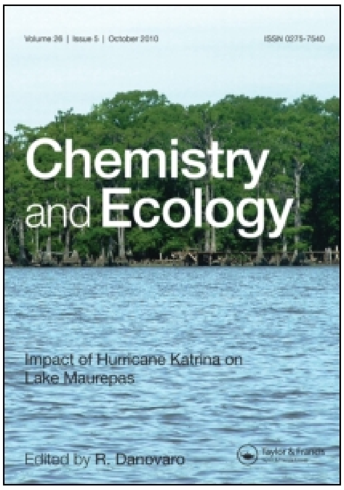

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P. Cellini Legittimo[a]; L. Ducceschi[b]; M. Martini[c]
[a] Department of Pharmaceutical Sciences, University of Florence, Florence, Italy [b] Department of Public Health, Epidemiology, and Environmental Analytical Chemistry, University of Florence, Florence, Italy [c] Department of Earth Sciences, University of Florence, Florence, Italy

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HOLLY (*ILEX AQUIFOLIUM* L.) AS ZINC AND CADMIUM ACCUMULATIVE INDICATOR IN BIOGEOCHEMICAL PROSPECTING

P. CELLINI LEGITTIMO[a,*], L. DUCCESCHI[b] and M. MARTINI[c]

[a] *Department of Pharmaceutical Sciences, University of Florence, via Gino Capponi 9, 50121 Florence, Italy;*
[b] *Department of Public Health, Epidemiology, and Environmental Analytical Chemistry, University of Florence, via Gino Capponi 9, 50121, Florence, Italy;*
[c] *Department of Earth Sciences, University of Florence, via Giorgio La Pira 4, 50121 Florence, Italy*

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The results of previous investigations in mineralized and sterile areas of Tuscany (Italy) appeared to point out the special aptitude of holly (*Ilex aquifolium* L.) in concentrating cadmium and zinc. In the present paper this behaviour has been verified in a mineralized area of Lombardy (Northern Italy).

Samples of leaves, twigs and barks from holly trees were collected, and metal contents (Zn, Cd, and Pb) have been determined by differential pulse anodic stripping voltammetry (DPASV). For comparison, some samples of wood anemone (*Anemone nemorosa* L.) and soil were also analyzed.

The data obtained confirm a preferential cadmium and zinc accumulation by holly (up to 16 and 1400 $\mu g\ g^{-1}$ dry weight, respectively) also depending on different vegetal organs and vegetative stages of plants. Highest contents of lead have been recovered in the outer portion of bark.

Keywords: Holly; heavy metals; cadmium and zinc accumulative indicator; biogeochemical prospecting

INTRODUCTION

The use of vegetal species in biological methods of prospecting for ore deposits has been in progress with successful results during the last

*Corresponding author.

decades (Brooks, 1983, 1993; Kovalevsky, 1984; Ernst, 1993). In fact, the distribution of heavy metals in natural soils can greatly affect the vegetation and the concentration of a given element in the whole plant, or in some of its parts (e.g., leaves, twigs), can be related to geochemical anomalies (Cole, 1991; Rogers and Dunn, 1993; Reeves *et al.*, 1995). Consequently, plants can be used in a process termed "*phytoextraction*", that is a more recent term which includes both "*phytoremediation*" and "*phytomining*", of metals from soils (Chaney *et al.*, 1995; Nanda Kumar *et al.*, 1995). All these procedures are based on the properties of *bioaccumulation* of trace elements displayed by selected vegetal species known as *bioaccumulators* or *accumulative indicators* (Wittig, 1993).

However, for many elements a small proportion of their total contents in the soil is bioavailable, due to several factors such as pH, Eh, drainage, the nature of clay minerals, antagonistic effects of other ions, and the presence of complexing agents (Brooks, 1983; Streit and Stumm, 1993). Generally, root systems of tree species are able to take up elements from bigger volumes of soil with respect to herbaceous plants, producing mean concentration values among the humic layer and the horizons below.

Different plant species had been tested in a mineralized area of Tuscany (Italy) characterized by a complex polymetallic sulphide assemblage (Cellini Legittimo *et al.*, 1995); holly (*Ilex aquifolium* L.) leaves and twigs appeared as enriched significantly in cadmium, acting as a pathfinder of zinc mineralizations. The present paper takes into account the data obtained for a mineralized area of Lombardy (Italy), in order to verify to which extent the previous results can be confirmed. Moreover, a comparison with wood anemone (*Anemone nemorosa* L.) has been done since this plant has been considered by Tyler (1976) as a cadmium-hyperaccumulator according to the significant cadmium concentrations in root samples. Metal concentrations in roots from holly and wood anemone have not been considered because possible root contamination from soil particles can bias the analytical data (Markert, 1996).

AREA OF STUDY

The area investigated in located is the western part of the Gorno mining district (Brembana Valley, Lombardic Prealps).

Several ore bodies have been here recognized and exploited since the Roman age.

According to the availability of holly plants, our attention has been mainly focused on Paglio Pignolino mine (Fig. 1), as well as Mount Vaccareggio-Mount Pedrozio ore body, located about 2 km to the east. Both deposits were actively exploited for lead and zinc sulphides until the 1930s; significant exploitation of fluorite has been also subsequently carried out at Paglio Pignolino.

The mineralizations of the Gorno district are located near the Ladinian/Carnian boundary, represented by deposits of carbonate platform (Esino Limestone) and transitional facies (Breno Formation, Calcare Metallifero sequence, Gorno Formation). In particular, Paglio Pignolino ore body is genetically linked with the sediments of Calcare Metallifero Bergamasco (Assereto *et al.*, 1977; Ridge, 1990).

Several mineral species have been reported from these deposits: fluorite, sphalerite, galena, pyrite, marcasite, copper and antimony

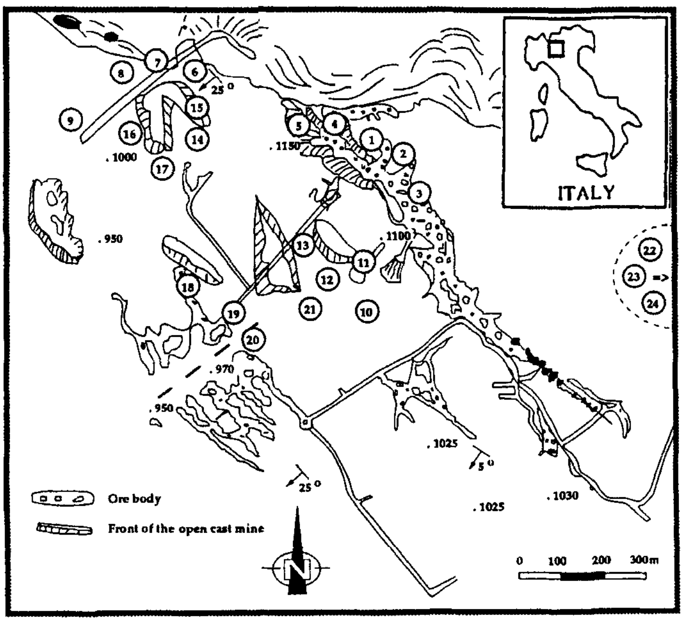

FIGURE 1 Schematic map of the area investigated (from Assereto *et al.*, 1977; modified).

sulphosalts, barite and alteration minerals (smithsonite, emimorphite, cerussite, covellite, malachite, limonite, gypsum) (Assereto *et al.*, 1977; Appiani *et al.*, 1993, 1994 a,b); greenockite is also reported (Boscardin *et al.*, 1970).

Mining activity was abandoned about ten years ago.

In this area the atmospheric pollution due to industrial or other human activities is very low, and therefore the foliar absorption of heavy metals can be considered negligible.

SAMPLING AND ANALYTICAL PROCEDURE

The collection of samples has been mainly carried out in April 1995 at Paglio Pignolino ore deposit, in an approximately circular area, at an elevation between 1000 and 1200 m (Fig. 1).

The sampling points have been selected according to the distribution of holly plants, at intervals of about 50m. Two-year old twigs, together with leaves, at a height of about 2m, have been collected from different points of the canopy of approximately 20-year old trees; bark samples have been picked at breast height (about 130 cm) according to Karandinos *et al.* (1985), distinguishing outer and inner portions at thicknesses in the interval of 0–1 mm and 1–3 mm, respectively. Where wood anemone was found at the same place, aerial parts have been collected, together with samples of soil from a depth of about 20 cm. Holly plants in the area of Mount Vaccareggio-Mount Pedrozio grow apart from the mineralization, on lithologically different and barren substratum (Assereto *et al.*, 1977); three samples of holly and one sample of wood anemone and soil have been collected from this site.

Lattice gloves and steel scissors have been used for sampling operations. In the laboratory, following a careful mechanical cleaning with moistened filter paper, the plant samples were desiccated at 60°C for 48 hours and finely ground; a carefully weighted aliquot of about 150 mg was subsequently digested using a 4:1 mixture of concentrated nitric and perchloric acids up to 200°C.

Soil samples were dehydrated at 105°C for 24 hours, passed through a 2mm-mesh sieve and then metals extracted by aqua regia

(3:1 mixture of hydrochloric and nitric acids), soil: solvent = 1:8 w/v; an aliquot of this extract was then treated with perchloric acid and mineralized, up to 200°C. All the acids were Suprapur grade (Merck).

After mineralization and addition of acetic buffer at pH 4.7, the concentrations of zinc, cadmium and lead, were determined by the technique of Differential Pulse Anodic Stripping Voltammetry (DPASV), with a polarographic analyzer (AMEL model 473).

Standard calibration curves were used for each metal in the same supporting electrolyte and in the same range of concentrations of the samples. The addition of known aliquots of heavy metals to the significant number of samples before the mineralization, produces similar analytical results with respect to the use of calibration curves.

Metal contents were also determined in a BCR Reference Material (olive leaves, BCR number 62). The precision of the method was estimated from ten replicate determinations. As shown in Table I, the values agree with the certified values.

Soil pH was measured using the standard procedure (soil: water = 1:2.5 w/w). The analytical data, obtained as mean values of three determinations for each sample, are given in micrograms per gram of dry weight.

RESULTS AND DISCUSSION

a) Metals Concentration in Leaves, Twigs and Barks

The results of the distribution of zinc, cadmium, and lead in leaves and twigs of holly samples are given in Table II; values in brackets pertain

TABLE I Metal contents ($\mu g\ g^{-1}$ dry wt) certified and obtained in BCR 62 Reference Material (olive leaves) with their 95% confidence interval

Metal	*Number Samples*	*Obtained Value ($\mu g\ g^{-1}$)*	*Certified Value ($\mu g\ g^{-1}$)*
Zinc	10	16.7 ± 0.8	16.0 ± 0.7
Cadmium	10	0.10 ± 0.04	0.10 ± 0.02
Lead	10	25.9 ± 1.1	25.0 ± 1.5
Copper	10	47.4 ± 1.5	46.6 ± 1.8

TABLE II Concentrations of zinc cadmium and lead in holly samples collected in the mineralized area of Brembana Valley. Height (H) and diameter (D) of holly trunks, and corresponding ratio (H/D) are also indicated

Sample no.	*Height m*	*Diameter m*	*H/D*	*Concentration (µg g⁻¹ dry wt)* *Zn* *Leaf*	*Zn Twig*	*Cd Leaf*	*Cd Twig*	*Pb Leaf*	*Pb Twig*
Paglio Pignolino									
(1)	10	0.150	67	839	792	3.2	4.7	0.45	3.0
(2)	6	0.130	46	516	436	2.0	4.2	0.34	1.0
(3)	10	0.150	67	262	202	1.6	2.1	1.7	0.45
(4)	3	0.055	55	825	548	6.0	3.6	0.22	4.3
(5)	2.5	0.055	45	919	656	7.2	6.3	0.67	1.2
(6)	3	0.065	46	853	680	2.5	4.7	0.99	2.6
7	7	0.145	48	322	394	2.1	2.9	0.05	0.94
(8)	6.5	0.120	54	785	616	4.7	5.8	0.06	1.2
(9)	11	0.130	85	839	731	7.2	5.6	1.7	1.0
10	14.5	0.215	67	760	534	3.7	4.5	0.34	2.0
(11)	10	0.120	83	370	333	1.6	2.0	0.22	0.90
(12)	5	0.075	67	712	572	5.7	6.4	0.45	1.2
(13)	8	0.110	73	786	412	3.3	4.5	0.22	1.8
(14)	10	0.160	62	841	525	2.9	3.8	0.21	2.2
15	11	0.120	92	838	725	7.5	10.9	0.30	1.9
16	3.5	0.080	44	1050	938	10.2	8.7	0.60	4.1
(17)	3	0.080	38	894	581	2.6	2.0	0.04	4.7
18	5	0.085	59	544	484	2.6	3.5	0.12	0.04
19	5	0.065	77	812	512	3.3	3.5	0.04	0.60
20	6	0.085	71	1338	975	10.0	5.7	0.04	1.2
21	5	0.080	62	906	388	6.2	3.0	0.04	1.4
Mount Vaccareggio-Mount Pedrozio									
(22)	3	0.060	50	276	199	1.8	1.2	0.36	0.27
23	4	0.095	42	288	344	3.2	3.6	0.05	0.05
24	3	0.045	67	354	260	2.4	2.8	2.7	1.8

( ) = Stump main sprout.

to samples collected from the main sprouts of stump. In the same table are also reported the height and diameter of the plants sampled and the ratio between height and diameter (H/D).

By considering zinc concentrations in holly samples, higher values are observed in leaves with respect to twigs. Cadmium concentrations on the contrary are slightly higher in twigs (about 70% of samples) especially for the lowest values found in leaves. These features are shown in Figure 2 using the Acropetal Coefficient (AC), defined as the ratio between the concentration of an element in a particular plant organ and the concentration of the same element in a reference or

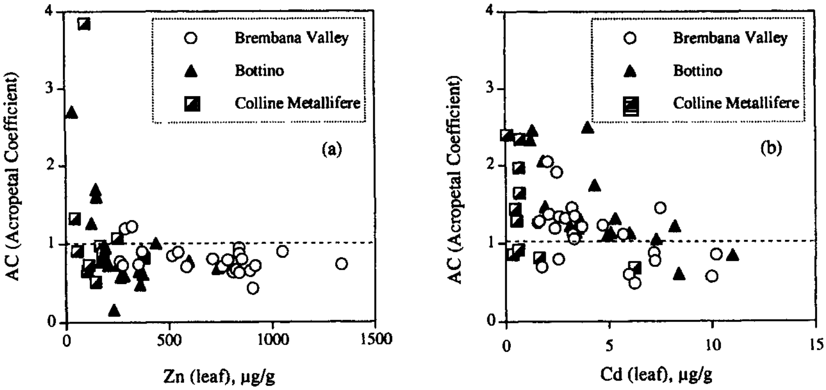

FIGURE 2 Variation of Acropetal Coefficient (AC) values for zinc and cadmium in holly samples from different mineralized areas. The AC values (twig/leaf) are plotted as a function of the zinc (a) and the cadmium (b) content in the leaf.

standard organ of the same species (Brooks, 1983). In the same figure the values obtained in two mineralized areas of Tuscany, Bottino and Colline Metallifere (Cellini Legittimo *et al.*, 1995) are also reported and a similar behaviour can be observed.

Lead appears as accumulated mainly in twigs with respect to leaves (mean concentrations of 1.67 and 0.48 $\mu g\ g^{-1}$, respectively); for comparison, at Bottino lower absolute values of lead in both organs have been observed with mean concentrations of 1.34 $\mu g\ g^{-1}$ in twigs and 0.28 $\mu g\ g^{-1}$ in leaves (Cellini Legittimo *et al.*, 1995). Finally, at Colline Metallifere the lowest values of lead have been observed (mean concentrations of 0.42 $\mu g\ g^{-1}$ in twigs and 0.05 $\mu g\ g^{-1}$ in leaves, unpublished data). On the other hand, the value of Pb_{twig}/Pb_{leaf} ratio increases in the order Brembana Valley < Bottino < Colline Metallifere.

No significant differences pertaining to single trees or to the main sprouts of stump have been observed in the trend of accumulation of zinc, cadmium and lead.

The concentrations determined in bark of holly plants are shown in Table III. Zinc and cadmium appear to accumulate in bark samples, especially in the inner portions. Lead, on the other hand, mainly accumulates in the outer portions of bark where, for increasing lead concentrations and in spite of scattering data, a relatively lower values of cadmium and zinc in respect to inner bark contents can be observed (Fig. 3).

TABLE III Concentrations of zinc, cadmium and lead in holly bark samples from the mineralized area of Brembana Valley

Sample no.	*Concentration ($\mu g\ g^{-1}$dry wt)*					
	Zn Bark		*Cd Bark*		*Pb Bark*	
	Outer	*Inner*	*Outer*	*Inner*	*Outer*	*Inner*
Paglio Pignolino						
(1)	825	1195	5.8	6.9	n. d.	1.8
(2)	334	828	3.2	6.7	27.0	0.05
(3)	453	644	2.1	3.0	1.6	0.05
(4)	666	884	2.8	3.9	9.6	0.05
(5)	800	1125	7.9	9.7	23.1	0.90
(6)	619	950	11.0	16.7	6.8	1.2
7	612	722	5.1	5.5	22.2	0.60
(8)	956	1481	7.0	6.2	0.8	0.90
(9)	631	1044	8.2	10.2	5.8	0.30
(10)	203	788	1.2	5.5	11.8	0.05
(11)	650	781	4.3	5.9	13.8	0.45
(12)	794	612	9.8	6.0	3.5	0.05
(13)	386	831	4.5	8.3	12.6	0.30
14	638	631	4.2	8.6	30.0	1.5
15	912	1106	6.5	8.3	3.1	0.05
16	962	1025	10.4	7.5	4.0	1.8
(17)	1131	969	4.8	5.1	7.7	0.05
18	794	634	5.0	3.4	2.6	2.1
19	653	806	4.3	3.0	0.07	0.24
20	744	994	9.0	9.8	7.3	0.05
21	794	825	8.4	7.9	3.7	0.15
Mount Vaccareggio-Mount Pedrozio						
(22)	547	547	2.9	2.9	1.1	0.15
23	185	472	1.8	3.8	7.6	0.30
24	769	859	4.3	4.5	0.15	0.05

( ) = Stump main sprout.

A similar distribution for zinc and cadmium in respect to lead, can be observed among different vegetal organs as well in Figure 4, where the ratios of mean values of the metals concentration among outer bark and the other organs are shown. Consequently, a systematic response of holly vegetal organs to the concentration in soil of these metals can be hypothesized.

b) The Influence of Vegetative Characters

Holly samples here investigated have been collected from plants of different dimensions. According to the procedure suggested for

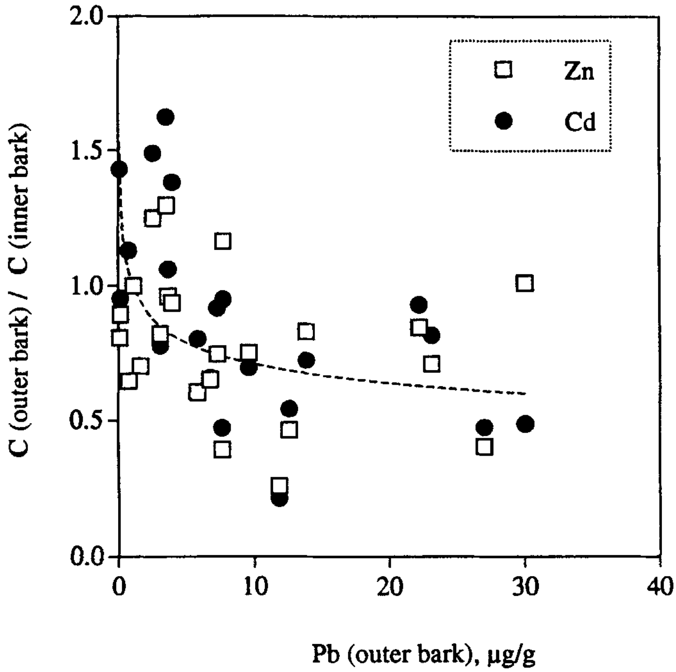

FIGURE 3 Distribution of zinc and cadmium ratios in different portions of the bark *vs.* lead concentration in outer bark samples of holly from Brembana Valley.

environmental gradients (Bacon and Zedaker, 1986; Mazzoleni, 1991), a similar consideration has been attempted by means of height/diameter ratio of holly trunks; these can give an idea of different edaphic conditions in the area considered (mainly soil humidity and luminosity) which characterized the growth of the vegetation.

The holly trees here considered fall in two different groups with height/diameter ratios above and below 65, and 75 as 47 mean values respectively (Tab. IV). The mean values for different metals in leaves, twigs and bark, pertaining to these groups, are also shown.

According to these data, zinc, cadmium and lead display a common behaviour showing higher levels in leaves and lower contents in bark at a greater extent of height/diameter ratios.

This behaviour is particularly evident for lead.

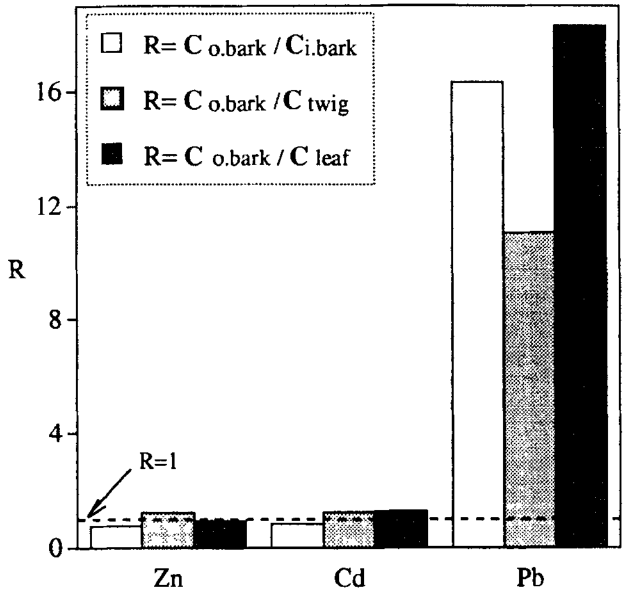

FIGURE 4 Mean value of the ratios (*R*) of zinc, cadmium and lead contents (*C*) among different holly organs collected in Brembana Valley.

TABLE IV Zinc, cadmium and lead mean values for each sampled holly organ according to vegetative conditions. Group 1 and Group 2 are related to different mean values of Height/Diameter (H/D) ratios

Vegetal organ	*Zinc, cadmium and lead mean values ($\mu g\ g^{-1}$ dry wt)*					
	Zn		*Cd*		*Pb*	
	Group 1[a]	*Group 2*[b]	*Group 1*	*Group 2*	*Group 1*	*Group 2*
Leaf	649	687	4.0	4.6	0.35	0.77
Twig	526	526	3.8	4.8	1.9	1.3
Outer bark	665	620	5.5	5.4	11.2	6.0
Inner bark	816	783	6.5	6.4	0.72	0.16

[a] H/D (mean value) = 47.
[b] H/D (mean value) = 65.

c) Comparison Between Holly and Wood Anemone

Table V shows the analytical results for wood anemone and soil samples collected in the same sites where holly plants have been investigated. In spite of the relatively small number of wood anemone and soil samples in comparison with holly samples, significant

TABLE V Soil pH values and zinc, cadmium and lead concentrations in wood anemone and soil samples from Brembana Valley

Sample no.	*Metal concentrations (μg g^{-1} dry wt)*						
		Zn		*Cd*		*Pb*	
	Soil pH	*Anemone*	*Soil*	*Anemone*	*Soil*	*Anemone*	*Soil*
1	5.7	980	284	3.6	1.6	1.2	6.4
8	6.3	328	31	2.1	0.92	1.5	77
9	5.1	862	382	4.8	3.1	3.4	32
10	6.1	350	36	1.1	0.32	0.51	1.8
19	5.4	391	29	1.2	0.08	1.6	311
23	5.6	488	7.2	1.9	0.05	1.0	0.40

concentrations of zinc and cadmium in wood anemone are observed, with lower cadmium values with respect to holly samples. Wood anemone and holly can accumulate zinc and cadmium. In particular for cadmium, at concentration of this metal in soil of 0.005 – 0.10 μg g^{-1}, in

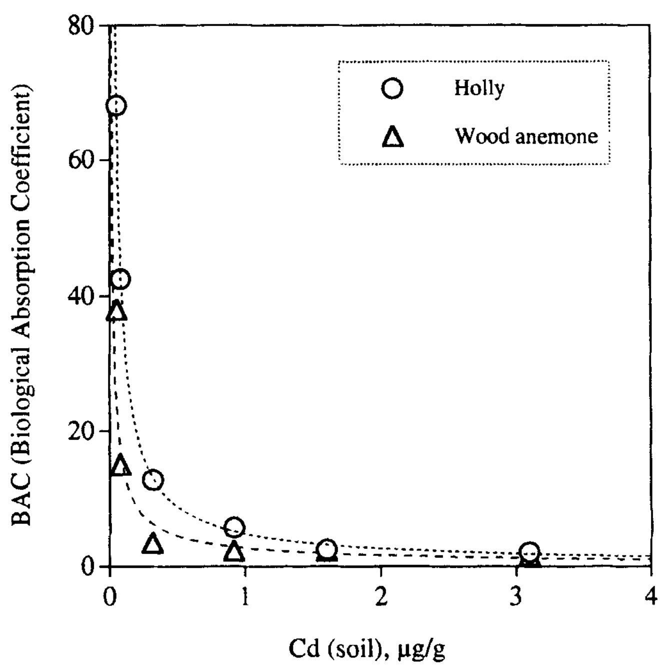

FIGURE 5 Biological Absorption Coefficient for cadmium in holly (leaf and twig, mean values) and wood anemone *vs*. cadmium concentration in soils from Brembana Valley.

the range of crustal average (Adriano, 1990), values of biological absorption coefficient (BAC) (defined as the ratios of metal concentrations in plants and soils (Kovalevsky, 1969) above 10 have been found (Fig. 5). At increasing metal concentrations in soil, lower but still significant BAC values are observed, and this behaviour can be useful in biogeochemical applications. It is also interesting to remark that both vegetal species considered show for cadmium, BAC plots similar to those attempted for essential elements.

The acidity of soil appears as influencing the bioavailability of metals; accordingly, significant differences have been observed for zinc in holly plants growing in some mineralized areas on soils at different pH values (Fig. 6). In the same figure, BAC values for wood anemone are plotted too.

The values of Zn/Cd ratios in soil samples are spread over a large interval, probably because different mineralogical composition of

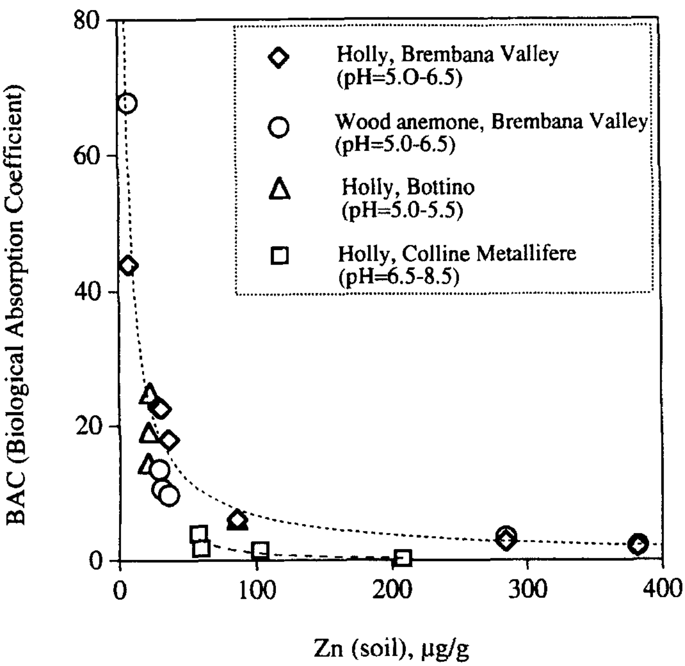

FIGURE 6 Biological Absorption Coefficient for zinc in holly (leaf and twig, mean values) and wood anemone *vs.* zinc concentration in soils from different mineralized areas. Influence of soil pH (Bottino and Colline Metallifere, unpublished data).

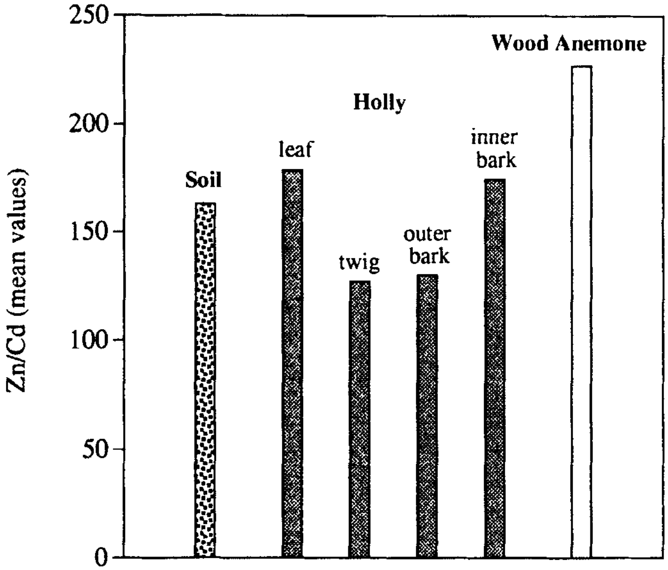

FIGURE 7 Comparison of Zn/Cd ratios among holly different organs, wood anemone, and soil from Brembana Valley.

the mineralization here considered (e.g., sphalerite, greenockite, smithsonite, emimorphite). However, if the mean value of Zn/Cd ratio for soil is compared with those obtained for wood anemone and different holly organs (Fig. 7), a close fitting response is given by the leaf and the inner bark of holly.

CONCLUSIONS

The data obtained during this investigation and their comparison with abnormal accumulation of trace metals by plants allows us to confirm the role of holly in biogeochemical prospecting as an accumulative indicator of zinc and cadmium. Both metals show a similar distribution pattern by leaves, twigs, and holly barks, even if in variable extent and with highest contents in the inner bark portions. The lead behaviour is different in respect to zinc and cadmium. In fact, lead accumulation is very low in holly organs except in the outer bark portions where an antagonistic effect among this metal and the other metals considered could exist. On the other hand, zinc, cadmium, and lead contents in holly follow a similar trend depending on the vegetative stages of different plants, with higher levels in leaves and lower contents in bark at greater extent of height/diameter ratios. In spite of this last feature, the holly bark, taken as a whole, appears the best accumulative vegetal organ of zinc, cadmium, and lead. The soil acidity also influences the element amounts in plants and then the pH value remains a very important parameter for metals bioavailability.

The comparison between holly and wood anemone has shown a preferential cadmium accumulation by holly. Furthermore, the herbaceous wood anemone has a root system smaller in respect to holly and its identification is more difficult, especially out of its bloom season. On the other hand, holly is a more spread species. However, the results obtained suggest an interesting role of both species in the biogeochemical field, as useful sensors of geochemical anomalies.

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