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(with emphasis on trace elements)

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this Portuguese region. The quantity and variety of minerals present in the honeys was determined by instrumental neutron activation analysis (INAA) and the relationships to its contents in the soil and plants investigated. Results were also compared to the reported by Freitas *et al.*

Biomonitoring Metal Deposition in an Old Abandoned Mine Area

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The São Domingos mine, first exploited in Roman times, was a major sulphur pyrites and copper mine, with both underground and open sky exploitations. Since the closing of the mine, in 1966, there have been no attempts to manage metal soil contamination and acid waters existent in the area. In addition, the air contamination by metals was never assessed, because since the mine was closed there have been no other contamination sources in the area. However, metal soil contamination is expected to have an environmental impact on vegetation, animals, and other ecosystem components through soil particle resuspension and deposition after wind or rain action. In the present study, a biomonitoring survey was performed to determine the levels of metals in the vegetation, covering not only the areas of extraction of ore bodies, but also ore processing plants and mine product transportation. The sampling was performed twice in 39 sites, with one-year interval to assess temporal variations in the contamination levels. The selected biomonitor was an epiphytic lichen *Ramalina fastigiata* (Pers.) Ach., sampled on *Quercus ilex* L. and *Olea europaea* L. trees. Each sample was analysed for total element concentration of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. Cellular fractions (surface, wall-bound and intracellular) of Ca, K and Mg were also determined using a sequential extraction protocol to determine possible cell-membrane malfunction/disruption in lichens caused by metal contamination. Results, analysed using multivariate data analysis and geostatistical interpolation, show that there is a spatial gradient for As, Pb, Cu and Fe, with higher values observed near the exploitation area and the ore processing industrial plant. For the other elements, such as Cr, Mn, Ni, V, Zn, the distribution seems to be independent of the mining impact, resulting probably from natural contamination by soil particles. In general, the distribution pattern of the elements is similar when comparing results from both sampling dates, revealing a persistent environmental contamination over time. On the other hand, it appears that the contamination levels did not have a significant impact on the membrane permeability of the lichen organisms.

Atmospheric Pollutant Monitoring by Neutron Activation Analysis of Epiphytic Lichens

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The epiphytic lichen *Canoparmelia texana* was used to monitor atmospheric pollution in the São Paulo

metropolitan area, SP, Brazil and to obtain distribution maps of element concentrations. From July 2003 to February 2004, lichens were collected from the bark of trees in 23 sites all near automatic monitoring stations belonging to the Environmental Protection Agency of the State of São Paulo and analyzed by instrumental neutron activation analysis. For the analyses, lichen samples were first cleaned by examining them under a stereomicroscopy to remove foreign materials, and then immersed in purified water for about 3-5 minutes. Then the samples were freeze-dried and ground to a powder using a vibratory micro mill. Samples and synthetic element standards were irradiated for long and short periods at the IEA-R1 nuclear research reactor under thermal neutron flux of about $5 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$. The induced gamma activities of the irradiated samples and standards were measured using a HGe detector coupled to a gamma ray spectrometer. Concentrations of the elements As, Ba, Br, Cl, Co, Cr, Cs, Fe, K, La, Mn, Mo, Na, Rb, Sb, Sc, Se, U and Zn were determined in the lichens and these results were submitted for sampling sites classification by cluster analysis. The cluster analysis confirmed the site groups of different levels of pollution due to industrial and vehicular emissions. In the distribution maps of element concentrations, higher concentrations of elements were observed in the vicinity of industries. High concentrations of elements found in lichens from the Santo André sites may be associated to the emissions from industries and a petrochemical complex located in this region. The highest concentration of Co found in lichens from São Miguel Paulista site is probably due to the emission from a metallurgical processing plant that produces this element. The accuracy and the precision of the results were evaluated by the analyses of certified reference materials IAEA 336 Lichen and INCT-MPH-2 Mixed Polish Herbs. Results obtained for these certified reference materials showed a good precision and a good agreement with the certified values for most elements. (Acknowledgments to FAPESP and CNPq).

Heavy Metals Effects on the Physiology and Anatomy of Lichen Photobionts: Isolated Cultures of *Trebouxia* or Lichenized from *Ramalina farinacea* (Spain) and *Usnea amblyoclada* (Argentina)

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Some lichens are sensitive to heavy metals such as Cu, Zn, Pb, Cd or Hg. The absorption of these pollutants by lichen photobionts produces mainly a photooxidative stress. The principal objectives of this study were to assess differences in physiological and anatomical responses to Cu and Pb of two lichen species, *Ramalina farinacea* (L.) Ach. and *Usnea amblyoclada* (Mull. Arg.) Zahlbr., and isolated photobionts of *Trebouxia erici* Ahmadjian (SAG 32.85 = UTEX 911). Solutions of CuSO_4 and $\text{Pb}(\text{NO}_3)_2$ were applied to colonies of algae growing on cellulose acetate disks, in agar culture medium, whereas 50 mg of lichen thalli were incubated in 50 ml of solutions for 30 min. Heavy metal concentrations used were 5, 2.5, 1 and 0.1 mM. Both, algae and lichens, were maintained for 48 h at 20°C under a 12h photoperiod and $30 \text{ mol m}^{-2} \text{ s}^{-1}$ artificial irradiance. As well, two weeks old algal disks were changed to agar cultures medium containing heavy metals solutions, and maintained there for a week. Modulated chlorophyll *a* fluorescence emission measurements were carried out in laboratory using a portable fluorometer PAM-2000 (Walz, Germany). In lichen thalli, a significant decrease in the activity of the photosystem II was evaluated, as shown by the reduction in the values of F_v/F_m (maximal quantum yield) and Φ_{PSII} (quantum efficiency of PSII). This reduction seems to be correlated to the increasing metal concentrations and was higher in *R. farinacea* than in *U. amblyoclada*.