Evaluation of geochemical baselines and metal enrichment factor values through high ecological quality reference points: a novel methodological approach

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Abstract:

In this study, we propose a new approach to estimate geochemical local baselines and enrichment factor values for metals in riverine sediments. The goal is to describe catchment areas characterized by intensive and spread anthropogenic activities, for which it is challenging to identify undisturbed sites to utilize as reference. The case study is the Nestore river basin (Central Italy). Our approach is based on the use of ecological quality as a criterium to select the reference points in the normalization processes of metal baselines. The rationale is to assume that the sediments with a better environmental quality are anthropogenically least impaired. On these grounds, we detected geochemical local baselines and enrichment factor values of various metals (Ca, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, and Zn). Also, this approach allowed to highlight a major level of pollution for the most downstream site of Nestore river and its left tributaries.

Keywords: Environmental Contamination, Metal, Riverine Sediment, Baseline, Enrichment factor, Normalization

Introduction

Metal pollution of the aquatic environment represents a serious problem worldwide, owing to ubiquity, high persistence, and toxicity of these contaminants. Numerous studies have demonstrated that, during transportation in the riverine system, metals are distributed between the aqueous phase and the sediments through dissolution, precipitation, and sorption phenomena (Abdel-Ghani and Elchaghaby 2007). Sediments are important sinks for metals and play a significant role in the remobilization of metal contaminants in freshwater systems. Furthermore, sediments contaminated by metals are considered source of adverse health effects for biota (Ali et al. 2016). For these reasons, the concentration of metals in sediments are widely used as environmental indicators for the assessment of anthropogenic metal pollution in the aquatic ecosystem (Islam et al. 2015). To this respect, in order to discriminate between anthropogenic pollution and natural sources, it is very important to estimate the geochemical background concentrations of various metals (Pardo et al. 1990; Boughriet et al. 1992; Yu et al. 2001; Klavins et al. 2000).

Geochemical and statistical methods can be used to estimate the geochemical background concentrations. The average shale contents, crust contents and preindustrial background levels of the metals have been used as reference background levels (Turekian et al. 1961). However, these data cannot be used as reference points, because the regional background levels of metals in a sediment may also depend on local geological characteristics (Jiang et al. 2013). Furthermore, due to rapid population growth and to industrialization and urbanization processes occurred in the last two centuries, no pure and local natural background are easily available for metals in the sediments. For these reasons Darnley (1997) suggested the use of geochemical baseline to value the anthropogenic metal pollution in the aquatic ecosystem. The term geochemical baseline represents background conditions that contain a certain degree of human impact and often it has a value that is slightly higher than the natural geochemical background (Teng et al. 2009).

The regression technique and the enrichment factor (EF) are the statistical methods commonly used for the purpose (Matschullat et al. 2000). They both consider the naturally occurring relationships between metals and reference elements, which are not influenced by anthropogenic activities (e.g. Al and Fe).

When the enrichment factor and the normalization method are used, the choice of the reference elements must be done in accordance with a set of conditions. In particular, the reference element must be resistant to chemical weathering and unsusceptible to natural processes such as redox changes. It must be unsusceptible to adsorption–desorption effects, to the soil-forming process and must be geochemically stable or inert, with only small deviations from the natural distribution. Furthermore, a reference element should come from natural parent materials, such as crustal rocks. Finally, the mass fraction of the other elements must variate with that of the reference element (Teng et al. 2009; Schiff and Weisberg 1999). In these normalization approaches it is important to ensure that the information used to develop the interpretative tool is representative of different sediments and allows to evaluate areas suspected to be contaminated. Therefore, it is necessary to utilize, as reference points, anthropogenically undisturbed sediments (Schropp and Windom 1988). However, this aspect presents difficulties when the anthropogenic activities around the riverine basin are intensive and spread.

The objective of our study was to test a new approach for detecting undisturbed sediments based on the identification of areas of high ecological quality by means of macroinvertebrate biomonitoring (Rosenberg and Resh 1993; Bonada et al. 2006; Fabrizi et al. 2010). In running waters, macroinvertebrate taxa are benthic and, because they live at close contact with the sediments, they are considered effective bioindicators of sediment quality where metal pollutions are suspected. With this methodological approach we decided to detect geochemical local baselines and enrichment values of various metals (Cd, Co, Mo, Ni, Pb, Cu, Sb, Se, Cr, Mn, Zn, Ca, and Sr) in the sediments of Nestore river basin (Central Italy, see Figure 1), used as application example. The Nestore river is a right tributary of the Tiber river, it is affected by numerous sources of pollution resulting from urbanization, industry, agriculture, and extensive livestock production, for which it is very difficult to identify anthropogenically undisturbed sediments to utilize as reference points. We recently used bio-ecological data and water and sediment chemistry parameters from the Nestore river basin to demonstrate that taxonomic and functional composition of macroinvertebrates assemblages can be used to determine the anthropogenic disturbance level of freshwater ecosystems even in chemical pollution conditions, in particular due to metal contamination of sediments (Pallottini et al. 2015; Pallottini et al. 2017a, b). The advantage of using biological endpoints over chemical quality assessment criteria is that a biological test responds to the effects of all contaminants present at their actual bioavailability and detects possible synergistic effects (Di Veroli et al., 2010; 2014). The environmental conditions mainly influence the trophic network of living organisms (Apostolico et al.,

2016) which are good bioindicators regarding the evaluation of the metal contamination (Goretti et al., 2018).

This biological approach allowed to evaluate the ecological enrichment factors of Nestore river basin sediments and to identify the sites with higher quality. We supposed that the sediments of these sites were the anthropogenically least impaired and decided to test them as reference points in the normalization processes of metal geochemical baselines.

Materials and Methods

Study area

The Nestore river is a right tributary of the Tiber river, it has a drainage basin area of 1,116 km^2 and a total length of about 48 km (Figure 1). It receives water from two left tributaries, the Genna (stream length about 23 km) and Caina (31 km) streams, and two right tributaries, the Fersinone (25 km) and Calvana (18 km) streams (Figure 1) (Lorenzoni et al. 2004). The left tributaries drain gravel and sandy soils forming a complex system of fluvio-lacustrine deposits of Plio-Pleistocene (Villafranchian) age, the right tributaries, on turn, flow on turbiditic terraines of the Macigno del Mugello formation (Jacobacci et al. 1970). This formation consists of well stratified quartz-feldspar sandstones with variable mica and dolomite amounts (Pandeli et al. 1994). Variably thick lenses and levels of calcareous members (calcarenites and calcirudites, limestones, marly limestones) are present in the formation especially in the lower section of Fersinone and the whole Calvana stream till their confluence in the Nestore river.

The Nestore river (sites 1-6) and its left tributaries, Caina stream (site 7) and Genna stream (site 8) flow through urbanized and agricultural areas draining civil sewage, run-off from agricultural and wastewater from livestock farms, on the other side the right tributaries, Fersinone (site 9) and Calvana (site10) streams, flow through sparsely populated areas.

In particular, the site 8 is located about 0.7 km before the confluence with the Nestore river and about 2 km downstream from the wastewater treatment plant (biodigester) from the swine farms (40,000 heads, at full capacity), whereas a glass factory is located upstream of site 2 (ARPA Umbria 2010).

Sampling campaign

The survey extended from March 2010 to October 2010 and consisted of four seasonal samplings (winter WI, spring SP, summer SU and autumn AU) related to hydrologic features of Mediterranean streams, characterized by high (e.g. winter and spring) and low (up to dry, i.e. summer and autumn) flow conditions. Ten sampling sites were selected: six sites along the Nestore river (sites 1–6) and four sites on its main tributaries (site 7: Caina stream; site 8: Genna stream; site 9: Fersinone stream; site 10: Calvana stream) (Figure 1). Site 9 was dry in autumn, whereas site 10 was dry both in summer and autumn. During each seasonal sampling campaign, samples of the superficial layer of sediments (3–5 cm) were collected by means of a hand dredge. Sediment samples (500 g) were preserved in Pyrex glass bottles and stored at -18 °C (MATT and APAT 2005).

Detection of anthropogenically least impacted sediments

In our previous studies on the Nestore river basin (Pallottini et al., 2015; 2017a,b), we pointed out that the anthropogenic metal contamination of sediments influenced the quali-quantitative composition of macroinvertebrate communities (37,249 specimens belonging to 91 taxa from ten sampling sites). First (Pallottini et al. 2015), we used self-organizing maps (SOM, neural networks) to bring out patterns of benthic macroinvertebrate diversity concerning river pollution of the Nestore river basin (Cereghino and Park, 2009). We introduced metal concentrations in sediments into a SOM previously trained with macroinvertebrate data, and we found out a co-variation in macroinvertebrate community structure and some metals. We divided sites into clusters characterized by similar macroinvertebrate assemblages, and we noticed that clusters with higher ecological quality were associated with a lower concentration of some metals considered. In Pallottini et al. 2017a, we examined the associations between functional traits of macroinvertebrates and heavy metals in sediments of the Nestore river basin. Functional traits were used to assess the stream environmental conditions by characterizing the peculiar taxa assemblages based on biological, physiological, and ecological attributes (Usseglio-Polatera et al., 2001; Tachet et al., 2010). The functional traits did well at detecting disturbance associated with sediment pollution, and there was a definite shift of trait combinations from impacted to least impacted sites. In particular, the dominant trait types, like eutrophic (trophic status), polysaprobic (saprobity), absorber, and deposit feeder (feeding habits), characterized the benthic communities of the sites with higher metal contamination in sediments. Successively (Pallottini et al. 2017b), we designed a multimetric index based on macroinvertebrate as well as physicochemical variables of water and metals of sediments to distinguish non-impacted sites from impacted sites of Nestore river basin. We determined the metric selection for the multimetric index construction based on the response of macrobenthic communities to metal contamination of sediments.

All of these studies on the Nestore river basin allowed us to identify sites 1, 9, and 10 as those characterized by higher ecological quality and, therefore, with low levels of anthropogenic metal contamination of sediments. Thus, we considered the sediments of these sites as the least impacted and used them as reference points in the normalization processes.

Metals analysis

Concentration of metals (Cd, Co, Mo, Ni, Pb, Cu, Sb, Se, Cr, Mn, Zn, Al, Fe, Ca, Sr, and V) in the sediment samples were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Ultima 2, HORIBA Scientific) equipped with ultrasonic nebulizer (CETAC Technologies, U-5000AT) after sample acid microwave digestion. All sediment samples were analysed in double and the ICP measures were replicate three times for each sample. Commercially produced (ICP multi-element standard solution IV CertiPUR® , VWR Merck Chemicals and Reagents) standard solutions (1.000 $mg^{.}L^{-1}$) in nitric acid were used to prepare appropriate elemental calibration standards. Ordinary least-squares regression model (OLSR) was used for calibrations. Linearity between intensity and concentration ($R^2 > 0.999$) was observed for traces metals in the range 0.01–1.00 mg/L and for mayor elements in the range 0.01–5.00 mg/L. For quantifying Al, Ca, Fe, Mn, and Zn the digested solutions were diluted 1:100 using ultrapure water (18MΩ). Experimental repeatability was calculated by performing three replicates analyses of three multi-element standards solutions (0.01, 0.10, and 1.00 mg/L). The metal RSDs obtained by the repeatability test were good and were in the range 0.5-10.0% . Detection limits, estimated by calibration curve, were in the range 0.8–2 mg kg^{-1}, working at a radiofrequency power of 1,000W. The accuracy of the method was obtained using standard reference material (IAEA soil -7, Trace Elements in Soil, Vienna, Austria). The metal concentrations were in satisfactory agreement with the certified value and recovery fell in the range of 80-130%. Zn, Al, and Cu showed lower recovery. Analytical quality control included analysis of digestion reagent blank with each batch of 6 samples. All laboratory glassware was soaked in 10% HNO_3 for 24h, then rinsed before use with ultrapure water (Analytical Methods Committee, 1994; MATT and APAT, 2005; UNICHIM, 2001; EURACHEM 1998).

The sediments were air-dried, disaggregated using a mortar and pestle to pass through a 2-mm mesh sieve, dried at 105°C for 24 h and digested as follows: 8 mL of ultrapure nitric acid (Millipore Suprapur®, 65%) and 2 ml of ultrapure solution of hydrogen peroxide (Carlo Erba Reagents, 30-32%) were added to 0.250 g of sediment samples and digested in Mars Microwave Oven, working at a power of 800W. Microwave digestion consisted of two steps: 130°C (200 psi) for 1 min, 180°C (300 psi) for 10 min. The mixture was cooled, filtered (Whatman Grade No. 42, particle retention 2.5 mm) and diluted with ultrapure water to 50 mL.

We note that this method is not a total digestion technique for most samples. However, this type of acid digestion dissolves mostly the elements that could become “environmentally available.” The elements firmly bound in silicate structures, those that are not easily dissolved by the present procedure are usually also not mobile in the environment.

Mean Enrichment factor (EF)

The enrichment factor (EF) for each element was calculated to evaluate the degree of anthropogenic metals contamination in sediments using the formula proposed by Selvaraj et al. (2004) as stated below:

$$EF = \frac{\left({C_x}/{C_{reference\ metal}}\right) site_{2-8}}{\left({C_x}/{C_{reference\ metal}}\right) reference\ site}$$

where:

$C_x/C_{reference\ metal}$ is the ratio between the annual mean concentrations of the examined metal and of the reference metal (Al) in the considered site (sites 2-8) and in the reference site (annual mean values of sites 1, 9, and 10), respectively.

The degree of enrichment was interpreted based on the method proposed by Birch and Davies (2003):

EF<1 “no enrichment”, 1≤EF<3 “minor enrichment”, 3≤EF<5 “moderate enrichment”, 5≤EF<10 “moderately severe enrichment”, 10≤EF<25 “severe enrichment”, 25≤EF<50 “very severe enrichment” and EF> 50 “extremely severe enrichment”.

EF values were also interpreted as suggested by Zhang and Liu (2002): if 0.5≤EF≤1.5, it indicates that the metal could be mainly from natural weathering process, and if EF>1.5, it indicates that the metal is from anthropogenic sources or a greater percentage of the metal is from non-natural weathering process.

Regression technique

The linear regression relationships between an examined metal and the reference elements (Al, Fe, and V) were calculated using the principle of normalization (Newman and Watling 2007; Colizza et al. 1996):

$$C_M = a + bC_N$$

where:

C_M represents the baseline concentration of the investigated metal, C_N indicates the concentration of the reference element in the reference site (mean values of the sites 1, 9, and 10), and *a* and *b* are the coefficient and constant of the regression equation, respectively.

Results

The annual mean metals concentration in sediments sampled at sites 1-10, are reported in Table 1, while seasonal site concentrations are showed in Online Resource 1 (ESM1).

The metals present in highest quantity were Ca, Al and Fe. Al and Fe had mean concentrations of the same order of magnitude. Ca and Sr, elements of terrigenous and biogenic origin, showed a downstream gradient of concentrations, which increased from site 1 to site 6 along the Nestore river, in every season.

Cd concentrations were higher than the limit of detection only in summer and autumn. The majority of measured concentrations of Mo and Sb were under the limit of detection.

Enrichment Factor (EF)

The enrichment factor is usually evaluated using Al or Fe. In the present study, Al was used to compute the EF since it represents the quantity of alumino-silicates, which is the predominant carrier phase for adsorbed metals sediments (Alexander et al. 1993). In Table 2 are reported the enrichment factors values obtained using Al for normalization and the values obtained averaging the concentrations of sites 1, 9 and 10 as reference points. We did not use the metal concentration values of site 9 in autumn and site 10 in summer and autumn to calculate the mean values because in 2010 they were dry.

The values of enrichment factors for Ca, Co, Cu, Mn, Pb, Sr, and Zn (Table 2) showed an increase along a downstream gradient of the Nestore river (sites 2-6). High EF values of the same metals were usually observed at the Caina (site 7) and the Genna (site 8) streams (Fig. 2).

The enrichment factors of Mo, Sb and Cd were not calculated because the majority of measured concentrations were under the limit of detection.

Regression-technique

A significant number of metals have been used as normalisers in previous literature, including aluminum (Schropp et al. 1990; Daskalakis and O’Connor 1995; Cooke and Drury 1998; Weisberg et al. 1999; Roach 2005), iron (Daskalakis and O’Connor 1995; Schiff and Weisberg 1999; Tanner et al. 2000), lithium (Loring 1990, 1991; Aloupi and Angelidis 2001; Veinott et al. 2001), vanadium (Chaoyang and Hailong2005) rubidium (Grant and Middleton 1990), and caesium and cobalt (Matthai and Birch 2001).

In the present work, we selected aluminum, iron and vanadium. Aluminum is considered to be the most suitable normaliser since it is a major constituent of fine-grained alumino-silicates (clays), with which trace metals are associated. Iron oxides, which serve as a host for metals, are usually associated with sediments in quantities related to the sediment surface area. Consequently, the concentration of iron often exhibits a strong positive correlation to concentrations of trace metals in sediment (Newman and Watling, 2007). Vanadium shows a strong positive association with organic matter which serves as a matrix on particle surfaces for complexing metals (El-Moselhy 2006).

Linear regressions were performed for ten metals (Ca, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, and Zn) using as alternative reference elements aluminium, iron and vanadium. The coefficient of the regression was used as criteria for selection of the final normalizer. Data beyond 95% confidential intervals of the linear regression equations were removed as the outliers in the calculation of the baseline. Since site 9 in October and site 10 in October and August were dry, their metal concentration values were not used to calculate the linear regressions, similarly to the EF calculation. Also, we did not perform linear regressions for Mo, Sb and Cd, because the majority of measured concentrations were under the limit of detection.

Aluminum was found to be the most suitable normaliser for Ca and Sr, iron for Mn and Zn, and vanadium for Cu, Co and Ni. No statistically significant reference element was selected for Pb, Se and Cr.

Linear regression analyses, mean values and linear ranges of the baseline concentrations are presented in Table 3. The linear regressions were considered for $P<0.05$ with a relatively higher R^2 value. If compared with estimate composition of the Upper Continental Crust (UCC) (Rudnick and

Gao, 2003), our mean baseline values resulted being lower than UCC concentrations for Cu, Co, Sr, and Mn, higher for Ca and similar for Zn and Ni.

Discussion

The sites under study showed mean metal concentrations characterized by high relative standard deviations, revealing a wide range of seasonal variation (Table 1). Such high seasonal variability in sediment metal is consistent with the dynamics of the hydrological regime of the river basin, characterized by wet-winter and dry-summer. Nevertheless, the enrichment factors calculated on average concentration made possible to assess the quality of the sediments according to the degree of metal pollution and to discriminate between anthropogenic and natural sources. There was no (EF<1) or minor (1≤EF<3) enrichment for all metals at the sites 2-8, except for Pb which showed a moderate EF=3.2 enrichment at site 8.

According to Zhang and Liu (2002), the EF values revealed that all sites were contaminated from anthropogenic sources (EF>1.5) at least for one metal, except sites 4 and 5. Sites 6, 7 and 8 were the most contaminated sites. EF values greater than 1.5 were observed for Ca, Co, Cu, Mn, Pb, Sr and Zn in sediments of site 8 and for Cu, Pb and Zn at sites 6 and 7. This evidence is consistent with the level of anthropogenic disturbance, that characterize the Nestore catchment.

The left tributaries, Caina (site 7) and Genna (site 8) streams, flow in urbanized and agricultural areas draining civil sewage, run-off from agricultural and wastewater from livestock farms. In particular the site 8 is located about 2 km downstream from the wastewater treatment plant (biodigester) from the swine farms (40,000 heads, at full capacity). The sites 2 and 3 presented an

EF=1.6 for Zn and Cr, respectively, above the threshold of 1.5 for anthropogenic disturbance. This condition is probably a consequence of the wastewater of a glass factory located upstream of these sites.

The baselines calculated (sites 1, 9 and 10) using the regression technique were dependent on the reference elements selected (Table 3 and Figure 1s). The impossibility of finding a suitable reference element (i.e. Al, Fe or V) for Pb, Se and Cr may indicate a widespread contamination of all sites of Nestore river basin by external sources, such as atmospheric deposition of Pb and Se produced by the combustion of fossil fuel and by the use of metal contaminated fertilizers (Se, Pb and Cr) in agricultural activities (Jiang et al. 2013; Belon et al. 2012; Nacke et al. 2013). Moreover, the weaker relationships observed (i.e. Co, Mn, and Zn) could be due to the partial digestion method used, because the elements firmly bound in silicate structures are not easily dissolved by this procedure.

The mean values obtained for each metal by linear regression were used as baselines allowing to identify the anthropogenically disturbed sites. Mean metal concentrations (Table 1) higher than the baseline values were observed for Ca at sites 6, 7 and 8, for Cu at sites 2, 3, 6, 7 and 8, for Mn at sites 7 and 8, for Ni at sites 2 and 6, for Sr at sites 7 and 8, for Co and Zn at all sites.

To estimate the level of accordance between the two statistical methods used, we correlated for the sites 2-8 the mean enrichment factors versus the ratios between the mean metal concentrations and the mean baseline values (Table 4 and Figure 3). The good regression coefficients obtained (with the exception of Ni, for which both methods identified no or minor enrichment at all sites) indicated that the baseline values found by the regression technique were in agreement with the calculated enrichment factors and confirmed a major level of disturbance for sites 6, 7 and 8, minor for sites 2 and 3 respect to the least disturbed sites 4 and 5.

In addition, the mean enrichment factors were also calculated using the upper continental crust (UCC) as reference background levels and Al, as reference element (Table 5). Compared to UC the sediment mean composition revealed a significant enrichment in Ca, Cu, Ni, Pb, and Zn in respect to Al amounts (Table 4). Sites 7-10 were also enriched in Sr, while sites 1, 7 and 8 showed some Mn excess. Comparison between the sampling sites showed in fact a severe enrichment in Ca at all the Nestore tributaries (sites 7-10), and some moderately severe and severe enrichment of the same element along the Nestore river (e.g., site 4, 5 and 6). Cu and Pb, in turn, were strongly enriched only at site 8, though they showed moderately severe enrichment also at site 6, 7 and 5, the latter with reference only to lead. Moderately severe enrichment values were also revealed for Zn at site 2, 6, 7 and 8. A moderate enrichment was detected at all sites for Ni, at sites 1, 7 and 8 for Mn and at sites 7-10 for Sr.

In summary, the mean enrichment factors calculated using the upper continental crust values were much higher than those calculated with our new approach for all metals (except for Se) in all sites, including sites 1, 9 and 10. The latter, characterized by a high ecological quality, should have shown only minor enrichments and this therefore demonstrated the inadequacy of the use of the UCC content as reference background levels for Nestore river basin sediments.

Conclusions

Geochemical baseline concentrations of various metals (Ca, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, V and Zn) in surface sediments of Nestore river basin were estimated by the methods of enrichment factors and normalization process (regression technique). Sites located within the basin and characterized by high ecological quality were used as reference points.

According to the calculated enrichment factors, sites 6 (Nestore river), 7 (Caina stream) and 8 (Genna stream) were the most contaminated sites. EF values greater than 1.5 were observed for Ca, Co, Cu Mn, Pb, Sr, and Zn in sediments of site 8 and for Cu, Pb and Zn at sites 6 and 7. The sites 2 and 3 presented EF values equal to 1.6 for Zn and Cr, respectively.

The baseline values found by the regression technique were in agreement with the calculated enrichment factors and confirmed a major level of disturbance for sites 6, 7 and 8 and minor for sites 2 and 3.

These results are consistent with the presence and the geographical distribution of industrial, zootechnical and agricultural activities in the area of the study (ARPA Umbria 2010).

On the other side the enrichment factors calculated using UCC content as reference background levels were usually much higher ($1.1 \leq EF \leq 22$) than those obtained with our approach ($0.2 \leq EF \leq 3.2$), also for sites 1, 9 and 10 characterized by a high ecological quality.

In conclusion, our results showed that sediments with high ecological quality can be considered suitable reference points for baseline studies. They can also be used to assess the metal pollution level in river basins characterised by intensive and widespread anthropogenic activities.

Since the values of ecological quality are acquired relatively easily and inexpensively, our approach could be applied in regional monitoring and remediation programs to identify in a quick and cheap way the reference points for the determination of the metal geochemical baselines in order to recognize with a best accuracy anthropogenically disturbed areas in riverine system.

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FIGURE CAPTIONS

Fig. 1 Nestore river basin (Umbria, Central Italy) and location of the sampling sites

Fig. 2 Correlation of the mean Enrichment Factors vs ratios between the mean metal concentrations and the mean baseline values for sites 2-8 of the Nestore river basin

TABLE CAPTIONS

Table 1 Mean concentrations (mg/kg) ± standard deviation of metals in sediments of the Nestore river basin sites

Table 2 Mean Enrichment factors of metals in sites 2-8 of the Nestore river basin sites

Table 3 Linear regression equations for various heavy metals on the reference elements and the corresponding calculated baseline concentrations (mg/kg) in the sediments of the Nestore river basin

Table 4 Ratios between the mean metal concentrations and the mean baseline values for sites 2-8 of the Nestore river basin

Table 5 Mean Enrichment factors of metals in sites 1-10 of the Nestore river basin sites calculated using the Upper Continental Crust as the reference background levels and Al, as the reference element

Evaluation of geochemical baselines and metal enrichment factor values through high ecological quality reference points: a novel methodological approach

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Abstract:

In this study, we propose a new approach to estimate geochemical local baselines and enrichment factor values for metals in riverine sediments. The goal is to describe catchment areas characterized by intensive and spread anthropogenic activities, for which it is challenging to identify undisturbed sites to utilize as reference. The case study is the Nestore river basin (Central Italy). Our approach is based on the use of ecological quality as a criterium to select the reference points in the normalization processes of metal baselines. The rationale is to assume that the sediments with a better environmental quality are anthropogenically least impaired. On these grounds, we detected geochemical local baselines and enrichment factor values of various metals (Ca, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, and Zn). Also, this approach allowed to highlight a major level of pollution for the most downstream site of Nestore river and its left tributaries.

Keywords: Environmental Contamination, Metal, Riverine Sediment, Baseline, Enrichment factor, Normalization

Introduction

Metal pollution of the aquatic environment represents a serious problem worldwide, owing to ubiquity, high persistence, and toxicity of these contaminants. Numerous studies have demonstrated that, during transportation in the riverine system, metals are distributed between the aqueous phase and the sediments through dissolution, precipitation, and sorption phenomena (Abdel-Ghani and Elchaghaby 2007). Sediments are important sinks for metals and play a significant role in the remobilization of metal contaminants in freshwater systems. Furthermore, sediments contaminated by metals are considered source of adverse health effects for biota (Ali et al. 2016). For these reasons, the concentration of metals in sediments are widely used as environmental indicators for the assessment of anthropogenic metal pollution in the aquatic ecosystem (Islam et al. 2015). To this respect, in order to discriminate between anthropogenic pollution and natural sources, it is very important to estimate the geochemical background concentrations of various metals (Pardo et al. 1990; Boughriet et al. 1992; Yu et al. 2001; Klavins et al. 2000).

Geochemical and statistical methods can be used to estimate the geochemical background concentrations. The average shale contents, crust contents and preindustrial background levels of the metals have been used as reference background levels (Turekian et al. 1961). However, these data cannot be used as reference points, because the regional background levels of metals in a sediment may also depend on local geological characteristics (Jiang et al. 2013). Furthermore, due to rapid population growth and to industrialization and urbanization processes occurred in the last two centuries, no pure and local natural background are easily available for metals in the sediments. For these reasons Darnley (1997) suggested the use of geochemical baseline to value the anthropogenic metal pollution in the aquatic ecosystem. The term geochemical baseline represents background conditions that contain a certain degree of human impact and often it has a value that is slightly higher than the natural geochemical background (Teng et al. 2009).

The regression technique and the enrichment factor (EF) are the statistical methods commonly used for the purpose (Matschullat et al. 2000). They both consider the naturally occurring relationships between metals and reference elements, which are not influenced by anthropogenic activities (e.g. Al and Fe).

When the enrichment factor and the normalization method are used, the choice of the reference elements must be done in accordance with a set of conditions. In particular, the reference element must be resistant to chemical weathering and unsusceptible to natural processes such as redox changes. It must be unsusceptible to adsorption–desorption effects, to the soil-forming process and must be geochemically stable or inert, with only small deviations from the natural distribution. Furthermore, a reference element should come from natural parent materials, such as crustal rocks. Finally, the mass fraction of the other elements must variate with that of the reference element (Teng et al. 2009; Schiff and Weisberg 1999). In these normalization approaches it is important to ensure that the information used to develop the interpretative tool is representative of different sediments and allows to evaluate areas suspected to be contaminated. Therefore, it is necessary to utilize, as reference points, anthropogenically undisturbed sediments (Schropp and Windom 1988). However, this aspect presents difficulties when the anthropogenic activities around the riverine basin are intensive and spread.

The objective of our study was to test a new approach for detecting undisturbed sediments based on the identification of areas of high ecological quality by means of macroinvertebrate biomonitoring (Rosenberg and Resh 1993; Bonada et al. 2006; Fabrizi et al. 2010). In running waters, macroinvertebrate taxa are benthic and, because they live at close contact with the sediments, they are considered effective bioindicators of sediment quality where metal pollutions are suspected. With this methodological approach we decided to detect geochemical local baselines and enrichment values of various metals (Cd, Co, Mo, Ni, Pb, Cu, Sb, Se, Cr, Mn, Zn, Ca, and Sr) in the sediments of Nestore river basin (Central Italy, see Figure 1), used as application example. The Nestore river is a right tributary of the Tiber river, it is affected by numerous sources of pollution resulting from urbanization, industry, agriculture, and extensive livestock production, for which it is very difficult to identify anthropogenically undisturbed sediments to utilize as reference points. We recently used bio-ecological data and water and sediment chemistry parameters from the Nestore river basin to demonstrate that taxonomic and functional composition of macroinvertebrates assemblages can be used to determine the anthropogenic disturbance level of freshwater ecosystems even in chemical pollution conditions, in particular due to metal contamination of sediments (Pallottini et al. 2015; Pallottini et al. 2017a, b). The advantage of using biological endpoints over chemical quality assessment criteria is that a biological test responds to the effects of all contaminants present at their actual bioavailability and detects possible synergistic effects (Di Veroli et al., 2010; 2014). The environmental conditions mainly influence the trophic network of living organisms (Apostolico et al.,

2016) which are good bioindicators regarding the evaluation of the metal contamination (Goretti et al., 2018).

This biological approach allowed to evaluate the ecological enrichment factors of Nestore river basin sediments and to identify the sites with higher quality. We supposed that the sediments of these sites were the anthropogenically least impaired and decided to test them as reference points in the normalization processes of metal geochemical baselines.

Materials and Methods

Study area

The Nestore river is a right tributary of the Tiber river, it has a drainage basin area of 1,116 km^2 and a total length of about 48 km (Figure 1). It receives water from two left tributaries, the Genna (stream length about 23 km) and Caina (31 km) streams, and two right tributaries, the Fersinone (25 km) and Calvana (18 km) streams (Figure 1) (Lorenzoni et al. 2004). The left tributaries drain gravel and sandy soils forming a complex system of fluvio-lacustrine deposits of Plio-Pleistocene (Villafranchian) age, the right tributaries, on turn, flow on turbiditic terraines of the Macigno del Mugello formation (Jacobacci et al. 1970). This formation consists of well stratified quartz-feldspar sandstones with variable mica and dolomite amounts (Pandeli et al. 1994). Variably thick lenses and levels of calcareous members (calcarenites and calcirudites, limestones, marly limestones) are present in the formation especially in the lower section of Fersinone and the whole Calvana stream till their confluence in the Nestore river.

The Nestore river (sites 1-6) and its left tributaries, Caina stream (site 7) and Genna stream (site 8) flow through urbanized and agricultural areas draining civil sewage, run-off from agricultural and wastewater from livestock farms, on the other side the right tributaries, Fersinone (site 9) and Calvana (site10) streams, flow through sparsely populated areas.

In particular, the site 8 is located about 0.7 km before the confluence with the Nestore river and about 2 km downstream from the wastewater treatment plant (biodigester) from the swine farms (40,000 heads, at full capacity), whereas a glass factory is located upstream of site 2 (ARPA Umbria 2010).

Sampling campaign

The survey extended from March 2010 to October 2010 and consisted of four seasonal samplings (winter WI, spring SP, summer SU and autumn AU) related to hydrologic features of Mediterranean streams, characterized by high (e.g. winter and spring) and low (up to dry, i.e. summer and autumn) flow conditions. Ten sampling sites were selected: six sites along the Nestore river (sites 1–6) and four sites on its main tributaries (site 7: Caina stream; site 8: Genna stream; site 9: Fersinone stream; site 10: Calvana stream) (Figure 1). Site 9 was dry in autumn, whereas site 10 was dry both in summer and autumn. During each seasonal sampling campaign, samples of the superficial layer of sediments (3–5 cm) were collected by means of a hand dredge. Sediment samples (500 g) were preserved in Pyrex glass bottles and stored at -18 °C (MATT and APAT 2005).

Detection of anthropogenically least impacted sediments

In our previous studies on the Nestore river basin (Pallottini et al., 2015; 2017a,b), we pointed out that the anthropogenic metal contamination of sediments influenced the quali-quantitative composition of macroinvertebrate communities (37,249 specimens belonging to 91 taxa from ten sampling sites). First (Pallottini et al. 2015), we used self-organizing maps (SOM, neural networks) to bring out patterns of benthic macroinvertebrate diversity concerning river pollution of the Nestore river basin (Cereghino and Park, 2009). We introduced metal concentrations in sediments into a SOM previously trained with macroinvertebrate data, and we found out a co-variation in macroinvertebrate community structure and some metals. We divided sites into clusters characterized by similar macroinvertebrate assemblages, and we noticed that clusters with higher ecological quality were associated with a lower concentration of some metals considered. In Pallottini et al. 2017a, we examined the associations between functional traits of macroinvertebrates and heavy metals in sediments of the Nestore river basin. Functional traits were used to assess the stream environmental conditions by characterizing the peculiar taxa assemblages based on biological, physiological, and ecological attributes (Usseglio-Polatera et al., 2001; Tachet et al., 2010). The functional traits did well at detecting disturbance associated with sediment pollution, and there was a definite shift of trait combinations from impacted to least impacted sites. In particular, the dominant trait types, like eutrophic (trophic status), polysaprobic (saprobity), absorber, and deposit feeder (feeding habits), characterized the benthic communities of the sites with higher metal contamination in sediments. Successively (Pallottini et al. 2017b), we designed a multimetric index based on macroinvertebrate as well as physicochemical variables of water and metals of sediments to distinguish non-impacted sites from impacted sites of Nestore river basin. We determined the metric selection for the multimetric index construction based on the response of macrobenthic communities to metal contamination of sediments.

All of these studies on the Nestore river basin allowed us to identify sites 1, 9, and 10 as those characterized by higher ecological quality and, therefore, with low levels of anthropogenic metal contamination of sediments. Thus, we considered the sediments of these sites as the least impacted and used them as reference points in the normalization processes.

Metals analysis

Concentration of metals (Cd, Co, Mo, Ni, Pb, Cu, Sb, Se, Cr, Mn, Zn, Al, Fe, Ca, Sr, and V) in the sediment samples were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Ultima 2, HORIBA Scientific) equipped with ultrasonic nebulizer (CETAC Technologies, U-5000AT) after sample acid microwave digestion. All sediment samples were analysed in double and the ICP measures were replicate three times for each sample. Commercially produced (ICP multi-element standard solution IV CertiPUR® , VWR Merck Chemicals and Reagents) standard solutions (1.000 $mg{\cdot}L^{-1}$) in nitric acid were used to prepare appropriate elemental calibration standards. Ordinary least-squares regression model (OLSR) was used for calibrations. Linearity between intensity and concentration ($R^2 > 0.999$) was observed for traces metals in the range 0.01–1.00 mg/L and for mayor elements in the range 0.01–5.00 mg/L. For quantifying Al, Ca, Fe, Mn, and Zn the digested solutions were diluted 1:100 using ultrapure water (18MΩ). Experimental repeatability was calculated by performing three replicates analyses of three multi-element standards solutions (0.01, 0.10, and 1.00 mg/L). The metal RSDs obtained by the repeatability test were good and were in the range 0.5-10.0% . Detection limits, estimated by calibration curve, were in the range 0.8–2 mg kg^{-1}, working at a radiofrequency power of 1,000W. The accuracy of the method was obtained using standard reference material (IAEA soil -7, Trace Elements in Soil, Vienna, Austria). The metal concentrations were in satisfactory agreement with the certified value and recovery fell in the range of 80-130%. Zn, Al, and Cu showed lower recovery. Analytical quality control included analysis of digestion reagent blank with each batch of 6 samples. All laboratory glassware was soaked in 10% HNO_3 for 24h, then rinsed before use with ultrapure water (Analytical Methods Committee, 1994; MATT and APAT, 2005; UNICHIM, 2001; EURACHEM 1998).

The sediments were air-dried, disaggregated using a mortar and pestle to pass through a 2-mm mesh sieve, dried at 105°C for 24 h and digested as follows: 8 mL of ultrapure nitric acid (Millipore Suprapur®, 65%) and 2 ml of ultrapure solution of hydrogen peroxide (Carlo Erba Reagents, 30-32%) were added to 0.250 g of sediment samples and digested in Mars Microwave Oven, working at a power of 800W. Microwave digestion consisted of two steps: 130°C (200 psi) for 1 min, 180°C (300 psi) for 10 min. The mixture was cooled, filtered (Whatman Grade No. 42, particle retention 2.5 mm) and diluted with ultrapure water to 50 mL.

We note that this method is not a total digestion technique for most samples. However, this type of acid digestion dissolves mostly the elements that could become “environmentally available.” The elements firmly bound in silicate structures, those that are not easily dissolved by the present procedure are usually also not mobile in the environment.

Mean Enrichment factor (EF)

The enrichment factor (EF) for each element was calculated to evaluate the degree of anthropogenic metals contamination in sediments using the formula proposed by Selvaraj et al. (2004) as stated below:

$$EF = \frac{\left(C_x / C_{reference\ metal}\right) site_{2-8}}{\left(C_x / C_{reference\ metal}\right) reference\ site}$$

where:

$C_x/C_{reference\ metal}$ is the ratio between the annual mean concentrations of the examined metal and of the reference metal (Al) in the considered site (sites 2-8) and in the reference site (annual mean values of sites 1, 9, and 10), respectively.

The degree of enrichment was interpreted based on the method proposed by Birch and Davies (2003):

EF<1 “no enrichment”, 1≤EF<3 “minor enrichment”, 3≤EF<5 “moderate enrichment”, 5≤EF<10 “moderately severe enrichment”, 10≤EF<25 “severe enrichment”, 25≤EF<50 “very severe enrichment” and EF> 50 “extremely severe enrichment”.

EF values were also interpreted as suggested by Zhang and Liu (2002): if 0.5≤EF≤1.5, it indicates that the metal could be mainly from natural weathering process, and if EF>1.5, it indicates that the metal is from anthropogenic sources or a greater percentage of the metal is from non-natural weathering process.

Regression technique

The linear regression relationships between an examined metal and the reference elements (Al, Fe, and V) were calculated using the principle of normalization (Newman and Watling 2007; Colizza et al. 1996):

$$C_M = a + bC_N$$

where:

C_M represents the baseline concentration of the investigated metal, C_N indicates the concentration of the reference element in the reference site (mean values of the sites 1, 9, and 10), and *a* and *b* are the coefficient and constant of the regression equation, respectively.

Results

The annual mean metals concentration in sediments sampled at sites 1-10, are reported in Table 1, while seasonal site concentrations are showed in Online Resource 1 (ESM1).

The metals present in highest quantity were Ca, Al and Fe. Al and Fe had mean concentrations of the same order of magnitude. Ca and Sr, elements of terrigenous and biogenic origin, showed a downstream gradient of concentrations, which increased from site 1 to site 6 along the Nestore river, in every season.

Cd concentrations were higher than the limit of detection only in summer and autumn. The majority of measured concentrations of Mo and Sb were under the limit of detection.

Enrichment Factor (EF)

The enrichment factor is usually evaluated using Al or Fe. In the present study, Al was used to compute the EF since it represents the quantity of alumino-silicates, which is the predominant carrier phase for adsorbed metals sediments (Alexander et al. 1993). In Table 2 are reported the enrichment factors values obtained using Al for normalization and the values obtained averaging the concentrations of sites 1, 9 and 10 as reference points. We did not use the metal concentration values of site 9 in autumn and site 10 in summer and autumn to calculate the mean values because in 2010 they were dry.

The values of enrichment factors for Ca, Co, Cu, Mn, Pb, Sr, and Zn (Table 2) showed an increase along a downstream gradient of the Nestore river (sites 2-6). High EF values of the same metals were usually observed at the Caina (site 7) and the Genna (site 8) streams (Fig. 2).

The enrichment factors of Mo, Sb and Cd were not calculated because the majority of measured concentrations were under the limit of detection.

Regression-technique

A significant number of metals have been used as normalisers in previous literature, including aluminum (Schropp et al. 1990; Daskalakis and O’Connor 1995; Cooke and Drury 1998; Weisberg et al. 1999; Roach 2005), iron (Daskalakis and O’Connor 1995; Schiff and Weisberg 1999; Tanner et al. 2000), lithium (Loring 1990, 1991; Aloupi and Angelidis 2001; Veinott et al. 2001), vanadium (Chaoyang and Hailong2005) rubidium (Grant and Middleton 1990), and caesium and cobalt (Matthai and Birch 2001).

In the present work, we selected aluminum, iron and vanadium. Aluminum is considered to be the most suitable normaliser since it is a major constituent of fine-grained alumino-silicates (clays), with which trace metals are associated. Iron oxides, which serve as a host for metals, are usually associated with sediments in quantities related to the sediment surface area. Consequently, the concentration of iron often exhibits a strong positive correlation to concentrations of trace metals in sediment (Newman and Watling, 2007). Vanadium shows a strong positive association with organic matter which serves as a matrix on particle surfaces for complexing metals (El-Moselhy 2006).

Linear regressions were performed for ten metals (Ca, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, and Zn) using as alternative reference elements aluminium, iron and vanadium. The coefficient of the regression was used as criteria for selection of the final normalizer. Data beyond 95% confidential intervals of the linear regression equations were removed as the outliers in the calculation of the baseline. Since site 9 in October and site 10 in October and August were dry, their metal concentration values were not used to calculate the linear regressions, similarly to the EF calculation. Also, we did not perform linear regressions for Mo, Sb and Cd, because the majority of measured concentrations were under the limit of detection.

Aluminum was found to be the most suitable normaliser for Ca and Sr, iron for Mn and Zn, and vanadium for Cu, Co and Ni. No statistically significant reference element was selected for Pb, Se and Cr.

Linear regression analyses, mean values and linear ranges of the baseline concentrations are presented in Table 3. The linear regressions were considered for $P<0.05$ with a relatively higher R^2 value. If compared with estimate composition of the Upper Continental Crust (UCC) (Rudnick and

Gao, 2003), our mean baseline values resulted being lower than UCC concentrations for Cu, Co, Sr, and Mn, higher for Ca and similar for Zn and Ni.

Discussion

The sites under study showed mean metal concentrations characterized by high relative standard deviations, revealing a wide range of seasonal variation (Table 1). Such high seasonal variability in sediment metal is consistent with the dynamics of the hydrological regime of the river basin, characterized by wet-winter and dry-summer. Nevertheless, the enrichment factors calculated on average concentration made possible to assess the quality of the sediments according to the degree of metal pollution and to discriminate between anthropogenic and natural sources. There was no ($EF<1$) or minor ($1\leq EF<3$) enrichment for all metals at the sites 2-8, except for Pb which showed a moderate $EF=3.2$ enrichment at site 8.

According to Zhang and Liu (2002), the EF values revealed that all sites were contaminated from anthropogenic sources ($EF>1.5$) at least for one metal, except sites 4 and 5. Sites 6, 7 and 8 were the most contaminated sites. EF values greater than 1.5 were observed for Ca, Co, Cu, Mn, Pb, Sr and Zn in sediments of site 8 and for Cu, Pb and Zn at sites 6 and 7. This evidence is consistent with the level of anthropogenic disturbance, that characterize the Nestore catchment.

The left tributaries, Caina (site 7) and Genna (site 8) streams, flow in urbanized and agricultural areas draining civil sewage, run-off from agricultural and wastewater from livestock farms. In particular the site 8 is located about 2 km downstream from the wastewater treatment plant (biodigester) from the swine farms (40,000 heads, at full capacity). The sites 2 and 3 presented an

EF=1.6 for Zn and Cr, respectively, above the threshold of 1.5 for anthropogenic disturbance. This condition is probably a consequence of the wastewater of a glass factory located upstream of these sites.

The baselines calculated (sites 1, 9 and 10) using the regression technique were dependent on the reference elements selected (Table 3 and Figure 1s). The impossibility of finding a suitable reference element (i.e. Al, Fe or V) for Pb, Se and Cr may indicate a widespread contamination of all sites of Nestore river basin by external sources, such as atmospheric deposition of Pb and Se produced by the combustion of fossil fuel and by the use of metal contaminated fertilizers (Se, Pb and Cr) in agricultural activities (Jiang et al. 2013; Belon et al. 2012; Nacke et al. 2013). Moreover, the weaker relationships observed (i.e. Co, Mn, and Zn) could be due to the partial digestion method used, because the elements firmly bound in silicate structures are not easily dissolved by this procedure.

The mean values obtained for each metal by linear regression were used as baselines allowing to identify the anthropogenically disturbed sites. Mean metal concentrations (Table 1) higher than the baseline values were observed for Ca at sites 6, 7 and 8, for Cu at sites 2, 3, 6, 7 and 8, for Mn at sites 7 and 8, for Ni at sites 2 and 6, for Sr at sites 7 and 8, for Co and Zn at all sites.

To estimate the level of accordance between the two statistical methods used, we correlated for the sites 2-8 the mean enrichment factors versus the ratios between the mean metal concentrations and the mean baseline values (Table 4 and Figure 3). The good regression coefficients obtained (with the exception of Ni, for which both methods identified no or minor enrichment at all sites) indicated that the baseline values found by the regression technique were in agreement with the calculated enrichment factors and confirmed a major level of disturbance for sites 6, 7 and 8, minor for sites 2 and 3 respect to the least disturbed sites 4 and 5.

In addition, the mean enrichment factors were also calculated using the upper continental crust (UCC) as reference background levels and Al, as reference element (Table 5). Compared to UC the sediment mean composition revealed a significant enrichment in Ca, Cu, Ni, Pb, and Zn in respect to Al amounts (Table 4). Sites 7-10 were also enriched in Sr, while sites 1, 7 and 8 showed some Mn excess. Comparison between the sampling sites showed in fact a severe enrichment in Ca at all the Nestore tributaries (sites 7-10), and some moderately severe and severe enrichment of the same element along the Nestore river (e.g., site 4, 5 and 6). Cu and Pb, in turn, were strongly enriched only at site 8, though they showed moderately severe enrichment also at site 6, 7 and 5, the latter with reference only to lead. Moderately severe enrichment values were also revealed for Zn at site 2, 6, 7 and 8. A moderate enrichment was detected at all sites for Ni, at sites 1, 7 and 8 for Mn and at sites 7-10 for Sr.

In summary, the mean enrichment factors calculated using the upper continental crust values were much higher than those calculated with our new approach for all metals (except for Se) in all sites, including sites 1, 9 and 10. The latter, characterized by a high ecological quality, should have shown only minor enrichments and this therefore demonstrated the inadequacy of the use of the UCC content as reference background levels for Nestore river basin sediments.

Conclusions

Geochemical baseline concentrations of various metals (Ca, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, V and Zn) in surface sediments of Nestore river basin were estimated by the methods of enrichment factors and normalization process (regression technique). Sites located within the basin and characterized by high ecological quality were used as reference points.

According to the calculated enrichment factors, sites 6 (Nestore river), 7 (Caina stream) and 8 (Genna stream) were the most contaminated sites. EF values greater than 1.5 were observed for Ca, Co, Cu Mn, Pb, Sr, and Zn in sediments of site 8 and for Cu, Pb and Zn at sites 6 and 7. The sites 2 and 3 presented EF values equal to 1.6 for Zn and Cr, respectively.

The baseline values found by the regression technique were in agreement with the calculated enrichment factors and confirmed a major level of disturbance for sites 6, 7 and 8 and minor for sites 2 and 3.

These results are consistent with the presence and the geographical distribution of industrial, zootechnical and agricultural activities in the area of the study (ARPA Umbria 2010).

On the other side the enrichment factors calculated using UCC content as reference background levels were usually much higher ($1.1 \leq EF \leq 22$) than those obtained with our approach ($0.2 \leq EF \leq 3.2$), also for sites 1, 9 and 10 characterized by a high ecological quality.

In conclusion, our results showed that sediments with high ecological quality can be considered suitable reference points for baseline studies. They can also be used to assess the metal pollution level in river basins characterised by intensive and widespread anthropogenic activities.

Since the values of ecological quality are acquired relatively easily and inexpensively, our approach could be applied in regional monitoring and remediation programs to identify in a quick and cheap way the reference points for the determination of the metal geochemical baselines in order to recognize with a best accuracy anthropogenically disturbed areas in riverine system.

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FIGURE CAPTIONS

Fig. 1 Nestore river basin (Umbria, Central Italy) and location of the sampling sites

Fig. 2 Correlation of the mean Enrichment Factors vs ratios between the mean metal concentrations and the mean baseline values for sites 2-8 of the Nestore river basin

TABLE CAPTIONS

Table 1 Mean concentrations (mg/kg) ± standard deviation of metals in sediments of the Nestore river basin sites

Table 2 Mean Enrichment factors of metals in sites 2-8 of the Nestore river basin sites

Table 3 Linear regression equations for various heavy metals on the reference elements and the corresponding calculated baseline concentrations (mg/kg) in the sediments of the Nestore river basin

Table 4 Ratios between the mean metal concentrations and the mean baseline values for sites 2-8 of the Nestore river basin

Table 5 Mean Enrichment factors of metals in sites 1-10 of the Nestore river basin sites calculated using the Upper Continental Crust as the reference background levels and Al, as the reference element

Figure 1

Evaluation of geochemical baselines and metal enrichment factor values through high ecological quality reference points: a novel methodological approach

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Fig. 1 Graphic Program: QGIS 2.14 Essen

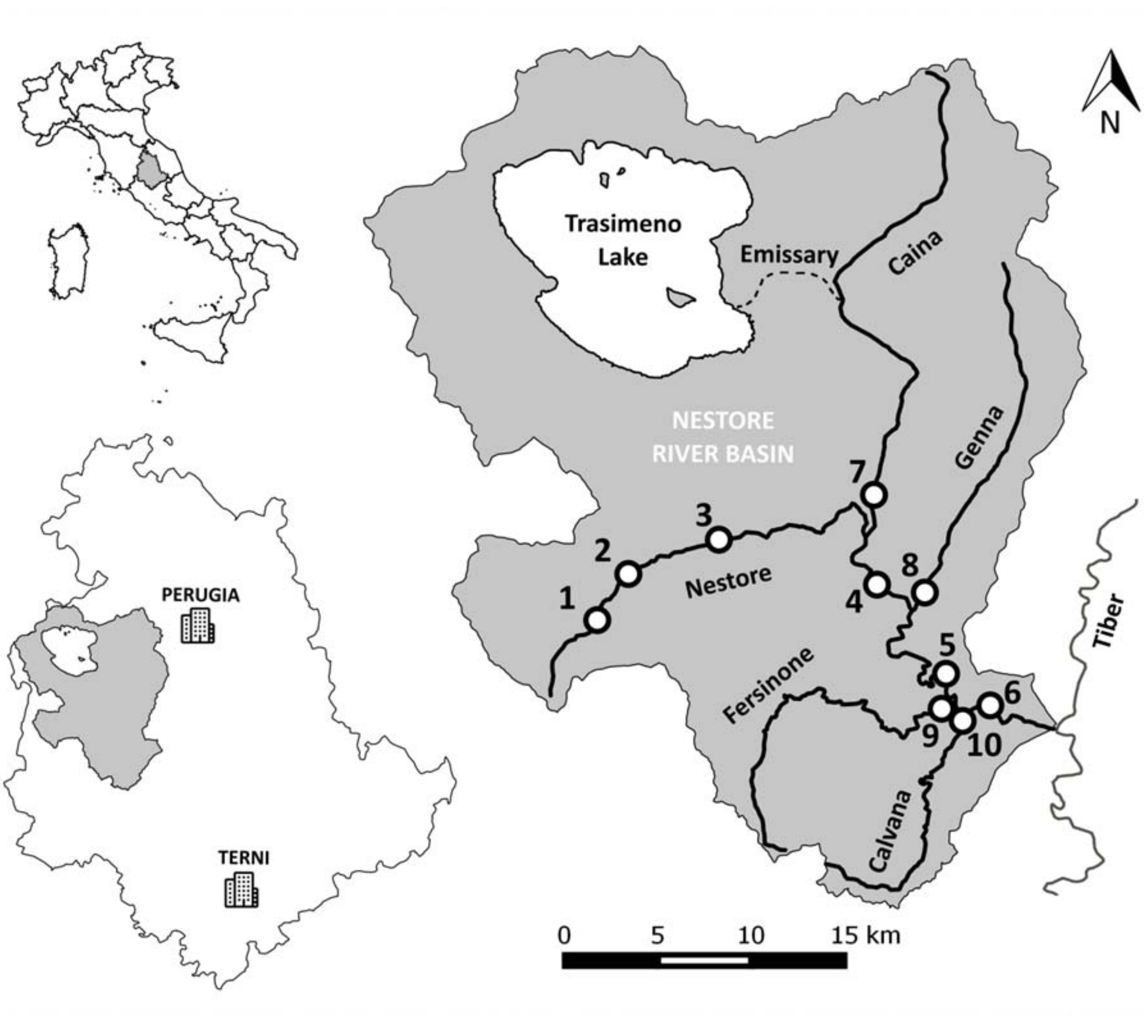

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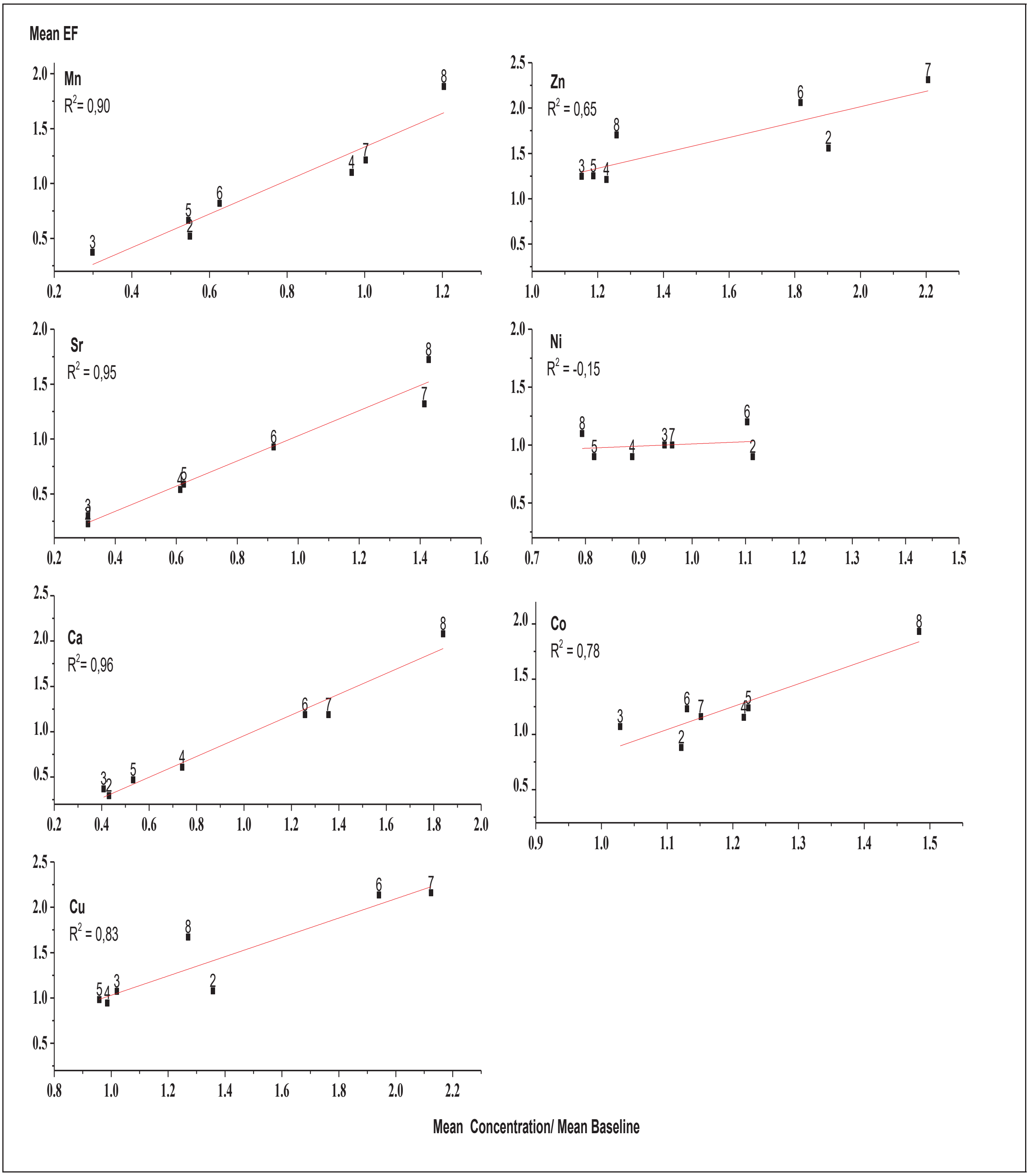
Fig. 2 Graphic Program: OriginPro 9.0 64bit



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Metal/Site	1	2	3	4	5
Al	20000 ± 1500	27300 ± 50100	20700 ± 2200	22700 ± 1800	21200 ± 7100
Ca	27200 ± 17800	31400 ± 5400	29800 ± 6800	53900 ± 11900	38800 ± 25300
Cd	1.1 ± 1.0	1.6 ± 1.0	1.3 ± 1.1	1.3 ± 1.1	1.7 ± 1.3
Co	7.4 ± 2.8	7.8 ± 1.8	7.1 ± 1.2	8.4 ± 1.3	8.5 ± 1.4
Cr	28.8 ± 7.2	38.3 ± 9.5	37.1 ± 16.2	15.8 ± 1.0	12.5 ± 3.4
Cu	9.8 ± 2.9	23.1 ± 9.5	17.4 ± 7.1	16.8 ± 7.1	16.3 ± 5.2
Fe	19200 ± 2500	26000 ± 6100	19900 ± 2600	21400 ± 2700	19200 ± 6300
Mn	617.3 ± 676.1	264.4 ± 212.2	143.7 ± 53.6	465.0 ± 228.7	262.6 ± 153.1
Mo	<1.95	<1.95	<1.95	<1.95	<1.95
Ni	42.5 ± 3.4	53.1 ± 15.2	45.3 ± 5.6	42.4 ± 5.2	39.0 ± 2.5
Pb	17.9 ± 6.9	25.0 ± 15.0	23.0 ± 8.5	22.0 ± 11.0	19.9 ± 12.3
Sb	1.4 ± 0.6	1.7 ± 0.7	1.5 ± 0.4	1.4 ± 0.2	1.4 ± 0.0
Se	3.1 ± 1.4	3.9 ± 0.4	2.5 ± 0.7	2.1 ± 1.0	2.4 ± 1.2
Sr	28.2 ± 6.0	59.6 ± 23.4	59.6 ± 35.9	117.7 ± 14.5	119.9 ± 48.3
V	33.6 ± 6.8	45.5 ± 10.4	31.9 ± 2.7	31.6 ± 7.0	36.7 ± 18.9
Zn	51.3 ± 8.8	129.2 ± 59.7	78.2 ± 18.6	83.3 ± 10.2	80.6 ± 14.4

Metal/Site	6	7	8	9	10
Al	19700 ± 1300	21300 ± 5300	16500 ± 1100	24600 ± 1100	22400 ± 2700
Ca	91600 ± 61000	98800 ± 12700	134000 ± 18100	145800 ± 8500	91400 ± 47300
Cd	2.0 ± 1.4	1.6 ± 1.1	2.1 ± 1.7	1.5 ± 1.3	1.8 ± 1.8
Co	7.8 ± 2.6	8.0 ± 2.0	10.3 ± 0.9	6.3 ± 1.6	7.3 ± 2.7
Cr	24.1 ± 6.9	18.6 ± 3.1	18.2 ± 1.3	19.7 ± 0.7	23.9 ± 2.1
Cu	33.1 ± 9.8	36.2 ± 14.2	21.6 ± 6.5	22.6 ± 5.1	29.4 ± 5.8
Fe	19800 ± 1300	21000 ± 5000	15700 ± 1000	23700 ± 1400	22600 ± 2600
Mn	301.2 ± 260.7	482.3 ± 109.7	579.3 ± 278.8	243.8 ± 68.4	275.4 ± 53.3
Mo	<1.95	<1.95	<1.95	<1.95	<1.95
Ni	52.6 ± 13.3	45.9 ± 6.3	37.9 ± 4.8	49.5 ± 4.4	55.4 ± 8.2
Pb	29.4 ± 19.5	33.5 ± 15.6	39.8 ± 20.3	22.6 ± 12.3	23.7 ± 10.7
Sb	1.5 ± 0.1	1.5 ± 0.2	1.5 ± 0.2	<1.4	<1.4
Se	3.3 ± 0.6	3.0 ± 0.7	1.8 ± 0.7	3.8 ± 1.2	4.7 ± 1.1
Sr	176.5 ± 45.5	271.3 ± 29.5	274.0 ± 35.4	406.3 ± 44.0	338.7 ± 54.7
V	32.3 ± 11.4	34.1 ± 10.7	22.2 ± 3.5	40.7 ± 4.3	42.7 ± 11.9
Zn	123.4 ± 25.7	149.8 ± 66.1	85.4 ± 15.0	77.1 ± 7.4	79.9 ± 22.6

Table 2

Evaluation of geochemical baselines and metal enrichment factor values through high ecological quality reference points: a novel methodological approach

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EF	**2**	**3**	**4**	**5**	**6**	**7**	8	**EF mean**
Ca	0.3	0.4	0.6	0.5	1.2	1.2	2.1	0.9
Co	0.9	1.1	1.2	1.2	1.2	1.2	1.9	1.2
Cr	1.3	1.6	0.6	0.5	1.1	0.8	1.0	1.0
Cu	1.1	1.1	0.9	1.0	2.1	2.2	1.7	1.4
Fe	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0
Mn	0.5	0.4	1.1	0.7	0.8	1.2	1.9	0.9
Ni	0.9	1.0	0.9	0.9	1.2	1.0	1.1	1.0
Pb	1.2	1.5	1.3	1.2	2.0	2.1	3.2	1.8
Se	0.9	0.7	0.6	0.7	1.0	0.8	0.7	0.8
Sr	0.2	0.3	0.5	0.6	0.9	1.3	1.7	0.8
V	0.9	0.9	0.8	1.0	0.9	0.9	0.8	0.9
Zn	1.6	1.2	1.2	1.3	2.1	2.3	1.7	1.6

Table 3

Evaluation of geochemical baselines and metal enrichment factor values through high ecological quality reference points: a novel methodological approach

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Table 3. Linear regression equations for various metals on the reference elements and the corresponding calculated baseline concentrations (mg/kg) in the sediments of the Nestore river basin

Element	**Formula**	**R^2**	**Baselines**		
			Min	**Max**	**Mean**
Ca	= -379220 + 208900*Al	0.82	11100	153000	73840
Co	= -0.3 +1890*V	0.48	5.4	11.2	6.9
Cu	= -14.4 + 8070*V	0.73	6.5	34.5	17.0
Mn	= 3150 -1270*Fe	0.57	196	1630	481.1
Ni	= 15.2 + 8330*V	0.80	38.2	68.9	47.7
Sr	= -1100 + 590*Al	0.65	24.2	479.1	191.9
Zn	= -57.8 + 58.4*Fe	0.48	41.7	118.3	67.9

* correlations were statistically significant at $p < 0.05$.

Table 4

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Metal/Site	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Ca	0.4	0.4	0.7	0.5	1.3	1.4	1.8
Co	1.1	1.0	1.2	1.2	1.1	1.2	1.5
Cu	1.4	1.0	1.0	1.0	1.9	2.1	1.3
Mn	0.5	0.3	1.0	0.5	0.6	1.0	1.2
Ni	1.1	0.9	0.9	0.8	1.1	1.0	0.8
Sr	0.3	0.3	0.6	0.6	0.9	1.4	1.4
Zn	1.9	1.2	1.2	1.2	1.8	2.2	1.3

Table 5

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EF	1	2	3	4	5	6	7	8	9	10	EF mean
Ca	3.5	3.1	4.0	6.5	6.0	12.2	13.6	21.8	15.9	10.3	9.7
Co	1.8	1.3	1.6	1.8	2.0	1.9	1.8	3.0	1.2	1.5	1.8
Cr	1.4	1.4	1.8	0.7	0.6	1.2	0.9	1.1	0.8	1.1	1.1
Cu	1.6	2.6	2.8	2.4	2.7	5.4	5.7	14.0	3.0	4.3	4.4
Fe	2.2	2.2	2.2	2.2	2.1	2.3	2.3	2.2	2.2	2.3	2.2
Mn	4.4	1.2	0.9	2.7	2.0	2.0	3.1	4.8	1.3	1.6	2.4
Ni	3.9	3.5	4.1	3.4	3.6	4.9	4.1	4.2	3.7	4.6	4.0
Pb	4.2	4.1	5.3	4.5	4.9	7.1	7.8	11.5	4.4	5.2	5.9
Se	0.3	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2
Sr	0.3	0.5	0.7	1.2	1.5	2.0	3.1	3.8	3.8	3.5	2.1
V	1.3	1.2	1.2	1.1	1.3	1.2	1.2	1.0	1.2	1.4	1.2
Zn	2.9	5.2	4.4	4.2	4.5	7.1	8.2	5.9	3.6	4.1	5.0