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Bioaccumulation of Copper, Iron, and Zinc by *Pinus halepensis* (Miller)

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Differences in the response to heavy metal toxicity have been reported. Deciduous species seem to be more sensitive than conifers (Arduini et al., 1996) and portuguese dominant flora is essentially non-deciduous.

Although conifers were well represented in our ancient flora since Carboniferous ±320 million years ago (Pais, 1986, 1987) the landscape was man-modified with the introduction of both exotic species (*Eucalyptus* spp.) or with the formation of homogeneous or mixed stands in areas where these species were not naturally dominant which is the case of Serra do Monsanto, the green lung of Lisbon. This forest is dominated by conifer trees of the *Pinus* genus, such as *P. pinea*, *P. pinaster* and *P. halepensis* although *Cupressus* spp and *Eucalyptus* spp were also frequently observed.

Since this green area is crossed by several heavy traffic roads and particularly an important highway (A5) which links Lisbon to Cascais a study was undertaken in order to know the accumulation degree of some heavy metals (Cu, Fe and Zn) by the bark, needles and cone scales of *Pinus halepensis* (Miller) as well as in adjacent soils.

This is particularly relevant because *P. halepensis* cones remain on the tree for some years after they have ripened and the municipality initiated in 1998/1999 a programme to reduce the tree density per ha and cut off several *P. halepensis* trees. Dry weight and height of pine cones were also taken into account and cone scales were also removed, in order to evaluate the metal levels easily leached by water.

MATERIALS and METHODS

Bark (exterior layer with approximately 1cm thickness), needles and cones of *Pinus halepensis* (Miller) and soil from the top layer (0-2cm) from the adjacent areas were collected in four sampling points near the A5 a heavy traveled highway with an average daily traffic volume of 77201 (data from first semester of 2003). Samples were dried to constant weight. The

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determination of weight and height in dry cones was performed and classified according to the development state - closed, almost closed and totally open.

Plant material and soils were digested by the acid mixture HNO_3-$HClO_4$ (4:1) according Reboredo (1988), Cu, Fe, and Zn being determined by atomic absorption spectrometry (Unicam 939 model) fitted with deuterium background corrector. The operating conditions were those recommended by the manufacturer.

Water soluble levels were monitored in the bark and cone scales – one gram was shaken for 12h. at 50°C with 50ml of bidistilled water and the supernatant carefully removed and filtered through a Whatman GF/C fiber glass filter to a final volume of 50ml.

The adjustment of data through a linear regression was performed and the determination coefficient (R^2) was also achieved.

RESULTS AND DISCUSSION

Copper and zinc levels in soils exhibited a different pattern; for Cu, Station 1>Station 3>Station 4, Station 2, while for Zn Station 4>Station 2>Station 1>Station 3 (Table 2); however, the levels observed never exceed the critical limits. For iron a large variation in the soil levels was observed – between 0.8% and 2.4% (Table 2).

Threshold values for various soils whatever was their use, were for Cu 30-60 mg/kg and for Zn 100-200 mg/kg (Roth, 1997). Due to the large amounts of iron in the soils and the large variations generally observed no critical limit is generally defined.

The highest mean values of Cu, Fe and Zn in needles and bark of *Pinus halepensis* were generally observed in Station1, the closest local to the highway. Generally, the bark contains more Fe and Zn than the needles, except for Cu.

According to the Foliar Expert Panel (1995) Cu levels >7-20 μg/g, Fe levels >200-500 μg/g and Zn >50-100 μg/g are regarded as excessive. In needles of *P.halepensis* the high Cu level is 9.8 μg/g while for the bark the high Fe and Zn levels were 766 μg/g and 54.9 μg/g (Table 2), the last mean value being far above the upper critical limit which is 500 μg Fe/g.

Calculating the ratio soil concentration/tissue concentration for Cu and Zn using threshold values (TV) we have the following ratios (Table 1). These ratios do not give us valuable information generally in terms of a defined pattern of metal accumulation. Nevertheless, for copper soil/bark ratio a

Table 1. Soil/bark and soil/needle ratios for Cu and Zn.

	Bark		Needle	
	Cu	Zn	Cu	Zn
Station 1	4.0	1.7	3.2	3.4
Station 2	4.1	5.2	2.0	6.8
Station 3	6.3	3.4	6.3	4.6
Station 4	4.4	4.7	2.5	4.9
TV	4.3 – 3.0	2.0	4.3 – 3.0	2.0

certain and interesting constancy was observed. The ratios fall in the upper limit except a single case in which this limit is clearly exceeded (Table 1). There seems to exist a direct relationship between the Cu soil levels presented in the soil and those observed in the bark which is confirmed by statistical analysis.

When washing experiments were conducted with the bark, levels recovered differ greatly according to the element. Only 5.2% maximum of the total bark Fe was leached by bidistilled water, while for Cu those percentages vary between 21.9 and 42.4%. For Zn we have a minimum in Station 3 (only 9.0%) and a maximum in Station 1 (49.8%) – Table 2.

Cone scales were also removed and leached with bidistilled water and the results presented in Table 3. Taking into account the dry weight of each cone it was possible to evaluate the bulk of Cu, Fe and Zn, whatever was their maturity degree. The metal load of pine cones varies greatly as expected, with some extreme values in cones number 5 and 1.

When total mean values of Cu, Fe and Zn in soils were plotted against the average concentrations incorporated in the bark, obtained by the difference between the total mean value *minus* the water extractable mean value, R^2 positive values were observed (Figure 1). However, only in the case of Cu a high R^2 was detected (0,9561).

Several authors observed that the heavy metal content is high in areas very close to important highways (Crump and Barlow, 1980; Tong and Farrell, 1991), which agree with our findings.

Although carboxylic groups of the bark compounds may bind several metals (Sjöstrom, 1993) it is the porous structure of the bark which allows the particulate matter to adhere easily (Laaksovirta et al., 1976).

It seems that the studied elements are bound by different ways. Apparently, Fe is strongly bound to the bark of *P.halepensis* due to the low percentages recovered after leaching experiments. Copper is moderatly removed by water although Österas (2004) observed that the element is firmly bound to both the wood and the bark of Norway spruce after

Table 2. Average values of Cu, Fe, and Zn in the different compartments and sampling points.

Metal	Sample	Station 1	Station 2	Station 3	Station 4
Cu	Soil	31.8 ± 3.5	15.5 ± 0.4	27 ± 3.7	16.9 ±1.3
	Bark	7.9 ± 1.1	3.8 ± 0.4	5.2 ± 1.0	3.8 ± 0.4
	Bark *	2.0 ± 0.3 (25%)	1.6 ± 0.2 (42.4%)	1.1 ± 0.3 (21.9%)	1.5 ± 0.3 (39.5%)
	Needles	9.8 ± 0.3	7.8 ± 0.6	5.2 ± 1.0	6.8 ± 0.7
	Scales *	0.28	0.66	0.33	0.5
Fe	Soil**	2.4 ± 0.38	0.84 ± 0.12	2.2 ± 0.2	1.0 ± 0.2
	Bark	766 ± 102	180 ± 34.7	159 ± 24.2	160.0 ± 19.5
	Bark *	31.6 ± 6.8 (4.1%)	7.8 ± 0.7 (4.3%)	8.0 ±1.8 (5%)	8.4 ± 1.6 (5.2%)
	Needles	134 ±11.8	71.9 ± 14.1	86.2 ± 20.2	59.6 ± 6.1
	Scales *	4.0	7.3	2.9	2.4
Zn	Soil	93.8 ± 7.4	116 ± 14.3	83.5 ± 11.4	176 ± 21.8
	Bark	54.9 ± 7.9	22.4 ± 3.5	24.2 ± 2.8	37.1 ± 4.3
	Bark *	27.3 ± 5.6 (49.8%)	3.1 ± 0.41 (13.7%)	2.2 ±0.51 (9%)	9.2 ± 1.3 (24.8%)
	Needles	27.9 ±5.4	17.1 ± 2.8	18.1 ± 3.0	35.8 ± 5.8
	Scales *	1.3	1.5	0.9	0.45

The values are expressed as µg/g dry weight ± standard deviation or (**) as %/g dry weight ± standard deviation. (*) Mean values detected after leaching by distilled water. In brackets is the percentage of total content; n=3 except for scales n=2.

Table 3. Characterization of some pine cones and the water extractable levels in the scales.

	Station 1		Station 2		*Station 3*		*Station 4*	
Cone number	*6*	*5*	*1*	*13*	*4*	*11*	*10*	*12*
Dry weight *	21.8	6.9	32.3	18.8	18.1	21.4	23.2	15.1
Height **	7.6	7.6	9.9	9.4	7.3	8.4	7.8	8.1
Cu scale content ***	0.39	0.18	0.9	0.42	0.26	0.4	0.3	0.7
Probable Cu content of the whole cone★	8.5	1.2	29.1	7.9	4.7	8.6	7.0	10.6
Fe scale content ***	4.2	3.7	10.8	3.8	2.9	2.9	2.2	2.5
Probable Fe content of the whole cone★	91.6	25.5	349	71.4	52.5	62.1	51.0	37.8
Zn scale content ***	1.2	1.4	1.8	1.2	1.1	0.7	0.4	0.5
Probable Zn content of the whole cone★	26.2	9.7	58.1	22.6	19.9	15.0	9.3	7.6

*dry weight in grams; **height in cm; ***µg/g dry weight; ★Mean values expressed in µg.

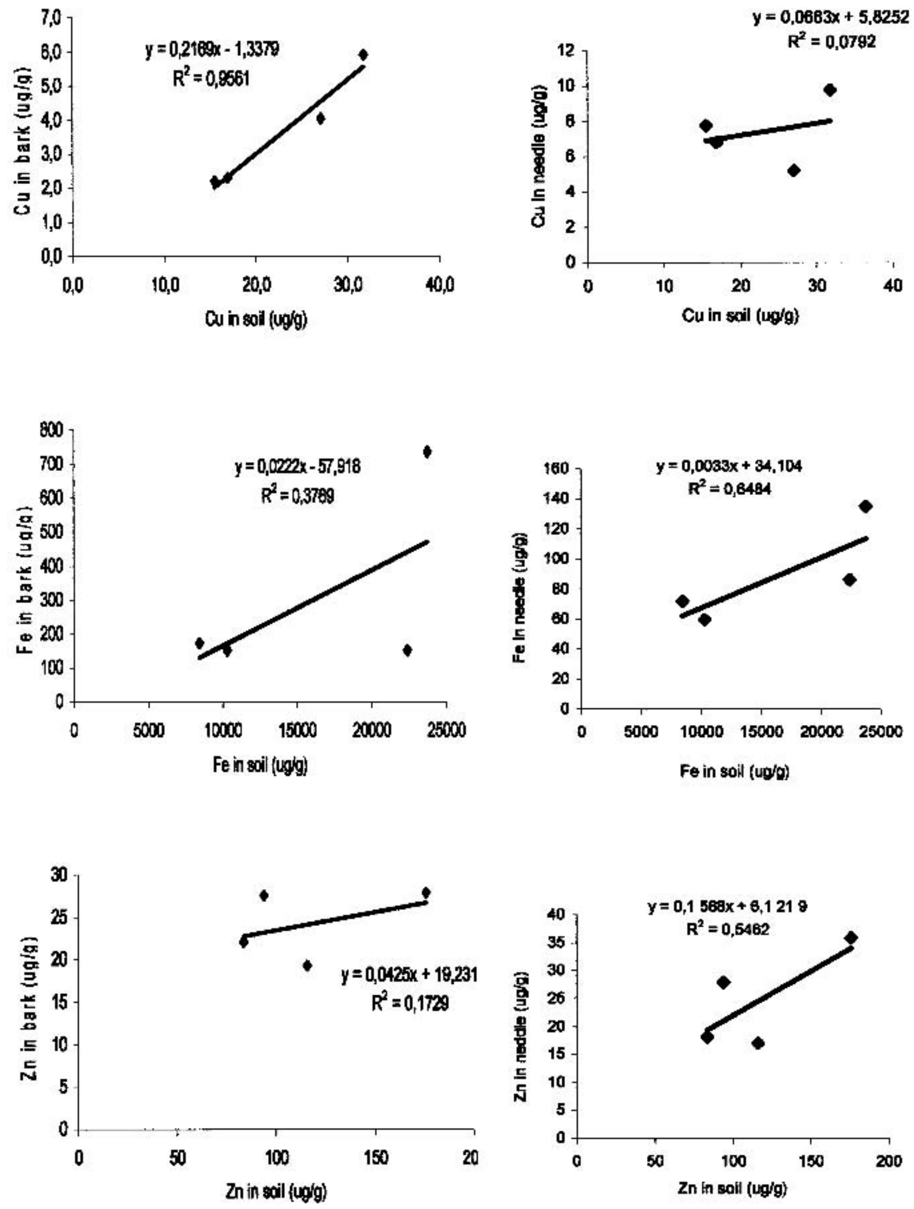

Figure 1. Cu, Fe and Zn mean values in soils *Vs.* mean values incorporated in the bark of *P. halepensis* in different Stations - mean values incorporated were obtained by the difference between the total mean value minus the water extractable mean values (left column) and Cu, Fe and Zn mean values in soils *Vs.* mean values in needles of *P. halepensis* in different Stations (right column).

extraction with EDTA, while Tong and Farrell (1991) observed that 58% of the Cu in the pine needles (*Pinus strobus*) was readily leached by water.

Concerning Zn, the degree of water leaching changes from weak (9% in Station 3) to good (49.8% in Station 1); a different pattern was observed by Österas (2004) - Zn was loosely bound after extraction with EDTA.

Apart the differences among species and within compartments in the same species, the dry deposited particles on the leaves contained significant fractions larger than 5 μm in diameter (Salomons and Forstner, 1984) and the relative solubility of trace metals increases with the decrease of particle size.

Our data confirm the bark as the main receptor target of atmospheric pollution as also noted by Laaksovirta et al., (1976) and Hagemeyer and Lohrie, (1995), although tree rings of *Pinus echinata* may also furnish valuable information (Baes and McLaughlin, 1988). Comparatively with needles and scales, bark may function also as a source of pollutants to the soils (due to the leaching process by rainfall).

The variations of Cu in Europe show that the Cu levels in needles of pine and spruce stands in Bulgaria are above the critical levels of 7 μg Cu/g, occurring the same in Italy in many cases. Otherwise, the wide variation of the average values among different countries is particularly relevant in the case of Cu and Pb (Rademacher, 2003). In our study the Cu levels of *P.halepensis* needles are above the critical levels in 50% of the cases.

Taking into account the Fe and Zn content of all the needles and leaves of all the trees of the beech, oak, pine and spruce groups from different european countries, it can be concluded that the highest mean values for Fe (>150 μg/g) are found in Greece and Spain and the lowest (<50 μg/g) in Sweden, Austria and Finland. For Zn the highest mean values (>40 μg Zn/g) are found in Poland and Finland while the lowest (±25 μg/g) in France, Belgium, United Kingdom, Portugal and Czech Republic (Rademacher, 2003).

The calculation of metal load in pine cones of *Pinus halepensis*, is based on the assumption that the concentration within the cone scales are identical, which may not be the case. It seems the deposition of metals was independent from the maturity degree of the cone – mature cones with large areas (as it happens for cone number 10) may support the same load (or even less) than young and almost closed cones (cone nº1), which explain the large variations observed (Table 3).

Our soil/bark ratios are in the upper limit generally in 75% of the cases indicating that soil Cu load may be well correlated with the bark content which is assessed by the statistical analysis - R^2 (0,9561). When the concentration of the soil increases also does the bark concentration. Although positive but weak R^2 values were observed for Fe and Zn (Figure 1), high positive values were detected for the same elements when soil/needle ratios were correlated (Figure 1) but not for copper.

Since heavy metals accumulate over a long period we may monitor the long-term accumulation by the same trees and in the same area especially in target compartments even knowing that ten European countries have reduced their total emissions of fine particulates by more than 20% during the period 1990-2000, while Portugal and Greece increased their emissions (10%) in the same period (European Environment Agency, 2003). Emissions of Cd, Hg and Pb have also been reduced through all over the Europe although no data is available for our country.

REFERENCES

Arduini I, Godbold DL, Onnis A (1996) Cadmium and copper uptake and distribution in mediterranean tree seedlings. Physiol Plantarum 97: 111-117

Baes CF, McLaughlin SB (1988) Trace metal uptake and accumulation in trees as affected by environmental pollution. In: Effects of atmospheric pollutants on forests, wetlands and agricultural ecosystems. TC Hutchinson and KM Meema edit., Springer-Verlag (Berlin, Heidelberg).

Crump DR, Barlow PJ (1980) A field method of assessing lead uptake by plants. Sci Tot Environ 15 : 269-274

EC-UN/ECE (1995) Foliar Expert Panel. Symposium paper ICP-forest, Wien, 6-8 November.

European Environment Agency (2003) Air Pollution in Europe, 1999-2000, Topic Report, 84 pp.

Hagemeyer J, Lohrie K (1995) Distribution of Cd and Zn in annual xylem rings of young spruce trees (*Picea abies* L. Karst.) grown in contaminated soil. Trees 9: 195-199

Laaksovirta K, Olkkonen H, Alakuijala P (1976) Observations on the lead content of lichen and bark adjacent to a highway in southern Finland. Environ Pollut 11: 247-255

Osteras AH (2004) Interactions between calcium and heavy metals in Norway spruce. Ph D Thesis, Stockholm University, 52 pp.

Pais J (1986) Evolution de la vegetation et du clima pendant le Miocene au Portugal. Ciências da Terra, Lisboa, nº 8, 179-191

Pais J (1987) Macrorestos de Gimnospérmicas dos diatomitos de Rio Maior (Portugal). Edições Delta, Lisboa, pg 51-66.

Rademacher P (2003) Atmospheric heavy metals and forest ecosystems. Work Report of the Institute for World Forestry, Hamburg, 19 pp.

Reboredo F (1988) Alguns aspectos sobre a acumulação de Fe, Cu e Zn por *Halimione portulacoides* (L.) Aellen. Ph D Thesis, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, 165 pp

Roth L (1997) Landesanstalt fur Umweltschutz Baden-Wurttemberg, Grenzwerte. Kennzahlen zur Umweltbelastung und in der EG (Tabellenwerk). Ecomed. Verlag, Lansdsberg. 480 S.

Salomons W, Forstner U (1984) Metals in the hydrocycle. Springer-Verlag (Berlin, Heidelberg).

Sjostrom E (1993) Wood chemistry. Fundamentals and Applications. 2nd edition Academic Press, Orlando, Florida.

Tong STY, Farrell PM (1991) The concentration profile of heavy metals in an urban forest. Environ Technol 12: 79-85