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Biomonitoring of nutrient and toxic element concentrations in the Sarno River through aquatic plants

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ABSTRACT

The Sarno River is considered the most polluted river in Europe and one of the ten most polluted rivers in the world. So far, its quality has been usually evaluated by water and sediment analyses of either inorganic or organic pollutants. However, a biomonitoring approach would be of paramount importance in the evaluation of river quality, since it integrates pollutant temporal fluctuations, as in the case of discontinuous inputs from urban, industrial and agricultural activities. To this end, a passive biomonitoring study of the Sarno River was carried out, using two native aquatic plants accumulators of inorganic pollutants. The spring area was monitored analysing the roots of the semi-submerged *Apium nodiflorum*, whereas the whole river course was monitored analysing the shoots of the submerged *Potamogeton pectinatus*. The information on the four macronutrient (Ca, K, Mg, P), the six micronutrient (Cu, Fe, Mn, Na, Ni, Zn) and the four toxic element (Cd, Cr, Pb, V) concentrations were separately combined in the Nemerow Pollution Index. Results evidenced a severe pollution degree of the Sarno River, attributable to toxic elements > micronutrients > macronutrients. In particular, the spring area showed high K concentrations, as well as high concentrations of several micronutrients and toxic elements. A generalized Zn contamination and a progressive macronutrient (above all Ca and P), micronutrient (above all Ni, Cu and Fe) and toxic element (above all Cr and Pb) accumulation toward the mouth was related to pollution from agricultural and urban activities. Industrial sources, especially tanneries along the Solofrana tributary, accounted for high Mn concentrations, whereas the volcanic origin of the substrate accounted for a generalized V contamination.

1. Introduction

The Sarno River, in southern Italy, is considered the most polluted river in Europe (Montuori et al., 2015; Pepi et al., 2016) and one of the ten most polluted rivers in the world (Cicchella et al., 2014). For geographical reasons, the Sarno River catchment basin hosts an area strongly developed under the urban, industrial and agricultural profiles. Indeed, the presence of surface and groundwater reservoirs makes the Sarno flatland ideal for numerous industrial activities, and the favourable climate, together with the high agronomic quality of its soil (constituted by volcanic and alluvial layers), makes the area one of the most fertile ones in Italy (De Pippo et al., 2006; Lofrano et al., 2015; Montuori et al., 2015).

The combination of high population density (up to 2200 inhabitants per km^2) (Cicchella et al., 2014) and the presence of highly polluting economic activities (Montuori et al., 2015) determined an extremely degraded environmental situation hindering all prospects of sustainable growth (Cicchella et al., 2014). The main pollutants affecting water quality are represented by heavy metals originating mainly from the industrial activities along the river path (Montuori et al., 2013). In addition, the area ranks 2nd in Italy for pesticide consumption, so that the river accounts as one of the main contributors of organochlorine pesticides, polychlorinated biphenyls (Montuori et al., 2014), and organophosphates pesticides (Montuori et al., 2015) to the Tyrrhenian Sea.

The main tributaries (Cavaiola and Solofrana) further detriment the middle and lower Sarno water quality, being contaminated by wastewater originating from furniture factories, ceramics, paints and tanning

Abbreviations: ANOVA, analysis of variance; NPI, Nemerow Pollution Index

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industries (184 leather tanneries principally along Solofrana tributary) as well as agricultural activities (Cicchella et al., 2014; Albanese et al., 2015; Basile et al., 2015; Lofrano et al., 2015; Montuori et al., 2015; Pepi et al., 2016). Moreover, the lower course of the Sarno River receives wastewater mainly from seasonal food processing and packaging, in particular from tomato industry accounting for 108 plants located along the river banks (De Pippo et al., 2006; Cicchella et al., 2014; Albanese et al., 2015). In addition, one of the largest pharmaceutical facilities in the world owned by Novartis Pharma (Cicchella et al., 2014), covering an area of about 201,000 m^2, is located at 200 m from the river mouth (Montuori et al., 2015).

Due to illegal discharges and deficiencies in sewage treatment systems, anthropogenic activities have significantly affected the quality not only of freshwater (Albanese et al., 2013a; De Pippo et al., 2006; Lofrano et al., 2015), but also of neighbouring land soils (Adamo et al., 2003), as well as of seawater and sediments of the Gulf of Naples (Montuori et al., 2013; Albanese et al., 2015; Lofrano et al., 2015), especially in relation to heavy metals (Basile et al., 2015). Irrigation activities employing river water and frequent flooding events further contaminate agricultural lands along its course (Albanese et al., 2015). The situation is worsened by the abundance of clay and organic matter in soils and sediments, which easily adsorb heavy metals, amplifying both the effects of contamination and the environmental risk (De Pippo et al., 2006; Albanese et al., 2013a). Epidemiologically, a noticeable increase in human cancer frequency in the local population was observed (De Pippo et al., 2006; Albanese et al., 2013b). As such, the Sarno River was declared by the Environment MiNISTry an "area with high risk of environmental crisis" (Ministry Council's Decree, 1995). For these reasons, studies on health risks associated with living near this river are increasing (Motta et al., 2008).

Even if the Sarno River recently received great attention and several depollution programs have been performed (Basile et al., 2015), a biomonitoring study of water quality based on bioaccumulation of inorganic pollutants was never carried out. Although river contaminants are usually analyzed in water or sediments, obtaining information on pollution gradients is challenging and expensive (Besse et al., 2012). Indeed, measures in water are affected by water discharge fluctuations and low residence time (Albanese et al., 2013a), and those in sediments by their heterogeneous physico-chemical properties (Baldantoni and Alfani, 2016) and solid transport. Accumulator aquatic plants allow overcoming such limitations, providing space- and time- integrated information on the environmental concentrations (Besse et al., 2012). Therefore, they become a key tool in aquatic systems monitoring, providing information on anthropogenic impacts in an accurate and inexpensive way (Baldantoni and Alfani, 2016; Debén et al., 2017). In this context, the use of selected rooted aquatic plants is especially promising, as they continuously absorb and accumulate inorganic pollutants from sediments and water, integrating pollution peaks (Baldantoni et al., 2005; Wang et al., 2014). Moreover, element concentrations in plant tissues shed light on both their bioavailability in the environment and their transfer through the food webs, constituting a bridge between the environmental pollution and its possible effects on the biota (Besse et al., 2012). The pivotal position of this point in current legislations on the topic, like the European Water Framework Directive (Directive 2000/60/EC), boosted the application of biomonitoring in aquatic ecosystems, which is increasingly recognised as an essential and complementary technique to chemical water and sediment analyses (Besse et al., 2012).

This research aimed at evaluating Sarno River quality in relation to both nutrient and toxic elements, using native accumulator plants through a passive biomonitoring approach. To reach this goal, two rooted aquatic plants, widely recognised and used as biomonitors in Mediterranean areas (Zurayk et al., 2001; Demirezen and Aksoy, 2004; Baldantoni et al., 2005; Peng et al., 2008; Baldantoni and Alfani, 2016), were employed according to their availability: *Apium nodiflorum*, in the area of the springs, and *Potamogeton pectinatus*, along the course. Biomonitoring involved the assessment of four macronutrients (Ca, K, Mg, P), six micronutrients (Cu, Fe, Mn, Na, Ni, Zn) and four toxic elements (Cd, Cr, Pb, V).

2. Materials and methods

2.1. The Sarno River and sampling sites

The Sarno River originates in Campania Apennine Chain (south-west Italy) and it is delimited in the north-west by Somma-Vesuvio Mountains and in the south-east by Lattari Mountains. It flows through the Sarno valley, reaching the Tyrrhenian Sea in the Gulf of Naples (Albanese et al., 2013a; Montuori et al., 2015). The river has a relatively short straight course (24 km) and a daily average flow rate of about 1 $m^3\ s^{-1}$ (Arienzo et al., 2001).

The river is supplied by three springs (Foce, Palazzo and Santa Marina), originating three separated streams joining in the Sarno River. Southwards, the river receives the waters from a concrete-lined canal collecting Cavaiola and Solofrana tributaries (Basile et al., 2015).

The area is characterized by a Mediterranean climate, with an average annual temperature of 17.2 °C and an annual rainfall amount of 1200 mm, mostly concentrated at the end of the summer (Albanese et al., 2013a; Cicchella et al., 2014). Since the Middle Bronze Age, the whole area is affected by continuous anthropogenic activities (Cicchella et al., 2014). The historical background, as well as an accurate description of the catchment area and of the environmental pressures are reported in Lofrano et al. (2015).

Sampling was performed downstream the three springs (1 − 3), as well as along the river course (4 − 9) to the mouth (Fig. 1). Unfortunately, the same accumulator plants were not found in all the sites due to their different ecology: *Apium nodiflorum* was used for spring biomonitoring and *Potamogeton pectinatus* for course biomonitoring. At each sampling site (with the exception of site 5), water samples were also collected for water physico-chemical characterization (Supplementary Table 1).

2.2. The plant species and sample collection

Sampling campaign was performed in the middle of the flowering season of both the species and before senescence, on 8th and 9th July 2015. According to the actual availability of accumulator plants along the Sarno River and taking into account the results of previous studies

Fig. 1. Map of the three sampling sites in the spring areas (1–3: blue circles) and the six sampling sites along the course (4–9: orange circles) of the Sarno River (image from Google Earth). Geographical coordinates of the sampling sites are also reported.

(Baldantoni et al., 2005; Peng et al., 2008; Baldantoni and Alfani, 2016), the roots of *Apium nodiflorum* and the shoots of *Potamogeton pectinatus* were employed.

Apium nodiflorum (L.) Lag. is a perennial semi-submerged macrophyte belonging to the Apiaceae family, with an Euri-Mediterranean distribution. It is a glabrous, prostrate plant up to 100 cm long, typically found in shallow streams throughout the Mediterranean region (Pignatti, 1982; Knees, 2003; Baldantoni and Alfani, 2016), where it spends most of the year in a semi-submerged state (Zurayk et al., 2001).

Potamogeton pectinatus L. is a perennial submerged macrophyte belonging to the Potamogetonaceae family, with a nearly cosmopolitan distribution; it has small stems and a scarcely developed vessel system (absorbing most of the elements directly from water), and lives in waters encompassing great variation in depth, flow regime, trophic status and salinity (Pignatti, 1982; Pilon and Santamaría, 2002; Baldantoni et al., 2005). It is highly tolerant toward eutrophic waters and it can be the only species of submerged macrophyte present in heavily polluted sites (Demirezen and Aksoy, 2004).

At each sampling site, 6–10 healthy and fully developed plants were randomly collected along the riverbanks within a 25 m stretch of the river. The roots of *Apium nodiflorum* and the shoots of *Potamogeton pectinatus* were separated and carefully rinsed in river water (at each sampling site) to remove sediments, organisms and other exogenous materials. Hence, the values reported in this study refer to internal and surface-adsorbed nutrient and toxic element concentrations. All of the roots or shoots from each sampling site were pooled together in order to obtain a representative sample per site.

2.3. The laboratory analyses

Plant samples were kept in oven at 75 °C overnight, then manually pulverized in china mortars, using liquid nitrogen, and finally oven-dried at 75 °C until constant weight. For the determination of the chemical element concentrations, plant material was digested, in triplicate, by an acid mixture in a microwave oven (Milestone Ethos, Shelton, CT, USA). In particular, subsamples of 250 mg were mineralized using 2 mL 50% HF (Sigma-Aldrich, Milano, Italy) and 4 mL 65% HNO_3 (Sigma-Aldrich, Milano, Italy). A 6-step mineralization program was employed: 250 W for 2′, 0 W for 2′, 250 W for 5′, 400 W for 5′, 0 W for 2′, 500 W for 5′. After digestion, the solutions were diluted using milli-Q water (Millipore Elix 10, Darmstat, Germany) to a final volume of 50 mL.

Fe, K, Mg, Mn, Na and Zn concentrations were determined by atomic absorption spectrometry, via flame atomization (PerkinElmer AAnalyst 100, Wellesley, MA, USA). Ca, Cd, Cr, Cu, Ni, P, Pb and V were determined by inductively coupled plasma optical emission spectrometry (PerkinElmer Optima 7000DV, Wellesley, MA, USA). Standard reference material (1575a Pine Needles from NIST, 2004) was also analyzed to check the method accuracy. The recovery percentage of each element in the standard reference material (ranging from 85% to 106%) was used to correct the quantification of the investigated elements in the plant samples. The method precision, calculated as relative standard deviation, based on $n = 9$ sequential measurements of the same sample for each element, ranged from 2% to 7%, depending on the element.

2.4. Data analysis

The one-way analysis of variance (ANOVA) was performed, followed by the Tukey HSD *post hoc* test ($\alpha = 0.05$), to evaluate the differences in element concentrations among sampling sites, separately for the spring areas and the river course. Homoscedasticity and normality of the residuals were assessed using the Breuch-Pagan and the Kolmogorov-Smirnov tests, respectively. The analyses were carried out using the R 3.1.1 programming environment (R Core Team, 2015) with functions from the “stats”, “agricolae” and “lmtest” packages.

For each species, the Nemerow Pollution Index (NPI) was calculated (Eq. (1)), according to Cai et al. (2015), separately for macronutrients (Ca, K, Mg, P), micronutrients (Cu, Fe, Mn, Na, Ni, Zn) and toxic elements (Cd, Cr, Pb, V), in order to evaluate the relative variation in their concentrations in plants near the springs and along the river course. Data from the Standard Reference Plant (Markert et al., 2015) were employed in calculating the NPI as reference values for each element.

$$NPI = \sqrt{\frac{\left(\frac{1}{n}\sum_{1}^{n}\frac{c_i}{c_{oi}}\right)^2 + \left(\frac{c_i}{c_{oi}}\right)^2_{\max}}{2}} \tag{1}$$

where c_i is the concentration of the *i*-element and c_{oi} is the concentration of the *i*-element in the Standard Reference Plant.

3. Results and discussion

3.1. Macronutrients

Macronutrients (Mg, K, Ca, and P) showed significant differences in their concentrations both among sites 1–3 in the spring areas and among sites 4–9 along the river course, as highlighted by the ANOVAs performed on the concentration values measured in *Apium nodiflorum* roots and in *Potamogeton pectinatus* shoots, respectively (Supplementary Table 2).

Among the spring areas, site 1 and site 3 were characterized by the highest Ca and P concentrations in *Apium nodiflorum* roots, respectively (Fig. 2). K concentrations (32.08–35.37 mg/g d.w.) did not significantly differ among the spring areas, and were ~ 4-fold higher than those measured in the roots of the same species collected in seven sites (6.58–9.13 mg/g d.w.) along the Irno River, an urban Mediterranean river in Campania Region, Italy. Conversely, Mg concentrations (5.98–6.62 mg/g d.w.) were halved in respect to those measured (11.43–15.38 mg/g d.w.) in the Irno River (Baldantoni and Alfani, 2016).

Apart from Mg, all the other macronutrient concentrations in *Potamogeton pectinatus* increased along the course of the Sarno River, with the highest values of K and Ca measured near the river mouth. There, Ca and P showed values more than 2-fold higher than those measured at site 4, immediately after the confluence of the streams from the three springs (Fig. 2).

Wastewater and other organic loads from urban and industrial activities, especially those related to tomato processing, as well as leaching from agricultural soils (see Lofrano et al., 2015 for a comprehensive overview), are likely responsible for the macronutrient concentrations, high even near the springs and increasing along the course.

3.2. Micronutrients

With the exception of Cu in the area of the three springs, all the other micronutrient (Fe, Mn, Na, Ni, and Zn) concentrations significantly ($P < 0.001$) differed both among the springs (sites 1–3) and along the course (sites 4–9) of the Sarno River (Supplementary Table 2).

In the spring area, *Apium nodiflorum* roots showed the highest concentrations of Na and Zn at site 1, of Ni at site 2 and of Fe and Mn at site 3 (Fig. 3). In particular, at site 2 Ni showed values ~ 2-fold higher than those measured near the other two springs and at site 3 Fe showed values 2- and 10-fold higher than those measured at sites 2 and 1, respectively (Fig. 3). These concentrations, compared with those measured in *Apium nodiflorum* collected in the Irno River (Baldantoni and Alfani, 2016), reported in brackets, were lower for Mn (233.9–1581.3 μg/g d.w.), comparable for Zn (0.24–1.44 mg/g d.w.), higher for Na (10.27–11.13 mg/g d.w.) and Fe (2.89–5.28 mg/g d.w.), and more than one order of magnitude higher for Ni (8.68–43.44 μg/g d.w.). Zn concentrations were always higher than the maximum value (0.22 mg/g d.w.) found in *Apium nodiflorum* collected in a Portuguese

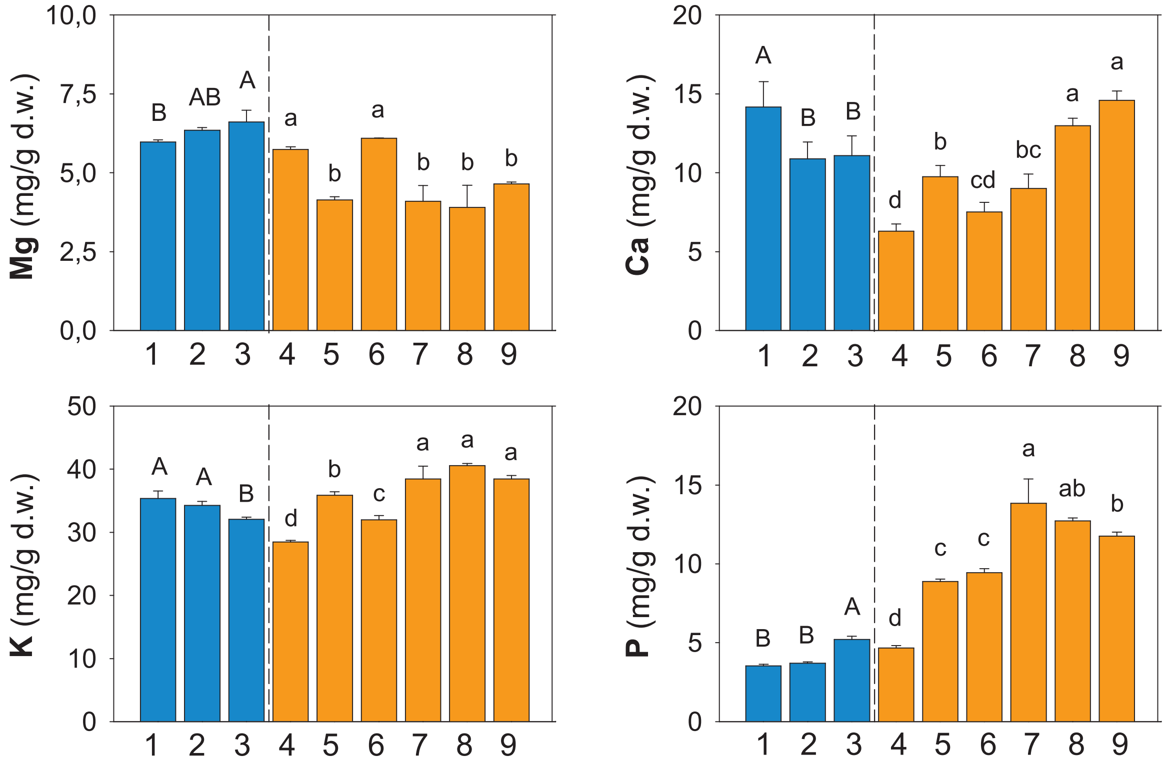

Fig. 2. Mean concentrations of Mg, K, Ca and P in *Apium nodiflorum* roots (blue bars) and in *Potamogeton pectinatus* shoots (orange bars). The error bars represent the standard deviations; different letters (capital for the spring area and small for the river course) on the bars show significant differences (for α = 0.05) among the sites, according to the Tukey HSD test.

site heavily affected by a large industrial complex mainly composed by chemical facilities (Moreira et al., 2011). Cu concentrations, which in proximity of the three springs of the Sarno River were comparable (88.96–96.22 µg/g d.w.), were also in the same order of magnitude of those measured in most sites (30.22–72.40 µg/g d.w.) of the Irno River (Baldantoni and Alfani, 2016). The high Cu concentrations found at sites 1 and 3 may be attributable to the agricultural activities extensively practised in their surroundings, since Cu is widely employed as fungicide even in organic farming (Simončič et al., 2017). An intensive vehicular traffic may explain the high Cu concentrations at site 2, coupled with the high Ni concentrations (Pan et al., 2017).

In the course of the Sarno River, for all the analyzed micronutrients with the exception of Mn, *Potamogeton pectinatus* shoots highlighted increasing trends in element concentrations from the site immediately after the confluence of the three spring streams (site 4) to the mouth (site 9), where the highest values of Ni, Cu and Fe were found (Fig. 3). There, these elements showed concentrations ~ 2-, 4- and 10-fold higher than those measured in *Potamogeton pectinatus* (2.29 µg/g d.w., 17.04 µg/g d.w. and 1.57 mg/g d.w., respectively) collected near an overflow channel of a wastewater treatment plant in Lake Averno, an urban lake in Campania Region, Italy (Baldantoni et al., 2005). Also Zn concentrations, showing little variations among sites 4–9 (0.10–0.19 mg/g d.w.), were on average 2-fold higher than those observed (0.09 mg/g d.w.) in the highest polluted site of Lake Averno (Baldantoni et al., 2005). Zn, but also Cu, showed higher concentrations than those measured by Demirezen and Aksoy (2004) in *Potamogeton pectinatus* collected in a Mediterranean marsh polluted by heavy metals; Ni concentrations, on the contrary, were comparable. In the Sarno River, the highest Mn concentrations were observed at site 6 (494.9 µg/g d.w.), beyond the confluence with the canal collecting Cavaiola and Solofrana tributaries. Downstream this site, Mn values decreased, reaching at the river mouth (site 9) a concentration (90.6 µg/g d.w.) 5.5-fold lower (Fig. 3). Unfortunately, our biomonitoring study could not be extended to Cavaiola and Solofrana tributaries due to the complete absence of aquatic plants, partially due to concrete lining of the riverbed. However, tanneries along Solofrana tributary likely account for the high Mn concentrations at site 6, since manganese oxides are usually employed during several stages of leather manufacture (Tariq et al., 2010).

Water chemical analysis (Supplementary Table 1) reflected the observed spatial trends particularly in the spring areas, where highest concentrations of Ni at site 2, as well as of Fe and Mn at site 3, were observed. Along the river course, the wider variability in the chemical composition of water, due to the anthropogenic inputs, does not allow to draw a clear pollution scenario, the results reflecting only element concentrations at the sampling time. As a general trend, however, seawater at the river mouths greatly affects water micronutrient concentrations, a process explaining the lower values of Cu, Fe, Mn, Ni and Zn at site 9 compared to site 8 (Supplementary Table 1). On the one hand, freshwaters contain higher amounts of dissolved metals than seawater (Nordberg et al., 2007). Therefore, the simple dilution of the freshwater with the seawater reduces metal concentrations at the river mouth. On the other hand, metals with a sufficiently high binding affinity to particles may be scavenged, especially in freshwater due to humic substances. The process is particularly effective in the estuaries due to the usually high suspended particulate matter content, and can be enhanced for some metals (e.g. Fe) by seawater due to salinity and ionic force (Bruland and Lohan, 2006). As a consequence, in the river mouths the anthropogenic metal pollution generally leads to an increase in sediment metal concentrations as well as to a greater metal availability for rooted aquatic plants (Nordberg et al., 2007).

3.3. Toxic elements

All the studied toxic elements (Cd, Cr, Pb, and V) showed significant differences ($P < 0.001$) both among sites 1–3 in the spring areas and sites 4–9 along the course of the Sarno River (Supplementary Table 2).

Whereas V and Cd showed the highest concentrations in *Apium nodiflorum* roots sampled at site 3, the highest concentrations of Pb and Cr were found in the roots collected at site 2, followed by those

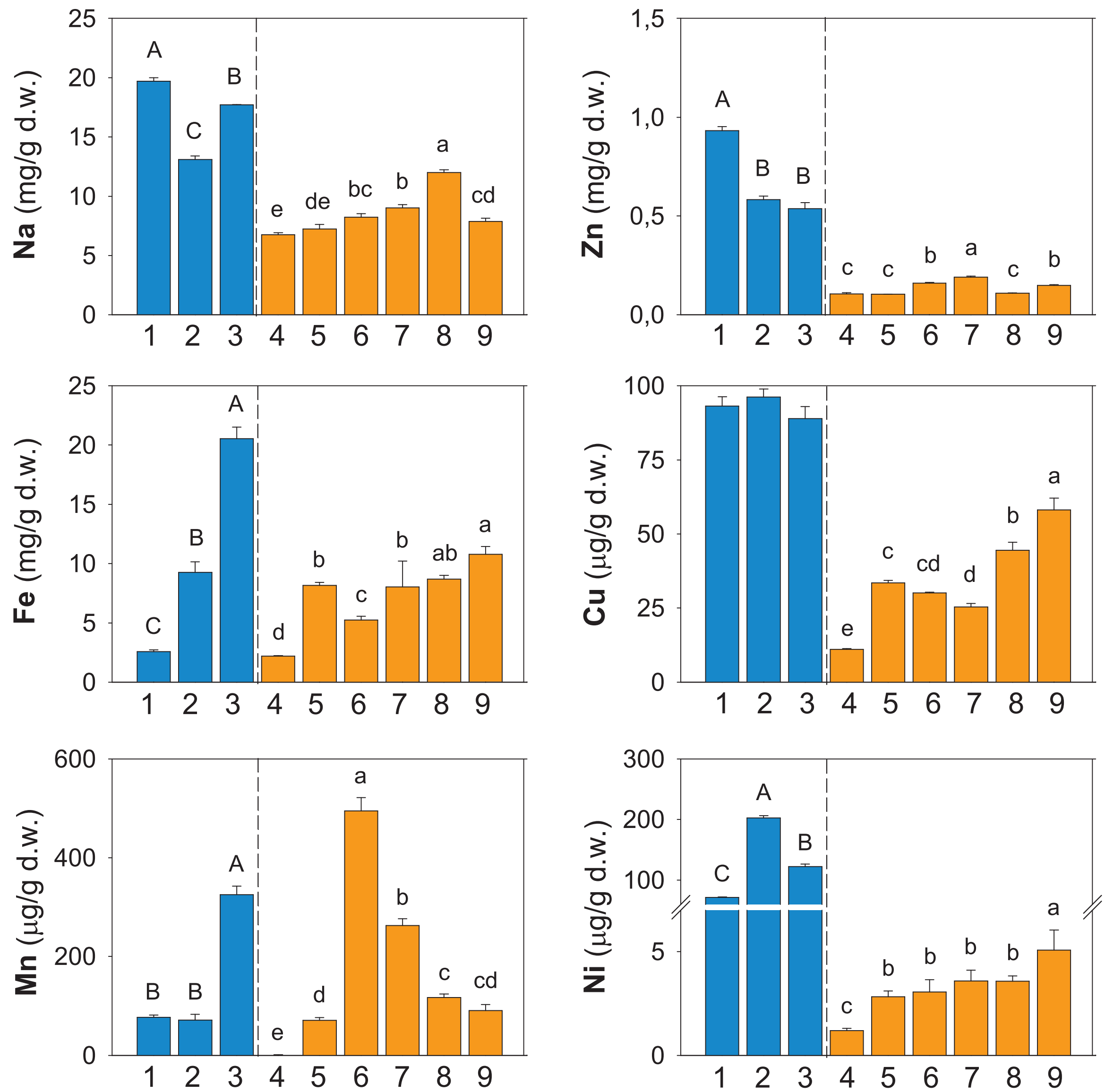

Fig. 3. Mean concentrations of Na, Fe, Mn, Zn, Cu and Ni in *Apium nodiflorum* roots (blue bars) and in *Potamogeton pectinatus* shoots (orange bars). The error bars represent the standard deviations; different letters (capital for the spring area and small for the river course) on the bars show significant differences (for $\alpha = 0.05$) among the sites, according to the Tukey HSD test.

collected at site 3 and then at site 1 (Fig. 4). The high V and Cd concentrations at site 3 are likely the result of the intensive agricultural activities in the surroundings (Parelho et al., 2014), whereas the high concentrations of Pb and Cr at site 2 are attributable to vehicular traffic in the urban area surrounding the site (Pan et al., 2017). Despite the site differences, all toxic elements in *Apium nodiflorum* roots showed concentrations in the same order of magnitude than those measured in the same species from the Irno River (Cd: 0.93–8.08 µg/g d.w., Cr: 2.43–4.70 µg/g d.w., Pb: 2.65–104.62 µg/g d.w., V: 14.52–38.80 µg/g d.w.), where severe anthropogenic impacts have been identified (Baldantoni and Alfani, 2016).

Whereas V did not show a clear accumulation trend along the course of the Sarno River, the other toxic elements (Cd, Cr, and Pb) progressively accumulated in *Potamogeton pectinatus* shoots, reaching the highest values at the river mouth (Fig. 4). Cd, that at sites 4–7 was undetectable (< 0.03 µg/g d.w.), reached the value of 0.07 µg/g d.w. at site 9, a low concentration however, if compared to those found in the same species collected in several sites of a Mediterranean marsh polluted by Cd, Cr and Pb (Demirezen and Aksoy, 2004). At the Sarno River mouth, the concentrations of Cr (27.27 µg/g d.w.) and Pb (15.96 µg/g d.w.) were, respectively, 10- and 3-fold higher than those measured in *Potamogeton pectinatus* collected in a highly polluted site of Lake Averno (Baldantoni et al., 2005): 2.84 µg/g d.w. for Cr and 4.58 µg/g d.w. for Pb. In addition, these two toxic elements showed concentrations always higher than the maximum values measured by Demirezen and Aksoy (2004) in the same species collected in a Mediterranean marsh polluted by these metals. V concentrations, high at all the sites from 5 to 9 (9.52–14.48 µg/g d.w.), were comparable to those observed in the Lake Averno (12.19 µg/g d.w.), where the effluents from a wastewater treatment plant drain into the volcanic lake (Baldantoni et al., 2005).

Among the toxic elements in water (Supplementary Table 1), only Cr reflected the trends highlighted by the aquatic plants both in the spring areas and in the river course, where, as for micronutrients, the highest concentrations were detected at the site right away before the mouth (site 8). Water Pb concentrations (Supplementary Table 1) were always lower than the limit of detection (1.0 µg/L).

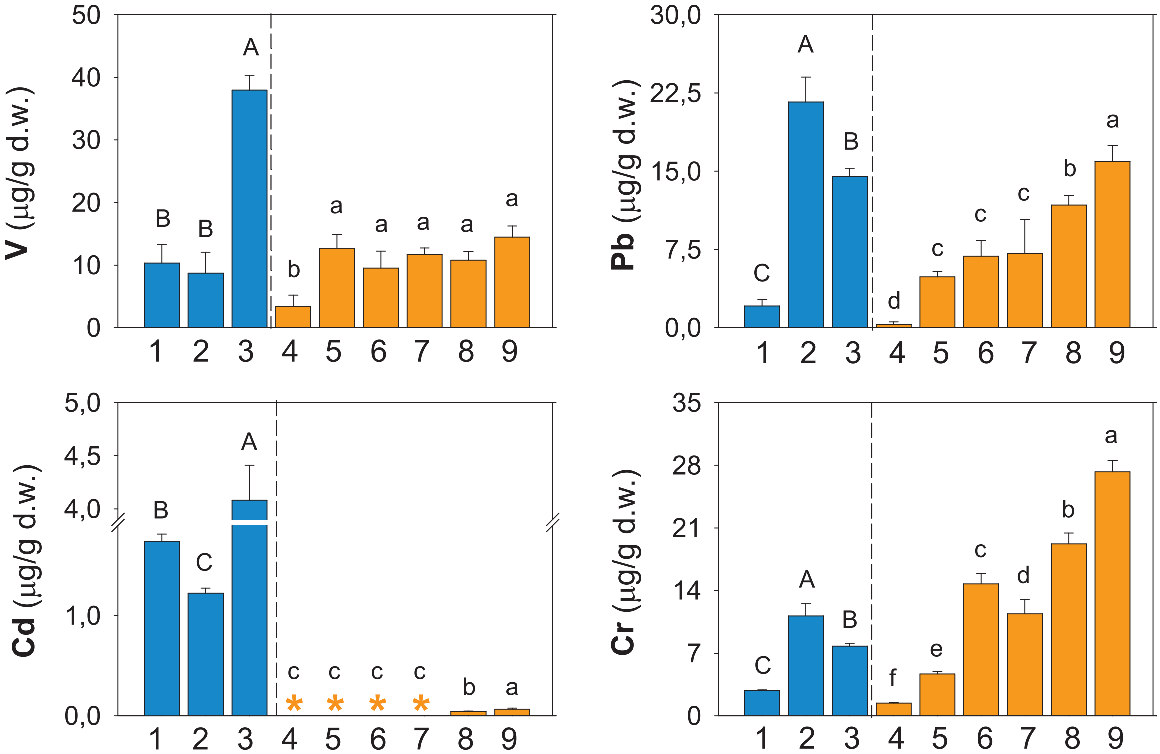

Fig. 4. Mean concentrations of V, Cd, Pb and Cr in *Apium nodiflorum* roots (blue bars) and in *Potamogeton pectinatus* shoots (orange bars). The error bars represent the standard deviations and the asterisks indicate undetectable values (limit of detection = 0.03 µg/g d.w.); different letters (capital for the spring area and small for the river course) on the bars show significant differences (for α = 0.05) among the sites, according to the Tukey HSD test.

3.4. Pollution gradients

The present biomonitoring study allowed reconstructing persistent pollution gradients in the Sarno River related to both nutrients and toxic elements. Element concentrations in *Apium nodiflorum* roots and *Potamogeton pectinatus* shoots reflect their actual bioavailability in the environment and are susceptible to be transferred through food webs, providing information on their possible effects on biota (Besse et al., 2012). In many sites, accumulator aquatic plants pointed out a severe pollution degree, which is especially evident considering that the observed concentrations were higher than in the most polluted sites reported in the studies employing the same accumulator species (Demirezen and Aksoy, 2004; Baldantoni et al., 2005; Moreira et al., 2011; Baldantoni and Alfani, 2016). The spring area was affected by high concentrations of macronutrients (above all K), micronutrients (above all Ni in site 2, and Fe in site 3) and toxic elements (above all Pb and Cr in site 2, and V and Cd in site 3). Consequently, and considering the continuous pollutant loads (Lofrano et al., 2015), a clear and progressive element accumulation along the course was detected, especially for Ca and P (Fig. 2), for Ni, Cu and Fe (Fig. 3), as well as for Cr and Pb (Fig. 4). The highest Mn pollution was detected, instead, downstream the confluence of the Cavaiola and Solofrana tributaries into the Sarno River (Fig. 3), due to industrial activities, mainly tanneries (Tariq et al., 2010), taking place along their course. The high Mn concentrations may further explain the morphological changes and the related Hsp70s induction observed after the confluence of the two tributaries in *Lemna minor*, employed as an active bioindicator in the Sarno River, and attributed by the authors to other pollutants in addition to those (Cd, Cr, Cu, Zn, Pb) measured in water (Basile et al., 2015). Tanneries also account for Cr concentrations (Bharagava and Mishra, 2018) at site 6 (Fig. 4) exceeding what expected from the trend in this element concentration along the course of the Sarno River, likely due to generalized sources related to urban activities. Finally, a generalized V and Zn contamination was found along the Sarno River course, from the confluence of the three spring streams to the mouth (Figs. 3 and 4). Whereas the volcanic substrate of the area accounts for the V concentrations pattern (Parelho et al., 2014), anthropogenic activities, especially vehicular traffic (Imperato et al., 2003), should be responsible for the generalized Zn contamination. The same scenario was observed in Lake Averno (Baldantoni et al., 2005), which shares a common substrate with the Sarno River.

In the evaluation of the ecological quality of an urban river subjected to several and different pollution sources, such as the Sarno River, a synthetic pollution index can be more informative than the individual element concentrations. On the one hand, the NPI values, being referred to the Standard Reference Plant (Markert et al., 2015), provide an estimation of the element accumulation degree by the studied plants. On the other hand, the NPI spatial variation provides insights on the environmental contamination by elements with similar ecophysiological behavior. In this context, the NPI, calculated separately in the two species for macronutrients (Fig. 5A), micronutrients (Fig. 5B) and toxic elements (Fig. 5C), highlighted increasing levels of pollution by each of the three element groups from site 1 to site 3, as well as from the confluence of the three spring streams (site 4) to river mouth (site 9). Moreover, the widest increase in NPI (Fig. 5C), and thus in environmental pollution, along the river course was observed for toxic elements (3.4–33.5), whereas the NPI for macronutrients showed the narrowest variations (3.3–6.8). However, the values of the NPI were everywhere higher than 3.0, indicating, according to Cheng et al. (2007), exceedingly high element accumulation by both the species in relation to the three element groups.

Our findings point out that the Sarno River represents an important source of inorganic pollutants (both nutrients and toxic elements) to the Tyrrhenian Sea, in addition to the organic pollutants (polychlorinated biphenyls, organochlorine and organophosphate pesticides), as previously reported (Montuori et al., 2014, 2015).

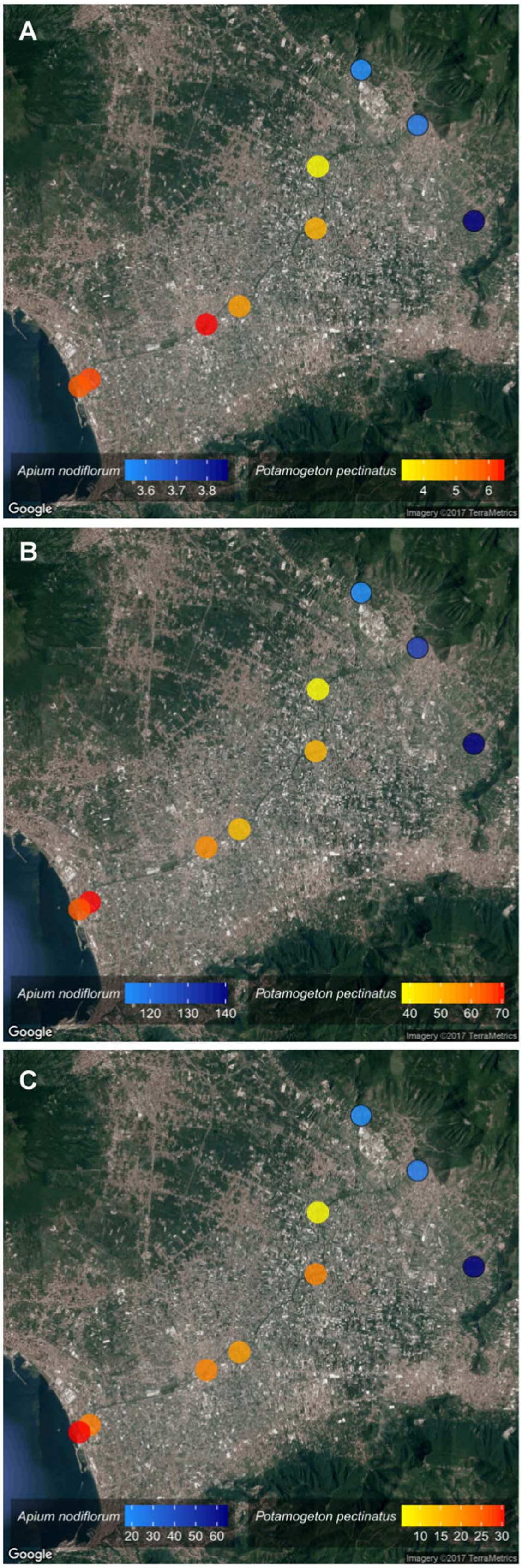

Fig. 5. NPI calculated for macronutrients (A), micronutrients (B) and toxic elements (C), expressed on colour scales separately for *Apium nodiflorum* roots and *Potamogeton pectinatus* shoots.

4. Conclusions

This research, based on the use of native accumulator aquatic plants, represents the first biomonitoring study of the Sarno River, one of the most polluted rivers in the world. Our findings highlighted a severe pollution degree of the river, mainly due to toxic elements and then to micronutrients and macronutrients. Differently to the measurement of these pollutants in water, the bioaccumulation study provided time integrated information, allowing the definition of persistent pollution gradients. Indeed, by integrating the temporal fluctuations of element concentrations in water and sediments, the contribution of local sources like the industrial, agricultural, and urban activities occurring along the river course, could be clearly highlighted and the actual effects on biota and food webs evaluated. The three spring areas were characterized by high concentrations of several toxic elements (Cd, Cr, Pb, V), micronutrients (Cu, Ni, Zn) and macronutrients (K). As a consequence, and in relation to the continuous pollutant loads affecting the whole river course, as well as to the contribution of two tributaries, a clear and progressive element accumulation toward the mouth was detected for the studied pollutants, with the exception of V and Zn contributing to a generalized contamination of the Sarno River.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.10.063.

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