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Impact of arsenic and antimony contamination on benthic invertebrates in a minor Corsican river

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Abstract

The chemical and biological characteristics of a Corsican river that drains contaminated waters and sediment from an abandoned realgar mine were studied. The concentrations of pollutants such as As and Sb were found to be notably high. For example, in the Presa River, downstream the realgar mine, the mean As concentrations in the water and in the sediment were 3010 μg $1^{-1}$and 9450 μg g^{-1} respectively. Species richness and abundance of benthic invertebrates decreased downstream the As mine. The disappearance of oligochaetes, leeches and a rarity of mayfly populations have been evidenced. On the contrary, a large increase in species belonging to stoneflies and gastropods was found. Some species like *Baetis cyrneus, Ephemerella ignita, Sericostoma clypeatum* and *Ibisia marginata* were more sensitive to As and Sb concentrations while others species like *Ancylus fluviatilis, Isoperla insularis, Hydropsyche cyrnotica, Caenis luctuosa* and *Silonella aurata* were less sensitive. The benthic invertebrates show different reactions in this contaminated environment, which could be explained by their feeding behaviours and certain morphological characteristics.

Introduction

A decline in biodiversity of benthic communities has often been related to metal pollution (Clements, 1994; Gibert et al., 1995; Kiffney & Clements, 1996; Hill et al., 1997), but As and Sb are not often studied (in contrast to Zn, Cu, Cd).

Arsenic, and to a lesser extent, antimony, are important environmental pollutants related to anthropogenic activities. Large quantities of arsenic compounds are released into the environment, particularly through mining operations and biocidal products; Sb chemistry is very similar to that of As (Migon et al., 1995).

As and Sb bioaccumulation has been demonstrated for aquatic invertebrates by Sanders & Cope (1968), Schuth et al. (1974) and Irgrolic et al. (1977).

Toxicity and bioaccumulation of arsenic are strongly dependent on its chemical state, in particular its chemical speciation (Moore & Ramamoorthy, 1984; Phillips, 1990). Trivalent and pentavalent inorganic ionogenic forms of As can exist dissolved in natural waters, as well as organic forms. According to Spehar et al.(1980) the inorganic form is the most toxic and its bioaccumulation is more significant.

$As^{(III)}$ is considered to be more toxic than $As^{(V)}$ (Fergusson & Gavis, 1972; Shannon & Strayer, 1989). $As^{(III)}$ probably reacts with SH groups in proteins while $As^{(V)}$, chemically similar to phosphate, is quickly assimilated by microorganisms (Elinder, 1984) and may inhibit primary production, especially if the external phosphate concentration is low (Sanders, 1979).

The toxicity of antimony is less well documented. The relative toxicity of $Sb^{(III)}$and $Sb^{(V)}$ is less known than that of As, but it seems that the oxidation state is a factor influencing excretion rate: $Sb^{(III)}$should be eliminated less quickly than $Sb^{(V)}$ (Migon et al., 1995). Since the chemistry is similar to that of As, fate and effect may also be comparable.

The present study addresses the impact of an abandoned realgar mine on the chemical and biological components of a minor Corsican river, especially the As and Sb sensitivity of benthic invertebrates.

Material and methods

Sampling sites

The Bravona river spring (central Corsica, Figure 1) is located on the Punta de Caldane slope (1700 m). The watershed is 140 km^2. After 38 km with a mean slope of 4.5%, the river reaches the Tyrrhenian Sea, nearby Aleria, on the eastern coast of Corsica. One of its affluents, the Presa river, crosses an abandoned realgar mine, close to the village of Matra. The deposit was discovered between 1880 and 1890. In 1913, the ore production reached its highest value, more than 4,000 tonnes per year. After a production of 30,000 tonnes of ore with 30% of As, the mine was abandoned in 1945.

Ten sampling sites were chosen (Figure 1): 6 are located on the axial course of the Bravona river, 3 on the Presa river (1 upstream the mine and 2 downstream) and 1 on a secondary affluent (the Alzillelo river). The stations B1, B2, P1 and Al were reference sites, B3, B4, B5 and B6 were slightly polluted sites and P2, P3 were more polluted sites. Mean monthly flow rate of the Bravona river (station B5) is given in Table 1.

Experimental

For all the stations, nine campaigns of water and sediment sampling and three campaigns of benthic fauna sampling were carried out (winter, spring and summer 1990). For each site, water samples were divided into 2 subsamples. The first one (plastic bottle) was used for measuring pH and Ca. The second one (Teflon bottles, FEP) was used for the measurement of metalloid concentrations. Prior to sampling, Teflon bottles were rinsed with Suprapur HNO_3 and demineralized water and then ultrasonically cleaned. Water samples were acidified with HNO_3 (1%).

Surface sediment (0–6 cm) samples were collected in Teflon bottles. Sediments were mineralized in a closed Teflon bottle and heated in a micro-wave oven. Ultrapur acids were used (HNO_3, H_2SO_4 and HF), and then a dilution in boric acid 6%.

As and Sb were measured by graphite furnace atomic absorption spectrophotometry, using the hydride generation method. The spectrophotometer was a Perkin-Elmer 3100 with EDL As and Sb lamps. The generation of hydrides was carried out with a Perkin Elmer FIAS 200; the rinsing solution was HCl 3% and the reducing solution was $NaBH_4$ 0.2% in NaOH 0.05%. The vector gas was argon (flow: 100 ml mn^{-1}). The cell temperature was 900 °C. Standard solutions were provided by Merck. Relative standard deviations were 0.5–5% and 1–10% for As and Sb respectively.

The completely automated hydride generation (quartz furnace atomic absorption spectrometric technic) was used for As chemical speciation. Briefly, subsamples (0.2 ml) of enzymatic extracts are introduced into the reaction flask with 100 ml of deionized water acidified by 1 ml acetic acid. Organotin compounds are reduced to stannanes by addition of an alkalyne sodium borohydride solution introduced via a peristaltic pump. Organotin hydrides flushed from the solution by the generated H_2 are then carried by a helium flow to a glass column packed with chromatographic material and cooled in liquid nitrogen. After removing the cryogenic trap the column is progressively warmed, first at ambient temperature, then by electrical heating, allowing sequential volatilization of the separated hydrides that are eluted and flushed by helium in a quartz furnace (950 °C) set in the light beam of an atomic absorption spectrometer.

Benthic macroinvertebrates were sampled quantitatively with a Surber sampler (catch net mesh: 0.25 mm, area sampled: 0.1 m^2). The substrate within the sampler was removed; large rocks were brushed then returned to the stream. The samples were preserved with formalin (5%). For each station, four standard samples (0.1 m^2) were taken. The ten locations, sampled on three occasions, rendered 120 samples of 0.1 m^2. Organism densities have been standardized to organisms m^{-2} in the Results.

Results

Chemistry

Mean values with their range are given in Table 2 for water and Table 3 for sediment.

In the 10 sampling sites, pH values were nearby neutrality and Ca concentrations varied little (30–45 mg l^{-1}). These values were closely correlated with granitic substrate. The values of pH do not exhibit great variations here and the effect of this parameter on As and Sb concentrations is insignificant. Concentrations of As and Sb are particularly high nearby the

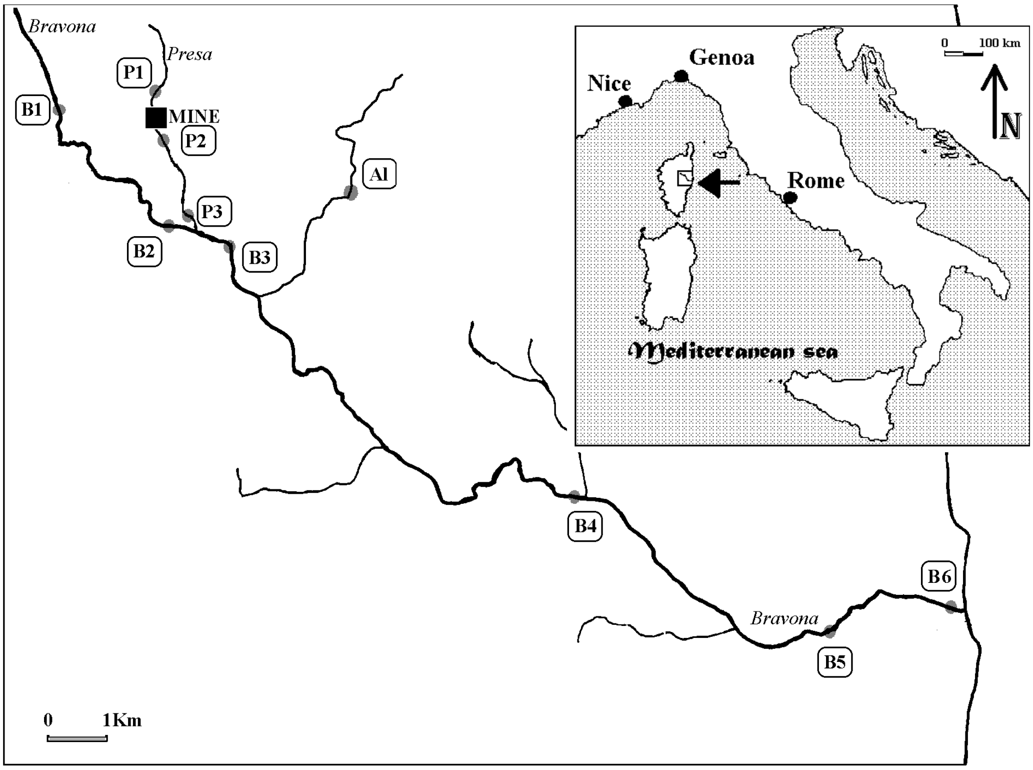

Figure 1. Location of sampling sites.

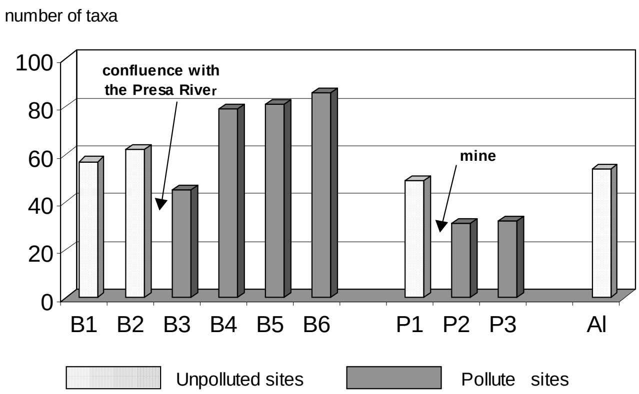

Figure 2. Taxonomic abundance in the ten sampling sites.

Table 1. Mean monthly flow ($m^3\ s^{-1}$) of Bravona river (sampling site B5, 1961–1988)

Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1.45	2.57	1.62	1.78	0.91	0.51	0.34	0.28	0.28	0.47	0.56	1.37

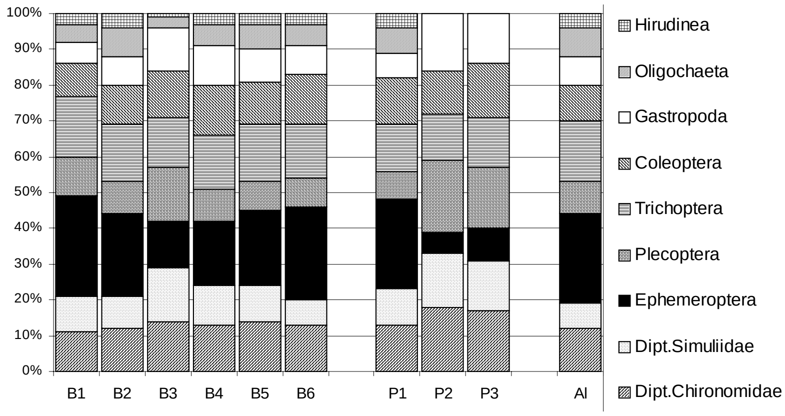

Figure 3. The relative abundance of the major faunistic groups in the sampling sites.

Table 2. Average concentration in the water of chemical variables of sampling sites

	B1	B2	B3	B4	B5	B6	P1	P2	P3	Al
pH	7.3	7.4	7.3	7.6	7.5	7.4	7.5	7.6	7.6	7.4
st. dev. (*N*=9)	0.23	0.11	0.05	0.22	0.31	0.18	0.16	0.09	0.06	0.19
Ca(mg $l^{-1)}$	30	31	30	36	40	42	30	32	30	31
st. dev. (*N*=9)	2.15	3.16	3.29	8.74	4.56	5.13	4.01	4.45	1.23	4.9
(μg l^{-1})	4	7	430	142	41	40	60	3010	2850	10
st. dev. (*N*=9)	0.12	1.68	22.08	12.26	2.09	2.89	6.54	69.07	60.54	0.66
Sb (μg l^{-1})	1	3	41	31	10	10	4	249	385	3
st. dev. (*N*=9)	0.58	1.31	6.39	6.97	3.03	2.56	0.63	11.7	18.89	0.58

st. dev. = standard deviation

ancient mine. In the Presa river, downstream the realgar mine (site P2), the mean As concentrations in the water and in the sediment were high: 3,010 μg $1^{-1}$and 9,450 μg g^{-1} respectively. In the Bravona river a dilution effect was observed. Table 2 shows the mean As concentration in the sites along the axial flow of the Bravona river. In the upper stretch (sites B1 and B2), the As concentration was low (4 and 7 μg 1^{-1}, respectively). After the confluence with the Presa river (sites B3), it increased sixtyfold (430 μg 1^{-1}). In the lower stretch (sites B5 and B6), the As concentrations (41 and 35 μg 1^{-1}respectively) were below directives formulated by French policies (DDASS, 1995), i.e. 50 μg 1^{-1}.

Chemical speciation analysis showed that $As^{(III)}$, the most toxic form, was predominant in sediment (91.2%) and $As^{(V)}$predominant in water (98.9%).

For the case of Sb, after crossing the mining spoils, the Presa river became very enriched (249–385 μg 1^{-1}). These values are 25–40 times higher than the maximum admitted concentration (DDASS, 1995). In the upper stretch of the Bravona (sites B1 and B2), the Sb concentration was low (1–3 μg 1^{-1}). After the confluence with the Presa river (site B3), it increased 14 fold (41 μg 1^{-1}). The values found in the lower flow of the Bravona river (10 μg 1^{-1}; sites B5 and B6) were close to the French directives (DDASS, 1995).

Benthic invertebrates

At the different stations 121 taxa were found. The sites situated along the axial flow of the Bravona river (B1 to B6) were the richest, 57 to 81 taxa respectively (Figure 2). The sites on the affluent rivers (P1 to P3 and Al) had the smallest number of taxa. The high As and Sb values caused a decrease in the specific communities of the benthic populations. In the Presa River, the taxonomic diversity decreased from 49 (site P1, i.e. upstream the mine) down to 31 (site P2, i.e. downstream the mine), because of the impact of the mine. The diversity of the benthic communities in the

Table 3. Average As and Sb concentrations in sediment (μg g^{-1})

	B1	B2	B3	B4	B5	B6	P1	P2	P3	Al
As	10	13	354	207	95	4	35	9450	3297	17
st.dev. (*N*=9)	0.58	1.52	7.57	8.51	3.05	4.04	1.52	81.71	89.72	1.54
Sb	16	6	40	35	20	5	13	1108	300	4
st. dev. (*N*=9)	1.53	0.58	1.15	3.05	2.64	0.72	0.53	44.91	11.15	0.58

Table 4. Density (organisms m^{-2}) of some macroinvertebrates in unpolluted and polluted sampling sites

	P1, B1, B2, Al	P2, P3
Leuctra geniculata	42	443
Silonella aurata	30	251
Isoperla insularis	18	160
Chloroperla apicalis hamulata	12	84
Hydrocyphon australis	21	72
Helicopsyche revelieri	4	51
Thremma sardoum	9	45

reference non-polluted sites (B1 and Al) ranged from 54 to 57 species.

As and Sb concentrations had also an impact on the specific composition of the populations along the axial flow of the Bravona river. Upstream the confluence with the Presa river (site B2), the taxonomic abundance value was 62, while downstream the confluence (site B3), only 45 taxa were found. Not only the number of taxa decreased but also the macroinvertebrate densities. The maximal abundance of some macroinvertebrates in unpolluted (P1, B1, B2, A1) and polluted (P2, P3) sampling sites is presented in Table 4.

Results concerning Insect larvae and Mollusca and Annelida phyla are presented separately. The relative abundance of the major faunistic groups, in the ten sites' samples, is presented in Figure 3.

Diptera, Trichoptera, Ephemeroptera, Plecoptera and Coleoptera represented 72% of the inventoried species of the ten sampling sites. In reference sites, the Diptera Order was important in the benthic community (18–23%), Chironomidae (Orthocladiinae, Tanypodinae, Tanytarsini) and Simuliidae being the best represented. But in polluted sites (P2, P3), the larvae of *Tipula* sp., *Clinocera* sp., *Ibisia marginata, Tanytarsini, Chironomini, Stratiomyidae* and *Chelifera* sp. were not found in benthic communities. Caddisflies, essentially represented by *Hydropsyche fumata, Rhyacophila tarda, Sericostoma clypeatum* and *Allogamus corsicus* constituted 13–17% of the aquatic fauna in unpolluted sites. In the sites situated downstream the mine, certain caddisflies, *Sericostoma clypeatum, Leptodrusus budtzi* and *Allogamus corsicus* were not found. In reference sites, mayflies represented 23–28% of the total effective. This group was dominated by four species: *Baetis ingridae*, *Baetis cyrneus, Heptagenia* sp. and*Caenis luctuosa*. However, in polluted sites mayflies represented only 6–8% of the benthic fauna and certain mayfly larvae (*Ecdyonurus* sp., *Heptagenia* sp., *Baetis cyrneus, Ephemerella ignita* and *Habrophlebia fusca*) were not found dowstream the mine. In unpolluted sites, Plecoptera group represented 8–11% of the aquatic fauna. The best represented species was *Protonemura bucolica*. In polluted sites, *Leuctra budtzi, Leuctra geniculata, Isoperla insularis* and*Chloroperla apicalis hamulata* populations survived despite pollution but *Protonemura bucolica* larvae are not found. In reference sites, the Coleoptera population (9–13% of total fauna) was dominated by *Esolus brevis* and *Stictonectes rufulus*. In polluted sites, *Yola bicarinata obscurior, Esolus brevis, Elmis maugetii fossulata, Hydraena subacuminata* and *Helichus substriatus* were, however, among Coleoptera the most sensitive species to high As and Sb concentrations. Moreover, in the Presa river, 50% of Coleoptera remained downstrean the mine and the larvae of *Hydrocyphon australis* were best represented.

Among Molluscs, gastropods represented 6–8% of the aquatic fauna in unpolluted sites. In polluted sites, gastropods represented 14–16% of the benthic fauna but *Potamopyrgus jenkinsi* and *Planorbis* sp. did not resist to contamination, while *Ancylus fluviatilis* and *Lymnaea peregra*, as well as the bivalve *Pisidium casertanum*, were less sensitive to high As and Sb concentrations.

The abundance of oligochaetes (5–8%), in references sites, shows the importance of allochthonous input of organic matter from the riparian zone; in lenitic facies, where decomposing detritic material deposits, the density of these worms reached 1280 organisms m^{-2}. But where the sites are polluted by As and Sb enrichment, oligochaetes disappeared, in particular *Eiseniella tetraedra*. The Hirudinea *Glossiphonia* sp. also disappeared from the polluted zone.

Actually, As and Sb pollution did not lead to an important decrease in abundance. In the highly polluted stations (P2 and P3), the density of the benthic communities varied from 2 600 to 3 800 organisms m^{-2}, while in the reference sites (B1, B2, Al, P1) the density ranged from 2900 to 4600 organisms m^{-2}.

In polluted sites, the abundance of *Protonemura buccolica, Leuctra geniculata, Isoperla insularis* and *Chloroperla apicalis hamulata* put the stoneflies at the top of the list with 17–20% of the total. Gastropods came second with 14–16%, then the true flies with 13–14%, the caddisflies with 13–14% and the mayflies (essentially the species *Caenis luctuosa*) with 6–9%. In these four fauna groups, the dominating species were I *soperla insularis, Choroperla apicalis hamulata, Ancylus fluviatilis, Lymnaea peregra*. Orthocladiinae, Simuliidae, *Hydropsyche cyrnotica, Tinodes agaricinus, Silonella aurata* and *Caenis luctuosa*. Hence these taxa were less sensitive to high As and Sb concentrations.

Discussion

Concentration of As and Sb

Mean As and Sb concentrations (3000 and 400 μg l^{-1} respectively) of the Presa river are compared with those of other polluted and non polluted running waters. For example, in non-polluted freshwater, Wagemann et al. (1978) found that mean As concentration ranged from 1.6 up to 68 μg l^{-1}. According to Elbaz-Poulichet et al. (1989), in the Rhône river (France), the mean As concentration level is 2 μg $l^{-1}$and in the Krka river (Croatia), which can be considered to be an unpolluted river, As concentration is 0.08 μg l^{-1}. In the U.S.A., in a river contaminated by mining activities (Coeur d'Alene, Idaho), the As concentration was close to 6 μg l^{-1} and the Sb concentration 8 μg l^{-1} (Mok & Wai, 1990).

The As and Sb concentration levels recorded here are higher than in other polluted rivers. However, high concentrations were oberved in polluted lakes; according to Moore et al. (1979a), water in Keg and Meg lakes (Canada) has mean As concentrations as high as 2000 μg l^{-1}, the highest value being 3400 μg l^{-1}.

Benthic invertebrates

According to Moore (1979) and Winner et al. (1980), the diversity of macroinvertebrate communities is not a reliable tracer of metal and metalloid pollution. Downstream the confluence with the Presa river, the Bravona lost 17 species (i.e. 27.5% of the total diversity observed in B2). Nevertheless, Moore et al. (1979a) have often observed a decrease in specific abundance of the aquatic communities in metal-polluted waters: they found 9 to 13 species in some Canadian lakes where the mean As concentration is 2000 μg l^{-1}, 44 in the lakes where levels are found to be below 20 μg 1^{-1}. Armitage (1980) reported that rivers where metal and metalloid contamination is high, exhibit a low diversity of benthic invertebrate communities. The As chemical speciation as well as the concentrations found in the sediment may have induced differences within the toxicity factor values.

In the Presa river, downstream the mine, As and Sb enrichment caused the oligochaetes to disappear. Moore (1979) found oligochaetes in Canadian lakes where As concentration in the water ranged between 200 and 750 μg 1^{-1}; they are totally lost when concentrations reach 2000 μg 1^{-1} (Moore et al., 1979b). So, it seems that the lethal threshold of the oligochaetes was exceeded at 2000 μg 1^{-1}(in the Presa River, As concentration was 3010 μg l^{-1}).

According to Mori (1997), the lack of Oligochaeta a well as the decresase in Chironomidae larvae could explain the lack of Hirudinea which feed on these organisms. In agreement Wagemann et al. (1978) have noted too the lack of Hirudinea in As-polluted Canadian lakes. A number of gastropods like *Ancylus fluviatilis, Lymnaea peregra* or *Pisidium casertanum* resist to contamination in the Presa river. According to Moore et al. (1979b), *P. casertanum* is indeed less sensitive to high As and Sb concentrations, since it is particularly abundant in Canadian lakes polluted by As.

According to Giudicelli (1968) and Orsini (1986), Insect larvae represented by Diptera, Ephemeroptera and Plecoptera groups are important within the benthic communities of Corsican rivers. The Diptera group represents 15–29% of the benthic community of the Presa river. This group is dominated by chironomids, less sensitive to high As and Sb concentrations. In polluted Canadian lakes, Moore (1979), Moore et al. (1979a) and Winner et al. (1980) have found that the impact of an environmental As enrichment leads to a decrease in specific richness and density of chironomid populations. Chironomids' life-cycles in running waters and in lakes are different which may explain this contrast.

For the Ephemeroptera group, a large number of mayfly species are not found downstream the mine of the Presa river. In Canada, Wagemann et al. (1978) have also observed a lack of mayflies in As-polluted lakes. In contrast, stoneflies like *Isoperla insularis* or *Chloroperla apicalis hamulata* survive in the Presa river despite the As and Sb pollution.

Resistance to metalloid pollution and the decrease in interspecific competition, due to the low variety of benthic populations in polluted zones, could explain the proliferation of the species *Leuctra geniculata, Silonella aurata, Helicopsyche revelieri* and *Isoperla insularis*. According to Cummins & Klug (1979), when a species is dominant in rivers polluted by metal or metalloids, this is due to its bioresistance and to a lower interspecific competition. Three factors may explain the sensitivity of species to As and Sb pollution which leads to their disappearance downstream the mine:

1. *Feeding behaviour and diet:* Benthic invertebrates which feed on organic substance and sediment (shredders-detritivores) swallow a high quantity of metals (Mathis & Cummins, 1973), while those that are grazers (herbivores or substrate scrapers) and those that feed on epilithic algal populations (e.g. diatoms), accumulate a lower quantity of metal (Williams et al., 1987).

   The mode of nutrition (grazer-scraper) and the presence of a shell are the two major factors in the proliferation of gastropods.

   The diet of *Isoperla insularis* and *Chloroperla apicalis hamulata* larvae (predatory invertebrates) may explain their proliferation. Indeed, Wagemann et al. (1978) underscore that As bioaccumulation decreases when the position of organisms in the food web is higher.
2. *The morphologic characteristics:* The thin layer of the cuticle and the absence of chitinised or hardened parts may be assumed to act as a physical barrier. These reasons may explain the resistance of *Asellus* sp. with the presence of chitinised parts, to high lead grades (Eyres & Pugh-Thomas, 1978).

   Among the species that disappear downstream the As mine are those which live in zones of low flow with a detritivorous diet and a thin cuticle (*Eiseniella tetraedra, Oligocheta* sp., *Sericostoma clypeatum, Allogamus corsicus, Glossiphonia* sp., *Tipula* sp., Chironomini).

   The absence of mayflies (*Baetis cyrneus, Ephemerella ignita, . . .*) could be explained by the fact that the larvae have external branchiae that are highly exposed to pollution; Förstner & Whittmann (1983) have found that the accumulation took place at the level of the tegument and branchiae.
3. *The habitat of species:* Benthic organisms that live in thin sediments (like *Eiseniella tetraedra, Ephemerella ignita, Habrophlebia fusca, Allogamus corsicus*), in lenitic zones and therefore in contact with the high sediment concentration, are more exposed than rheophilous species (like *Ancylus fuviatilis, Isoperla insularis, Protonemura buccolica*) that live in erosion substate (cobbles and pebbles).

   In fact, 82% of taxa which disappear upstream the As mine (like*Eiseniella tetraedra, Oligocheta* sp., *Allogamus corsicus, Tipula* sp., Chironomini) are mainly in sediments, or with a part of their life-cycle in the sediments, and thus in contact with high sediment concentrations.

So, rheophilous organisms, which are often grazers and predators in the rapid Corsican waters (Orsini, 1986), run a lower risk of bioaccumulation (Wagemann et al., 1978).

Conclusion

The concentrations measured in this study are very high compared to other studies and the impact on the invertebrates is significant. As and Sb concentrations are very high in the water, and in the sediments where the most toxic form (As $^{(III)}$) was predominant.

The present study has demonstrated that As and Sb contamination has a strong impact on the benthic invertebrate community, and that the vulnerablity of the individual species clearly depends on the place in the foodweb and on feeding behaviour. Other factors may explain the sensitivity of species to As and Sb concentrations, e.g. the morphologic characteristics (size of the cuticle, presence or absence of chitinised parts, external branchiae) and the habitat of the species (lenitic or lotic zones). In fact, all these factors are closely related.

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