Analyses of three native aquatic plant species to assess spatial gradients of lake trace element contamination

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Abstract

A bioaccumulation study was performed on Lake Averno, a volcanic lake close to Naples (southern Italy), to ascertain the degree of trace element contamination. Good information to assess spatial gradients of Cd, Cr, Cu, Fe, Ni, Pb, V and Zn contamination was provided by analysing *Phragmites communis* roots, *Najas marina* shoots, *Potamogeton pectinatus* leaves and surface sediments of Lake Averno collected from July 1999 to July 2000 at three sites in the littoral zone. The highest concentrations of the trace elements were measured in sediments and plants from the site where a nearby sewage outfall channel reaches the lake, the lowest at the site below woodland. Significant correlations were found between sediment and plant element concentrations. For almost all the elements, values 2–4-fold higher were found for the site affected by the sewage effluent. Among the plant species, *Ph. communis* is the most useful species in biomonitoring studies due to its ability to accumulate trace elements in the roots and its collectability throughout the year. Given the absence of systematic water quality monitoring, trace elements in plants, rather than sediments, provide a cost-effective means for assessing trace element accumulation in aquatic systems during plant organ lifespan.
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1. Introduction

Direct discharge or wet and dry air depositions of contaminants increase the concentrations of trace elements of aquatic systems, thus resulting in their accumulation in sediments (Dunbabin and Bowmer, 1992; Sinicrope et al., 1992). Aquatic plants absorb elements through roots and/or shoots (Schierup and Larsen, 1981; Pip and Stepaniuk, 1992; Jackson, 1998) and in water quality studies they may be useful biomonitors. Various species show different behaviour regarding the ability to accumulate elements in roots and/or shoots. Therefore, it is useful to identify the plant organs that absorb the greatest amounts of trace elements (Wells et al., 1980; St-Cyr and Campbell, 1994; Baldantoni et al., 2004). In aquatic systems, where pollutant inputs are discontinuous and pollutants are quickly diluted, analyses of plant tissues provide time-integrated information about the quality of the system.

The aim of this paper was to assess spatial gradients of trace element lake contamination by analysing native plant species sampled from three sites along the littoral zone of Lake Averno, a brackish volcanic lake near Naples (southern Italy). Data of trace element concentrations of lake sediments were also compared to plant concentrations. Recently the study area has been markedly affected by human activities. In Lake Averno three native plants, *Phragmites communis* Trin., *Najas marina* L. and *Potamogeton pectinatus* L., grow simultaneously in the same sites; in previous studies (Baldantoni et al., 2001, 2004) the best accumulator organs of these species were identified. Accordingly, *Ph. communis* roots, *N. marina* shoots and *P. pectinatus* leaves were used to determine the spatial distribution in the lake of some trace elements (Cd, Cr, Cu, Fe, Ni, Pb, V, Zn) released by human activities. When plant species were present throughout the study year, samplings of plants were repeated several times in order to confirm spatial trace element gradient contamination. Data of element composition of lake sediments are also reported. Finally, this paper aimed to highlight major common sources of trace element contamination in Lake Averno.

2. Materials and methods

2.1. The species

Three native aquatic vascular species rooted in the sediments were used to assess trace element contamination gradient among three sites in Lake Averno. The species were: *Ph. communis* Trin., *N. marina* L. and *P. pectinatus* L. These species grow together at the sampling sites and are widely distributed in the lake.

Ph. communis is a perennial and emergent macrophyte, characterised by a worldwide distribution in aquatic systems. It also lives in extreme environmental conditions, where there may be toxic trace elements, such as Cd, Pb and Zn (Van der Werff, 1991; Ye et al., 1997). Plants have a well developed root-rhizome system (Pignatti, 1982). *N. marina* is a herbaceous annual submersed macrophyte that lives in brackish and stagnant waters (Pignatti, 1982); it mainly absorbs elements directly from water through foliar surfaces (Lovett-Doust et al., 1997). *P. pectinatus* is a perennial submersed plant that lives in brackish (Cole, 1963) and in eutrophic waters, such as lakes (Spence, 1964; Jupp and Spence, 1977) and estuaries (Perkins and Abbott, 1972). *P. pectinatus* has small stems and

a scarcely developed vessel system; it absorbs most of the elements directly from water (Sculthorpe, 1967; Ho, 1979; Pignatti, 1982).

2.2. Study area

Lake Averno fills the mouth of one of several volcanic craters in the Campi Flegrei area near Naples (southern Italy). It is round, with a diameter of roughly 800–1000 m. Lake Averno is brackish because it is close to the sea (about 1 km). Recently, the entire area has been subjected to considerable human impact, such as construction works, recreation facilities and agriculture. In addition a channel, used as an overflow for a Naples sewage treatment plant, flows into the lake when rainfall is abundant.

The three sampling sites were A, B and C. Site A (40°50.384′N; 14°04.886′E) is at the bottom of a cultivated slope and near the point where the overflow reaches the lake. Site B (40°50.197′N; 14°04.724′E) is near the access road to the lake and near an artificial canal, that connects the lake to the sea. Site C (40°50.127′N; 14°04.388′E) is located below woodland and appears the least exposed site to human impact.

2.3. Sediment and plant samplings

Sediments were sampled on one occasion only, while plants were sampled several times, depending on their seasonal availability. In order to evaluate spatial variations, sediments and plants, at each of the three sampling sites, were collected.

Several sediment samples surrounding the plants were collected using a grab, at a depth of 0–10 cm in July 1999. At each sampling site at least ten healthy plants per species were collected and washed in lake water to remove sediment particles. Therefore, the element concentrations in the plant organs refer not only to tissue concentrations but also to adsorbed elements on organ surfaces. The plants were collected at the sites of the littoral zone of the lake, in different periods of the year, depending on their phenology and availability: *Ph. communis* roots were collected in October 1999, in January, April and July 2000, *N. marina* shoots in July and October 1999, and in July 2000, *P. pectinatus* leaves only in October 1999, since in the other months the plants were either absent or were completely covered by green algae. To avoid the introduction of metal contamination, polythene tools were used in sampling and storing the collected matrices (Allen, 1989).

2.4. Laboratory procedures and analyses

In the laboratory, *Ph. communis* roots, *N. marina* shoots and *P. pectinatus* leaves were separated from the collected plants, the sediment samples were sieved (2 mm) and all the matrices were oven-dried at 75 °C to a constant weight. The total concentrations of Cd, Cr, Cu, Fe, Ni, Pb, V and Zn were determined on the oven-dried plant and sediment samples ground into a fine powder by an agate mortar and pestle of Fritsch pulverisette and mineralised with HF (50%) and HNO_3 (65%) at a ratio of 1:2 (v/v) in a Milestone mls 1200 micro-wave oven (Baldantoni et al., 2004). In order to extract also the available fractions of the investigated elements in the sediments, diethylenetriamine–pentacetic acid (DTPA), $CaCl_2$ and triethanolamine (TEA) at pH 7.3 were used (Lindsay and Norwell, 1978). The

concentrations of each element in plant and sediment samples were measured by atomic absorption spectrometry (Spectr AA 20 – Varian), via graphite furnace (Cd, Cr, Cu, Ni, Pb and V) or via flame (Fe and Zn). In addition, the organic matter content, via loss on ignition (550 °C), and pH, in distilled water at a ratio of 1.0:2.5 (ww = sediment:water) via the electrometric method, were evaluated in the sediments. All the analyses were carried out on three subsamples.

2.5. Statistical treatments

The data were processed by statistical tests using the SigmaStat software package. The correlations were assayed between plant and sediment element concentrations as well as among the elements. The correlations between element concentrations were carried out only for *Ph. communis* roots, due to the high number of measurements. The correlations were evaluated by Spearman's test because of the non-normal distribution of the data set. Principal component analysis (PCA) was processed on element concentrations in the investigated species from the three sampling sites in order to highlight the distinction between the sites and the species according to element concentrations.

3. Results

At the first sampling, in July 1999, pH and organic matter were determined in the sediments. The sediment pH values were around neutrality and similar at all the sites (Table 1). The organic matter contents showed the highest values at the site B (Table 1). For the total element concentrations in the sediments, the highest values of Cr, Cu, Ni and Pb were detected at site A. At this site the available fractions of Cu, Fe, Pb and Zn were the highest. The sediment of site C showed the lowest values (Table 1); in particular, both total and available Cu concentrations were one order of magnitude lower than at site A. For all the sites, Cu and Zn exhibited the highest percentages (more than 10%) of the available fractions with respect to the total concentrations, the available Cd, Ni, Pb and V ranged from 2 to 7%, and the available Cr and Fe were lower than 0.3% (Table 1).

For each sampling, at site A *Ph. communis* roots showed concentrations of Pb and Zn on average twice those measured at sites B and C, where Pb and Zn concentrations were similar (Fig. 1). Except for a few samplings, also Cd, Cr, Cu, Fe and Ni concentrations in *Ph. communis* roots were highest at site A (Fig. 1). Differences were less considerable for Cd (Fig. 1). V concentrations in *Ph. communis* roots did not show a clear trend at the studied sites (Fig. 1).

N. marina shoots were collected only at sites B and C as the plants were always absent at site A. Generally, higher concentrations of all the studied elements were measured at site B than C (Fig. 2). The differences among the sites were particularly evident for Cu, Fe, Pb and V, whereas Cr, Ni and Zn exhibited less substantial or no differences between the sites (Fig. 2). For both *N. marina* and *Ph. communis* the temporal trend of the element concentrations was similar at the sites (Figs. 1 and 2).

P. pectinatus leaves showed a clear gradient of element concentrations according to the sampling sites: a decrease was observed from site A to B and then to C (Fig. 3). The

Table 1
Mean pH, organic matter (% dry wt.) and total (tot.) and available (av.) concentrations ($\mu g\ g^{-1}$ dry wt.) of Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in the sediments collected at sites A–C of Lake Averno on July 1999

	Site A	Site B	Site C
pH	7.3	7.8	7.7
Organic matter	4.32	5.40	4.09
Cd (tot. concentration)	0.40	0.44	0.41
Cd (av. concentration)	0.03	0.04	0.02
Cr (tot. concentration)	5.15	4.38	2.56
Cr (av. concentration)	0.01	0.01	0.01
Cu (tot. concentration)	75.87	66.51	6.05
Cu (av. concentration)	16.87	11.32	0.47
Fe (tot. concentration)	17580	17780	17180
Fe (av. concentration)	40.00	21.00	21.00
Ni (tot. concentration)	7.49	4.70	3.07
Ni (av. concentration)	0.25	0.27	0.12
Pb (tot. concentration)	92.57	69.09	47.37
Pb (av. concentration)	3.10	1.85	0.51
V (tot. concentration)	21.21	29.92	28.65
V (av. concentration)	0.59	3.74	1.17
Zn (tot. concentration)	118.67	149.00	126.67
Zn (av. concentration)	28.00	10.02	3.68

Standard deviations for the total sediment concentrations ranged from 0.3 to 9.1% of mean values, and for the available sediment concentrations from 0.2 to 16.7%.

broadest differences in element concentrations were found between site A and the other two sites: Cr, Cu, Ni and Zn showed values two- or three-fold higher (Fig. 3).

Among the studied trace elements, with the exception of V, significant positive correlations in *Ph. communis* roots were found (Fig. 4). The PCA in relation to the element concentrations in the studied plants (Fig. 5) showed a clear pattern both of sites and species. Site A appeared more dissimilar than sites B and C, above all for *Ph. communis*, while sites B and C were similar. In Fig. 5, the distribution of *Ph. communis* and *P. pectinatus* collected at site A depended on Cd, Cr, Pb and Zn, whereas that of *Ph. communis* at both sites B and C depended on Cu, Fe, Ni and V. The element concentrations in sediments were correlated ($P < 0.0001$) to those found in each plant species for each sampling date (Table 2).

4. Discussion

Most of the studied elements (Cr, Cu, Ni, Pb) present the highest total sediment concentrations at site A, where the available fractions of Cu, Fe, Pb and Zn are also the highest (Table 1). However, Pb total concentrations, at all the sites, are higher than the range of values (14–40 $\mu g\ g^{-1}$ dry wt.) reported by Förstner and Wittmann (1979) for uncontaminated sediments; particularly, at site A Pb concentrations are twice the maximum value of the range. Also Cu at sites A and B show concentrations higher than the background ranges (16–44 $\mu g\ g^{-1}$ dry wt.). Zn at all the sites exhibits concentrations
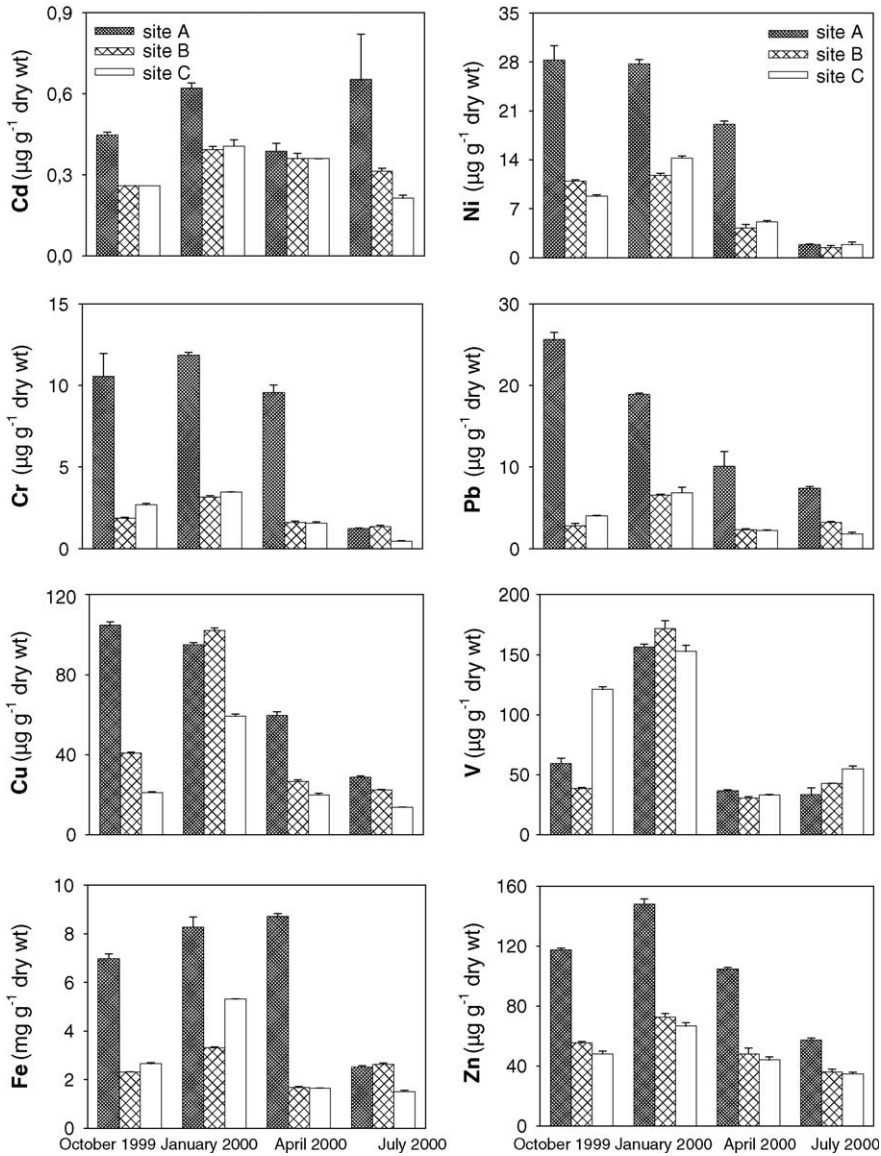
Fig. 1. Mean concentrations (±S.D.) of Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in *Ph. communis* roots at the three Lake Averno sites, sampled in October 1999 and in January, April and July 2000.

around the highest value of the range 7–124 $\mu g\ g^{-1}$ dry wt. (Förstner and Wittmann, 1979). Cu, Pb and Zn concentrations in the sediments of Lake Averno are comparable to those measured in freshwater sediments contaminated by urban and industrial activities (Welsh and Denny, 1980; Catallo et al., 1995; Avelar et al., 1997). The highest Pb and Cu concentrations at site A suggest that the channel leading from the sewage outfall is an
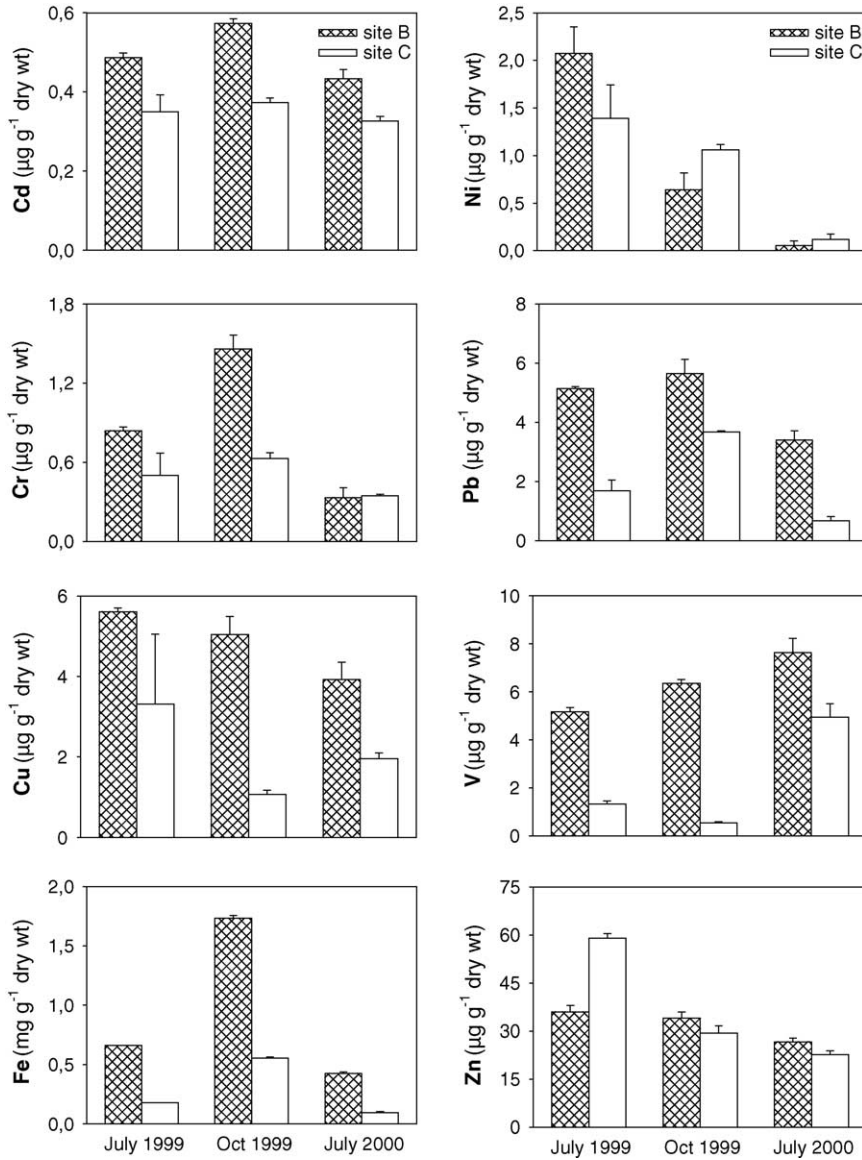
Fig. 2. Mean concentrations (±S.D.) of Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in *N. marina* shoots at sites B and C of Lake Averno, sampled in July and October 1999 and in July 2000.

important source of contamination in the lake. Agriculture on soils around the lake may also be responsible for high Cu concentrations measured in the sediments of Lake Averno, as reported also by Jones et al. (1991) for a lake in Wales.

The aquatic plants growing in the study area exhibit different trace element concentrations, depending on the plant organ, and both the sampling time and the sampling
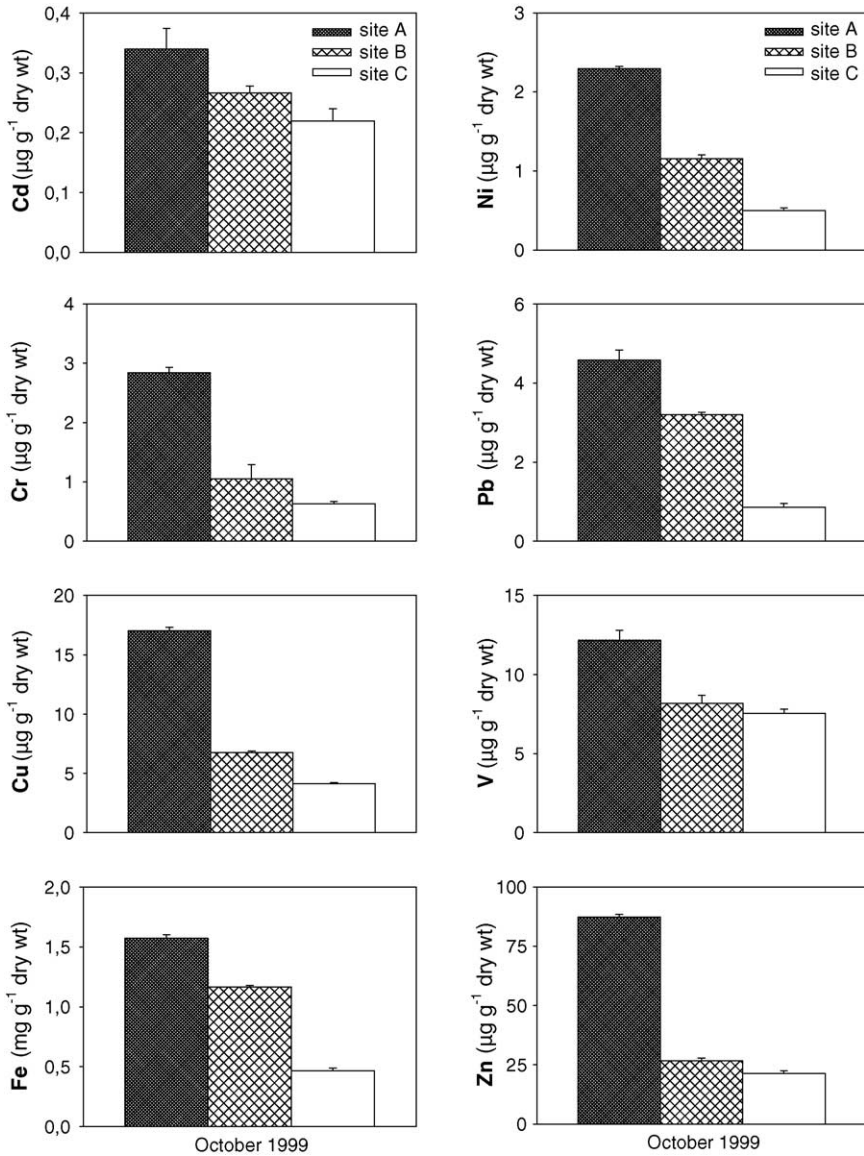
Fig. 3. Mean concentrations (±S.D.) of Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in *P. pectinatus* leaves at the three Lake Averno sites, sampled in October 1999.

sites. Roots of aquatic plants absorb from the sediments and accumulate high concentrations (Baldantoni et al., 2004). Even if shoots and/or leaves of submersed plants accumulate lower concentrations of trace elements than roots, they may reliably provide information chiefly on water quality over short periods equivalent to leaf age. Trace element accumulations in plant organs are integrated values of contaminant exposure in time. On

	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
Cd	1							
Cr	0.48	1						
Cu	0.69 *	0.81 ***	1					
Fe	0.58 *	0.87 ***	0.80 ***	1				
Ni	0.48	0.96 ***	0.81 ***	0.79 **	1			
Pb	0.80 ***	0.76 **	0.83 ***	0.90 ***	0.72 **	1		
V	0.19	0.53	0.47	0.53	0.49	0.41	1	
Zn	0.77 **	0.88 ***	0.93 ***	0.85 ***	0.88 ***	0.90 ***	0.43	1
	Cd	**Cr**	**Cu**	**Fe**	**Ni**	**Pb**	**V**	**Zn**

Fig. 4. Spearman correlation coefficients between the elements in *Ph. communis* roots at the three studied sites; [*]: $P < 0.05$, [**]: $P < 0.01$, [***]: $P < 0.001$.
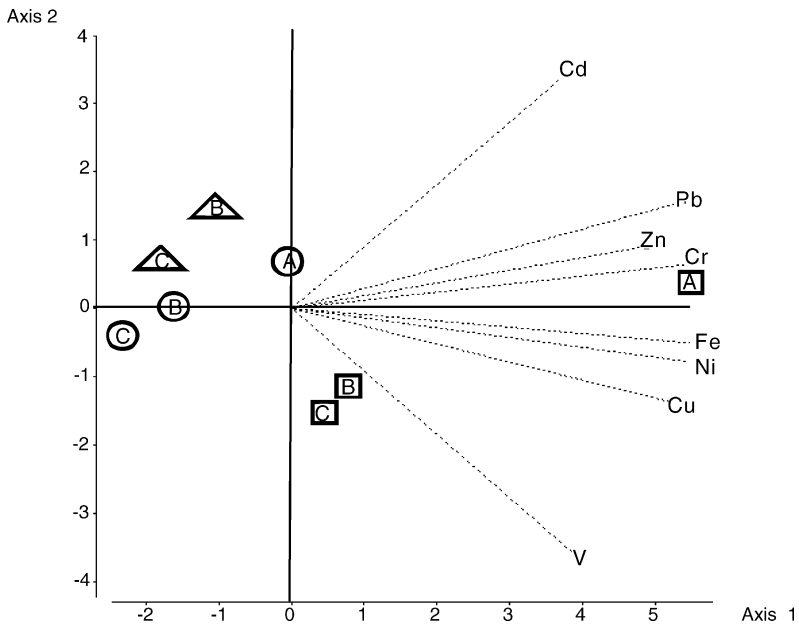

Fig. 5. Principal component analysis (PCA) on element concentrations in the species collected at the three Lake Averno sites (A–C). The eigenvalues are 79.21 and 12.35% for axes 1 and 2 respectively. (□) *Ph. communis* roots, (△) *N. marina* shoots, (○) *P. pectinatus* leaves.

Table 2
Spearman correlation coefficients between the element concentrations in sediments and in *Ph. communis* ($n = 24$), *N. marina* ($n = 16$) and *P. pectinatus* ($n = 24$) plants at the three studied sites ($P < 0.0001$)

Ph. communis				*N. marina*			*P. pectinatus*
October 1999	January 2000	April 2000	July 2000	July 1999	October 1999	July 2000	October 1999
0.82	0.77	0.86	0.87	0.94	0.91	0.86	0.91

the whole, good information to assess spatial gradients of trace element contamination in Lake Averno is provided by analysing plant organs collected at each studied site.

Except for a few samplings, at site A *Ph. communis* roots show higher concentrations of Cd, Cr, Cu, Fe, Ni, Pb and Zn than those measured at sites B and C, where trace element concentrations are similar (Fig. 1). The concentrations of Cu and Pb in *Ph. communis* roots from site A exceed the values (48 and 11 μg g^{-1} dry wt., respectively) reported by Hutchinson (1975) as average concentrations in aquatic plants. Moreover, Cr, Ni and Pb concentrations are on average higher than those (2.7 μg g^{-1} dry wt. for Cr, 10.0 μg g^{-1} dry wt. for Ni, and 11.3 μg g^{-1} dry wt. for Pb) measured in *Ph. australis* Trin. (Cav.) roots – a species that Pignatti (1982) considers the same as *Ph. communis* Trin. – collected in the Danube delta in Romania (Keller et al., 1998). Fe root concentrations at site A are on average higher than those reported by Hozhina et al. (2001) for *Ph. australis* roots collected in the vicinity of ore mining in south-western Siberia. Moreover, the high Fe as well as Cu concentrations in *Ph. communis* roots could be also due to their precipitation in iron plaques on root surfaces (Tanner, 1996; Batty et al., 2002).

N. marina, absent at site A, generally shows the highest trace element concentrations at site B, in particular for Cu, Fe, Pb and V (Fig. 2). Although for *N. marina* shoots the differences between sites B and C are more evident than for *Ph. communis* roots, these two species may be used as monitors of trace element temporal gradients, showing similar temporal trends in element concentrations. At each site variations in trace element concentrations among the samplings are greater for *Ph. communis* than for *N. marina* (Figs. 1 and 2). This may be explained by the fact that whereas plants of *N. marina* were collected twice in July, when the plants were growing at their peak, and once in October, plants of *Ph. communis* were collected at the beginning of the life cycle (April) and every 3 months, until their senescence. Indeed, the variations observed for *Ph. communis* roots could reflect the accumulation of the elements during 1 year, according to exposure time (Leendertse et al., 1996), whereas those observed for *N. marina* shoots could reflect the accumulation in a shorter exposure time (spring–autumn).

For *P. pectinatus* leaves, a decrease in element concentrations is evident from site A to B and then to site C (Fig. 3); the broadest differences in element concentrations are found between site A and the other two sites.

On the basis of the element concentrations, *P. pectinatus* leaves, as well as *N. marina* shoots, show greater differences between sites B and C than *Ph. communis* roots. All the three species highlight the same spatial trend of lake contamination, but each species shows various degrees of differences among the sites. *Ph. communis*, analysed for roots, probably shows trace element accumulation in the sediments, whereas *N. marina* and *P. pectinatus*, analysed for shoots and leaves respectively, reflect mainly element concentrations in water.

Trace element accumulation in sediments is the result of long-term exposure, whereas trace element concentrations in water mainly result from recent contamination.

The highest element concentrations at site A, as attested by analysing sediments and plants in Lake Averno and by the separation of site A from B and C in the PCA analysis (Fig. 5), suggest that the overflow channel from a sewage plant is the main source of metals in Lake Averno. Trace element analyses carried out on sewage sludge arriving at the Naples sewage treatment plant show values that do not usually exceed the concentrations stipulated by the law (D.L. 152/1999, D.L. 258/2000). Nevertheless, sediments and plants appear to accumulate high concentrations of trace elements, showing that, in time, the channel brings to the lake a conspicuous amount of pollutants. The significant positive correlations among the trace elements in *Ph. communis*, except for V (Fig. 4), as well as the similar element variations over time at each site (Fig. 1) reveal the same element source (Ansari et al., 1999). The slightly higher concentrations measured in the plants collected at site B than site C may be also linked to higher vehicular traffic at site B and/or to movement of lake waters and consequent mixing up of surface sediments, as at this site an artificial canal connects the lake to the sea. Analysis of plants confirms the initial hypothesis that site C is less exposed to human impact than the other two sites.

Analyses of metal concentrations in plant tissues often provide useful evaluation of point-source pollution in the water column. This is particularly true when monitoring studies regard habitats affected by various point sources of pollution (St-Cyr and Campbell, 2000). The correlations found between sediment and plants highlight the same effect of water contamination on the two matrices (Table 2). Given the absence of systematic water quality monitoring, trace element accumulation in plants rather than in sediments provides a cost-effective means for assessing short-term contamination that is within the lifespan of the plant organs in question.

In approaching biomonitoring studies it may be useful to investigate several species together, as suggested also by Osmolovskaia and Kurilenko (2001), in order to identify the best biomonitor. In our study, substantial information on the spatial distribution of trace elements in Lake Averno was provided by analysing shoots of *N. marina*, leaves of *P. pectinatus* and roots of *Ph. communis*. Among the investigated species, *Ph. communis* appears the best monitor because it may be easily collected and is present throughout the year.

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