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

E. Camizuli [a,*], F. Monna [a], R. Scheifler [b], P. Amiotte-Suchet [c], R. Losno [d], P. Beis [a], B. Bohard [a], C. Chateau [e], P. Alibert [c]

[a] UMR 6298, ARTéHIS, Université de Bourgogne—CNRS-culture, Boulevard Gabriel, Bat. Gabriel, 21000 Dijon, France
[b] UMR 6249, Chrono-Environnement, Université de Franche-Comté—CNRS, Place Leclerc, 25000 Besançon, France
[c] UMR 6282, Biogeosciences, Université de Bourgogne—CNRS, Boulevard Gabriel, Bat. Gabriel, 21000 Dijon, France
[d] UMR 7583, LISA, Universités Paris 7-Paris 12—CNRS, 61 avenue du Gal de Gaulle, 94010 Créteil Cedex, France
[e] Université de Bourgogne, UFR SVTE, Bat. Gabriel, 21000 Dijon, France

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ABSTRACT

This study seeks to determine to what extent trace metals resulting from past mining activities are transferred to the aquatic ecosystem, and whether such trace metals still exert deleterious effects on biota. Concentrations of Cd, Cu, Pb and Zn were measured in streambed sediments, transplanted bryophytes and wild brown trout. This study was conducted at two scales: (i) the entire Morvan Regional Nature Park and (ii) three small watersheds selected for their degree of contamination, based on the presence or absence of past mining sites. The overall quality of streambed sediments was assessed using Sediment Quality Indices (SQIs). According to these standard guidelines, more than 96% of the sediments sampled should not represent a threat to biota. Nonetheless, in watersheds where past mining occurred, SQIs are significantly lower. Transplanted bryophytes at these sites consistently present higher trace metal concentrations. For wild brown trout, the scaled mass and liver indices appear to be negatively correlated with liver Pb concentrations, but there are no obvious relationships between past mining and liver metal concentrations or the developmental instability of specimens. Although the impact of past mining and metallurgical works is apparently not as strong as that usually observed in modern mining sites, it is still traceable. For this reason, past mining sites should be monitored, particularly in protected areas erroneously thought to be free of anthropogenic contamination.

1. Introduction

Trace metals (TMs) persist in the environment, reaching potentially toxic levels for biota over time (Linde et al., 1998; Alibabić 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-Rybicka, 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

* Corresponding author. Tel: +33 (0)3 80 39 36 26; fax: +33 (0)3 80 39 57 87.
E-mail address: estelle.camizuli@u-bourgogne.fr (E. Camizuli).

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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 km^2, for a population of just over 70,000 inhabitants (Fig. 1b). Industries are very scarce, and

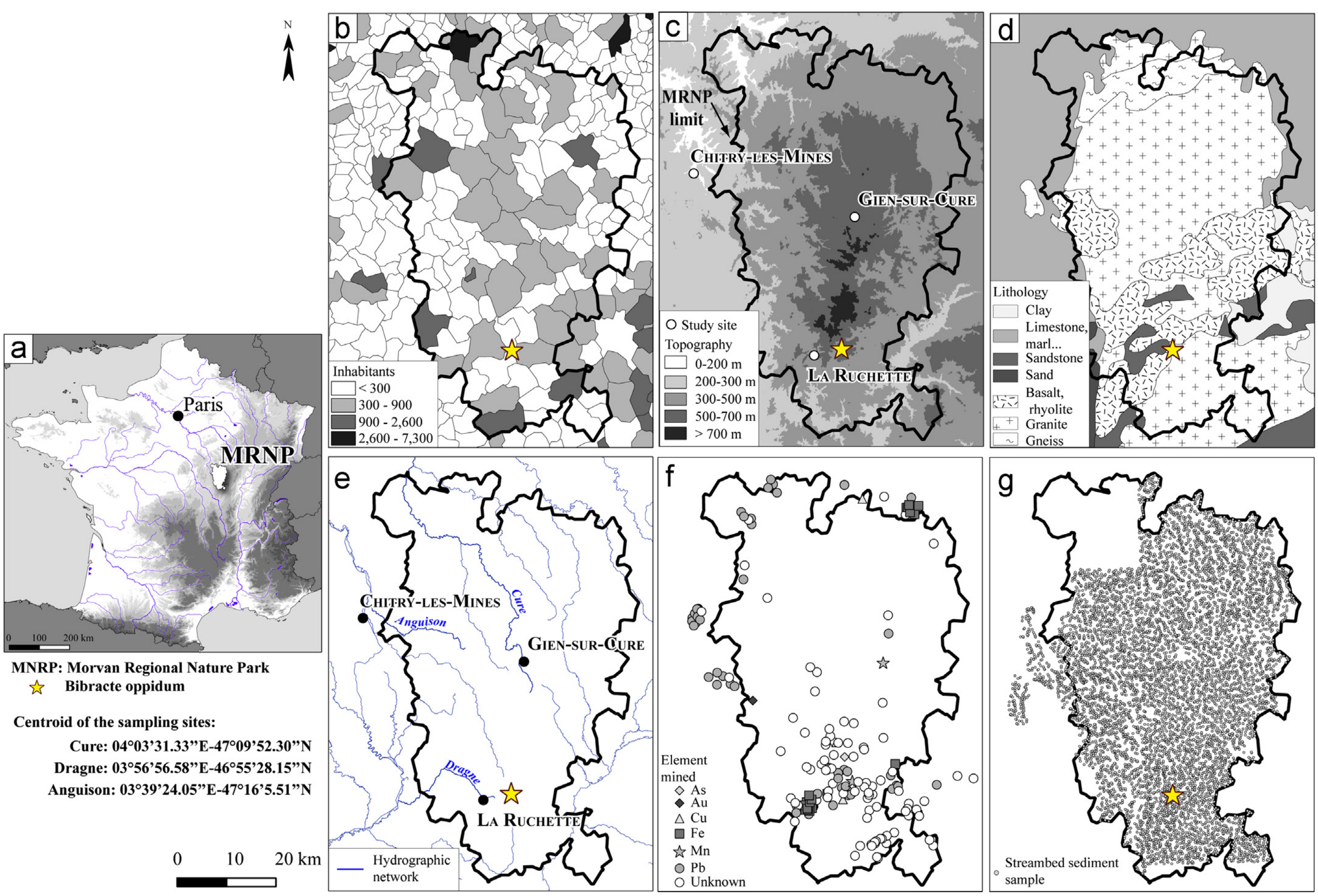

Fig. 1. (a) location of the MRNP in France, (b) numbers of inhabitants in Morvan localities, (c) digitalised elevation model, (d) lithological map, (e) simplified hydrographical network, (f) map of the mining sites, for which the nature of minerals exploited is indicated when known, (g) BRGM sampling location for streambed sediments.

the local economy is mainly centred on forestry, cattle breeding and the tertiary sector (Sirugue, 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 mineral deposits are: (i) hydrothermal mineralised quartz veins (with U, F–Ba, Pb–Zn–Ag, Sn–W), (ii) abundant polymetallic ores in NNW–SSE and NNE–SSW veins, and, (iii) 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; Jouffroy-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-sur-Cure. This reference watershed has a granitic substratum, is free of local mining, and purportedly non-contaminated. The second 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, limestone, marl and mineralised dolomite (F–Ba, Zn–Pb–Ag) substratum. Royal decrees attest that Chitry-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, because 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://sigminesfrance.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 the 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; Desrosiers et al., 2010). However, there is a lack of clear directives in France, and even in the European Union (Besten et al., 2003). As a result, the Probable Effect Levels (PELs), defined by the Canadian government, and frequently used in various contexts (Robson et al., 2006; Arienzo et al., 2013), were selected as reference values. These values correspond to the levels above which adverse biological effects are expected to occur frequently (CCME, 1999). The SQI approach does not necessarily imply that all compounds and elements are included in the calculation, although the most accurate results are obtained if this is the case. A minimum of four variables is nonetheless advised (CCME, 2001). Final SQIs are expressed as a percentage, thus permitting inter-sample comparisons. In this study, calculations are based on the seven elements measured by the BRGM for which PELs are available: As ($17\ \mu g\ g^{-1}$), Cd ($3.53\ \mu g\ g^{-1}$), Cr ($90\ \mu g\ g^{-1}$), Cu ($196.6\ \mu g\ g^{-1}$), Ni ($75\ \mu g\ g^{-1}$), Pb ($91.3\ \mu g\ g^{-1}$), Zn ($314.8\ \mu g\ g^{-1}$) (Marvin et al., 2004). Two factors were computed for each sample. The first is F_1:

$$F_1 = \frac{f}{n} \times 100,$$

where n represents the number of metals tested (here, $n=7$), and f the total number of metals for which the concentration measured exceeds the guideline level ($f \le 7$).

The second is F_2:

$$F_2 = \frac{\overline{nc}}{0.01 \times \overline{nc} + 0.01},$$

with $\overline{nc} = (\Sigma_{i=1}^{n} nc_i)/n$ where $nc_i = (C_i/PEL_i) - 1$ when $C_i > PEL_i$, and $nc_i = 0$, otherwise.

The variable C_i is the sample concentration of the metal i to be tested, PEL_i is the corresponding guideline value, and nc_i is non-compliance.

The SQI of a sample is:

$$\text{SQI} = 100 - \sqrt{\frac{F_1^2 + F_2^2}{2}}$$

Five SQI categories are defined: (i) [0;45] 'poor sediment quality', most concentrations are substantially higher than PELs; (ii)]45;60] 'marginal sediment quality', concentrations are frequently higher than PELs; (iii)]60;80] 'fair sediment quality', occasional metal excess; (iv)]80;95]'good sediment quality', most measurements are below PELs; (v)]95;100] 'excellent sediment quality', values are well below PELs (Marvin et al., 2004).

2.3. Bioindicators

2.3.1. Aquatic mosses

In May 2011, a large quantity of aquatic moss was collected from a non-contaminated zone of the River Sioule (La Sioule: 02°52.143′E, 45°43.782′N). Part of this moss was set aside to serve as the non-contaminated reference level. The remaining moss was immediately divided into samples, which were transplanted in clean plastic net bags, maintained in running water, into the three Morvan rivers under study (CUR, DRA, and ANG). The transplanted mosses were sampled in June, and again in July 2011. Both reference and transplanted samples were rinsed in river water (Sioule, CUR, DRA, or ANG), and stored in polyethylene bags. In the laboratory, all samples were rinsed with Milli-Q water in an ultrasonic bath to eliminate particles and debris. They were dried at 60 °C for 24 h to reach constant dry mass. Green leaves were crushed manually in an acid pre-cleaned agate mortar. About 100 mg of fine leaf powder was digested using 2 mL of concentrated HNO_3 of Suprapur© grade and 2 mL of Milli-Q water in a Savilex™ PTFE beaker, on a hot plate for three days. After appropriate dilution, concentrations of Cd, Cu, Pb, and Zn were measured using a Spectro ARCOS ICP-AES installed in a clean room and equipped with a CETAC U-5000A$^+$ ultrasonic nebuliser. Analytical quality was checked, using blanks, duplicates, and certified reference materials (CRMs): Peach Leaves NIST-1547 and lichen BCR-482. For CRM, recovery percentages of ~92–130% were obtained when metal concentrations were sufficiently above the LOD (Table S1). Duplicates indicate good analytical reproducibility, except for Zn in one duplicate (F06), for reasons which remain unknown (Table S2).

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

$$\text{CF}_M^i = \frac{C_M^i}{\overline{R_M}},$$

where C_M^i is the concentration of the metal M in the sample i, and $\overline{R_M}$ 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 (*S. trutta fario*) were caught by electrofishing in May 2011, along transects of up to 400 m. While more than 30 trout were caught at CUR and DRA, only 4 specimens were captured at ANG, 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 Peig 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_{SMA}, corresponding to the slope of the regression, as proposed by Peig and Green (2009, 2010). Then, the predicted body mass SMI_i was estimated for each individual i, with linear body length standardised to the size average ($\overline{size}$). Calculations for SMI_i follow the equation:

$$\text{SMI}_i = mass_i \times \left(\frac{\overline{size}}{size_i}\right)^{b_{\text{SMA}}},$$

where $mass_i$ is the body mass and $size_i$ is the body length of a given individual i, while b_{SMA} is the slope of the ln(*mass*) vs. ln(*size*) regression. A scaled liver index, SLI_i, was also computed in a similar manner, using liver mass instead of body mass. Fish age was estimated using the scalimetry method (Baglinière et al., 1985). Four bilateral morphometric traits were selected for FA estimation: (i) length of pectoral fins, (ii) length of pelvic fins, (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 mosses. For all geochemical measurements, analytical quality was checked, using blanks, duplicates, and certified materials: bovine liver BCR-185R, 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 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, lmodel2, 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: identify) 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 considered 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 trait, 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 trait, 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 trait 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 (Aleksander-Kwaterczak 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 Boussicault, 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.

Table 1
Descriptive statistics: min, max, median and skewness, for As, Cd, Cr, Cu, Ni, Pb and Zn concentrations for the entire Morvan (7369 data). Skewness after $\log_{10}$-transformation is between parentheses; % cen for percentages below the LOD; nc: not calculated. Data from http://sigminesfrance.brgm.fr.

	As (mg kg^{-1})	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Ni (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
Limit of detection	20	1	10	10	10	10	10
Min	< 20	< 1	< 10	< 10	< 10	< 10	< 10
Median	33	< 1	34	< 10	13	53	93
Max	1305	30	358	516	232	1817	1350
Skewness	9.3 (0.5)	nc	2.9 (−0.3)	12.7 (1.5)	4.2 (0.6)	9.5 (1.6)	5.1 (0.4)
% cen	24	93	2	52	39	0.01	0.04

3.2.2. *Aquatic mosses*

Average concentrations in native Sioule mosses are low: ~1 µg g^{-1} for Cd, 26 µg g^{-1} for Cu, 15 µg g^{-1} for Pb, and 115 µg 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 equilibrium 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 Carballeira, 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 bryophytes 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

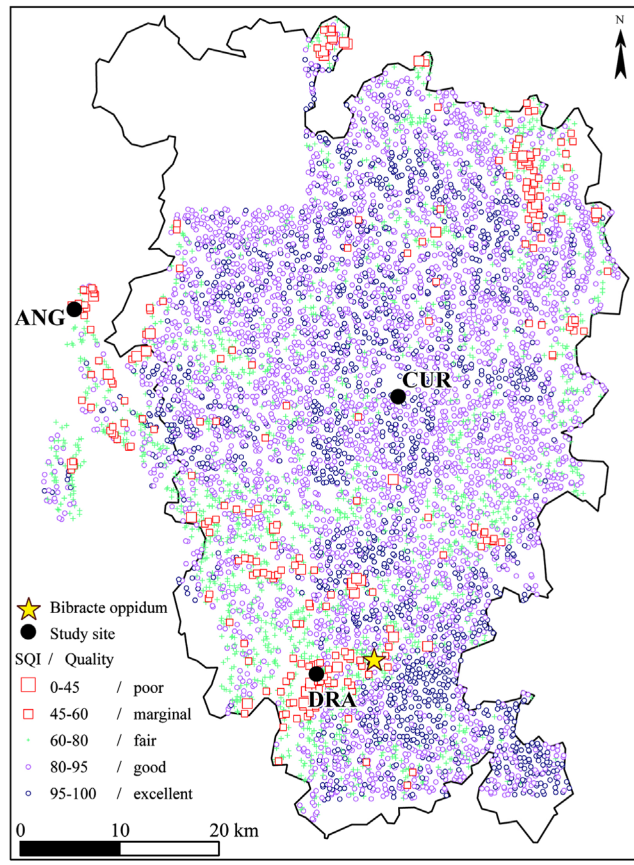

Fig. 2. SQI map computed from BRGM sediment analyses. CUR for Cure, DRA for Dragne, and ANG for Anguison.

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_{SMA} 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 SMI, are unexpectedly found at DRA, the most polluted site (Fig. 4), suggesting favourable habitat or abundant nutritional resources. However, if each sector is taken separately, both SLI and SMI correlate negatively with liver Pb concentrations (except for SMI in DRA), suggesting deleterious effects on fish condition or relative organ size (Fig. 4). These rather surprising results (i.e. better trout conditions at the most polluted site, and a negative relationship with Pb concentrations) need further investigation to be fully understood. It must be recalled that the home-range of the trout can reach up to ten

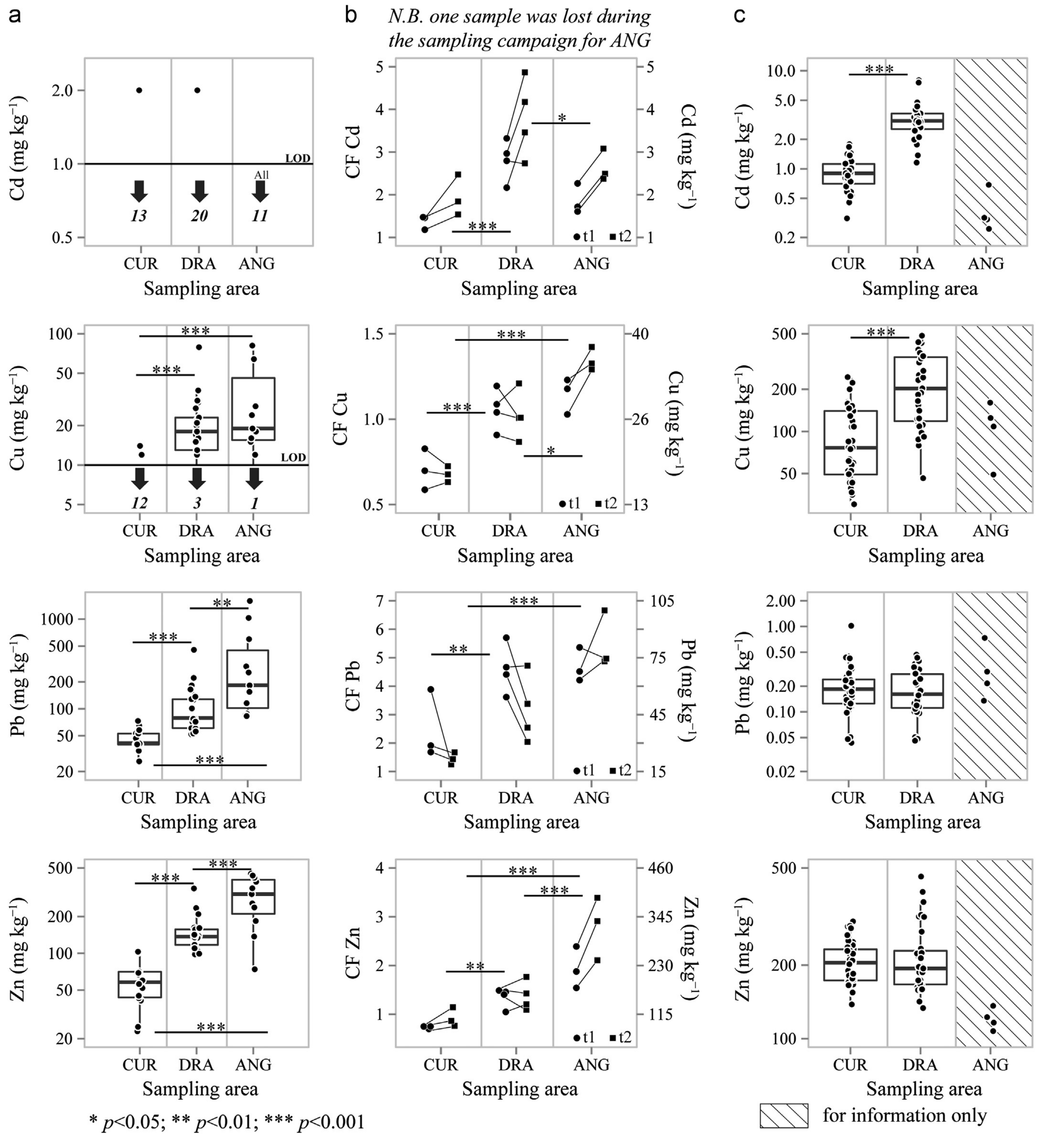

Fig. 3. Distribution of Cd, Cu, Pb, and Zn in (a) streambed sediments, (b) aquatic moss leaves, left y-axis for CF, right y-axis for concentrations (dry weight basis), and (c) trout liver concentrations (dry weight basis). The number of data below the LOD is reported under a bold line which represents 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.

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 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 ($F_{CUR/DRA}=1.99$, $p=0.03$) and for the operculum-eye distance ($F_{CUR/DRA}=2.05$, $p=0.03$). A third difference is observed for the pelvic fin, where FA10 is higher for the mining site, DRA ($F_{DRA/CUR}=1.80$, $p=0.0497$). 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 focused 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 of exposure or sector.

Best-fit models	*n*	LL	K	AICc	wic	Δ AICc null
$\log_{10}$(Cd fontinalis) ~ time + sector	20	24.43	5	−34.57	0.98	22.83
$\log_{10}$(Cu fontinalis) ~ sector	20	33.01	4	−55.35	0.83	29.34
$\log_{10}$(Pb fontinalis) ~ sector	20	12.68	4	−14.68	0.37	15.12
$\log_{10}$(Zn fontinalis) ~ sector	20	20.61	4	−30.55	0.37	26.36

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 5
Summary of the best-fit models using (a) TM concentrations in wild brown trout and (b) condition indices, as variables to be explained.

Best-fit models	*n*	LL	K	AICc	wic	Δ AICc null
(a) Liver TM concentration						
$\log_{10}$(Cd trout) ~ sector	68	25.89	3	−45.41	0.79	85.43
$\log_{10}$(Cu trout) ~ sector	68	−5.63	3	17.62	0.40	26.96
$\log_{10}$(Pb trout) ~ 1	68	−8.90	2	21.99	0.36	0.00
$\log_{10}$(Zn trout) ~ size	68	55.12	3	−103.9	0.43	3.00
(b) Condition indices						
SMI ~ sector + $\log_{10}$(Pb trout)	68	−197.8	4	404.31	0.92	17.93
SLI ~ sector + $\log_{10}$(Pb trout)	68	18.81	4	−28.98	0.37	16.84

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.

Best-fit model	*p*-value ANOVA	R^2	Adj R^2	Site ranking after post-hoc
$\log_{10}$(Cd fontinalis) ~ time + sector		0.80	0.77	
Time	$\boldsymbol{p=0.001}$			
Sector	$\boldsymbol{p=1.3\ 10^{-5}}$			CUR–ANG < DRA
$\log_{10}$(Cu fontinalis) ~ sector		0.83	0.81	
Sector	$\boldsymbol{p=3\ 10^{-7}}$			CUR < DRA < ANG
$\log_{10}$(Pb fontinalis) ~ sector		0.65	0.61	
Sector	$\boldsymbol{p=10^{-4}}$			CUR < DRA-ANG
$\log_{10}$(Zn fontinalis) ~ sector		0.80	0.78	
Sector	$\boldsymbol{p=10^{-6}}$			CUR < DRA < ANG

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

Site Label	Cure CUR	Dragne DRA	Anguison[a] ANG
Supposed contamination degree	−	+	++
Number of fishes analysed	37	31	4
Age (year)	1+ to 6+	1+ to 3+	2+ to 4+
Gender			
Female	20	15	0
Male	17	16	4
Weight (g, ww)	13–241 (51)	20–100 (47)	70–223 (152.5)
Length (mm)	102–292 (161)	118–194 (151)	174.5–264 (231.5)
Liver (g, ww)	0.17–2.78 (0.64)	0.16–1.55 (0.68)	0.70–3.55 (2.08)
SMI (g, ww)	45.33–67.86 (52.2)	52.00–75.71 (56.8)	54.52–59.38 (57)
SLI (g, ww)	0.44–1.48 (0.66)	0.44–1.24 (0.83)	0.55–0.81 (0.68)

ww: wet weight, SMI: scaled mass index, SLI: scaled liver index.

[a] for information only, not taken into account for the statistical model.

Table 6
ANOVA performed on the best-fit model to explain TM concentrations in wild brown trout, and summary of post-hoc tests.

TMs in trout	*p*-value ANOVA	R^2	Adj R^2	Site ranking
$\log_{10}$(Cd trout)~sector		0.72	0.72	
sector	$\mathbf{p < 2.2.10^{-16}}$			CUR < DRA
$\log_{10}$(Cu trout)~sector		0.35	0.34	
sector	$\mathbf{p = 1.16.10^{-7}}$			CUR < DRA
$\log_{10}$(Pb trout)~1		–	–	–
	–			
$\log_{10}$(Zn trout)~size		0.07	0.06	–
size	$\mathbf{p = 0.03}$			

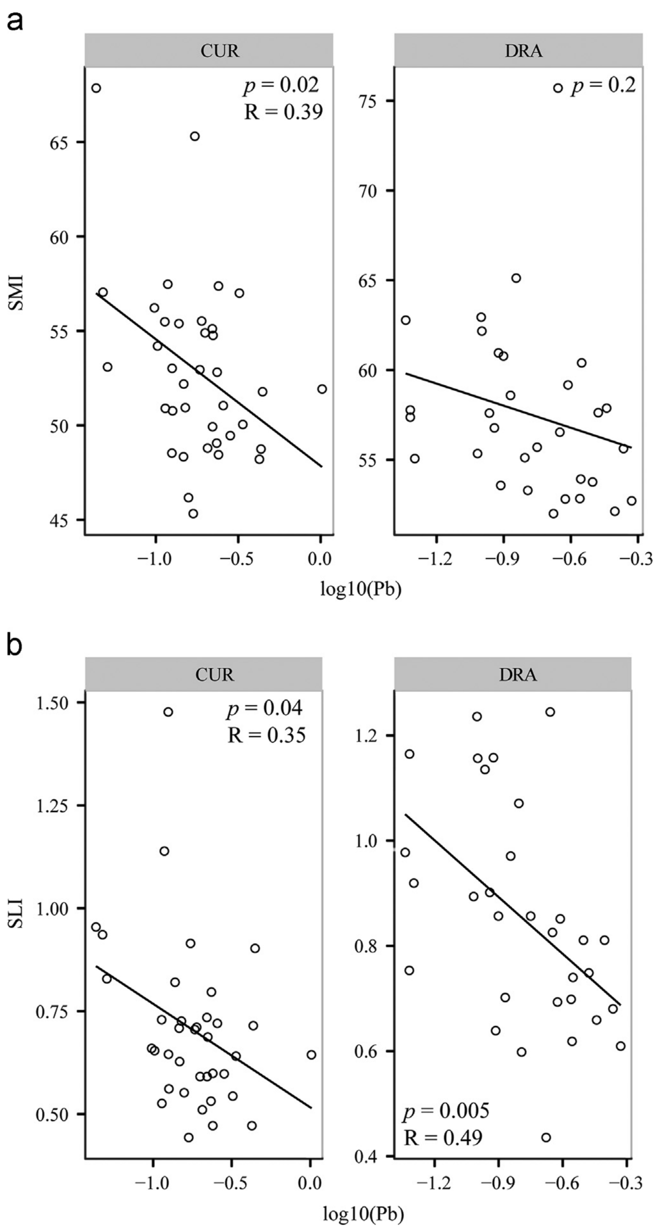

Fig. 4. Scaled mass index (a) and scaled liver index (b) vs. Pb concentrations in trout livers ($\log_{10}$-transformed) for each study area. CUR for Cure, DRA for Dragne.

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 of 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.

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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

Aleksander-Kwaterczak, U., Helios-Rybicka, E., 2008. Contaminated sediments as a potential source of Zn, Pb, and Cd for a river system in the historical metalliferous ore mining and smelting industry area of South Poland. J. Soils Sediments 9, 13–22.
Alibabić, V., Vahčić, N., Bajramović, M., 2007. Bioaccumulation of metals in fish of salmonidae family and the impact on fish meat quality. Environ. Monit. Assess. 131, 349–364.
Allenbach, D.M., 2011. Fluctuating asymmetry and exogenous stress in fishes: a review. Rev. Fish Biol. Fish 21, 355–376.

Almeida, J.S., Meletti, P.C., Martinez, C.B.R., 2005. Acute effects of sediments taken from an urban stream on physiological and biochemical parameters of the neotropical fish Prochilodus lineatus. Comp. Biochem. Physiol. Part C : Toxicol. Pharmacol. 140, 356–363.
Amiotte-Suchet, P., Gauthier, A., Henault, C., Bollache, L., 2011. Les relations entre qualité des cours d'eau et occupation du sol dans le Morvan: production et transfert de matière organique en solution. Bourgogne Nature, Hors-Sér. 9, 118–127.
Arienzo, M., Masuccio, A.A., Ferrara, L., 2013. Evaluation of sediment contamination by heavy metals, organochlorinated pesticides, and polycyclic aromatic hydrocarbons in the Berre Coastal Lagoon (Southeast France). Arch. Environ. Contam. Toxicol. 65, 396–406.
Audry, S., Grosbois, C., Bril, H., Schäfer, J., Kierczak, J., Blanc, G., 2010. Post-depositional redistribution of trace metals in reservoir sediments of a mining/smelting-impacted watershed (the Lot River, SW France). Appl. Geochem. 25, 778–794.
Audry, S., Schäfer, J., Blanc, G., Jouanneau, J.-M., 2004. Fifty-year sedimentary record of heavy metal pollution (Cd, Zn, Cu, Pb) in the Lot River reservoirs (France). Environ. Pollut. 132, 413–426.
Baglinière, J.-L., Bomassi, P., Bousquet, B., Chancerel, F., De Pontual, H., Dumas, J., Euzenat, G., Fontenelle, G., Fournel, F., Gayou, F., Luquet, J.-F., Maisse, G., Martin Ventura, J.-A., Marty, A., Nihouarn, A., Porcher, J.-P., Prevost, E., Prouzet, P., Pustelnik, G., Richard, A., Troadec, H., 1985. La détermination de l'âge par scalimétrie chez le Saumon atlantique (*Salmo salar*) dans son aire de répartition méridionale: utilisation pratique et difficultés de la méthode. Bull. Fr. Pêche Piscic. 298, 69–105.
Baxter, M., 2003. In: Everitt, Brian (Ed.), Statistics in archaeology, Arnold Applications of Statistics Series. Arnold, London, United Kingdom.
Besten, P.J. den, Deckere, E. de, Babut, M.P., Power, B., DelValls, T.A., Zago, C., Oen, A. M.P., Heise, S., 2003. Biological effects-based sediment quality in ecological risk assessment for European waters. J. Soils Sediments 3, 144–162.
Bleuel, C., Wesenberg, D., Sutter, K., Miersch, J., Braha, B., Bärlocher, F., Krauss, G.-J., 2005. The use of the aquatic moss *Fontinalis antipyretica* L. ex Hedw. as a bioindicator for heavy metals: 3. Cd^{2+} accumulation capacities and biochemical stress response of two Fontinalis species. Sci. Total Environ. 345, 13–21.
Bruns, I., Friese, K., Markert, B., Krauss, G.J., 1997. The use of *Fontinalis antipyretica* L. ex Hedw. as a bioindicator for heavy metals. 2. Heavy metal accumulation and physiological reaction of *Fontinalis antipyretica* L. ex Hedw. in active biomonitoring in the River Elbe. Sci. Total Environ. 204, 161–176.
Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach. Springer, New York, USA.
Casiot, C., Egal, M., Elbaz-Poulichet, F., Bruneel, O., Bancon-Montigny, C., Cordier, M.-A., Gomez, E., Aliaume, C., 2009. Hydrological and geochemical control of metals and arsenic in a Mediterranean river contaminated by acid mine drainage (the Amous River, France); preliminary assessment of impacts on fish (*Leuciscus cephalus*). Appl. Geochem. 24, 787–799.
Cauuet, B., Boussicault, M., 2006. Sondage d'une minière à la Pâture des Grangerands. In: Guichard, V. (Ed.), Rapport Annuel D'activités Scientifiques de Bibracte. Centre européen d'archéologie du mont Beuvray, Glux-en-Glenne, France, pp. 129–161.
CCME, 1999. Canadian environmental quality guidelines (Technical report). Canadian Council of Ministers of the Environment.
CCME, 2001. Canadian Water Quality Guidelines for the Protection of Aquatic Life: Canadian Water Quality Index 1.0 (Technical report). Canadian Council of Ministers of the Environment.
CCME, 2002. Canadian environmental quality guidelines—Summary Table (Technical report). Canadian Council of Ministers of the Environment.
Cenci, R.M., 2000. The use of aquatic moss (*Fontinalis antipyretica*) as monitor of contamination in standing and running waters: limits and advantages. J. Limnol. 60, 53–61.
Cesa, M., Bizzotto, A., Ferraro, C., Fumagalli, F., Nimis, P.L., 2006. Assessment of intermittent trace element pollution by moss bags. Environ. Pollut. 144, 886–892.
Claveri, B., Guérold, F., Pihan, J.C., 1995. Use of transplanted mosses and autochthonous liverworts to monitor trace metals in acidic and non-acidic headwater streams (Vosges mountains, France). Sci. Total Environ. 175, 235–244.
Deacon, J.R., Stephens, V.C., 1998. Trace elements in streambed sediment and fish liver at selected sites in the Upper Colorado River Basin, Water-Ressources Investigations Report. U.S. Geological Survey, Denver, Colorado (No. 98-4124).
Delfour, J., 2007. Géologie et gîtologie du Morvan. Règne Minér. Hors-Sér XIII, 9–15.
Desrosiers, M., Babut, M.P., Pelletier, M., Bélanger, C., Thibodeau, S., Martel, L., 2010. Efficiency of sediment quality guidelines for predicting toxicity: the case of the St. Lawrence river. Integr. Environ. Assess. Manag. 6, 225–239.
Elbaz-Poulichet, F., Dezileau, L., Freydier, R., Cossa, D., Sabatier, P., 2011. A 3500-year record of Hg and Pb contamination in a mediterranean sedimentary archive (The Pierre Blanche Lagoon, France). Environ. Sci. Technol. 45, 8642–8647.
Esteve, C., Alcaide, E., Ureña, R., 2012. The effect of metals on condition and pathologies of European eel (Anguilla anguilla): in situ and laboratory experiments. Aquat. Toxicol. 109, 176–184.
Foata, J., Quilichini, Y., Torres, J., Pereira, E., Spella, M.M., Mattei, J., Marchand, B., 2009. Comparison of arsenic and antimony contents in tissues and organs of brown trout caught from the River Presa polluted by ancient mining practices and from the River Bravona in Corsica (France): a survey study. Arch. Environ. Contam. Toxicol. 57, 581–589.
Fernández, J., Carballeira, A., 2000. Differences in the responses of native and transplanted mosses to atmospheric pollution: a possible role of selenium. Environ. Pollut. 110, 73–78.
Fernández, J.A., Aboal, J.R., Carballeira, A., 2000. Use of native and transplanted mosses as complementary techniques for biomonitoring mercury around an industrial facility. Sci. Total Environ. 256, 151–161.
Février, C., Party, J.-P., Probst, A., 1999. Acidification des eaux de surface et charges critiques d'acidité: le cas du massif des Ardennes françaises. Comptes Rendus Académie Sci. : Ser. IIA—Earth Planet. Sci. 328, 29–35.
Figueira, R., Ribeiro, T., 2005. Transplants of aquatic mosses as biomonitors of metals released by a mine effluent. Environ. Pollut. 136, 293–301.
Galán, E., Gómez-Ariza, J.L., González, I., Fernández-Caliani, J.C., Morales, E., Giráldez, I., 2003. Heavy metal partitioning in river sediments severely polluted by acid mine drainage in the Iberian Pyrite Belt. Applied Geochemistry 18, 409–421.
Gismera, M.J., Lacal, J., da Silva, P., García, R., Teresa Sevilla, M., Procopio, J.R., 2004. Study of metal fractionation in river sediments. A comparison between kinetic and sequential extraction procedures. Environ. Pollut. 127, 175–182.
Glass, D.C., Gray, C.N., 2001. Estimating mean exposures from censored data: exposure to benzene in the Australian petroleum industry. Ann. Occup. Hyg. 45, 275–282.
Goodyear, K.L., Ramsey, M.H., Thornton, I., Rosenbaum, M.S., 1996. Source identification of PbZn contamination in the Allen Basin, Cornwall, S.W. England. In: Proceedings of the 3rd International Symposium on Applied Geochemistry, Environmental Geochemistry Selected Papers 11, 61–68.
Gourault, C., Peraud, W., Camizuli, E., Guillaumet, J.-P., Hamm, G., Monna, F., Petit, C., 2012. Minéralogie de la mine du Prabis (Villapourçon, Nièvre, France). Bourgogne Nature 13, 93–116.
Has-Schön, E., Bogut, I., Kralik, G., Bogut, S., Horvatić, J., Čačić, I., 2008. Heavy metal concentration in fish tissues inhabiting waters of “Buško Blato” reservoar (Bosnia and Herzegovina). Environ. Monit. Assess. 144, 15–22.
Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5, 46–51.
Jouffroy-Bapicot, I., Pulido, M., Baron, S., Galop, D., Monna, F., Lavoie, M., Ploquin, A., Petit, C., Beaulieu, J.-L., Richard, H., 2007. Environmental impact of early palaeometallurgy: pollen and geochemical analysis. Veg. Hist. Archaeobotany 16, 251–258.
Kabata-Pendias, A., 2011. Trace Elements in Soils and Plants, 4th ed. CRC Press, Taylor & Francis Group, Boca Raton, USA.
Lambert, A., 2005. Les données géochimiques et alluvionnaires de l'Inventaire minier du territoire national, Constitution d'une Base de Données Exhaustive. BRGM, Orléans, France (Rapport final brgm no. RP-53546-FR).
Leleyter, L., Rousseau, C., Biree, L., Baraud, F., 2012. Comparison of EDTA, HCl and sequential extraction procedures, for selected metals (Cu, Mn, Pb, Zn), in soils, riverine and marine sediments. J. Geochem. Explor. 116–117, 51–59.
Linde, A.R., Sanchez-Galan, S., Izquierdo, J.I., Arribas, P., Maranon, E., García-Vázquez, E., 1998. Brown trout as biomonitor of heavy metal pollution: effect of age on the reliability of the assessment. Ecotoxicol. Environ. Saf. 40, 120–125.
MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31.
Martin, J.-M., Meybeck, M., 1979. Elemental mass-balance of material carried by major world rivers. Mar. Chem. 7, 173–206.
Martins, R.J.E., Boaventura, R.A.R., 2002. Uptake and release of zinc by aquatic bryophytes (*Fontinalis antipyretica* L. ex. Hedw.). Water Res. 36, 5005–5012.
Marvin, C., Grapentine, L., Painter, S., 2004. Application of a sediment quality index to the lower Laurentian great lakes. Environ. Monit. Assess. 91, 1–16.
Meybeck, M., 1986. Composition chimique des ruisseaux non pollués de France. Sci. Geol. Bull. 39, 3–77.
Miller, J.R., 1997. The role of fluvial geomorphic processes in the dispersal of heavy metals from mine sites. J. Geochem. Explor. 58, 101–118.
Monna, F., Camizuli, E., Nedjai, R., Cattin, F., Petit, C., Guillaumet, J.-P., Jouffroy-Bapicot, I., Bohard, B., Chateau, C., Alibert, P., 2014. Tracking archaeological and historical mines using mineral prospectivity mapping. J. Archaeol. Sci. 49, 57–69.
Monna, F., Camizuli, E., Revelli, P., Biville, C., Thomas, C., Losno, R., Scheifler, R., Bruguier, O., Baron, S., Chateau, C., Ploquin, A., Alibert, P., 2011. Wild Brown trout affected by historical mining in the Cévennes National Park, France. Env. Sci Technol. 45, 6823–6830.
Monna, F., Petit, C., Guillaumet, J.-P., Jouffroy-Bapicot, I., Blanchot, C., Dominik, J., Losno, R., Richard, H., Leveque, J., Chateau, C., 2004. History and environmental impact of mining activity in Celtic Aeduan territory recorded in a peat bog (Morvan, France). Environ. Sci. Technol. 38, 665–673.
Mouvet, C., 1992. Etude des Métaux Lourds sur les Mousses Aquatiques—Campagne 1991. BRGM, Orléans, France (Rapport BRGM no. R-35614).
Palmer, A.R., 1994. Fluctuating asymmetry analyses: a primer. In: Markow, T.A. (Ed.), Developmental Instability, Its Origins and Evolutionary Implications. Kluwer Academic Publishers, Dordrecht, (Netherlands), pp. 335–364.
Palmer, A.R., Strobeck, C., 2003. Fluctuating asymmetry analyses revisited. In: Polak, M. (Ed.), Developmental Instability: Causes and Consequences. Oxford University Press, New York, USA, pp. 279–319.
Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118, 1883–1891.
Peig, J., Green, A.J., 2010. The paradigm of body condition: a critical reappraisal of current methods based on mass and length. Funct. Ecol. 24, 1323–1332.
Quantum GIS Development Team, 2010. Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. 〈http:/qgis.osgeo.org〉.
R Development Core Team, 2008. R: A Language And Environment For Statistical Computing. R Development Core Team, Vienne, Austria.

Robson, M., Spence, K., Beech, L., 2006. Stream quality in a small urbanised catchment. Sci. Total Environ. 357, 194–207.
Samecka-Cymerman, A., Kolon, K., Kempers, A.J., 2002. Heavy metals in aquatic bryophytes from the Ore Mountains (Germany). Ecotoxicol. Environ. Saf. 52, 203–210.
Samecka-Cymerman, A., Kolon, K., Kempers, A.J., 2005. A comparison of native and transplanted *Fontinalis antipyretica* Hedw. as biomonitors of water polluted with heavy metals. Sci. Total Environ. 341, 97–107.
Sanchez-Galan, S., Linde, A.R., Izquierdo, J.I., García-Vázquez, E., 1998. Micronuclei and fluctuating asymmetry in brown trout (*Salmo trutta*): complementary methods to biomonitor freshwater ecosystems. Mutat. Res-Genet. Toxicol. Environ. Mutagen. 412, 219–225.
Siebert, A., Bruns, I., Krauss, G.J., Miersch, J., Markert, B., 1996. The use of the aquatic moss *Fontinalis antipyretica L. ex Hedw*. as a bioindicator for heavy metals: 1. Fundamental investigations into heavy metal accumulation in *Fontinalis antipyretica L. ex Hedw*. Sci. Total Environ. 177, 137–144.
Sirugue, D., 2008. Le Morvan, inventaire des paysages et du patrimoine naturel. Bourgogne Nature, Hors-Sér. 6, 134–207.
Vázquez, M.D., Wappelhorst, O., Markert, B., 2004. Determination of 28 Elements in Aquatic Moss *Fontinalis Antipyretica* Hedw. and Water from the Upper Reaches of the River Nysa (CZ, D), by ICP-MS, ICP-OES and AAS. Water Air Soil Pollut. 152, 153–172.
Vázquez, M.D., Fernández, J.Á., Real, C., Villares, R., Aboal, J.R., Carballeira, A., 2007. Design of an aquatic biomonitoring network for an environmental specimen bank. Sci. Total Environ. 388, 357–371.