauna samples were also rinsed repeatedly with deionised water to remove foreign material. After cleaning, all flora and fauna samples were refrozen to −10°C, freeze-dried and the dry samples homogenised using an IKA A11 basic mill (IKA, Staufen, Germany).

Sample analysis

Nitric acid (HNO_3) (Aristar, BDH) and hydrochloric acid (HCl) (Sigma Aldrich, Steinheim, Germany) were used for digestion of samples. Analytical standard solutions were prepared by diluting Accu Trace Reference standard ICP-MS-CAL3–1 (Merck, Newhaven, CT, USA) with high-purity deionised water (18 MΩ cm, Sartorius).

Flora and fauna samples were digested using a low-volume microwave-assisted acid digestion procedure.[31] Approximately

Table 2. Antimony and arsenic concentrations (dry weight) measured in the sediments, roots and leaves of riparian vegetation sampled from the Bakers Creek ecosystem
Mean ± s.e., $n = 5$

Species	Common name	Sample type	Antimony ($\mu g\,g^{-1}$)	Arsenic ($\mu g\,g^{-1}$)
Persicaria sp.	Smart weed	Sediment	180 ± 16	68 ± 8
		Root	142 ± 26	54 ± 21
		Leaf	5.4 ± 1.0	6.8 ± 1.0
Pennisetum clandestinum	Kikuyu grass	Sediment	267 ± 78	64 ± 15
		Root	99 ± 41	49 ± 24
		Leaf	5.1 ± 1.2	4.3 ± 0.9
Lomandra sp.	Sedge	Sediment	554 ± 158	176 ± 36
		Root	18 ± 5	8.7 ± 4.6
		Leaf	1.1 ± 0.4	<0.1[A]
Paspalum distichum	Water couch	Sediment	304 ± 84	59 ± 12
		Root	954 ± 228	70 ± 16
		Leaf	28 ± 4	3.8 ± 0.4
Juncus krussii	Juncus or maritime rush	Sediment	528 ± 145	74 ± 17
		Root	305 ± 45	120 ± 49
		Leaf	16 ± 4	2.7 ± 0.7

[A]Below detection limit. Detection limit for Sb = 0.03 $\mu g\,g^{-1}$ and As = 0.1 $\mu g\,g^{-1}$.

0.07 g of a freeze-dried sample was weighed into a 7-mL polyfluoroacetate (PFA) digestion vessel (AI Scientific, Sydney, Australia) and 1 mL concentrated HNO_3 added to each vessel (Aristar, BDH). Digestion was performed using an MDS-81D microwave oven (CEM Corp., Matthews, NC, USA) and a three-step program comprising 2 min at 600 W, 2 min at 0 W, and 45 min at 450 W. After digestion, the vessels were allowed to cool for ~60 min and diluted with high-purity deionised water (18 MΩ cm, Sartorius) and then transferred to 10-mL polyethylene vials.

Geological and seston samples were digested using a high-temperature extraction procedure.[32] Approximately 0.2 g of an oven-dried sample was weighed into a 55-mL polytetrafluoroethylene (PTFE) digestion vessel (CEM) to which 3 mL 1 : 2 (v/v) nitric–hydrochloric acid extraction solution was added. Samples were heated in a Microwave Assisted Reaction System (MARS) (CEM) using a four-step program: (1) ramp to 120°C over 10 min; (2) hold at 120°C for 5 min; (3) ramp to 150°C over 3 min; (4) hold at 150°C for 15 min. After digestion, samples were allowed to cool for ~60 min and diluted to 30 mL with high-purity deionised water (18 MΩ cm, Sartorius).

Total Sb and As concentrations were determined using a Perkin-Elmer Elan-6000 inductively coupled plasma mass spectrometer (ICP-MS) (PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA, USA). Signal intensity at *m/z* 121, 123, and 75, *m/z* 77 and 82 were monitored as cross-checks on *m/z* 75. Internal standards were added on-line to compensate for any acid effects and instrument drift. Additional detail may be found in Maher et al.[33]

Statistical analysis

Qualitative examination of residual plots and the Shapiro–Wilks W test was used to verify the assumptions of normality and homogeneity. The data were $\log_{10}$-transformed to meet assumptions of normality. A one-way ANOVA was used to detect significant differences in Sb and As concentrations. Significant differences were explored using an exhaustive multiple comparisons Tukey Kramer procedure. All mean values are reported with ±1 standard error of the mean. *Statistica* Version 7 (StatSoft, Tulsa, OK, USA) was used to perform all parametric tests.

Stream Invertebrate Grade Number Average Level 2 (SIGNAL 2) was used as a tool to assess the water quality of the Bakers Creek ecosystem.[34] SIGNAL (Stream Invertebrate Grade Number) is a simple biotic index based on river macroinvertebrates, developed initially for application in eastern Australia to assess water pollution and water quality. The SIGNAL score for a macroinvertebrate sample is calculated by averaging the pollution sensitivity grade numbers of the families present, and the more recent SIGNAL 2 incorporates correlation with water quality parameters. Grade numbers are set between 1 and 10, with grade 10 indicating a high sensitivity to pollution and grade 1 indicating a greater tolerance to pollution. A community with high numbers of a few low-grade taxa indicates a degraded aquatic habitat.

Results and discussion

The following discussion focusses on Sb because much less is known about its environmental behaviour and bioaccumulation. Comparisons with As were also undertaken. Mean Sb and As concentrations measured in environmental media and organisms sampled are presented in Tables 1 and 2.

Water and rhizosphere sediments

The mean Sb concentration measured in creek water was 381 ± 23 $\mu g\,L^{-1}$ whereas the mean As concentration was much lower at 46 ± 2 $\mu g\,L^{-1}$. These values significantly exceed background concentrations for the Macleay River Catchment (3 $\mu g\,L^{-1}$ and 4 $\mu g\,L^{-1}$ respectively) but are consistent with previous monitoring data for Bakers Creek (Straits Hillgrove Gold, unpubl. monitoring data).[24] Previous sampling over the 20 km from Hillgrove to the Macleay River has shown Sb concentrations in Bakers Creek waters to range from 125 to 1800 $\mu g\,L^{-1}$ (mean 730 $\mu g\,L^{-1}$) and As concentrations to range from 15 to 488 $\mu g\,L^{-1}$ (mean 82 $\mu g\,L^{-1}$).[24] According to current Australian drinking water guidelines, tolerable Sb and As concentrations are 3 $\mu g\,L^{-1}$ and 7 $\mu g\,L^{-1}$ respectively[35]

and the tolerable As concentration for recreational waters is $50\,\mu g\,L^{-1}$.[36] A guideline value for Sb concentration in recreational water has not been established. The current Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand (ANZECC/ARMCANZ) guidelines for fresh and marine water quality have established values of $24\,\mu g\,L^{-1}$ for As^{III} and $13\,\mu g\,L^{-1}$ for As^{V} for protection of 95% of species in freshwater.[36] No equivalent guidelines for Sb exist (although a low reliability value of $9\,\mu g\,L^{-1}$ is specified).[36] Based on these values, the water sampled from Bakers Creek is not suitable for human consumption and is of concern for maintenance of a healthy freshwater ecosystem.

Stream rhizosphere sediments had a mean Sb concentration of $777 \pm 115\,\mu g\,g^{-1}$ and a mean As concentration of $60 \pm 6\,\mu g\,g^{-1}$. Previous sampling of the active stream sediments of Bakers Creek over the 20 km from Hillgrove to the Macleay River and the first 25 km of the Macleay after the confluence has shown Sb concentrations ranging from 80 to $438\,\mu g\,g^{-1}$ (mean $270\,\mu g\,g^{-1}$) and As concentrations ranging from 86 to $329\,\mu g\,g^{-1}$ (mean of $180\,\mu g\,g^{-1}$).[24] Geochemical background concentrations for stream sediments of the Macleay River Catchment are $1.1\,\mu g\,g^{-1}$ for Sb and $7.9\,\mu g\,g^{-1}$ for As.[24] ANZECC/ARMCANZ interim sediment quality guideline (ISQG) values for Sb are $2\,\mu g\,g^{-1}$ (ISQG-Low) and $25\,\mu g\,g^{-1}$ (ISQG-High), and for As, $20\,\mu g\,g^{-1}$ (ISQG-Low) and $70\,\mu g\,g^{-1}$ (ISQG-High).[36] Antimony concentrations measured in stream sediments collected from Bakers Creek not only grossly exceed these guideline values but are greater than concentrations reported previously in the creek. Arsenic concentrations, although elevated, did not exceed the ISQG-High guideline value or previous monitoring data.

The anomalous Sb concentrations reported here compared with previous results may be an indication of the variability in the stream sediments within the creek. However, it must be noted that in the present study, the sediment samples were obtained from the plant rhizosphere. The presence of vegetation has been shown to significantly influence the concentration and accumulation of heavy metals in the rhizosphere of salt marsh soils where iron is significantly elevated compared with the bulk soil.[37]

The water and rhizosphere sediment concentrations reported here show that Bakers Creek is significantly contaminated by both Sb and As. Antimony and As enter the Bakers Creek subcatchment from the Hillgrove mining area through the following sources: (i) small-volume seepages high in Sb and As concentration from the Hillgrove mine, which include Sb- and As-rich hydrated Fe oxyhydroxide precipitates; (ii) natural weathering and erosion of mineralized outcrops exposed in the stream beds; and primarily from (iii) physical transfer, historic erosion and collapse of mineralized material from mine-waste dumps, scree, natural enriched sources and anthropogenically contaminated soil. Seepage flows are sourced from several mine openings, the tailings dam, below mine waste dumps and scree material. Although seepages are low in volume ($\sim$90 ML $year^{-1}$), Sb and As concentrations are high at $54.7\,mg\,L^{-1}$ and $7.2\,mg\,L^{-1}$ respectively.[24,25] It has been estimated that up to 7 Mt[24] of mine wastes have been produced at the Hillgrove mine site and as a result of pre-1970s waste disposal practices, the majority of this has been transported from mine dumps and scree slopes into Bakers Creek by high-energy fluvial processes. Sequential leaching experiments and equilibration of contaminated stream sediment with uncontaminated stream water have demonstrated the efficient mobilization of Sb and As from stream sediments (considered to be the major source of Sb and As into the water system) occurs.[24]

Aquatic autotrophs

Background Sb and As concentrations for aquatic algae samples collected from this region have been reported as 2.3 and $21\,\mu g\,g^{-1}$, respectively.[24] Both Sb ($96 \pm 13\,\mu g\,g^{-1}$) and As ($56 \pm 20\,\mu g\,g^{-1}$) concentrations in floating green macroalgae collected in Bakers Creek were significantly elevated above these background values but were comparable with other data reported for the creek.[24] Antimony and As concentrations were also significantly greater than the Sb and As concentration ranges generally reported for freshwater autotrophs (Sb: 0.1–$0.2\,\mu g\,g^{-1}$ [38] and As: 1–$9\,\mu g\,g^{-1}$).[39] Ashley et al.[24] have previously correlated algae uptake with the relative amounts of Sb and As in stream water. The data presented here suggest a lower uptake efficiency for Sb into algae compared with As when relative concentrations in algae (Sb : As, 2 : 1 mass/mass) are compared with relative water concentrations (Sb : As, 8 : 1 mass/mass). Sanchez-Rodriguez et al.[40] have shown that Sb had one of the lowest absolute metal algae concentrations as well as having a relatively low enrichment factor. Published studies of As concentrations in marine macroalgae[41,42] suggest that As uptake is not dependent on phosphorus or other elements required for photosynthesis but is a function of the individual algal species. In a recent review, Filella et al.[38] conclude that the little data available for Sb suggest Sb^{V} is the dominant species in freshwater and marine algae, with Sb^{III} converted to Sb^{V} in a detoxifying mechanism.

Two species of aquatic rooted plants, *Myriophyllum* and an unidentified emergent, were sampled. Antimony concentrations measured in the rhizosphere sediments, roots and leaves (Table 1) were significantly different (d.f. $= 2$, $F = 7.2$, $P = 0.009$; and d.f. $= 2$, $F = 8.5$, $P = 0.005$ respectively). A post hoc analysis revealed both plants accumulated Sb in the roots to concentrations similar to the rhizosphere sediments, but that leaf concentrations were significantly lower, suggesting some barrier hinders transfer from roots to leaves.

The ratio of analyte concentration in different plant components for both aquatic plant species is presented in Table 3. Antimony sediment to leaf concentration ratios were low at 0.24 for *Myriophyllum* and 0.31 for the unidentified emergent. Because of the high sediment concentrations, leaf Sb concentrations were significantly elevated (up to $212 \pm 30\,\mu g\,g^{-1}$) despite the low concentration ratios for Sb. Examination of the root and leaf Sb ratios for both plant species showed a similar pattern of antimony uptake and storage, with higher concentrations in roots than in leaves.

The highest As concentrations measured in the Bakers Creek ecosystem were present in the rooted aquatic autotrophs. Arsenic concentrations in leaves were greater than concentrations reported in other studies.[39,43] Arsenic concentrations measured in the sediments, roots and leaves of *Myriophyllum* and the unidentified emergent were significantly different (d.f. $= 2$, $F = 14.4$, $P = 0.0007$; and d.f. $= 2$, $F = 41.7$, $P < 0.0001$ respectively). A post hoc analysis revealed that the roots of both species had the highest As concentrations, being significantly higher than the sediment concentration. Transfer to the roots was efficient in both plants, with sediment to root As concentration ratios of up to 11.8 (Table 3). For both species, leaf concentrations were significantly lower than root concentrations ($<$50% of root concentrations). Relative uptake from roots to

Table 3. Antimony and arsenic concentration ratios in riparian vegetation and aquatic rooted plants
Concentration ratio expressed as concentration in receiving compartment divided by concentration in source compartment. N.D., not determined. Leaf concentration is below detection limit indicating no transfer to plant leaf

	Sediment to root		Root to leaf		Sediment to leaf	
	Sb	As	Sb	As	Sb	As
Persicaria spp.	0.83	0.95	0.04	0.19	0.03	0.10
Pennisetum clandestinum	0.47	0.76	0.13	0.26	0.03	0.07
Lomandra spp.	0.04	0.05	0.06	N.D.	0.002	N.D.
Paspalum distichum	3.68	1.42	0.04	0.07	0.11	0.08
Juncus krussii	0.05	1.60	0.05	0.03	0.03	0.04
Emergent macrophyte	0.76	11.8	0.54	0.47	0.31	4.7
Myriophyllum	0.66	9.2	0.84	0.14	0.24	0.54

leaves was lower in *Myriophyllum*, suggesting a limitation to the availability of As to leaves. However, mean As concentration in the unidentified emergent was high at $245 \pm 63\ \mu g\ g^{-1}$. Relative uptake of As by roots and leaves in both species was greater than for Sb, demonstrating that Sb has a lower uptake efficiency than As in the aquatic plants of Bakers Creek.

Metalloid uptake by aquatic autotrophs may be controlled by many factors including sediment mineralogy, organic matter, conditions such as pH, cation exchange, redox potential, plant species and the metalloid concentration.[44,45] The elevated Sb and As concentrations in the aquatic autotrophs could also be due in part to uptake or adsorption from water.[46,47] Previous studies have shown that Sb and As accumulate in the roots of aquatic plants with some barrier preventing transport to aboveground plant parts.[43] It is well known that aquatic plants can form a coating of iron oxide on their roots due to rhizosphere oxidation of ferrous iron to ferric oxide.[48–50] The iron plaque can influence the availability and uptake of nutrients and contaminants. It may sequester the contaminant and prevent movement into the plant[51] or it may serve to increase availability[37] depending on plant species. Significant relationships between iron and As in marine angiosperms have been identified.[52] However, the uptake process and the effect of iron plaque for Sb is not yet understood although Sb is known to sorb strongly to iron oxyhydroxides[53,54] and a positive relationship between ferric oxide and Sb in brown forest soils has been reported.[55] The sequestration of As onto root iron plaques may explain the high As root concentrations in the two aquatic plant species sampled at Bakers Creek. In comparison, relatively less Sb is associated with roots, suggesting that iron plaques may not be important for Sb in these plants or that Sb sequestration was less than As, if they were present. The presence of iron plaques on the plant roots was not confirmed in the present study.

Invertebrates

All invertebrates sampled showed elevated concentrations of Sb and As. Antimony concentrations were higher, reflecting the relative contaminant concentrations in the Bakers Creek system. Antimony concentrations in the sediment were significantly greater than those measured in the trophic groups (d.f. = 6, $F = 6.72$, $P = 0.00004$). However, there was no significant difference in Sb concentrations between the trophic groups (d.f. = 4, $F = 0.55$, $P = 0.70$) (Table 1, Fig. 2). Antimony concentrations were elevated in all organisms sampled except the gastropods, where concentrations were below detection limits ($<0.03\ \mu g\ g^{-1}$). The lowest measured mean Sb concentration

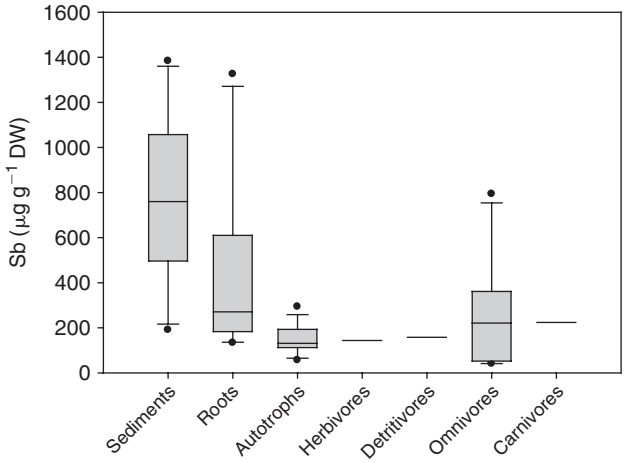

Fig. 2. Antimony concentrations ($\mu g\ g^{-1}$ DW, dry weight) measured in sediment, plant roots and trophic groups in the Bakers Creek ecosystem. Bar, mean; box, 25th and 75th percentiles; and whiskers, 5th and 95th percentiles.

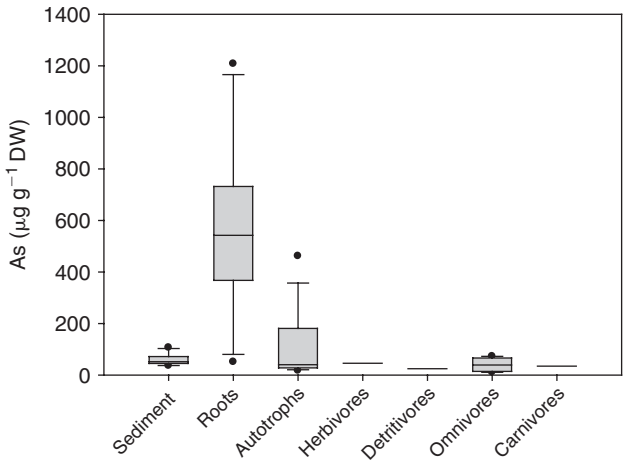

Fig. 3. Arsenic concentrations ($\mu g\ g^{-1}$ DW, dry weight) measured in sources and trophic groups in the Bakers Creek ecosystem. Bar, mean; box, 25th and 75th percentiles; and whiskers, 5th and 95th percentiles.

was in the flat-head gudgeons ($0.3 \pm 0.3\ \mu g\ g^{-1}$), with the worms having the highest mean Sb concentration ($316\ \mu g\ g^{-1}$) probably owing to enhanced exposure via skin contact and also relatively higher consumption of contaminated sediment.[56,57]

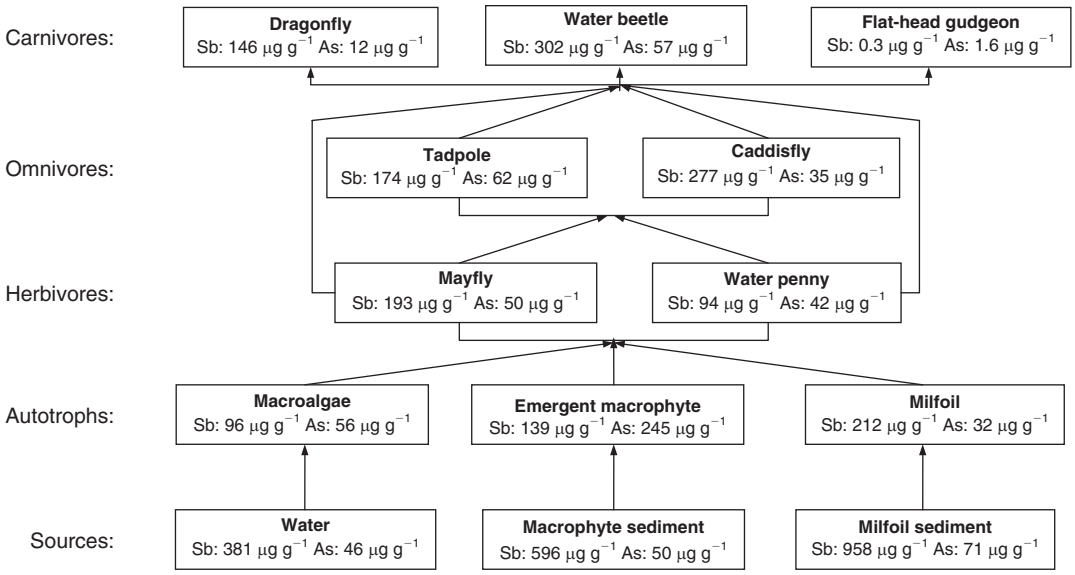

Fig. 4. Proposed hierarchical food web for the Bakers Creek ecosystem. Refer to Table 1 for Latin nomenclature of organisms.

Mean As concentrations measured in sediment and trophic groups (autotrophs (leaves), herbivores, detritivores, omnivores, and carnivores) were not significantly different (d.f. = 5, $F = 2.49$, $P = 0.053$) (Fig. 3). Similarly to Sb concentrations, the lowest mean As concentrations were again measured in the gastropods ($1.8\ \mu g\ g^{-1}$) and flat-head gudgeons ($1.6 \pm 0.4\ \mu g\ g^{-1}$). The tadpoles showed the highest mean As concentration ($62 \pm 2\ \mu g\ g^{-1}$).

Understanding metalloid transfer between organisms within an aquatic system is difficult. Food is potentially an important source of uptake but other exposure routes may also be significant[58] and distinguishing the relative importance of different sources may often prove difficult. The Sb and As water concentrations in Bakers Creek were extremely high and will be influencing the concentrations measured in organisms, e.g. through dermal adsorption, and offer one explanation for observing no significant difference between Sb and As concentrations in trophic groups. However, metalloid availability will differ among functional feeding groups. Filter feeders, collector gatherers, shredders and grazers are known to accumulate higher concentrations of certain metals compared with omnivores and predators.[59] The higher metalloid concentrations found in the worms in Bakers Creek are consistent with this observation, and concentrations in tadpoles may also be a result of specific functional feeding habit in the creek. Detritus, mainly formed from decaying aquatic and riparian vegetation, a major food component of an aquatic ecosystem, is consumed by caddisfly, Ephemeroptera (mayfly), Coleoptera (water pennies), oligochaetes and gastropods and may be one of the key entry points for metalloids into the food web.[59] However, detritus and periphyton samples collected from the Bakers Creek ecosystem were compromised during transportation to the laboratory and could not be included for analysis. Detritus normally has higher metalloid concentrations as degradation of fresh litter removes easily mineralized organic material, leaving metalloids concentrated in the remaining material. Thus detritus in this creek would be expected to contain high Sb and As concentrations based on Sb and As concentrations measured in the leaves of autotrophs and riparian vegetation (Tables 1 and 2).

A few studies have investigated the bioaccumulation of As in freshwater invertebrates,[39,60–62] but to date, bioaccumulation of Sb through a food chain has not been reported. Aquatic invertebrates may take up trace elements directly from water and sediments and act as a trophic link to higher food chain organisms. Understanding bioaccumulation is important for risk assessment to enable protection of all trophic levels in an ecosystem. We have attempted to understand Sb and As bioaccumulation for the Bakers Creek System by construction of a typical hierarchical food web (Fig. 4) including the organisms sampled. The proposed food web was based on the assumption that each organism consumed all species sampled from its targeted food group such that, for example, herbivores consumed each type of autotroph sampled. The food web shows that Sb and As were detected in upper trophic levels of the ecosystem. Biomagnification of Sb and As is not evident, with some organisms having lower Sb and As concentrations at higher trophic levels. Possibly some organisms have mechanisms to exclude or excrete Sb and As. Also, it seems that exposure to Sb and As via water is relatively unimportant for some organisms. At this stage, it is not possible to assess the toxicological effects of the metalloids in the invertebrates.

Aquatic invertebrates are often excellent bioindicators of trace element pollution in aquatic ecosystems and can be used to monitor and assess water quality.[34] In addition to feeding habits, exposure to contaminants and sensitivity has been linked to morphological characteristics (such as presence or absence of chitinised outer parts), size, habitat, location in the food chain, and life cycle.[63] Some populations decrease with increasing contamination levels (e.g. mayfly) whereas others are less sensitive (e.g. gastropods). Antimony contamination was reported to have a significant impact on a macroinvertebrate community in the Yesilirmak River, Turkey.[64] Exposure to arsenic is also known to induce behavioural responses in some invertebrates, for example phototactic behaviour.[65] Table 4 shows that Bakers Creek contained a range of organisms that can tolerate a wide range of environmental conditions. SIGNAL grades for the freshwater macroinvertebrates sampled range from 1 (gastropod) to the more sensitive 9 (Ephemeroptera) (Table 4). Antimony and As concentrations measured in Bakers Creek were above guideline values for protection of aquatic ecosystems and should impair ecosystem health.[36] However, caddisfly with a SIGNAL grade of 8, which is not commonly found in

Table 4. Macroinvertebrates sampled from the Bakers Creek ecosystem with SIGNAL grades

A SIGNAL (Stream Invertebrate Grade Number) grade is given for group, order and family levels. This indicates the pollution tolerance or intolerance for invertebrates within that group. A grade of 10 indicates a high sensitivity to pollution, a grade of 1 indicates a greater tolerance to pollution[34]

Taxa	Common name	SIGNAL grade
Odonata	Dragonfly	3
Coleoptera	Water beetle	5
Coleoptera (*Psephenidae*)	Water pennies	5
Trichoptera	Caddisfly	8
Oligochaeta	Worm	2
Gastropoda	Snail	1
Ephemeroptera	Mayfly	9

polluted waters, was the most abundant invertebrate sampled. Also, large numbers of mayfly (SIGNAL grade 9), which require water of good quality to breed, were sampled. The presence of macroinvertebrates with low ecological tolerances despite the elevated metalloid concentrations measured indicates that Sb and As concentrations are not obviously affecting the Bakers Creek ecosystem health and that the ecological guidelines may be conservative for this situation.

Riparian vegetation

Mean Sb and As concentrations measured in rhizosphere soils, roots and leaves of the five sampled species of riparian vegetation are presented in Table 2. Rhizosphere soil Sb and As concentrations were significantly greater than regional background concentrations ($1.1\,\mu g\,g^{-1}$ for Sb and $7.9\,\mu g\,g^{-1}$ for As).[24] Antimony concentrations were in general consistent with previous data for Bakers Creek[24] and greater than the ANZECC/ARMCANZ ISQG values.[36] Arsenic concentrations were lower than previously reported values.[24] They were, however, greater than the ISQG-Low guideline value and approaching the ISQG-High guideline value. Again, variability in sediment concentrations may account for differences with previous sampling at Bakers Creek.

Natural background Sb and As concentrations in terrestrial vascular plants growing in uncontaminated and unmineralised soils are typically low, 0.0001–$0.2\,\mu g\,g^{-1}$ dry mass for Sb and 0.01–$1.5\,\mu g\,g^{-1}$ dry mass for As.[16] Background concentrations in riparian vegetation sampled in the Bakers Creek region range from <0.01 to $0.22\,\mu g\,g^{-1}$ for Sb and 5.0 to $8.4\,\mu g\,g^{-1}$ for As.[24] For all species in the present study, leaf Sb concentrations were greater than area background values. However, As plant leaf concentrations fell within the background range.

For each species, Sb concentrations measured in the rhizosphere soils, roots and leaves of riparian vegetation were significantly different ($P < 0.05$). A post hoc analysis revealed for *Pennisetum clandestinum* and *Lomandra* spp. that rhizosphere soils had the highest mean Sb concentration followed by the roots. Leaf Sb concentrations were significantly lower than the root Sb concentrations, indicating that these plants maybe excluding Sb. For *Juncus krussii* and *Persicaria* spp., a post hoc analysis revealed no significant difference between the rhizosphere soils and roots, although antimony concentrations measured in the leaves were again significantly lower. A post hoc analysis for *Paspalum distichum* revealed that the roots had the highest mean Sb concentration, followed by the soil and leaves. The *P. distichum* appears to be accumulating Sb with its roots. The accumulated antimony, however, is not being transported to the leaves readily, although *P. distichum* did show the greatest leaf Sb concentrations ($28 \pm 4\,\mu g\,g^{-1}$). The concentration ratios (Table 3) indicate that uptake into *Lomandra* spp. was lowest with a rhizosphere soil to leaf Sb concentration ratio of 0.002, whereas uptake into *P. distichum* was greatest with a rhizosphere soil to leaf Sb concentration ratio of 0.11. Kabata-Pendias[66] states that typical Sb soil to plant transfer ratios are ~0.01, in agreement with the data here, except for *P. distichum*, which shows more efficient transfer. *P. distichum* showed the most efficient sediment to root transfer, but *P. clandestinum* showed the most efficient root to leaf transfer.

Previous studies have shown wide interspecies variability in plant Sb concentrations and uptake.[2,67] Hyperaccumulation has been reported for some species as well as extreme Sb concentrations in plants growing on grossly contaminated sites.[68–70] However, certain plant species are known to exclude Sb, e.g. *Cytisus striatus*.[70] Surface deposition can also account for a high proportion of the total Sb content of plants.[71] Uptake can be related to the form and bioavailability of Sb in the soil environment. Low plant uptake reported in highly contaminated mining areas is a result of low availability.[72] Relatively greater plant Sb concentrations were measured when Sb was present in a more bioavailable form.[28] The data reported here show no evidence of Sb hyperaccumulation but indicate limited transfer of rhizosphere soil-associated Sb to plant leaves. Rhizosphere soil-associated Sb transferred to the roots of *Juncus krussii*, *Persicaria* spp. and *Paspalum distichum* may not be readily transferred to the upper plant parts owing to presumably some form of exclusion mechanism. At this stage, the mechanism used by plants to take up and transport Sb is unknown. Antimony in the roots of *P. distichum* in particular may, as previously mentioned, be due to an association between Sb and iron plaque on the root surface[73] or to inadvertent uptake and storage of Sb in place of an essential nutrient such as phosphate. It is well known that As can act as a phosphate analogue but a recent study growing maize (*Zea mays*) and sunflower (*Helianthus annuus*) in hydroponic systems indicated that Sb is probably not taken up via the phosphate pathway.[74] In the Bakers Creek system, plant data suggest that a portion of the rhizosphere soil-associated Sb is present in a bioavailable form that may be accumulated in the roots of some species. However, the extent of uptake to the leaves depends on the plant uptake mechanisms and appears not to be efficient for the plant species sampled. Little is known about the phytotoxicity of Sb, with critical concentrations in rice plants estimated at $150\,mg\,kg^{-1}$ for Sb^{III} and $300\,mg\,kg^{-1}$ for Sb^{V} in plant tissues.[7] Based on the Sb concentrations measured in vegetation, and given that $100\,mg\,day^{-1}$ is chronically toxic to animals,[16] direct exposure to the sediment Sb (e.g. by consumption) would seem to pose a greater risk to the ecosystem fauna.

Arsenic concentrations measured in the rhizosphere soils, roots and leaves were significantly different ($P < 0.05$) for each species of riparian vegetation. A post hoc analysis revealed that for *Lomandra* spp., rhizosphere soil had the highest mean As concentration followed by the roots. Arsenic was not detected in the leaves, suggesting that *Lomandra* may be excluding As as well as Sb. For *Persicaria* spp., *P. clandestinum*, *P. distichum* and *J. krussii*, As concentrations in the rhizosphere soils and roots were not significantly different but As concentrations in the leaves were significantly lower. Ratios of rhizosphere soil to leaf As concentrations were 0.1 or less, with *Persicaria* spp. showing

the greatest ratio and leaf concentration ($6.8 \pm 1\ \mu g\ g^{-1}$). There was no evidence of As hyperaccumulation in the plant species studied. Despite the elevated soil As concentrations, soil–leaf As ratios were low (≤ 0.1), lower than those typical reported for As (approx. 0.2).[66] Most As accumulation occurred in the roots in agreement with other published studies.[75] Kabata-Pendias[66] states that soil-to-plant transfer for Sb is ~20 times lower than for As. However, in the current study, for the riparian vegetation species, As concentration ratios (Table 3) were no more than two to three times greater than or not significantly different to those for Sb.

Conclusions

Data collected during the present study have shown that the water and rhizosphere sediment of Bakers Creek contains significantly elevated levels of mining-derived Sb and to a lesser extent As. Antimony and As concentrations were elevated in aquatic autotrophs but data indicated that Sb uptake was lower than As uptake. Elevated Sb and As concentrations were measured in the macroinvertebrates in upper trophic levels and other organisms. Biomagnification was not evident. At this stage, it is not possible to definitively assess the toxicological effects of Sb and As on Bakers Creek invertebrates and further study is needed to assess the risk to the Bakers Creek ecosystem.

Antimony and As accumulation in the riparian vegetation was not evident. Even though rhizosphere soil Sb and As concentrations were elevated, transfer to the plant leaves was low for both metalloids. Arsenic rhizosphere soil to plant concentration ratios were up to two to three times greater than Sb ratios, depending on plant species. Elevated Sb concentrations were, however, present in the plant roots and further investigations into the role of iron plaque on plant roots will improve the current understanding of the plant uptake mechanisms for Sb. Based on the Sb and As concentrations measured in riparian vegetation, direct exposure to the rhizosphere sediment and soils, water ingestion and consumption of aquatic autotrophs appear to be the major routes of Sb and As uptake for the fauna of Bakers Creek.

The present study was the first part of a catchment-wide project investigating Sb and As bioavailability, and also mobilisation with river flow regime. Further work is also needed to investigate Sb and As speciation and toxicity in the ecosystem as well as the additional project data to fully understand the risks posed by the presence of Sb and As in the Bakers Creek ecosystem.

Acknowledgements

The authors would like to thank the NSW Environment Trust for funding the project and Straits Hillgrove Gold for allowing access to the site and providing logistical support. We thank Hugh Doyle for assistance in sample collection.

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Manuscript received 15 December 2008, accepted 12 February 2009