Impact of trace metals from past mining on the aquatic ecosystem:
A multi-proxy approach in the Morvan (France)

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1. Introduction

Trace metals (TMs) persist in the environment, reaching potentially toxic levels for biota over time (Linde et al., 1998; Alibabic et al., 2007). Elevated TM concentrations may result from human activity (e.g. emissions from urban and industrial areas), and also from the natural weathering of metal-enriched rocks and ore bodies (Aleksander-Kwaterczak and Helios-Rybička, 2008). In the case of mining, both natural and anthropogenic factors are involved; mineral deposits correspond to anomalous areas, while their exploitation may considerably extend metal dispersal. Mining and smelting activities often continue to affect the environment, even centuries after such sites have been abandoned (Casiot et al., 2009). River systems naturally drain mining waste (workshop grounds, slag heaps, and cleansing dumps), mobilising TMs by physical or chemical processes, and thus contaminating aquatic ecosystems for centuries, or even millennia (Miller, 1997). The first step in understanding how past mining still impacts ecosystems is by characterising TM geographic distribution (Deacon and Stephens, 1998). Trace metals may spread far from their place of origin under certain hydrological conditions (Audry et al., 2010). Analysing water chemistry is not completely satisfactory because water composition is affected by meteorological conditions. Streambed sediments are not true permanent sinks, but they do record the composite erosion products of terrains in the catchment area, including mines located upstream (Elbaz-Poulichet et al., 2011). Complex chemical procedures have been developed to approximate TM bioavailability (Gismera et al., 2004; Leleyter et al., 2012), but bioindicators have proved more valuable to assess environmental impacts, because they accumulate TMs over time (Bleuel et al., 2005). Two categories of bioindicators can be distinguished: sessile organisms yield information about contamination at the place where they live, while mobile organisms record contamination over a larger area (Cenci, 2000). Aquatic bryophytes, such as Fontinalis antipyretica, belong to the first category. They present several characteristics that make them...
suitable for monitoring studies (Bruns et al., 1997; Vázquez et al., 2004; Samecka-Cymerman et al., 2005): (i) they are essential members of food webs (Bleuel et al., 2005), and (ii) their cuticles are permeable to water, so that metals integrate their tissues by leaf surface and not by roots (Figueira and Ribeiro, 2005). These plants accumulate metals at levels several orders of magnitude higher than the water in which they live (Martins and Boaventura, 2002). Researchers have developed the so-called moss bag technique, which possesses the advantage of using a single species, with an adequate amount of material whatever the targeted site. Above all, time of exposure can be controlled precisely, making the interpretation of metal contents easier (Cesa et al., 2006). The wild brown trout (*Salmo trutta Fario*) belongs to the mobile category (up to ten kilometres, in our rivers, pers. comm. P. Berrebi). It is widely used as a sentinel species to monitor aquatic environments (Linde et al., 1998; Foata et al., 2009; Allenbach, 2011). This widespread predatory fish is situated at the top of the food web. Studies have already been undertaken on its tissues, providing a basis for comparison (Has-Schön et al., 2008; Monna et al., 2011). Fish liver is generally considered to be one of the best indicators of chronic exposure to TMs, because of its role in the accumulation, transformation and excretion of contaminants (Linde et al., 1998).

The present study focuses on the Morvan region. Today, this area is one of the least inhabited regions in France. It has nonetheless experienced several phases of mining and smelting of non-ferrous metals since at least the Bronze Age (Monna et al., 2004; Jouffroy-Bapicot et al., 2007). Even though no mining activity continued after the 20th century, mining may nevertheless have caused persistent local TM contamination. A sediment quality index (SQI; see Marvin et al. (2004) for details) was computed from TM concentrations (i.e. As, Cd, Cr, Cu, Ni, Pb and Zn) measured by the French Geological Survey (BRGM) in streambed sediments, to find new mineral deposits. After the SQI map had been used to provide a general overview of the Morvan, the map was combined with archaeological data to select three specific watersheds, differently affected by past mining (low, medium, and high contamination). For each watershed, four commonly investigated trace elements were studied: Cu and Zn (essential), and Cd and Pb (non-essential). Concentrations were measured in transplanted aquatic mosses (*F. antipyretica*) sampled twice, a month apart. Liver Cd, Cu, Pb and Zn concentrations were also measured in wild brown trout (*S. trutta*), caught at the same sites. Body condition indices were calculated, as they are well-known indicators of organism health (Peig and Green, 2010). Fluctuating asymmetry (FA) was measured on four bilateral traits, and used to quantify stress in wild brown trout (e.g. Sanchez-Galan et al., 1998; Monna et al., 2011). Higher degrees of asymmetry are expected in cases of strong environmental stress during early development (Allenbach, 2011). Such a scheme, where TM levels are measured in both abiotic and biotic compartments at the same sites, is suitable to assess metal transfers in ecosystems and their possible deleterious effects (Deacon and Stephens, 1998).

2. Materials and methods

2.1. Study area

2.1.1. The Morvan

The Morvan Regional Nature Park (MRNP) is a protected area (since 1970), situated in the north-eastern Massif Central, France (Fig. 1a). It covers 2814 km², for a population of just over 70,000 inhabitants (Fig. 1b). Industries are very scarce, and
the local economy is mainly centred on forestry, cattle breeding and the tertiary sector (Sirgue, 2008). The elevation ranges between 300 m and 900 m, a.s.l. (Fig. 1c). The massif is mainly composed of granitic rocks and volcano-sedimentary terrains, crosscut by several microgranitic and quartz veins (Fig. 1d). The three main types of granitic deposits are: (i) high andesitic to quartz monzodiorite; (ii) U–F–Ba, Pb–Zn–Ag, Sn–W; (iii) abundant polymetallic ores in NW–SW and NNE–SSW veins, and, (iv) stratiform F–Ba ore deposits in Early Mesozoic formations (Delfour, 2007). Recent studies on peat deposits have suggested that local metallurgy started as early as the Middle Bronze Age (ca. 1650 cal BC), with peaks during the Iron Age (Celtic occupation of the Bibracte oppidum) and Modern Times (Monna et al., 2004; Joffroy-Bapicot et al., 2007).

2.1.2. Watersheds

Three watersheds were selected to determine the impact of past mining on aquatic ecosystems (Fig. 1e). The first is the Cure (CUR), sampled near Gien-sure-Cure. This reference watershed has a granitic substratum, is free of local mining, and purportedly non-contaminated. The second contaminated watershed, near La Ruchette, the Dragne (DRA), has a non-calcareous detrital substratum, crosscut by numerous mineralised veins. It was exploited for pyrite and iron oxides from the 19th century to 1922 (Gourault et al., 2012). Radiocarbon dating for two charcoals trapped in iron slags provided dates between the 2nd and the 5th centuries AD (Monna et al., 2014). These clues suggest sporadic mining, at least since Antiquity. The third watershed is the Anguison (ANG), sampled near Chitry-les-Mines, with a clay, limonitic marl and mineralised dolomite (F–Ba, Zn–Ag substratum). Royal decrees attest that Chitrý-les-Mines was one of the largest silver-lead exploitations in France during the 15th and 16th centuries.

2.2. Geochemical database of streambed sediments

During the 1980s, the BRGM analysed thousands of surficial streambed sediments, to inventory mineral substances at the national scale (Lambert, 2005). Stream sediments represent composite erosion products of terrains outcropping in the catchment area. Their analysis may lead to the discovery of positive geochemical anomalies, which, in the Morvan at least, are not due to urban or industrial effluents but rather of low human population density. Positive geochemical anomalies tend rather to indicate natural metal enrichments, possibly due to the presence of ore bodies, combined or not with mechanical erosion or leaching of slag heaps, workshop grounds, and cleansing dumps, which may durably contaminate the aquatic environment, long after mining has ceased. The BRGM campaign collected 7369 samples in our study area (MRNP and ANG; Fig. 1f and g). Sediments were sieved at 125 μm, dried, and measured after near-total digestion for As, Cd, Cr, Cu, Ni, Pb and Zn by Direct-Current Plasma spectroscopy. Data and complementary information are available at http://sgmfinances.brgm.fr. Values below limits of detection (LOD) were kept for further analysis, because they contain useful information. As recommended in the environmental sciences, the values below detection (LOD) were kept for further analysis, because they contain useful information. As recommended in the environmental sciences, the values below LOD were replaced with the LOD divided by the square root of 2 (Hornung and Reed, 1990; Glass and Gray, 2001; Baxter, 2003).

The Sediment Quality Index (SQI or SeQI), developed by the Canadian Council of Ministers of the Environment (CCME, 2002) was chosen to describe the overall quality of streambed sediments, with regard to their metal contents. The underlying idea is to compute an index score for each sample, which takes into account the number of variables (here the metals) that exceed guideline values, as well as the magnitude of excess for each variable. Several countries have developed their own sediment guideline values (MacDonald et al., 2000; Desroisiers et al., 2010). However, there is a lack of clear directives in France, and frequently used in various contexts (Robson et al., 1992). The SMIs were measured using a Spectro ARCOS ICP-AES installed in a clean room and equipped with a CETAC U-5000A+ ultrafast nebuliser. Analytical quality was checked, using blanks, duplicates, and certified reference materials (CRMs): Peach Leaves NIST-1547 and lichen BCR-412. For CRM, recovery percentages of ~92%–110% were obtained when metal concentrations were sufficiently above the LOD (Table S1). Duplicates indicate good analytical reproducibility, except for Zn in one duplicate (96%), for reasons which remain unknown (Table S2).

Contamination factors for metal M and sample i, CI(M,i), were then computed as follows (Mouvet, 1992; Cesa et al., 2006):

$$\text{CI}(M,i) = \frac{C_{ia}}{C_{Na,M}}$$

where $C_{ia}$ is the concentration of the metal M in the sample i, and $C_{Na,M}$ is the average concentration of the metal M in the reference samples from the River Sioule (n=6).

2.3.2. Wild brown trout

A total of 72 fishes belonging to the same species (Salmo trutta fario) were caught by electrofishing in May 2011, along two transects of up to 400 m. While more than 30 trout were caught at CUR and DRA, only 4 specimens were captured at ANC, despite a substantial sampling effort. Trout were sacrificed and frozen at −20 °C until analysis. Body length, wet body and liver weights were measured in the laboratory and used to calculate scaled mass indices (SMIs), as described in Peg and Green (2009, 2010). First, a standard major axis (SMA) regression of ln(mass) vs. ln(size) was used on the entire dataset to extract the $b_{mass}$, corresponding to the slope of the regression, as proposed by Peg and Green (2009, 2010). Then, the predicted body mass SMI was estimated for each individual size, with linear body length standardised to the size average (SLs). Calculations for SMI follow the equation:

$$\text{SMI} = \text{mass}, \left( \frac{\text{SLs}}{\text{b}_{mass}} \right)$$

where mass, is the body mass and size(s), is the body length of a given individual size, while $b_{mass}$ is the slope of the ln(mass) vs. ln(size) regression. A scaled liver index, SLs, was also computed in a similar manner, using liver mass instead of body mass. Fish age was estimated using the stratigraphy method (Baglinière et al., 1985). Four bilateral morphometric traits were selected for FA estimation: (i) length of pectoral fins, (ii) length of pelvic fins, and (iii) distance between snout and the anterior edge of the eye, and (iv) distance between the posterior edge of the eye and the posterior edge of the operculum. All measurements were performed twice to assess measurement error.

Livers were dried, ground, dissolved (~50 mg), and measured, following the procedure described above for masses. For all geochemical measurements, analytical quality was checked, using blanks, duplicates, and certified reference materials: bovine liver BCR-1858, peach leaves NIST 1547, lichen BCR-482, and fish protein DORM-3 (Table S1). For CRM, recovery percentages varied from 70% to 125% when metal concentrations were sufficiently above the LOD, except in two cases for Pb. As with

$$\text{Peig} = \left( \frac{\text{mass}}{C_{Na,M}} \right)$$

with $\text{mass}$, is the body mass and $C_{Na,M}$ is the average concentration of the metal M in the reference samples from the River Sioule (n=6).
moss samples, duplicates were all acceptable when sufficiently above the LOD (Table S3).

Chemical characterisation of the waters was established on the basis of the existing literature for the Morvan, considering comparable substratum (Meybeck, 1986; Amiotte-Suchet et al., 2011; and water agency data), together with in-stream measurements of pH and conductivity at each station, using WTW portable devices, in June and July 2011.

2.4. Data processing and statistical treatment

2.4.1. Data processing

The Quantum GIS free software was used for mapping (Quantum GIS Development Team, 2010). The smart, imodel2, and pgirmess packages in the R free software were used for statistical treatment (R Development Core Team, 2008).

2.4.2. Sediments

Inter-site comparisons were made using an ANOVA performed on log_{10}-transformed concentrations (except for Cd because of the number of data below the LOD). When \( p < 0.05 \), pairwise comparisons were made using Tukey post-hoc tests.

2.4.3. Aquatic mosses

General linear models (distribution: Gaussian, link: identity) were computed to investigate to what extent sector and exposure time may explain moss log_{10}-transformed TM concentrations (because the distributions are skewed). The best model was selected among all possible candidates, using the bias-corrected version of the Akaike Information Criterion (AICC). The model with the smallest AICC was compared as the best-fit model, except when the difference between AICC was less than 2. In that case, the simplest model was retained, following the parsimony principle (Burnham and Anderson, 2002). The best-fit model was checked graphically for homogeneity of variance and normality, and tested with ANOVA. For \( p \) values below 0.05, pairwise comparisons were made, using Tukey post-hoc tests to rank the sectors in terms of TM concentrations.

2.4.4. Wild brown trout

Pairwise comparisons between the three sites are problematic because only 4 specimens could be caught at ANG. Further statistical treatments therefore excluded this site. To test whether TM concentrations in trout liver vary according to biological variables (age, mass, size, and gender) and river sector (CUR or DRA), TM concentrations were modelled using biological variables and sector as explanatory variables. First, univariate models were tested and then multivariate models combining a biological variable and sector were built and tested. General linear models (distribution: Gaussian, link: identity) were used. A similar approach was used to test whether condition indices (SMI and SLI) vary according to biological variables, sector and liver TM concentrations. Multivariate models were therefore composed of the selected biological variable, sector, and liver TM concentration.

Statistical assessment of FA was performed following the procedure recommended by Palmer and Strobeck (2003). Individual FA levels were estimated for each trout, using absolute asymmetry. Linear models were then computed to assess the relationship between absolute asymmetry values and liver TM concentrations. Sample FA levels were estimated for each trout, using between-sides variances corrected for measurement error, or FA10, obtained from the results of linear mixed models with sides (fixed) * individuals (random) (see Palmer (1994) for details about calculation). Fisher tests were then performed for each trout studied to explore inter-site differences.

3. Results and discussion

3.1. Risk mapping in the Morvan

Metal concentrations in streambed sediments vary widely and can reach extremely high values: 1300 mg kg^{-1} for As, 1817 mg kg^{-1} for Pb and 1350 mg kg^{-1} for Zn (Table 1). They exhibit asymmetric distributions, which tend to be more compatible with normal law once log_{10}-transformed, as shown by changes in skewness (Table 1).

It is not possible to estimate Cd distribution because of the number of data below the LOD. Streambed sediments sampled in areas where industrial and mining activities took place in the recent past may reach much higher metal concentrations: e.g. Pb and Zn concentrations up to 3309 mg kg^{-1} and 11,153 mg kg^{-1}, respectively, in the Mala Panew River catchment (Poland), which drains historical mining/smelting sites and a still active Zn smelter (Aleskander-Kwerczak and Helios-Rybicka, 2008). The maximum values measured in the Morvan rivers are more comparable to those observed close to past mining sites: e.g. the Tinto River (Spain) and the Lot River (France) for Pb (Audry et al., 2004; Galán et al., 2003), or the Allen Basin (England) for Zn (Goodyear et al., 1996).

More than 70% of the 7369 Morvan samples belong to the good or excellent categories (Fig. 2). This percentage reaches ~96% if the fair category is included, suggesting that the majority of Morvan sediments should not present a threat to biota. However, three particular clustered areas, characterised by low SQIs, can readily be distinguished in Fig. 2. The first is located east and northeast of the MRNP, and corresponds to geological contact between sedimentary and endogenous rocks. Another anomalous NE–SW band, including Bibracte, can be noticed. This area presents a high mineral potential, exploited by former societies, at least for iron and silver lead (Monna et al., 2014). At Bibracte, mining works extending underneath the walls of the oppidum indicate local metal exploitation, dating at least from Celtic occupation (Cauuet and Bouissicault, 2006). The third zone, with poor and marginal SQIs, is located west, outside the MRNP, near the mines of Chitry-les-Mines. The SQIs are therefore consistent with the mining history of the area. It is worth recalling that the method does not take into account metal speciation, which is known to have a drastic influence on toxicity (e.g. As species). The use of SQIs must therefore be seen as a screening tool, allowing a huge amount of data to be aggregated and sorted rapidly, thus highlighting areas where metal contents in sediments could present a risk. After this step, a change in both scale and tools is necessary, to better characterise deleterious effects on biota. For this purpose, sessile and mobile biomonitors (mosses and fishes) were studied at three specific areas.

3.2. Evaluating impact on biota for the three watersheds selected

3.2.1. Characterising abiotic parameters

Three watersheds were selected for their mining history and their SQI values: (i) the non-contaminated watershed, CUR, with a median SQI at 89 (\( n = 14 \)), a value suggesting good environmental quality; (ii) the ferrous-dominated mining site, DRA, median SQI\( = 66 \) (\( n = 21 \)), fair quality; and (iii) the silver–lead mining site, ANG, median SQI\( = 51 \) (\( n = 11 \)), marginal quality (Table S4). For three elements (Cu, Pb and Zn) out of four, concentrations in the CUR sediments (the reference, non-contaminated site) are, as expected, always significantly lower than for the two contaminated sites (Fig. 3a). The highest Pb and Zn concentrations are found at ANG, the Ag/Pb mining site, where sphalerite is found in association with galena. No difference in Cu concentrations is detected between the two mining sites. The results obtained using BRGM sediment data from the 1980s therefore attest the continuing impact of past mining. The highest Cu and Zn concentrations (81 and 447 mg kg^{-1}; Table S4) are just above the levels usually noted worldwide for pristine streambed sediments (50 and 240 mg kg^{-1}, respectively (Martin and Meybeck, 1979). However, Pb concentrations reaching 1590 mg kg^{-1} are far above the value for non-contaminated sediments (40 mg kg^{-1}). No inference can be drawn for Cd because most of the data are below the LOD (Fig. 3a).

All waters belong to the bicarbonate calcic/sodic type. Their pH values range from 6.8 to 7.4. Alkalinity indicates their low sensitivity to acidification (Février et al., 1999). The high conductivity and alkalinity at ANG is due to the nature of the partly calcareous substratum. The low dissolved organic carbon content (~5 mg L^{-1}) is typical of Morvan streams, which drain deciduous or mixed vegetation (Amiotte-Suchet et al., 2011). The similarity in chemical characteristics observed for the three rivers (circum neutral pH, low dissolved organic carbon content, and balanced water composition) is an asset for further inter-site comparison.
contaminated by mine ef
higher than those found by Figueira and Ribeiro (2005) in rivers (Samecka-Cymerman et al., 2005) (Table S5). Our Pb values are even
gender ratios are balanced (‘all metals, sector is the most in
principle, sector alone is retained for the Pb and Zn concentrations. For
S6 for model selection results). However, following the parsimony
sector and exposure time as explanatory variables (Table 2, see Table S7 for model selection results): liver
Cu concentrations were best modelled with sector alone
(Table 5a, see Table S7 for model selection results): liver
concentrations are lower at CUR than at DRA, the contaminated
site (Table 6). No factors explain liver Pb concentrations, while Zn
concentrations are best fitted by a model including fish size alone.

Three different indices that may be indicative of toxic effects of
TMs on trout were studied: SMI, SLI, and FA. The \( B_{24b} \) values at the
95% confidence level were 2.93 ± 0.10 for SMI and 3.22 ± 0.29 for SLI.
The SMIs are best fitted by a model including sector and liver Pb
concentration, as are the SLIs when the parsimony principle is applied
(Table 5b and Table S8). Better trout conditions, in terms of SLI and
SMA values at the

Fig. 2. SQI map computed from BRGM sediment analyses. CUR for Cure, DRA for Dragne, and ANG for Anguison.

3.2.2. Aquatic mosses
Average concentrations in native Sioule mosses are low: 
~1 \( \mu \)g g\(^{-1}\) for Cd, 26 \( \mu \)g g\(^{-1}\) for Cu, 15 \( \mu \)g g\(^{-1}\) for Pb, and 115 \( \mu \)g g\(^{-1}\)
for Zn, and comparable to those reported in Poland for control
samples (Samecka-Cymerman et al., 2005). Transplanted moss CFs
vary between 1.2 and 4 for Cd, 0.6 and 1.4 for Cu, 1.3 and 6.7 for Pb,
and between 0.7 and 3.4 for Zn. Values < 1 are observed in the non-
contaminated site of CUR, indicating a net balance of ~ 40% of
copper and ~ 30% of zinc after one month. Interestingly, Pb
concentrations measured in bryophytes for the present study are similar to those reported in other areas contaminated by past mining
industries (Samecka-Cymerman et al., 2002), or municipal sewage
(Samecka-Cymerman et al., 2005) (Table S5). Our Pb values are even higher than those found by Figueira and Ribeiro (2005) in rivers
contaminated by mine effluents. Concentrations of Cd, Cu, and Zn are
comparable to values reported in natural environments (e.g. Galician
rivers; Vázquez et al., 2007), and far below those from rivers
identified as highly contaminated by Cu (Cenci, 2000), and Zn
(Siebert et al., 1996).

In the Morvan samples, sector is the only explanatory variable for
Cu. Concentrations of Cd, Pb and Zn in mosses are best explained using
sector and exposure time as explanatory variables (Table 2, see Table S6 for model selection results). However, following the parsimony
principle, sector alone is retained for the Pb and Zn concentrations. For
all metals, sector is the most influential factor (Table 3) but CFs barely
reach ‘suspected contamination’ (2 < CF < 6 as defined by Mouvet (1992)), even for the mining sites. The Cd concentrations in tissues also
increased significantly during the experiment, suggesting that equilib-
rium was not reached, at least after one month. The Cu, Pb and Zn
concentrations in mosses follow the same pattern as in sediments
(CUR < DRA < ANG). The Cd pattern is slightly different (CUR–
ANG < DRA), but cannot be compared to sediments because of data
below the LOD. Such low but coherent variations highlight the
excellent sensitivity of aquatic mosses, as previously noted by several
authors (Fernández and Carballa, 2000; Figueira and Ribeiro, 2005;
Samecka-Cymerman et al., 2005; Cesa et al., 2006). It is well known
that plants possess a strong ability to adapt to variable chemical
properties of the environment (Kabata-Pendias, 2011). Native bryo-
phytes could therefore have developed adaptive mechanisms to
protect themselves against toxic element accumulation, in relation to
local environmental parameters (Claveri et al., 1995). Samecka-
Cymerman et al. (2005) found that transplanted F. antipyretica
accumulate significantly more trace elements than native mosses. In
this study, the use of transplants allows standardisation of experimental
material in terms of physiological conditions (as in Fernández et al., 2000) and thus makes inter-site comparison possible.

3.2.3. Trout biomonitoring
Age structures differ between sites (\( \chi^2 = 14.76, p = 0.01 \)), while
gender ratios are balanced (\( \chi^2 = 0.05, p = 0.8 \) (Table 4). Liver Cd
and Cu concentrations were best modelled with sector alone
(Table 5a, see Table S7 for model selection results): liver
concentrations are lower at CUR than at DRA, the contaminated
site (Table 6). No factors explain liver Pb concentrations, while Zn
concentrations are best fitted by a model including fish size alone.

Three different indices that may be indicative of toxic effects of
TMs on trout were studied: SMI, SLI, and FA. The \( B_{24b} \) values at the
95% confidence level were 2.93 ± 0.10 for SMI and 3.22 ± 0.29 for SLI.
The SMIs are best fitted by a model including sector and liver Pb
concentration, as are the SLIs when the parsimony principle is applied
(Table 5b and Table S8). Better trout conditions, in terms of SLI and
SMA values at the
kilometres in these rivers, so that the sediment concentrations at the place of capture might not properly characterise the true metal levels to which fishes have been exposed during their lifetimes.

Concerning developmental instability in trout, a set of preliminary tests was performed for all traits measured, as recommended by Palmer (1994). Tests did not suggest that directional asymmetry, antisymmetry, or relationships between asymmetry and trait size, could significantly bias FA estimates (for details, see Table S9). First, no significant correlations between absolute asymmetry distribution and TM concentrations measured in specimens were found (Table S10), except for the snout-eye length for Cd \( (p=0.007) \) and Zn \( (p=0.03) \) and for the eye-operculum length for Cd \( (p=0.02) \). When compared to

Fig. 3. Distribution of Cd, Cu, Pb, and Zn in (a) streambed sediments, (b) aquatic moss leaves, left y-axis for CF, right y-axis for concentrations (dry weight basis), and (c) trout liver concentrations (dry weight basis). The number of data below the LOD is reported under a bold line which represents the limit of detection. For mosses, closed circles correspond to collection in June 2011 \((t_1)\), and closed squares to collection in July 2011 \((t_2)\). Significant pairwise differences are represented by straight lines, and stars correspond to \( p \)-values. CUR stands for Cure, and DRA for Dragne. The fishes captured in ANG (for Anguison) were not included in statistical analysis, because of the small sample size \((n=4)\). Results for ANG are plotted in the hatched section of the graph, for information.
measurement error, FA is always significant (Table S11). No clear relationship between levels of developmental instability and TM concentrations can however be observed. Among the four traits measured, three exhibit significant differences in FA indices (FA10) between sites (Fig. S1): the highest FA10 values are surprisingly observed twice for the non-contaminated site, CUR: for the pectoral fin (F CUR/DR A = 1.99, p = 0.03) and for the operculum-eye distance (F CUR/DR A = 2.05, p = 0.03). A third difference is observed for the pelvic fin, where FA10 is higher for the mining site, DRA (F CUR/ang = 1.80, p = 0.0497). These heterogeneous results could indicate that other parameters, such as food access or water quality, might be involved.

The situation is therefore less clear for trout than for aquatic mosses. While the highest Cd concentration in the Morvan trout (8.03 mg kg⁻¹) is far below the maximum values (81.3 mg kg⁻¹) reported by Deacon and Stephens (1998) in mining land-use sites, concentrations for essential elements (i.e. Cu and Zn) remain quite comparable. In the Morvan, TM concentrations in sediments do not reach levels reported in a similar study undertaken in the Cévennes (Monna et al., 2011). There, the impact of past mining on wild brown trout was visible in terms of developmental stability and liver metal concentrations. In the Morvan, the mines were much smaller, and generally more ancient. Some focus on iron, whose exploitation is expected to be less contaminating than the non-ferrous ores mined in the Cévennes. The trout response in the Morvan is thus not as straightforward as in the Cévennes, especially for FA, which does not exhibit any clear pattern related to metal contamination. Decreases in SMIs and SLIs are nonetheless systematically observed when liver Pb concentrations increase. Such similar negative correlations between condition indices and TM concentrations have previously been identified (Esteve et al., 2012), and are generally explained by environmental stress, leading to a loss of energy reserves stored in the liver as glycogen or lipids (Almeida et al., 2005). It is worth recalling that correlation does not imply causality. Another pollutant or any other confounding factor, correlated with Pb, but not monitored by the BRGM, nor measured in trout, could be responsible for the results observed.

Table 2
Summary of the best-fit models explaining TM concentrations in Fontinalis antipyretica as a function of time or sector.

<table>
<thead>
<tr>
<th>Best-fit model</th>
<th>n</th>
<th>LL</th>
<th>K</th>
<th>AICc</th>
<th>wic</th>
<th>ΔAICc null</th>
</tr>
</thead>
<tbody>
<tr>
<td>log₁₀(Cd fontinalis) ~ time + sector</td>
<td>20</td>
<td>24.43</td>
<td>5</td>
<td>34.57</td>
<td>0.98</td>
<td>22.83</td>
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<td>log₁₀(Pb fontinalis) ~ sector</td>
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<td>1</td>
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<td>0.83</td>
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<td>log₁₀(Zn fontinalis) ~ sector</td>
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<td>12.68</td>
<td>4</td>
<td>14.68</td>
<td>0.37</td>
<td>15.12</td>
</tr>
<tr>
<td>log₁₀(Cu fontinalis) ~ sector</td>
<td>20</td>
<td>20.61</td>
<td>4</td>
<td>30.55</td>
<td>0.37</td>
<td>26.36</td>
</tr>
</tbody>
</table>

n: sample size, LL: maximised log-likelihood, K: number of estimated parameters, AICc: corrected Akaike Information Criterion, wic: Akaike weight of the model, ΔAICc null: difference between AICc of the model considered and AICc of the null model.

Table 3
ANOVA performed on the best-fit model to explain TM concentrations in aquatic mosses, and summary of post-hoc tests.

<table>
<thead>
<tr>
<th>Best-fit model</th>
<th>p-value</th>
<th>R²</th>
<th>Adj R²</th>
<th>Site ranking after post-hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>log₁₀(Cd fontinalis) ~ time + sector</td>
<td>p = 0.001</td>
<td>0.80</td>
<td>0.77</td>
<td>CUR-ANG &lt; DRA</td>
</tr>
<tr>
<td>log₁₀(Cu fontinalis) ~ sector</td>
<td>p = 1.3 × 10⁻⁵</td>
<td>0.83</td>
<td>0.81</td>
<td>CUR &lt; DRA &lt; ANG</td>
</tr>
<tr>
<td>log₁₀(Pb fontinalis) ~ sector</td>
<td>p = 3 × 10⁻⁷</td>
<td>0.65</td>
<td>0.61</td>
<td>CUR &lt; DRA-ANG</td>
</tr>
<tr>
<td>log₁₀(Zn fontinalis) ~ sector</td>
<td>p = 10⁻⁴</td>
<td>0.80</td>
<td>0.78</td>
<td>CUR &lt; DRA &lt; ANG</td>
</tr>
<tr>
<td>log₁₀(Zn fontinalis) ~ sector</td>
<td>p = 10⁻⁶</td>
<td>0.80</td>
<td>0.78</td>
<td>CUR &lt; DRA &lt; ANG</td>
</tr>
</tbody>
</table>

Table 4
Main biological characteristics of trout and condition indices, results in terms of range and median between parentheses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Cure</th>
<th>Dragne</th>
<th>Anguis</th>
<th>SMI</th>
<th>SLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label</td>
<td>CUR</td>
<td>DRA</td>
<td>ANG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supposed contamination degree</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Number of fishes analysed</td>
<td>37</td>
<td>31</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>1+ to 6+</td>
<td>1+ to 3+</td>
<td>2+ to 4+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>17</td>
<td>16</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Weight (g, ww)</td>
<td>13–241 (51)</td>
<td>20–100 (47)</td>
<td>70–223 (152.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (mm)</td>
<td>102–292 (161)</td>
<td>118–194 (151)</td>
<td>174.3–264 (2315)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (g, ww)</td>
<td>0.17–2.78 (0.64)</td>
<td>0.16–1.55 (0.68)</td>
<td>0.70–3.55 (2.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMI (g, ww)</td>
<td>45.33–67.86 (52.2)</td>
<td>52.00–75.71 (56.8)</td>
<td>54.52–59.38 (57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLI (g, ww)</td>
<td>0.44–1.48 (0.66)</td>
<td>0.44–1.24 (0.83)</td>
<td>0.55–0.81 (0.68)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ww: wet weight, SMI: scaled mass index, SLI: scaled liver index. 
* for information only, not taken into account for the statistical model.
4. Conclusion

The thousands of streambed sediment analyses conducted by geological surveys as exploration tools for mineral substances can nowadays be advantageously used to calculate SQIs, as a rough surrogate for the impact of polluted sediments on biota. In the Morvan, the overall quality of streambed sediments is quite good, but SQIs have to be supplemented by in-situ biomonitoring when the true effect on biota is sought. Environmental monitoring using transplanted aquatic mosses is sensitive and easy to manage: significant accumulations of TMs are noticed where past mining took place. With the exception of fish condition indices, which are negatively correlated with liver Pb concentrations, trout do not respond to past mining as clearly as bryophytes. The absence of any clear impact on fishes, more particularly on the early development of individuals, can be linked to the sporadic and ancient character of Morvan mining/metallurgical activity, which nowadays induces only moderate contamination, and/or to various confounding factors, including the mobility of the trout themselves. This issue requires further investigation.

The multi-proxy approach followed here, which combines geochemical, biological, archaeological and historical data, seems adequate to study the persistent impact of past mining on present-day aquatic environments. This approach is particularly useful when building new management plans in a protected area, since all records of some past mining sites and their possible impact on health may have been lost.

Acknowledgments

We are grateful to the FEDER (No. 34328), the Regional Council of Burgundy (No. 2010-9201AA0050S04060), the University of Burgundy (No. 2010-BQR-068), and Bibracte for funding the study. The Regional Nature Park of the Morvan is thanked for its continuing support. The French Ministry of Research is thanked for the Ph.D. grant of Estelle Camizuli. We wish also to thank Claude Gourault, Gilles Hamm and the Aquabio team for their precious assistance during the sampling campaigns. The French Water Agency is thanked for having kindly provided the water data. We are grateful to Ellen K. Silbergeld, whose judicious comments helped to improve the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2014.07.008.

References


Table 6

ANOVA performed on the best-fit model to explain TM concentrations in wild brown trout, and summary of post-hoc tests.

<table>
<thead>
<tr>
<th>TMs in trout</th>
<th>p-value ANOVA</th>
<th>R²</th>
<th>Adj R²</th>
<th>Site ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>log₁₀(Cd trout)−sector</td>
<td>p &lt; 2.2.10⁻¹⁶</td>
<td>0.72</td>
<td>0.72</td>
<td>CUR &lt; DRA</td>
</tr>
<tr>
<td>log₁₀(Cu trout)−sector</td>
<td>p = 1.16.10⁻⁷</td>
<td>0.35</td>
<td>0.34</td>
<td>CUR &lt; DRA</td>
</tr>
<tr>
<td>log₁₀(Pb trout)−1</td>
<td>0.07</td>
<td>0.06</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>log₁₀(Zn trout)−size</td>
<td>p = 0.03</td>
<td>0.07</td>
<td>0.06</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Fig. 4. Scaled mass index (a) and scaled liver index (b) vs. Pb concentrations in trout livers (log₁₀-transformed) for each study area. CUR for Cure, DRA for Dragne.

Table 6

ANOVA performed on the best-fit model to explain TM concentrations in wild brown trout, and summary of post-hoc tests.


