ATMOSPHERIC NITROGEN AND SULPHUR DRY DEPOSITION INTO FOREST STANDS

C. MARQUES CORREIA[1], J. CONSTANTIN[1], A. IBROM[1], G. GRAVENHORST[2],

[1] Department of Biological and Environmental Engineering,
University of Trás-os-Montes e Alto Douro, Portugal
[2] Faculty of Forestry and Forest Ecology, University of Göttingen, Germany

Abstract

In this study the dry input of atmospheric particles into forest stands is quantified. The chosen method, a wash-off-method using the natural leaf surfaces as collectors of the dry deposition, was standardized on spruce (*Picea abies* (L.) Karst.) and experimentally applied to other tree species, forest stands, and climatic regions. The field measurements were achieved predominantly in a spruce stand at Solling (Germany) and in a pine and eucalyptus stand at Herdade da Espirra (Portugal). To judge the trend of air pollution unaffected by normal meteorological deviations, continuing measurements were taken for several years at the main research site at Solling. At this site, the concentration developments of NO_3^-, NH_4^+, and SO_4^{2-} show an average annual pattern that varies in distinction according to the components. A comparison between the research sites in Germany and Portugal shows that the ion-balance of the aerosol at Solling is strongly dominated in the fine fraction by ammonium and sulphate, while at Herdade da Espirra the composition of ions is dominated by sea salt particles. Dry particle deposition constitutes an important part of the total matter input into forest ecosystems. Just the nitrogen input into Solling only by dry deposition (from particle, mist, and gas deposition) with about 30 kg N ha^{-1} a^{-1} already exceeds the critical load of 20 kg N ha^{-1} a^{-1} by far. Only a quick and drastic reduction of sulphur and nitrogen emissions could stop the further increase of the nutrient imbalance and the progressing acidification of this ecosystem.

1. INTRODUCTION

Evaluation of the ambient concentrations of substances in the lower atmosphere is important, but those values alone do not allow deeper conclusions on the damaging impacts of these compounds on a forest stand. More significant is the amount of deposition an ecosystem is exposed to. Forest ecosystems are particularly important for the removal of atmospheric aerosols. The forest canopy structure and geometry, together with the surface roughness properties favour that trees act as an important sink for particles from the atmosphere.

In order to evaluate the maximum tolerable deposition rates with critical loads, below which there will be no damage to a forest ecosystem, it is necessary to quantify the input of atmospheric pollutants on stand level. Until now, a weakness of all investigations on the total deposition rate into ecosystems has been that the portion of the nutrients and harmful substances dry deposition could only be assessed inadequately and hardly separated from the wet deposition. Thus far, within dry deposition, that of gaseous acid compounds has received far more attention in research despite the importance of the dry deposition of particles. The complexity of deposition processes of particles implies the difficulty to find appropriate methods for field measurements.

Together with wet deposition, particle dry deposition is responsible for delivering atmospheric loads of such compounds as SO_4^{2-}, NO_3^- and NH_4^+ to ecosystems. High deposition loads of sulphur and nitrogen compounds can have strong effects on the structure and function of the ecosystem (among others e.g. [1]). This paper summarizes data specific to sulphur and nitrogen air concentrations and particles dry deposition measured at three different forest stands.

2. MATERIALS AND METHODS

The field measurements of this study were carried out predominantly in a Norway spruce stand (*Picea abies* (L.) Karst.) at Solling (51°46’ N, 9°35’ E), a north German low mountain range using a 50 m high research tower. In Portugal, the field measurements were achieved in a pine (*Pinus pinea* L.) and eucalyptus (*Eucalyptus globulus* Labill.) stand at Herdade da Espirra (38°38' N, 8°36' W).

A wash-off-method for the direct on-site-measurement of the dry deposited trace material on living branches was chosen. The ion exchange processes occurring on natural branches with a wash-off-method were quantified through immediate sequential washings. At the beginning of a measuring period randomly selected, branches from the sun crown were washed on the tree in a Teflon bag by steady manual shaking. At the end of each sampling period the measuring twigs were then washed four times immediately sequential by four minutes, respectively, with demineralised water in Teflon bags. For the **conifers**, the element specific portion of leaching in the first washing can be extrapolated by simplex-logarithmic transformation of the element concentrations from the sequential washings two to four; for **eucalyptus,** the arithmetic mean of the extractions two to four was calculated [2]. Additionally at Solling, during each sampling period in 1993 and 1994, ten of the measuring twigs were washed with an acidified washing solution from demineralised water that was acidified by 0.05 M hydrogen chloride (HCl) to a pH-value of 4.2. Leaf surface areas were determined by an automatic image analysing system DIAS (**D**igital **I**mage **A**nalysis **S**ystem, Fa. Delta-T Devices Ltd, Cambridge, England) [3] and, afterwards, the leaves were dried to determine the dry weight for each measuring period.

The atmospheric concentrations of particles in Solling were measured continuously above the canopy by a six stage cascade impactor (*Sierra*-Model) and by a Teflon filter pack system. In each stand, the atmospheric aerosol concentration was measured with an impactor in close proximity to the selected branches during each sampling period.

All samples were analysed for pH and electrical conductivity. The samples were analysed for the water soluble parts of Ca^{2+}, $Mg^{2+,}$ and Mn^{2+} with an Inductive-Coupled Plasma Atomic Emission Spectroscope; the heavy metals ions Cd^{2+}, Cu^{2+} and Pb^{2+} were determined by Atomic Absorption Spectrometry. The ascertainment of the soluble ions Na^+, K^+, NH_4^+, Cl^-, NO_3, and SO_4^{2-} was carried out with an ion chromatograph.

In order to calculate also the gas dry deposition of NO_3^-, NH_4^+, and SO_4^{2-} ,a resistance model was used [2]. Evaluation, statistical analysis, and graphical representation were performed with SAS (**S**tatistical **A**nalysis **S**ystem).

3. RESULTS AND DISCUSSION

3.1. Atmospheric concentrations

The concentrations of trace substances (NO_3^-, NH_4^+ and SO_4^{2-}) at Solling have not changed significantly during the last five years [2]. On this site, the concentration developments of nitrate, ammonium, and sulphate show an average annual pattern that varies in distinction according to the components.

The annual variation of the nitrate concentrations indicates a pronounced maximum in early spring from February to April and a second one slighter in late autumn (November). The minima are found during the summer months May to October and in December/January. The lowest concentrations are measured in December. In the aerosol at Solling, nitrate is found in considerable fractions in both the coarse and fine mode (bimodal distribution). The seasonal variation is characteristic for nitrate fine particles that are mainly balanced by ammonium. A strong positive correlation between these two trace elements is also indicated by a Pearson correlation coefficient of r = 0.87 within a confidence belt of 99.9%. Therefore a decrease in the concentrations of NO_3^- during the warmer months can be observed as a result of a rise in the volatilisation of NH_4NO_3 in the secondary aerosol. However, with coarse particles similar processes do not occur because nitrate is mainly associated with sodium, forming a more stable compound [4,5].

The annual trend of the ammonium concentrations indicates a maximum in March/April. A more or less pronounced secondary maximum was observed in autumn. Ammonium itself is not emitted; the whole ammonium is just converted in the atmosphere out of ammonia. The high ammonium concentrations during spring and autumn indicate an increased conversion from NH_3-N to NH_4-N in the air. The ammonium concentration in the particles is strongly correlated with the sulphate concentration (r = 0.80 within a confidence belt of 99.9%). But the molar ratio from ammonium to sulphate exceeds in numerous measurements a factor of 2. In fact the excess of ammonium at both measuring sites (in Germany: NH_4^+/SO_4^{2-} = 1.18 eq/eq; in Portugal: NH_4^+/SO_4^{2-}= 1.24 eq/eq) indicates the presence of nitric acid (HNO_3) in the aerosol, which also associates with ammonium.

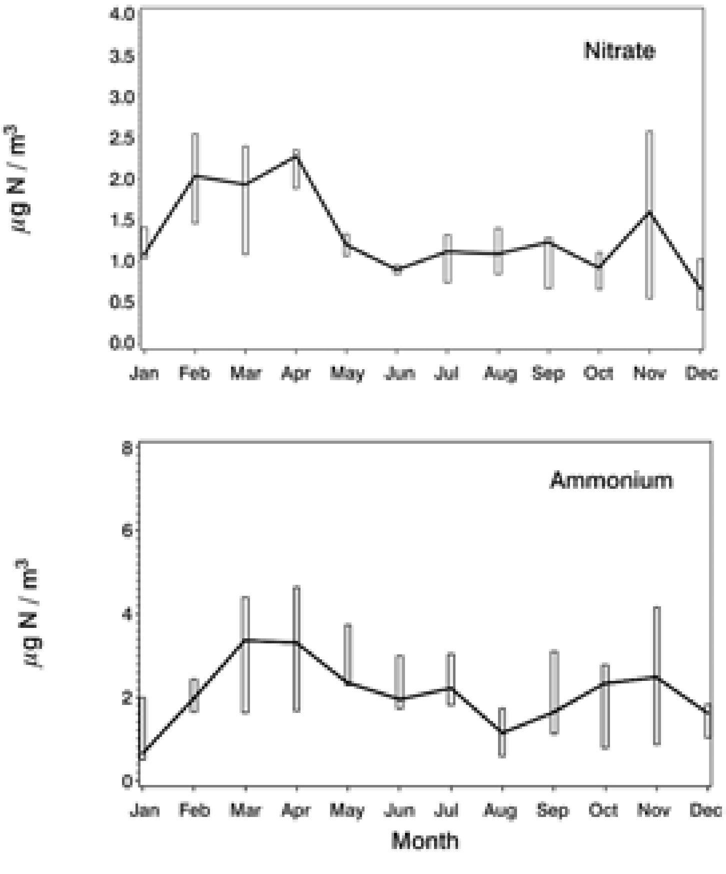


FIG. 1. Seasonal variation of air concentrations of nitrate (sampling with impactor for 67 measuring periods) and ammonium (with filter pack system for 84 measuring periods) at Solling, shown by monthly averages. Boxes represent the 25th and 75th percentile.

TABLE I. MEAN AIR CONCENTRATIONS OF SEVERAL TRACE ELEMENTS IN THE AEROSOL OF THE F1 STAND AT SOLLING (GERMANY) AND AT HERDADE DA ESPIRRA (PORTUGAL)

Measuring site	Trace element		
	NO_3^- (μg N m^{-3})	NH_4^+ (μg N m^{-3})	SO_4^{2-} (μg S m^{-3})
Solling	1.12	3.90	3.80
Herdade da Espirra	0.51	0.85	0.78

He electrolytic composition at Solling is thus dominated by ammonium and sulphate. The other ions are significantly lower concentrated. Only 26% of the equivalents account for other ions. A share of 4% for the cation equivalents is contributed to hydrogen ions. This small share of acids refers to neutralization and buffer capacities of the aerosol. However, at Herdade da Espirra, the ion composition is dominated by sea salt particles. Already 57% of the sum of the equivalents account for sodium and chloride and just 24% for ammonium and sulphate. This may be due to the short distance to the near Atlantic (30 km inland, while for Solling the shortest distance to the coast is 250 km). Lower values for nitrate indicate a small loading of traffic (Table I). The concentrations of particles at Herdade da Espirra have been found to lie considerably beyond the respective figures in less industrialized Portuguese coastal areas [6,7]. The aerosol of Solling can be characterized as a transition between a background aerosol and a continental one that contains a greater particle number [2].

Although it was shown in a differentiated rating of the atmospheric pollution [2] that the average air concentrations of the gaseous trace compounds SO_2, NO_2, and NH_3 lay beyond those concentrations known as physiologically harmful, the high input rates of SO_4^{2-} and NH_4^+ into the studied ecosystems during the dry seasons reveal that, besides the already existing critical levels for SO_2 and NH_3 there should be long term critical levels for the air concentrations of SO_4^{2-} and NH_4^+, too. The atmospheric concentrations of HNO_x and NO_3 aerosol, which are not limited by any critical levels or guidelines, are more important for the stability of these ecosystems than the NO_x concentrations (as NO and NO_2), for which legally binding limits do exist in both countries, and critical levels have been suggested.

3.2. Dry particle deposition

The quantification of atmospheric particle deposition is of great importance for understanding the impacts of these inputs into ecosystems, for determining when critical loads are exceeded, and for demonstrating, by analysis of multiannual trends, the efficiency of emissions reduction policies on the regional scale.

For Solling, a comparison between results of former measurements by throughfall experiments and those obtained in this study with acidified washing solution (of the same pH 4.2), shows a significant reduction of the atmospheric sulphate inputs (Table II). In course of the desulphurization of coal-fired power stations in the middle 1980s, the input rates for sulphur strongly decreased. Between 1980 and 1994, it is observed a considerable reduction of nitrate deposition as well. This can be caused in part by the observed alteration in nitrate mass size distributions. During the measurements of Höfken et al. [8] the median mass diameter (MMD) for nitrate was calculated as 2.4 μm, while a MMD of 1.9 μm during the deposition measurements of this study was found. This shifting to smaller particle sizes, which deposit slower, could be responsible for the large difference in the nitrate inputs between this study and the results reported in [8].

TABLE II. MEAN DEPOSITION OF SEVERAL TRACE ELEMENTS BY DRY PARTICLE DEPOSITION INTO THE F1 SPRUCE STAND AT SOLLING (GERMANY), CALCULATED FROM WASH-OFF-MEASUREMENTS WITH ACIDIFIED WASHING SOLUTION (WITH ONE STANDARD ERROR INDICATED IN PARENTHESES). FOR COMPARISON RESULTS OF FORMER MEASUREMENTS BY THROUGHFALL EXPERIMENTS AT THE SAME SITE ARE SHOWN

Solling	Trace element (kg/ha · 0.5a)		
	NO_3-N	NH_4-N	SO_4-S
1993/94 [3]	2.5 (±0.1)	1.6 (±0.5)	1.3 (±0.2)
1990/91 [2]	2.6	1.8	1.6
1980 [1]	9.0	2.6	28.8

1) The calculated median of the H^+-concentrations in open air precipitation corresponded to a pH of 4.1–4.2 [8].

2) Annual data were halved here for temporal adjustment; for sulphate particles, Ref. [3] assumed a deposition velocity of 0.5 cm s^{-1}; the median of the measured H^+-concentrations in open air precipitation corresponded to a pH of 4.2.

3) This study. The acidified washing solution was adjusted to pH 4.2. This complies with the calculated median of the H^+-concentrations of the open air precipitation at Solling for 1989–1991 [3].

Figure 2 shows the particle transfer rates for sulphur in the spruce stand F1 at Solling. The atmospheric inputs of sulphur by wet and dry deposition are of almost the same magnitude with a slight predominance of the dry deposition (58% of the total deposition). Gas deposition attains a share of nearly 50% in the dry sulphur deposition; the particle input on it reaches 20%. The sulphur balance at Solling is negative. The annual loss of stocks for sulphur of –17 kg ha^{-1} a^{-1} is shown as calculated balance item in Figure 2. This indicates that the ecosystem is at a stage of sulphate solution following a progressive soil acidification (for further explanation, see [10,11,12]).

The total nitrogen input into the forest ecosystem Solling is clearly dominated with 67% by the dry deposition (Marques et al., 2000, submitted). Just the particle deposition contributes with 27% of the whole dry deposition to the matter balance at this stand. Therefore it was and remains necessary to pay more attention to the (dry) deposition of particles. The nitrogen balance at Solling is positive. The annual accumulation of stocks for nitrogen by 19 kg ha^{-1} a^{-1} corresponds to 42% of the total deposited nitrogen. The total nitrogen input into the F1 spruce stand at Solling greatly exceeds the critical load for managed forests on acidic habitats of 20 kg N ha^{-1} a^{-1} (according to [14]) with respect to the critical load calculated [15] for Solling of just over 14 kg N ha^{-1} a^{-1}. These results indicate a very unstable situation for this whole forest ecosystem. Nitrogen input by dry deposition alone (from particle, mist, and gas deposition) at about 30 kg N ha^{-1} a^{-1} is already far in excess of the critical load, and this is without even considering the additional load by wet deposition which amounts to 15 kg N ha^{1} a^{-1}. These findings are of greatest ecological importance, as the damage to the stability of the forest ecosystem caused by increased nitrogen input is considerable. Only a quick and drastic reduction of sulphur and nitrogen emissions could counteract this increasing nutrient imbalance and progressive acidification of this ecosystem. There is no alternative to the reduction of acid compounds and nitrogen by air purification policy.

In the spruce stand at Solling the electrolytic composition in the dry deposition is dominated by ammonium resp. nitrate. Although NH_4^+ is the prevalent form of nitrogen in the atmosphere, NO_3^- dominates the dry deposition fluxes. By contrast to their air concentrations, nitrate is deposited in higher amounts than sulphate. This inversion is based on the fact that NH_4^+ and SO_4^{2-} are mainly associated with fine particles; both are found in the aerosol at Solling with a MMD of 0.5 µm, while nitrate with a MMD of 1.9 µm is deposited much more effectively on the needle surfaces than fine particles. The deposition rate observed in Solling for NO_3-particles lies in the order of magnitude of the coarse. particles (Table III). Since the deposition rate is heavily dependent on the particle size distribution, it is perfectly conceivable in the case of bimodal distributed particles that the coarse fraction is deposited more quickly and this leads to these higher values. By contrast, at Herdade da Espirra, the ion composition in the dry deposition is dominated by sea salt particles, like it was also shown for the atmospheric aerosol. According to other studies as well (e.g. [16]) the deposition of sulphur and nitrogen were substantially altered by tree species and stand structure, which is in agreement with our observations. The found differences between the two conifer stands can partly be explained by the density of the trees in the population, as the all-sided leaf area index in the spruce stand was found to be 20.5 compared to 5.7 m^2/m^2 in the pine wood. The leaf properties like size, form, orientation, and surface roughness have a strong influence on the different capacities, especially in capturing fine particles. Higher micro roughness favours the capture of a large amount of aerosol. The reasons for this are micro turbulences of the air stream in the sphere of the quasi-laminar boundary layer around the leaves.

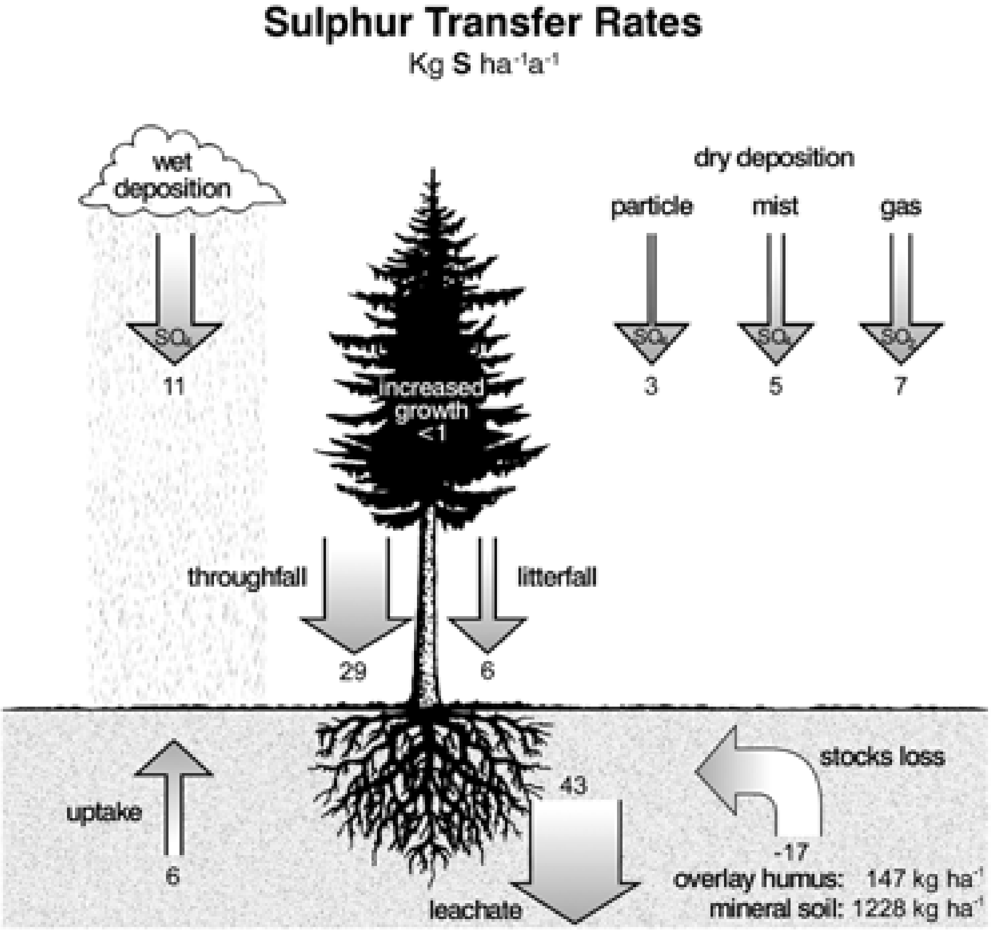


FIG. 2. Sulphur transfer rates in the spruce stand F1 at Solling. Dry particle and gas deposition (this study); mist deposition according to [9]; wet deposition and throughfall [3]; Niedersächsische Forstliche Versuchsanstalt [NFV], 1998, written communication); internal cycle [10–12]; soil leaching and stocks [10], stocks change as calculated balance item.

TABLE III. MEAN DEPOSITION OF SEVERAL TRACE ELEMENTS AT SOLLING (GERMANY) AND AT HERDADE DA ESPIRRA (PORTUGAL) (WITH ONE STANDARD ERROR INDICATED IN PARENTHESES)

Measuring site	Tree species	Trace element (μg m^{-2} h^{-1})		
		NO_3-N	NH_4-N	SO_4-S
Solling	*Picea abies*	54.3 (±14.6)	33.8 (±7.5)	26.6 (±2.6)
Herdade da Espirra	*Pinus pinea*	6.4 (±0.7)	—	24.7 (±5.4)
	Eucalyptus globulus	4.1 (±1.9)	9.8 (±3.2)	8.1 (±1.4)

4. CONCLUSIONS

For the characterization of the fluxes onto the different parts of the crown during particle deposition, wash-off measuring techniques are quite useful. With this improved technique to quantify the dry deposition of particles into forests, the input pathways and input rates can be assessed more detailed in space and time.

A comparison between the research sites in Germany and Portugal shows that the ion-balance of the aerosol at Solling is strongly dominated in the fine fraction by ammonium and sulphate, while at Herdade da Espirra the composition of ions is dominated by sea salt particles. It was found significantly higher rates of dry deposition fluxes of NO_3^- and SO_4^{2-} in the studied coniferous woods (*Picea abies* (L.) Karst. and *Pinus pinea* L.) than in the eucalyptus stands (*Eucalyptus globulus* Labill.). The spruce wood proved to be the more efficient sink for all components. The observed differences between the two conifers could also be explained by the density of trees in the population, because the all-sided leaf area index found in the spruce stand amounted to 20.5 compared to 5.7 m^2/m^2 found in the pine wood.

The dry particle deposition flux constitutes an important share of the total atmospheric flux into the forest ecosystem. The nitrogen input into Solling only by dry deposition (from particle, mist, and gas deposition) with about 30 kg N ha^{-1} a^{-1} already exceeds the critical load of 20 kg N ha^{-1} a^{-1} by far, and this is without even considering the additional load by wet deposition which amounts to 15 kg N ha^{-1} a^{-1}. These findings are of greatest ecological importance, as high deposition loads of nitrogen and sulphur compounds contribute to the damage to the stability of the forest ecosystem. Only a quick and drastic reduction of sulphur and nitrogen emissions could stop the further increase of the nutrient imbalance and the progressing soil acidification of this ecosystem.

ACKNOWLEDGEMENTS

The authors would like to thank Luis Gabriel for illustrations assistance. We also wish to express our appreciation to the working group of Prof. João Pereira at the Department of Forestry Engineering, Technical University of Lisbon, and to Prof. Rosa Paiva and her working group at the Faculty of Sciences and Technology, New University of Lisbon, for their help and necessary research facilities. This work has mainly been supported by the "Junta Nacional de Investigação Científica e Tecnológica" (**JNICT**), Government Department of Sciences and Technology, Portugal.

REFERENCES

[1] SMITH, V.H., TILMAN, G.D., NEKOLA, J.C., Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution **100** (1999) 179–196.

[2] MARQUES, M.C., Eintrag von luftgetragenen partikelgebundenen Spurenstoffen in Wälder durch trockene Deposition, Berlin: Wissenschaftlicher Verlag Berlin (1999).

[3] IBROM, A., Die Deposition und die Pflanzenauswaschung (Leaching) von Pflanzennährstoffen in einem Fichtenbestand im Solling. Dissertation. Institut für Bioklimatologie, Universität Göttingen. (Berichte des Forschungszentrums Waldökosysteme der Universität Göttingen, Reihe A, Band 105) (1993).

[4] HARRISON, R.M., PIO, C.A., Major Ion Composition and Chemical Associations of Inorganic Atmospheric Aerosols. Environmental Science and Technology **17** (1983) 169–174.

[5] HARRISON, R.M., PIO, C.A., Size-differentiated composition of inorganic atmospheric aerosols of both marine and polluted continental origin. Atmospheric Environment **17** (1983) 1733–1738.

[6] PIO, C.A., SANTOS, I.M., ANACLETO, T.D., NUNES, T.V., LEAL, R.M., Particulate and gaseous air pollutant levels at the Portuguese west coast. Atmospheric Environment **25A** (1991) 669–680.

[7] PIO, C.A., CASTRO, L.M., CERQUEIRA, M.A., SANTOS, I.M., BELCHIOR, F., SALGUEIRO, M.L., Source assessment of particulate air pollutants measured at the Southwest European coast. Atmospheric Environment **30** (1996) 3309–3320.

[8] HÖFKEN, K.-D., GEORGII, H.-W., GRAVENHORST, G., Untersuchungen über die Deposition atmosphärischer Spurenstoffe an Buchen- und Fichtenwald. Berichte des Instituts für Meteorologie und Geophysik, Universität Frankfurt am Main; Bericht Nr. 46 (1986).

[9] CONSTANTIN, J., Stoffeinträge in ein Fichtenwaldökosystem durch Deposition luftgetragener Partikel und Nebeltröpfchen. Dissertation. Institut für Bioklimatologie, Universität Göttingen. (Berichte des Forschungszentrums Waldökosysteme der Universität Göttingen, Reihe A, Band 106) (1993).

[10] MEIWES, K.J., Der Schwefelhaushalt eines Buchenwald- und eines Fichtenwaldökosystems im Solling. Dissertation. Institut für Bodenkunde und Waldernährung, Universität Göttingen. (Göttinger Bodenkundliche Berichte, Band 60) (1979).

[11] MATZNER, E., Der Stoffumsatz zweier Waldökosysteme im Solling. Habilitationsschrift. Institut für Bodenkunde und Waldernährung, Universität Göttingen. (Berichte des Forschungszentrums Waldökosysteme der Universität Göttingen, Reihe A, Band 40) (1988).

[12] ULRICH, B., Nutrient and Acid-Base Budget of Central European Forest Ecosystems. In: Godbold, D.L.; Hüttermann, A. (Eds.): Effects of Acid Rain on Forest Processes. New York: Wiley-Liss, (1994) pp. 1–50. (Wiley Series in Ecological and Applied Microbiology).

[13] BREDEMEIER, M., Stoffbilanzen, interne Protonenproduktion und Gesamtsäurebelastung des Bodens in verschiedenen Waldökosystemen Norddeutschlands. Dissertation. Institut für Bodenkunde und Waldernährung, Universität Göttingen. (Berichte des Forschungszentrums Waldökosysteme der Universität Göttingen, Reihe A, Band 33) (1987).

[14] UN ECE, Manual on Methodologies and Criteria for Mapping Critical Levels/Loads and Geographical Areas where they are exceeded. Berlin: Umweltbundesamt. (Texte 71/96 UN ECE Convention On Long-range Transboundary Air Pollution) (1996).

[15] VRIES, W. DE, POSCH, M., OJA, T., OENE, H. VAN; KROS, H., WARFVINGE, P., ARP, P.A., Modelling critical loads for the Solling spruce site. Ecological Modelling **83** (1995) 283–293.

[16] WILPERT, K. V., ZIRLEWAGEN, D., KOHLER, M., To what extent can silviculture enhance sustainability of forest sites under the immission regime in Central Europe? Water, Air, and Soil Pollution **122** (2000) 105–120.

EVALUATION OF SOME PLANT AND ANIMAL SPECIES FOR MONITORING ATMOSPHERIC MANGANESE

G. KENNEDY[1], J. ZAYED[2],

[1] Nuclear Engineering Institute, Ecole Polytechnique, Montreal, Quebec, Canada
[2] Department of Occupational and Environmental Health, University of Montreal, Montreal, Quebec, Canada

Abstract

Methylcyclopentadienyl manganese tricarbonyl (MMT) has been used as an additive in gasoline in Canada since 1976. Its combustion products are emitted from automobiles as particulates containing Mn. As a complement to measuring Mn contamination using air particulates collected on filters, various plant and animal species were studied as potential biomonitors. Six species of trees were studied as historical indicators, using the Mn concentrations in the annual rings of wood. Trees sampled near a Mn refinery showed increased Mn levels but no increase was observed at the contamination level produced by automobiles. Oats, beans and dandelions were also studied as indicators of Mn from automobiles. They were grown at sites with traffic densities of <20,000 vehicles/day and 132,000 vehicles/day. The stems and fruit of the oat plants showed higher Mn concentrations relative to control plants. Common pigeons were also evaluated as monitors of Mn contamination. In a laboratory environment, pigeons were exposed to manganese-oxide dust at high concentrations (239 mg m^{-3}) for up to 13 weeks. Other pigeons were captured from rural and urban environments with different traffic densities and different estimated atmospheric Mn levels. The highly exposed laboratory pigeons had significantly higher Mn concentrations in their lungs relative to the control group (0.9 mg kg^{-1} vs. 0.2 mg kg^{-1}), but not in blood nor other organs. With the possible exception of oat plants, none of the potential biomonitors studied here had sufficient sensitivity to detect atmospheric Mn at the levels arising from the combustion of MMT in automobiles.

1. INTRODUCTION

Methylcyclopentadienyl manganese tricarbonyl (MMT: $C_9H_7MnO_3$) is an organometallic derivative of manganese (Mn) added to gasoline as an octane booster [1,2]. Since its introduction in Canada in 1976, the use of MMT increased substantially until it completely replaced tetraethyl lead in 1990 [3]. In an internal combustion engine MMT burns to form various Mn compounds, which are emitted mainly as fine particulates (86% less than 1 mm), containing Mn sulphates and Mn phosphates [4,5]. Many studies have been conducted in the Montreal area in order to evaluate environmental contamination and human exposure to Mn from the combustion of MMT [6-10]. The majority of these studies involved the collection of particulates on filters, often using portable personal air samplers, and the results usually showed increased Mn concentrations related to high traffic density, although they are low compared to typical industrial contamination. A study using snow [11] revealed Mn deposition decreasing with increasing distance from a busy road.

As a complement to the collection of air particulates on filters, our group has also investigated the use of biomonitors to measure Mn contamination. Trees appeared to be an attractive choice because the amount of Mn fixed in the annual growth rings of wood could potentially provide an historical record of Mn contamination prior to the beginning of the study. Five species of trees were sampled, using an incremental corer, at a site 1 km from a Mn refinery located 30 km southwest of Montreal and at less-polluted control sites [12]. At the polluted site it was estimated from soil analyses that the annual Mn deposition was at least 35 g m^{-2} during the 20 years of operation of the refinery. Wood from the trees was analysed for Mn using neutron activation analysis (NAA). In spite of the high Mn contamination, only one of the five species of trees, hemlock (Tsuga canadensis, a conifer), showed wood Mn levels significantly higher than in the control trees during the years of operation of the refinery, and these levels varied substantially among the five hemlock trees sampled [12].

Trees were also evaluated as monitors at the much lower levels of Mn contamination produced by automobiles. Spruce trees (Picea pungens) growing at various distances from a busy road in Montreal showed no significant differences in Mn concentrations in the wood [13]. The results with trees may be explained by the fact that, since Mn is an essential element in plants [14], Mn uptake from the soil is fairly substantial and almost independent of the total Mn concentration in the soil; uptake depends on availability and other factors [15,16].

Oats (Avena nova) and beans (Phaseolus vulgaris) were also examined as indicators of Mn from automobiles [17]. They were grown at a non-exposed control site, at a lightly exposed site near a road with traffic density <20,000 vehicles/day and at a highly exposed site 250 m from a road with traffic density 132,000 vehicles/day. After 68 days growth, the roots, stems, leaves and fruits were analyzed for Mn by NAA. For the bean plants, only the stems and leaves showed slightly increased Mn concentrations at the exposed sites relative to the controls. For the oat plants, the stems, leaves and fruit showed increased Mn concentrations at the exposed sites relative to the controls but there were no significant differences as a function of traffic density [17]. It should be noted that, at 250 m from the road, the atmospheric Mn concentration is only of the order of 0.1 mg m-3. In another study [18], dandelions (Taraxacum officinale) were grown at sites 10 m, 50 m and 100 m from a road with traffic density 135,000 vehicles/day. No significant differences were found in the Mn concentrations in the roots, stems, leaves and flowers as a function of distance from the road [18].

A laboratory study was carried out to determine the bioaccumulation and toxicity of Mn_3O_4 in the common feral pigeon (Columba livia) [19]; the results could also be useful for assessing the value of pigeons as a potential biomonitor of Mn pollution in cities. They are therefore evaluated here for that purpose. Pigeons are fairly sedentary and their exposure in the urban environment could be similar to that of the human population. The study is described below.

2. EXPERIMENTAL

A total of 22 pigeons were housed in stainless-steel cages, 8 in the control group and 14 in the exposed group. Those in the exposed group were exposed 7 h/day, 5 days/week in inhalation chambers to Mn_3O_4 dust generated by a Fluidized Bed Aerosol Generator. The mean Mn concentration in the inhalation chambers was 239 mg m^{-3} and 98% of the particles were less than 1 mm. Blood samples were taken every 2 weeks for Mn analysis. At the end of weeks 5, 9 and 13, four exposed pigeons and two controls were sacrificed and dissected in preparation for analysis. Further experimental details are given in [19].

Mn concentrations were determined in blood and tissue samples by neutron activation analysis. NAA is ideal for these analyses because it has high sensitivity for Mn (typical detection limit 0.01 mg kg-1) and no sample dissolution is needed. Typical analysis time is 5 minutes for tissue samples and 30 minutes for blood.

3. RESULTS AND DISCUSSION

The Mn concentrations measured in the blood of the exposed and control groups of pigeons are shown in Fig. 1. No significant difference between the two groups was observed. The Mn concentrations measured in the various tissues of the pigeons are shown in Table I. When comparing the pigeons sacrificed after 5, 9 and 13 weeks of exposure with the controls, it can be seen that there are no significant differences for most of the tissues. For bone, it was originally thought that the differences between the control and exposed pigeons may be significant [19], but it is now thought that the apparent difference was due to the control group having slightly below normal bone Mn. In the lung, Mn is significantly higher in the exposed groups than in the control group. This is easily understood to be due to the fact that there is a direct pathway to the lungs for inhaled Mn, while Mn must be transported to all other organs by the blood, which is not elevated in Mn in the exposed pigeons. Although Mn is higher in the lungs of all the exposed groups relative to the controls, it does not seem to increase with increasing exposure time, contrary to what might be expected. The apparent decrease with increasing exposure time might be due to differences among individual pigeons. It appears that the lungs of pigeons are reliable indicators of elevated atmospheric Mn, but the indication is qualitative rather than quantitative.

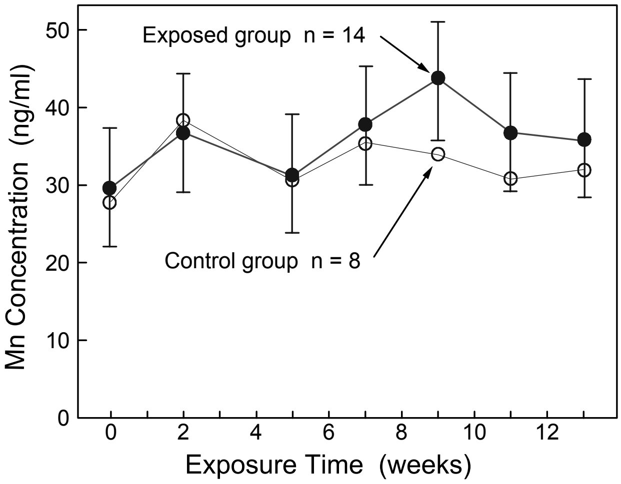


FIG. 1. Mn concentrations in the blood of pigeons exposed to Mn_3O_4 dust. Data from Ref. [19].

TABLE I. MANGANESE IN TISSUES OF PIGEONS EXPOSED TO Mn_3O_4 BY INHALATION FOR THREE DIFFERENT PERIODS OF TIME. THE OBSERVED MEAN AND STANDARD-DEVIATION ARE GIVEN FOR EACH GROUP. DATA TAKEN FROM REF. [19].

(µg Mn / g of wet tissue)

Tissue	control group (n = 6)	5 weeks (n = 4)	9 weeks (n = 4)	13 weeks (n = 4)
Brain	0.46 ± 0.07	0.54 ± 0.01	0.53 ± 0.03	0.59 ± 0.02
Lung	0.19 ± 0.05	1.24 ± 0.38	0.89 ± 0.13	0.58 ± 0.16
Liver	2.86 ± 0.72	2.44 ± 0.78	2.79 ± 0.60	3.61 ± 1.18
Intestine	1.49 ± 0.92	2.20 ± 1.40	1.85 ± 0.66	2.16 ± 1.04
Pancreas	1.66 ± 0.48	2.09 ± 0.47	1.66 ± 0.29	1.85 ± 0.20
Kidney	6.11 ± 1.98	5.23 ± 0.73	4.90 ± 1.12	7.65 ± 1.37
Muscle	0.67 ± 0.11	0.74 ± 0.04	0.68 ± 0.05	0.66 ± 0.12
Feces	57.77 ± 15.66	65.05 ± 32.93	77.61 ± 31.94	52.87 ± 8.08
Bone	1.74 ± 0.33	2.57 ± 0.26	3.07 ± 1.13	3.02 ± 0.29

In another study [20], pigeons were captured from rural and urban environments with different traffic densities within 100 m (4,900 vs. 75,000 vehicles/day) and different estimated atmospheric Mn levels (0.03 mg m^{-3} vs. 0.06 mg m^{-3}). No significant differences were seen in the amounts of Mn in their lungs. The urban pigeons had slightly higher Mn levels in their liver and feces [20]; this may be the effect of long term exposure to Mn by inhalation or oral ingestion.

4. CONCLUSION

The wood of hemlock trees and the lungs of pigeons were found to be reliable indicators of the presence of atmospheric Mn at levels of the order of 100 mg m^{-3} associated with significant industrial pollution, although neither was very quantitative. With the possible exception of oat plants, none of the potential biomonitors studied here had sufficient sensitivity to detect atmospheric Mn at levels of the order of 0.1 mg m^{-3} arising from the combustion of MMT in automobiles.

REFERENCES

[1] COOPER, W.C., The Health Implications of Increased Manganese in the Environment Resulting from the Combustion of Fuel Additives: a Review of the Literature, J. Toxicol. Environ. Health 14 (1984) 23–46.

[2] JAQUES, A.P., Environment Canada National Inventory of Sources and Emissions of Managanese–1984. Report EPS 5/MM/1, Environmental Analysis Branch, Environmental Protection, Conservation and Protection, Ministry of Supply and Services Canada, Ottawa, Ontario, Canada (1987) 26–37.

[3] The Royal Society of Canada, Lead in the Canadian Environment: Science and Regulation. Final Report. (1986) Commission on Lead in the Environment, Ottawa, Ontario.

[4] ARDELEANU, A., LORANGER, S., KENNEDY, G., GAREAU,L., ZAYED, J., Emission Rates and Physicochemical characteristics of Mn Particles Emitted By Vehicules using MMT as Octane improver, Water Air Soil Pollut. 115 (1999) 411–427.

[5] ZAYED, J., BANDE, H., Characterization of Manganese containing particles collected from exhaust emissions of automobiles running with MMT additive, L'Espérance, G., Env Sc Technol. 33 (1999) 3341–3346.

[6] LORANGER, S., ZAYED, J., Environmental and Occupational Exposure to Manganese: A Multimedia Assessment, Int. Arch. Occup. Environ. Health 67 (1995) 101–110.

[7] LORANGER, S., ZAYED, J., Environmental Contamination and Human Exposure to Airborne Total and Respirable Manganese in Montreal, J. Air Waste Manage Assoc. 47 (1997) 983–989.

[8] SIERRA, P., LORANGER, S., KENNEDY, G., ZAYED, J., Occupational and environmental exposure of automobile mechanics and nonautomative workers to airborne manganese arising from the combustion of methylcyclopentadienyl manganese ticarbonyl (MMT), Am. Ind. Hyg. Assoc. J. 56 (1995) 713–716.

[9] ZAYED, J., GÉRIN, M., LORANGER, S., SIERRA, P., BÉGIN, D., KENNEDY, G.,Occupational and environmental exposure at garage workers and taxi drivers to airborne manganese arising from the use of MMT (Methylcyclopentadienyl Manganese Tricarbonyl) in unleaded gasoline, Am. Ind. Hyg. Assoc. 55 (1994) 53–58.

[10] ZAYED, J., MIKHAÏL, M., LORANGER, S., KENNEDY, G., L'ESPÉRANCE, G., Exposure of Taxi drivers and Office Workers to Total and Respirable Manganese in an Urban Environment, Am. Ind. Hyg. Assoc. J. 57 (1996) 376□380.

[11] LORANGER, S., TÉTREAULT, M., KENNEDY, G., ZAYED, J., Atmospheric manganese and other trace elements in urban snow near an expressway, Environ. Pollut. 92 (1996) 203□211.

[12] KENNEDY, G., BERGERON, S., Tree Rings as Monitors of Heavy Metal Air Pollution Histories, J. Radioanal. Nucl. Chem. 151 (1991) 337–343.

[13] FORGET, E., COURCHESNE, F., KENNEDY, G., ZAYED, J., Response of Blue Spruce (Picea pungens) to Manganese Pollution from MMT, Water, Air and Soil Pollut. 73 (1994) 319–324.

[14] ADRIANO, D.C., Trace Elements in the Terrestrial Environment, Springer-Verlag, New York (1986) 533 pp.

[15] BJERRE, G.K., SCHIERUP, H.H., Influence of waterlogging on availability and uptake of heavy metals by oats grown in different soils, Plant and Soil 88 (1985) 45–56.

[16] BJERRE, G.K., SCHIERUP, H.H., Uptake of six heavy metals by oats as influenced by soil type and additions of cadmium, lead, zinc and copper, Plant and Soil 88 (1985) 57–69.

[17] BRAULT, N., COURCHESNE, F., LORANGER, S., KENNEDY, G., ZAYED, J., Bioaccumulation of manganese by plants: Influence of MMT as a gasoline additive, Sci. Total Environ. 153 (1994) 77–84.

[18] NORMANDIN, L., KENNEDY, G., ZAYED,J., Potential of dandelion (Taraxacum officinale) as a bioindicator of manganese arising from the use of metylcyclopentadienyl manganese tricarbonyl in unleaded gasoline, The Science of the Total Environment 239 (1999) 165–171.

[19] SIERRA, P., CHAKRABARTI, R., TOUNKARA, R., LORANGER, S., KENNEDY, ZAYED, G., Bioaccumulation of Manganese and its Toxicity in Feral Pigeons (Columbia livia) Exposed to Manganese Oxide Dust (Mn3O4), Environ. Res. 79 (1998) 94–101.

[20] LORANGER, S., DEMERS, G., KENNEDY, G., FORGET, E., ZAYED, J., The pigeon (Columba livia) as a monitor of atmospheric manganese contamination from mobile sources, Arch. Environ. Contam. Toxico. 27 (1994) 311–317.

ACCUMULATION OF TRACE METALS AND PAHS IN THE MOSS TORTULA MURALIS HEDW. IN THE TERRITORY OF FERRARA (NORTHERN ITALY)

R. GERDOL, F. ALBERGHINI, L. BRAGAZZA, R. MARCHESINI
Department of Biology, University of Ferrara, Ferrara, Italy

A. MEDICI, P. PEDRINI
Department of Chemistry, University of Ferrara, Ferrara, Italy

S. BENEDETTI, A. BOVOLENTA, S. COPPI
A.R.P.A. Emilia-Romagna, Area Specialistica Fitofarmaci, Ferrara, Italy

Abstract

Concentrations of trace metals and polycyclic aromatic hydrocarbons (PAHs) were determined in tissue of Tortula muralis, with the objective of assessing whether a common acrocarpous species could be used for monitoring pollution levels in an urban area in northern Italy and its surroundings. Based on a multivariate treatment of the data by principal component analysis (PCA) we found that trace metals and PAHs had different patterns of concentration reflecting differences in emission sources and/or transport mechanisms. Most metals had peak concentrations some km downwind from an industrial area, thus reflecting mid-distance transport as particulate. Pb was mainly derived from car engines and peaked within the urban area as an effect of slow-moving traffic. All PAHs presumably underwent short-distance transport and were, therefore, mostly concentrated close to emission sources, the latter mainly consisting in diesel engine exhausts.

1. INTRODUCTION

Mosses rely on atmospheric inputs for most of their nutrients, which makes them useful indicators of atmospheric deposition based on the accumulation of pollutants in their tissues [1]. Especially pleurocarpous mosses and Sphagnum mosses have been since long used for monitoring atmospheric deposition of trace metals [2] and, since more recently, of organic pollutants such as PCBs and PAHs [3,4]. Pleurocarpous mosses and peat mosses often form extensive carpets and hummocks in forests, heathlands, and ombrotrophic bogs. Therefore, pleurocarpous moss species have been successfully employed for extensive surveys of atmospheric heavy-metal deposition [5].

Pleurocarpous mosses are pollution-sensitive, usually being rare or even absent in areas subjected to heavy human pressure. For this reason, alternative techniques such as moss transplants on logs and/or dried moss in nylon bags have been used instead of living moss tissues for monitoring deposition levels in areas, such as city centres, where natural populations of pleurocarpous mosses are totally lacking [6]. Some acrocarpous mosses, conversely, tolerate much higher levels of air pollutants and can even been found in heavily polluted urban areas. However, acrocarpous species have only rarely been used as biomonitors of atmospheric deposition [7].

In this study we aimed at testing whether the acrocarpous moss Tortula muralis, one of the most common species in anthropogenic areas in northern Italy, could effectively be used for monitoring deposition levels of airborne trace metals and organic pollutants in urban areas.

2. MATERIALS AND METHODS

2.1. Study area and collection of moss samples

Ferrara is a medium size town (ca 130,000 inhabitants) situated in NE Italy, ca 100 km SW of Venice. The climate is cool-submediterranean with a mean yearly temperature of ca 13.5°C and a total annual precipitation of ca 600 mm. The prevailing winds blow from the West, and secondarily from the North-East. We did not have any detailed list of all potential sources of atmospheric pollution at our disposal. Anyway, major sources of emission could be easily identified, including:

1. Local traffic, as well as house-heating plants in the urban area.
2. Chemical plants and waste incinerators situated within a large industrial area ca 3 km NW of town's centre.
3. Heavy traffic, mostly concentrated in the southern surroundings of the urban area.
4. High-speed traffic, mostly concentrated in a motorway situated ca 5 km W of town's centre with a N-S orientation.

Samples were collected from Tortula muralis cushions, mostly growing on walls, at 25 sites homogeneously spread over a ca 10 × 6 km wide area. The sampling area included all major sources of emission listed above, as well as a residential area to the NE. All samples were collected within 15 days in February 1999, always over ca 1.5 m aboveground in order to avoid possible contamination from water splashes.

2.2. Tissue extraction and chemical analyses

Sample treatment. The apical green tissue, 0.8 cm long on the average, was carefully detached from the moss cushion, cleaned without washing, and dried at 40°C for 72 h. A subsample of moss tissue was dried at 105 °C for 24 h for determining the weight loss between 40 °C and 105 °C.

Metal analysis. Ca 0.5 g of moss tissue were wet digested in 65% nitric acid after adding 1 ml H_2O_2. Concentrations of trace metals in the solution were determined by ICP-MS (VG Plasma Quad 2-Plus).

PAH analysis. Ca 5 g of moss were milled and extracted with n-hexane in an ultrasonic bath for 30 minutes. The extract was filtered and injected into a GC/MS system (HP 5973) equipped with a capillary column SE54, 30 m × 0.5 mm I.D. 0.25 mm. Quantitation was carried out by deuterated internal standard method.

Data treatment. A matrix was built up including the concentrations of all trace metals and PAHs in the 25 Tortula samples. This matrix was subjected to a non-centred principal component analysis (PCA) in order to synthesize the correlation coefficients among the original variables into a reduced set of new variables resulting from the numerical computations, the latter being performed using the CANOCO software [8]. The concentrations values were interpolated among neighbouring sites by the kriging method and the resulting values graphically displayed onto a schematic map using the SURFER software [9].

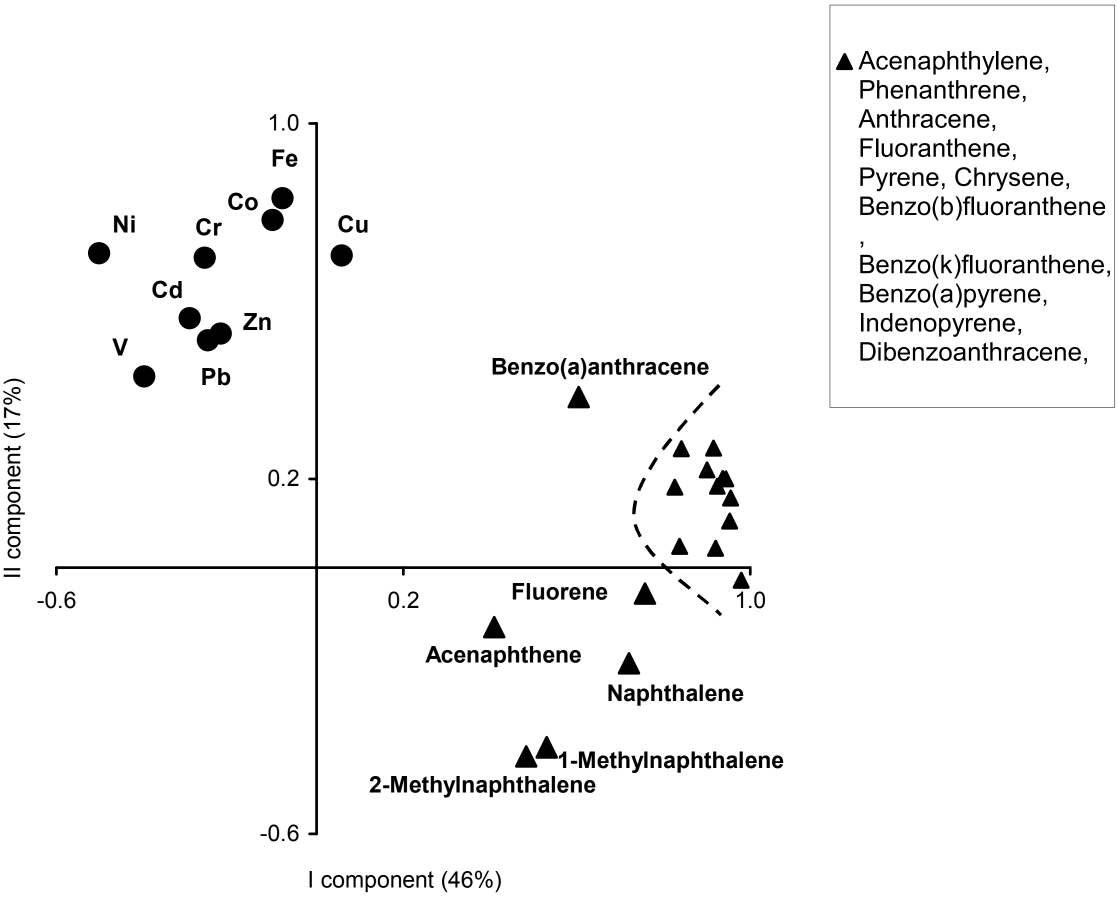


FIG. 1. Joint display of trace metals and PAHs based on their loadings on the first two principal components. All PAHs listed in the box have very similar loadings to the right of the dashed line. The figures in brackets indicate the percentage of variance accounted for by each PCA axis.

3. RESULTS AND DISCUSSION

The loadings of the original variables on the first two PCA axes, cumulatively accounting for ca 63% of the total variance, clearly separated all trace metals on one side from all PAHs on the other side (Fig. 1). This indicates that trace metals and PAHs were emitted from differing sources and/or experienced differing transport patterns.

Concentrations of Co, Cr, Fe, and Ni were highest in the eastern area (see, e.g. the map of Ni concentrations in Fig. 2). Since all those metals are mostly supplied to the atmosphere from the industrial area, with no other remarkable point sources outside the industrial area, the observed pattern must reflect a medium-range transport of metals as particulate.

Pb had peak concentrations in the urban area (Fig. 3). Indeed, vehicles represent the overall biggest source of Pb emissions. Lead is mostly emitted by combustion of leaded gasoline, which is still being used by a considerable percentage of circulating cars in Italy. The high Pb concentrations in the town’s centre were presumably determined by comparative traffic speed rather than by absolute traffic flow. Indeed, once emitted, most of the lead is readily deposited close to the point source, its concentrations increasing when traffic is slow-moving [10] and rapidly declining even at a very short distance from point emission sources [7].

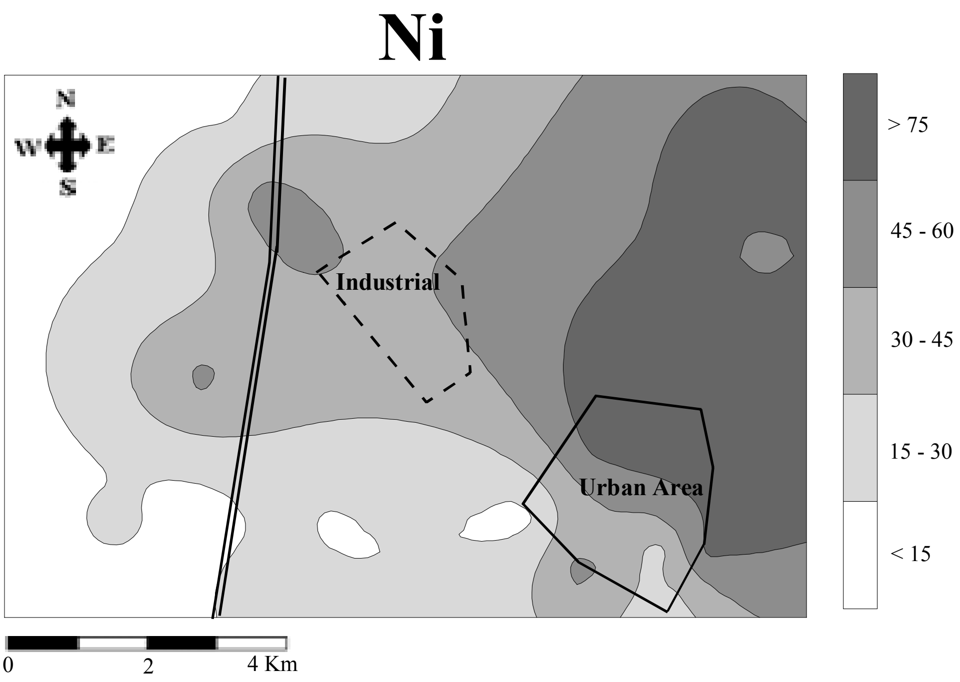


FIG. 2. Map of Ni concentrations in Tortula muralis.

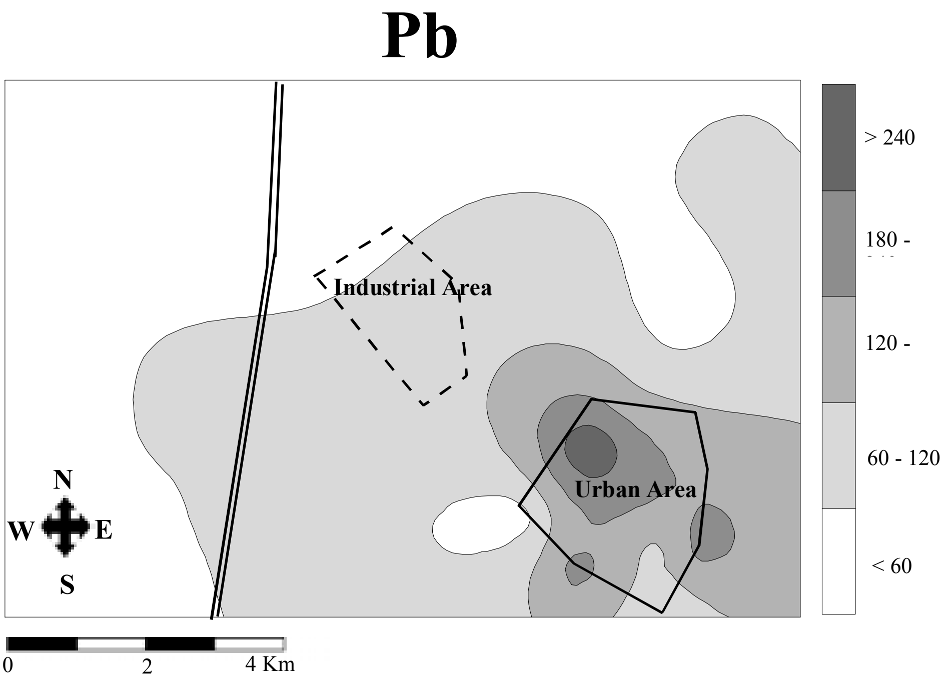


FIG. 3. Map of Pb concentrations in Tortula muralis.

By contrast, zinc derives from additional sources besides car exhausts, such as tyre wears and incinerators. This accounts for less distinct peak Zn concentrations in the urban area relative to Pb.

Among the PAHs, highest concentrations were found for fluoranthene, pyrene, benzo(a)anthracene, and phenanthrene and, lowest concentrations for fluorene, acenaphtene, and acenaphthylene. Contrary to metals, all of the PAHs had much the same geographic pattern of concentration, with peak values in the southern part of the area (Fig. 4), where density of heavy traffic is highest. Our findings are consistent with literature data, pointing to diesel engine exhaust as the main source of PAHs [11], although combustion of other organic materials such as wood and coal [12] may play a certain role in supplying PAHs into the atmosphere.

In conclusion, we found that the geographic pattern of trace metal and PAH concentrations in tissue of Tortula muralis observed in the urban area of Ferrara and its surroundings could be consistently explained based on available information on emission sources and transport mechanisms of metals and organic pollutants as wells. Therefore, this acrocarpous moss can be usefully employed as biomonitor of atmospheric deposition in urban areas.

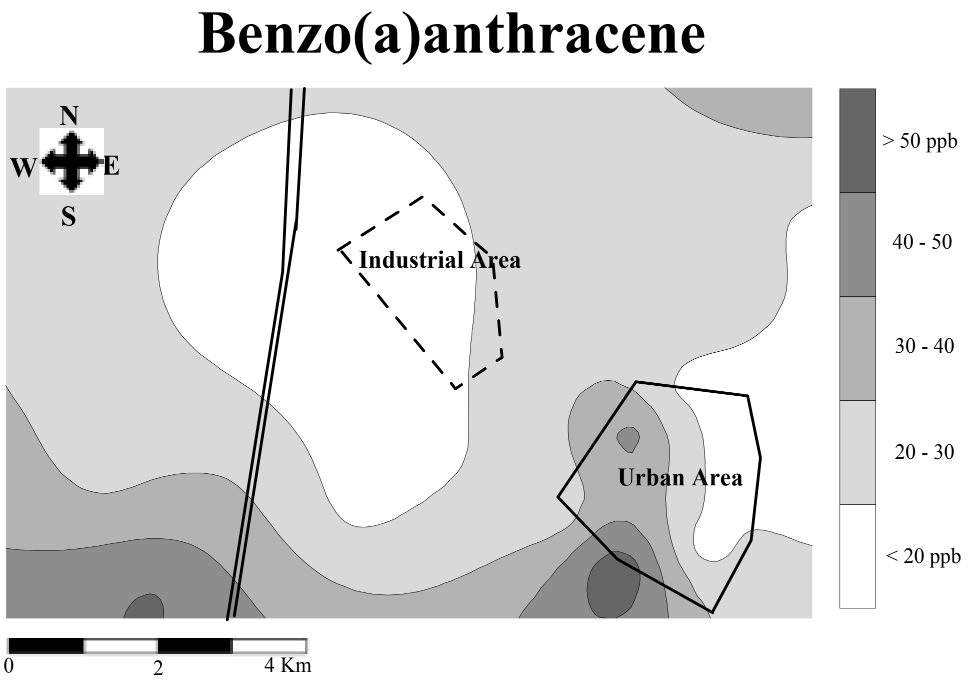


FIG. 4. Map of benzo(a)anthracene concentrations in Tortula muralis.

REFERENCES

[1] TYLER, G., Bryophytes and heavy metals: a literature review. Bot. J. Linn. Soc. 104 (1990) 231–253.

[2] RÜHLING, Å., TYLER, G., Ecology of heavy metals — a regional and historical study. Bot. Not. 122 (1969) 248–259.

[3] HIMBERG, K.K., PAKARINEN, P., Atmospheric PCB deposition in Finland during 1970s and 1980s on the basis of concentrations in ombrotrophic peat mosses (Sphagnum). Chemosphere 29 (1994) 431–440.

[4] SANDERS, G., JONES, K.C., HAMILTON, TAYLOR J., PCB and PAH fluxes to a dated UK peat core. Environ. Poll 89 (1995) 17–25.

[5] RÜHLING, Å., STEINNES, E., Atmospheric heavy metal deposition in Europe 1995–1996. Nord Environment 15 (1998) 1–66.

[6] GOODMAN, G.T., ROBERTS, T.M., Plants and soils as indicators of metals in the air. Nature 231 (1971) 287–292.

[7] PEARSON, J., WELLS, D.M., SELLER, .J., BENNETT, A., SOARES, A., WOODALL, J., INGROUILLE, M.J., Traffic exposure increases natural 15N and heavy metal concentrations in mosses. New Phytol 147 (2000) 317–326.

[8] TER BRAAK., C.J.F., SMILAUER, P., CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Ordination (Version 4). Microcomputer Power (Ithaca, NY, USA) (1998), 352 pp.

[9] KECKLER, D., SURFER for Windows. User's guide. Golden Software Inc., Golden, CO, U.S.A., (1994).

[10] BURTON, M.A.S., Terrestrial and aquatic bryophytes as monitors of environmental contaminants in urban and industrial habitats. Bot. J. Linn. Soc. 104 (1990) 267–280.

[11] ZHIQIANG, Q., SIEGMANN, K., KELLER, A., MATTER, U., SCHERRER, L., SIEGMANN, H.C., Nanoparticle air pollution in major cities and its origin. Atmos. Environ. 34 (2000) 443–451.

[12] LEE, H.K., WRIGHT, G.J., AND SWALLOW, W.H., Use of concentration ratios of polycyclic aromatic hydrocarbons (PAHs) and lead to determine source-origins of PAH in sediments and cockles. Int. J. Environ. Stud. 55 (1998) 211–218.

A EUROPEAN SURVEY OF ATMOSPHERIC HEAVY METAL DEPOSITION IN 2000–2001

A. RÜHLING
University of Lund, Department of Ecology, Lund, Sweden

Abstract

The background, administration and current status of the monitoring programme "European Survey of Atmospheric Heavy Metal Deposition" is described. All European states are invited to join the survey 2000–2001. The co-ordination and responsibility for the survey will gradually be transferred from the Nordic Council of Ministers to the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation).

1. BACKGROUND AND DESCRIPTION

Atmospheric Heavy Metal Deposition in Europe is the title of a monitoring project under the Nordic Council of Ministers. It has to a large extent been developed and is presently carried out by the Nordic Working Group on Monitoring and Data (NMD) under the Council. The concept and technique of analysing deposition of heavy metals (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, V, and Zn) in mosses (mainly Hylocomium splendens, Pleurozium schreberi) was introduced in the Nordic countries in the late 1960's.

Most mosses growing on the forest floor receive their nutrients mainly from dust-fall and precipitation. Several studies have shown that a survey of the metal concentration in mosses is a valuable means of identifying sources of airborne pollution and mapping the metal deposition. Analysis of heavy metals in mosses has also become a regularly used tool in monitoring practices. It has also been shown that there are possibilities to transfer the concentrations of heavy metals measured in the mosses to area deposition values. This can primarily be achieved by using corresponding measurements of metals contained in bulk precipitation.

The moss technique was first tested on an international scale in 1980 as a joint Danish-Swedish initiative. In 1985, 1990, and 1995 followed similar surveys of moss sampling, each time involving an increasing number of participating countries. In the years 1995–96, some 30 European countries took part in the project. A total of 64,000 measurements were made. Countries such as Iceland, Belorussia, and Bulgaria joined the survey thus completing the picture of heavy metal deposition in all Europe.

2. ADMINISTRATION AND ECONOMICS

The NMD Secretariat administrated the 1995–96 survey, and final reports were published in the Nordic Council of Ministers Nord-series. Costs for co-ordination and quality assurance work in the laborious phase of inviting the participants, intercalibrating national laboratories, sampling mosses, and assuring proper delivery of comparable data from all over Europe, amounted to some 40-60,000 USD/year. In the succeeding years of final checking, producing the maps, interpreting the results and summing it all up before the next survey, the annual expenses have been slightly less. The non-recurrent charge of printing the final reports is in the region of 15,000 USD.

The NMD Working Group has financed the project co-ordination, as well as some sampling and laboratory work in e.g. participating countries of the former Soviet Union and in connection with the intercalibration process. As for the rest of the work involved, it has been nationally organised, performed and financially supported, integrated into national monitoring strategies and budgets.

The costs for each participating country of sampling and producing the required data will naturally vary considerably. National project management, therefore, in the past years, has run into some difficulties particularly in the countries of Eastern and Central Eastern Europe.

3. CURRENT STATUS

All European states were invited to join the 2000 Survey and the response was positive from at least the participants of the 1995 survey.

NMD is financing the initiation phase of the 2000 survey, but has not committed itself to fulfil the entire process ending around 2003–2004. The activities of the NMD Working Group is not intended to run routine environmental monitoring field work, rather to develop methods and e.g. monitoring programmes in order to harmonise the practises of several co-operating countries. Today, the project, comprising the concept itself and performing the entire procedure, seems well developed, quality assured, tested many times over, and found useful for estimating heavy metal deposition and accumulation of at least ten various elements on a continental scale. Consequently, NMD considers its work done, and would preferably pass the entire concept into the hands of a respected international organisation in the field of environmental monitoring. The project has also grown out of its original frames of being a strictly Nordic affair, and is getting increasingly heavy to run by a relatively small Nordic working group alone. It is desirable these surveys continue in the future, so that we can get an uninterrupted series of European surveys displaying the development of heavy metal occurrence and the success of steps taken to reduce long-range transportation of air pollutants.

We thus feel that the revelations and outcome of the project are most valuable in monitoring the state of environment, in finding many relations of causes and effects, and also in assessing the effects of protective measures taken. The long-range scale makes international comparisons and transboundary conclusions possible, which in turn facilitates and intensifies the efforts of finding solutions to common problems simultaneously affecting a large number of states.

In a recent development, we have agreed to incorporate the project into the work-plan of the Working Group on Effects of the UN/ECE convention on Long-Range Transboundary Air Pollution (CLRTAP). The results of the year 2000 moss survey would be reported to the CLRTAP through the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation).

At the beginning of December 1999 the Executive Body for the UN/ECE Convention accepted the inclusion of an additional item in the workplan of the ICP Vegetation — "Preparations for taking over responsibility for the European survey on heavy metals in mosses". In doing so it recognised the importance and relevance of the European survey to future work under the Convention regarding the transport and effects of heavy metals. It accepted that the ICP Vegetation was a suitable body for undertaking the long-term management of the survey and endorsed the future work planned. It will be kept informed of progress through the report of the Working Group on Effects, and the chairperson of the ICP will be responsible for taking action through the Programme Centre and the Task Force.

It is also worth noting that the Executive Body accepted that the development and broadening of the ICP's activities would be better reflected by changing its name to "ICP on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation)".

The sampling of mosses has so far been a nationally organised and funded activity, and in some extensive countries this has implied a considerable effort to get a representative coverage of heavy metal deposition in the area. Future sampling is probably still best done and financed by the participating countries themselves, thus ensuring continuity of local biological and technical knowledge.

Publication of results is naturally an important link in the entire process. The map producing technique is well developed, and finding the right forum for the results of the survey will easily lead to a wide distribution of the findings for decision-makers and others to grasp. The maps of previous surveys have encountered great interest, and have widely been cited in e.g. international state of environmental reports, as they are very perspicuous and spontaneously reflect large-scale press-state relations. Viewed as a monitoring time series, the response aspects, for instance, emissions cuts will readily become obvious.

For work on conventions, critical loads and corresponding international environmental agreements, the results of the project are invaluable, the looks at past conditions and in a time continuum being as important as any. The present trend in Europe of displaying the state of the environment we share in a large scale to enhance responsibility thinking and public awareness, will stress the need of data and images such as those produced by the project.

4. THE SURVEY 2000–2001

Due to organisational and financial problems in several countries the sampling time has been extended to also include 2001. This time we do not intend to make an intercalibration between the laboratories. Instead, the national project leaders are asked to ensure that the analytical quality is satisfactory. For this purpose, we plan to distribute a reference sample with known heavy metal content. A few control samples will also be distributed to be included in the routine analytical work and reported on.

Even if it takes plenty of time to make all necessary controls of the analytical work, this is mainly a routine task. Much more difficult to overcome is the problem that it is hardly possible to use the same moss species from the north to the south of Europe. There were few problems when the measurements were limited to northern Europe with dominating coniferous or mixed forests. Here are mosses as Hylocomium splendens and Pleurozium schreberi very abundant. Unfortunately we still do not have data material enough to be able to recalculate the metal concentration found in one moss species to another species with sufficient accuracy or to calculate moss concentrations to deposition values. What we have done up to now is instead to check that the species used have an acceptable similarity in their uptake of heavy metals.

The need to distinguish anthropogenic and natural sources must be addressed in the survey. An attempt will be made to objectively assess these contributions. Appropriate methods may involve assessing wet and dry deposition at some test sites or sequential extractions of metals from moss to distinguish particle and cell associated metals. Also interspecies calibration should be extended to all participating countries in an attempt to convert values by regression analysis to appropriate concentrations in one species (preferably Pleurozium) or to area deposition values.

BIOLOGICAL FLUXES CONVERSION: A NEW BIOMONITORING TOOL FOR ATMOSPHERIC METALS AND TRACE ELEMENT DEPOSITION

G.A. GROSS[1], J.F. FÉRARD[2], F. CARROT[1], A. MAUL[3], J.M. DUCRUET[4],
P. CODDEVILLE[5], P. BÉGUINEL[6], S. AYRAULT[1], L. GALSOMIÈS[7]

[1] Laboratoire Pierre Süe, CEA-CNRS, CEA de Saclay, Gif-sur-Yvette, France
[2] EBSE, Campus Bridoux, Metz, France
[3] STID, IUT Metz University, France
[4] Laboratoire de Bioénergétique, CEA de Saclay, Gif-sur-Yvette, France
[5] Département de Chimie et Environnement, Ecole des Mines de Douai, Douai, France
[6] SRSE, CEA de Saclay, Gif-sur-Yvette, France
[7] ADEME, Département Air et transport, Paris, France

Abstract

A new active biomonitoring tool named "moss plate" was elaborated with standardization and quality criteria aiming to keep alive mosses for a short term period of two months. The chlorophyll fluorescence induction method (Fv/Fm parameter) on three moss species (*Pleurozium schreberi, Pseudoscleropodium purum, Eurhynchium praelongum*) has demonstrated the buffering action of the water presence for the biological activity stability of mosses. The two more resistant species were selected and exposed on four different sites with parallel exposure of monthly total atmospheric deposition gauges ("Owen" gauges) for three successive periods of two months. The "moss plate" allows relative site (Ba, Cs, Sb, Sr, U) and species (Pb, Sb, Ti, V) comparisons and, two months are enough for a significant enrichment (Ba, Cd, Pb, Sb, Ti, U, V). The coarse and sedimentable particles were separated into different size classes (>1 mm; 1000–200 µm ; 200–40 µm ; 40–20 µm). Dry depositions and transplanted mosses were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for a stock ($\mu g.g^{-1}$) — fluxes ($\mu g.m^{-2}.t^{-1}$) conversion experiment. Good explicative models were elaborated for Ba, Pb, Sb, and Ti (R^2>70%) including qualitative (stations, moss species) and quantitative variables (particles size classes in fluxes term). Models put in evidence the qualitative in site local influence of sedimentable particles on the moss content. Entire and intact shoots of *Pseudoscleropodium purum* were analysed by detected X ray emission by photon (SXRF) to locate the *in vivo* distribution of the multielemental enrichment along the moss stem. The Pb, Ti, Cl, Se short term enrichment on the moss is mainly localized in the old plant parts.

1. INTRODUCTION

Because of their large geographic repartition, mosses have been increasingly used for active and passive biomonitoring purposes with aims of spatial and temporal assessment of atmospheric depositions [1,2].

Their bioaccumulation properties make them very efficient indicators for atmospheric metals and trace element depositions [1,3]. Root and cuticule absence make them find their nutritive elements in wet and dry atmospheric deposition [4,5]. This capture is supposed to be mainly due to cation exchange capacity and surface structures with minimum diffusive resistance of elements [6,7] but poorly data concern dry depositions even more the coarse and sedimentable particles capture [8,9]. The exact relationship between the moss content and the dry deposition is still not known even with the standardized active biomonitoring method of "moss bags" [10,11]. Some authors agree with the efficiency of "moss bags" mainly for the dry deposition [10,12,13]. But, this active biomonitoring method is also used for the high surface contact and high cation exchange capacity of the moss species. But, these transplanted mosses do not keep a biological activity during exposure.

This paper reports on a new active biomonitoring tool named, "Moss Plate" in reference to the well-known "Moss Bags" method, and presents the relationships between the moss content and the dry atmospheric deposition, precisely the coarse and sedimentable fraction (>20 µm). This experiment is composed of three different parts with firstly, the active biomonitoring tool elaboration and tests, secondly the moss content data conversion into fluxes term, and thirdly, the *in vivo* distribution of elemental enrichment along the moss shoot.

2. MATERIALS AND METHODS

2.1. "Moss Plate" construction and exposition design

The "Moss Plate" is a three sub-unit tool (defined area of 3×95 cm^2) divided in two parts (Fig. 1.). The upper part of 12 cm height contains the biological material coming from a natural and young population of mosses (with two annual segments) sampled on a background area. A fresh sub-sample of mosses of a 95 cm^2 surface is installed in each sub-unit. They keep their natural individual density and vertical position as moss cushion. The high edges allow only a vertical atmospheric deposition onto the moss, by the circular and open area, and prevent from lateral capture, wind influence or rapid air drying. The mean material of each sub-unit corresponds to 1 g dried weight (for all species tested). A fine nylon net separates the two parts preventing from direct contact between mosses and water. The controlled water is stored (350 ml of a known water) in the downpart reserve keeping a constant level controlled by an outside gauge. To prevent from material losses, a large mesh size polyamide net is placed above the triplicate. The transplants are then installed on platforms to prevent from soil dust contaminations and to expose mosses to a similar atmospheric contact height as bulk collectors (1.1 m high) (Fig.1.). All measurements and tests (induction fluorescence of chlorophyll, stock-flux experiment and *in vivo* element distribution study) were made with mosses exposed in this design.

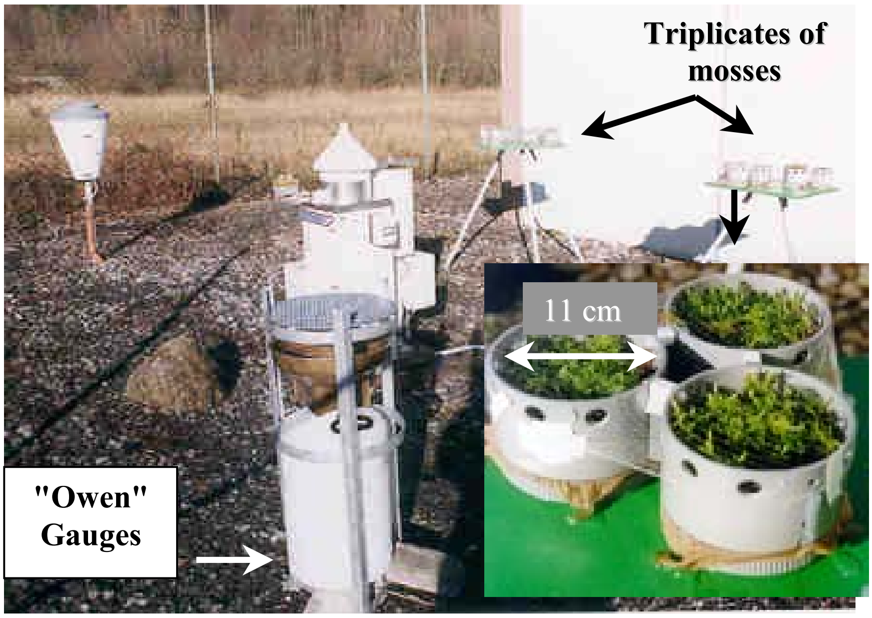


FIG. 1. Picture and enlargement of the "Moss plates" construction and exposition design on continuous monitoring station (station "R", MERA network, France). The transplants with a defined area and water tank (350 ml controlled water) are three sub-unit tools with natural population of mosses (enlargement picture of Pseudoscleropodium purum) installed on platforms (1.1 m high). This tool keeps alive mosses for two months in a natural and vertical position as moss cushion. Parallel exposure of total atmospheric deposition gauges ("Owen" gauges) (diameter 290 mm, height 100 mm) for the sedimentable and coarse dry atmospheric depositions (>1 mm; 1000–200 µm; 200–40 µm; 40–20 µm).

2.2. Chlorophyll Fluorescence Induction

Before transplantation on different sites, the viability of mosses for two months was tested in order to assess the water presence necessity and to choose the two more appropriate and resistant moss species.

During summer 1998, *Pleurozium schreberi* (Ps), *Pseudoscleropodium purum* (Sp), and *Eurynchium praelongum* (Ep) were installed in transplants with and without water and exposed in an open area. At t = 0, 2, 4, and 8 weeks, two chlorophyll fluorescence induction measurements were made on each transplant sub-unit. This method has proved to be a sensitive indicator of physiological stress in plants [14,15]. Chlorophyll fluorescence was measured in laboratory after a dark-adapted period of two hours. Green upper parts of five mosses were cut into pieces and put in the analytical chamber with 0.5 ml of water. Fluorescence was excited by a 634 nm Helium-Neon laser after a 120 and 60 seconds period time at 20°C and 10°C. At 20°C, two flashes induced the fluorescence. Monochromator scanning (1 nm optical bandwith) and recording were driven by a home-made computer program. The whole induction kinetic was followed with a modulated PAM fluorimeter (walz, Germany), under a continuous illumination (634 nm, 135 $\mu E.m^{-2}.s^{-1}$) and 5000 $\mu E.m^{-2}.s^{-1}$, 1 s light pulses. The key parameter Fv/Fm ratio (variable and maximal fluorescences) was calculated. MANOVA data treatment determined the significant controlled factors and interactions.

2.3. Parallel exposition of transplanted mosses and total deposition gauges

2.3.1. Stock-Flux conversion experiment

The two moss species *Pleurozium schreberi* (Ps) and *Pseudoscleropodium purum* (Sp) have proved to be appropriate for such transplant tool. “Moss plates” and monthly total atmospheric deposition gauges (“Owen” Gauge) were both exposed on four continuous air quality monitoring stations, 2 background and 2 polluted stations. The “blank” and remote stations (“D” and “R”) are part of the MERA network (Atmospheric Deposition Measurements). The two polluted stations (“CE” and SV”) are proximity stations, mainly influenced by dry particulate depositions and car exhausts. These stations are part of the environmental controlled network of the Atomic Energy Center (CEA, France). The bulk collector consisted of a polyethylene funnel (diameter 290 mm; vertical board 100 mm) and a polyethylene bottle (15 l). The funnel-bottle system was maintained by solid external framework. After t = 0, 1, and 2 months of cumulative exposure, one triplicate of each species and each station was removed from their platform and, mosses of each sub-unit were prepared for ICP-MS analysis. Total atmospheric deposition gauges were removed to the laboratory and prepared for ICP-MS analysis. From autumn 1998 to summer 1999, three exposure periods of two months (overall 6 month exposure) were conducted. Data statistical treatments were achieved through MANOVA procedure for the “Moss Plate” results, and multiple regression analysis for the stock-flux conversion.

2.3.2. Biological Material for SXRF experiment

During the summer exposition, intact two-years old shoots of *Pseudoscleropodium purum* were removed from the transplants of stations “D” and “SV” for the *in vivo* analysis by SXRF. At t=0 and after t = 1 and 2 months, three two years old's moss shoots were randomly sampled in each sub-unit of the transplant and prepared for *in vivo* analysis by SXRF.

SXRF data were analysed by MANOVA and regression analysis (SXRF-ICP-MS semi-quantitative conversion).

2.3.3. Preparation and analysis

2.3.3.1. ICP-MS

2.3.3.1.1. Transplanted Mosses

After t = 0,1, and 2 months of cumulative exposure in the four stations, moss species of each sub-unit were separately prepared and analysed for ICP-MS analysis. Samples were dried 24 hours at 40°C, milled and mineralised (200 mg portion in PTFE vessels by micro-wave with successive addition of ultra-pure reagents HNO_3, H_2O_2, HF, HNO_3, H_2O). A 200 mg dried moss portion over the 1 g total dried weight of one sub-unit has shown to be representative and homogeneous of the transplant material (coefficient of variation <20%). Solutions were diluted in 50 ml with high purity water (18.2 MΩ/cm), internal standards (In, Be, Re at 10 ng/ml) were added and analysis were performed by ICP-MS with a VG Plasmaquad II Plus. Quality control and reproducibility of results were ensured by comparison with standard reference materials Lichen 336 IAEA, Rye Grass CRM 281 BCR, M1 and M2 reference material [16]. Final elements concentrations are expressed in $\mu g.g^{-1}$ on a dry weight basis. Detection limits and results reproducibility have been published [17].

2.3.3.1.2. Bulk Collectors

After a monthly exposition (t = 1, 2 months) in the four stations, total atmospheric deposition gauges were removed to the laboratory where their content was weighted and homogenized (shaking for 2 hours). The particle content was divided into four size classes by passing through nylon sieves with different mesh sizes (>1 mm; 1000–200 µm; 200–40 µm; 40–20 µm). Samples were dried 24 hours at 60 °C and the whole size class particle amount (<70 mg) was mineralised by a wet digestion method, with successive addition of ultra-pure reagents (HNO_3, HF, $HClO_4$) on sandy bath of 150 °C for 3 hours. During a cooling period (cold bath, 15 min), H_2O_2 was added and put at 90°C for 30 min. Then, teflon pots were opened and two successive HNO_3 additions and evaporations allowed chlorine removing. Mineralised samples were diluted in 50 ml with high purity water (18.2 MΩ/cm), internal standards (In, Be, Re at 10 ng/ml) were added and analysis were performed by ICP-MS with a VG Plasmaquad II Plus. Quality control and reproducibility of results were ensured by comparison with standard reference material (Coal Fly Ash 1633a SRM).

2.3.2. SXRF Experimental arrangement

The moss gametophyte shoots with fully developped annual segments (two years old) were randomly chosen and kept in ultra-pure polyethylene plastic bags in a refrigerator (4°C) to avoid any dry out event. Just before SXRF experiments, moss shoots were fixed on a plastic sample-holder structure for *in vivo* analysis. In parallel, the sample rests were analysed by ICP-MS for concentration analysis.

Synchrotron induced X ray fluorescence analysis was performed on beam line D15 at LURE DCI (Laboratory for Electromagnetic Radiation Use), Orsay (France).

Relativistic positron, stored in a ring, radiates energy resulting in an intense white X ray beam, highly collimated and polarized. In the experimental hutch, this beam is monochromated and cut to a 300 micron diameter spot by a platinum collimator. An energy of 14 keV was chosen to enhance sensitivity for Pb determination. A laser beam is used to simulate the X ray beam path for proper positioning of the sample which is set on a three axis (x, y, z) remote controlled stage and observed with a microscope equipped with a CCD camera. The fluorescence spectrum is detected with a Si(Li) detector of 150 eV energy resolution set at 90 from the incident beam in order to minimize scattering. Acquisition life time was typically 600s. Three point acquisitions by annual segments were made on each two-years old individual to obtain a two-dimensional semiquantitative mapping of element repartition along identified annual segments. Data treatment was achieved through VIRESYME [18] which is a program used for the deconvolution of X ray fluorescence spectrum.

2.4. Statistics

For the chlorophyll fluorescence induction data, the influence of controlled factors (water, species, time) was tested through MANOVA procedure.

For the "Moss Plates" data, the significant influence of controlled factors (species, stations, time) were tested through MANOVA and the stock-fluxes conversion models were assessed with multiple regression analysis (qualitative variables as stations and species, and quantitative variables as class size particles fluxes, time). Binary encoding allowed the models to take account on the qualitative variables. The "bulk collectors" data were tested by MANOVA for the controlled factors (stations, size classes). The SXRF data were analysed with MANOVA for the controlled factors (time, annual segment, station). Possible semi-quantitative conversions between SXRF and ICP-MS were tested through different fitted models (linear, logarithmic). In these statistical analysis, variables were first transformed (logarithmic, square root functions) if necessary to satisfy the underlying conditions of variance and regression analysis. Multiple comparison procedures were applied to determine which means were significantly different from the others (Tukey's honestly significant differences (HSD) procedure.

All statistical analysis was performed with StatGraphics Plus® software.

3. RESULTS AND DISCUSSION

3.1. Chlorophyll induction Fluorescence: Viability of the "Moss Plates"

MANOVA determines significant effects of the three controlled factors (water, species, and time) and interactions (water×time) (W×T) and (species×time) (S×T) (Table I, Figs. 2a, and 2b). The "Water" effect indicates the necessity of the water presence in the transplant to sustain the PSII biological activity of the three moss species exposed. The PSII activity of lichen has shown to be dependent on the water presence for rapid restoration after an unfunctionnal state [15]. The activity of PSII is more susceptible to heavy metal ions induced damage compared to that of PSI [14,19] and it could be more influenced by atmospheric depositions. For the "Species" effect, the *pseudoscleropodium (Ps)* species has a higher biological activity than the two others. *Ps* is a more light resistant moss than Sp or *Eurhynchium praelongum (Ep)* which are shade species [20]. This significant difference can be explained by the ecology of Ps but could also reveal a probable difficult adaptation of two other species for a two months exposition in an open area.

The (W×T) interaction **(**Fig.. 2a) shows the buffering role of water for the biological activity of mosses exposed for two months. Indeed, the watered transplants have a relative constant activity whereas the unwatered's be more variable with time. At t = 2 and 8 weeks, the Fv/Fm ratios are different for both transplants. A drought period has preceded the t = 2 weeks measurements and a rainy period has overlaped the t = 4 weeks measurements. The water presence in the "moss plate" buffers the effects of external meteorological conditions on the chlorophyll fluorescence induction. With a rain event, the low biological activity of mosses comes back rapidly to a normal level. For the (S×T) interaction (Fig. 2b), the Ps species has a higher and more stable activity than the two others. Sp and Ep show parallel time evolution with a rapid recuperation after the drought period (rain event). For the stock-flux conversion, the selected moss species were *Pleurozium schreberi* (stable biological activity) and *Pseudoscleropodium purum* (fast biological recovery).

TABLE I. SUMMARY OF THE MULTIFACTOR ANALYSIS OF VARIANCE FOR FV/FM FOR THE CONTROLLED FACTORS A: "WATER" (WITH WATERED OR UNWATERED MOSS PLATES); B: "SPECIES" (MOSS SPECIES TESTED WITH *EURHYNCHIUM PRAELONGUM* (Ep), *PLEUROZIUM SCHREBERI* (Ps) AND *PSEUDOSCLEROPODIUM PURUM* (Sp)); C: "TIME" (t = 0, 2, 4, 8 WEEKS, NOT DETAILED HERE) AND THE INTERACTIONS (AxB) (FIG. 2B); (AxC) (FIG. 2A); (BxC) AND (AxBxC). SINCE 5 p-VALUES ARE LESS THAN 0.05, THESE FACTORS HAVE A STATISTICALLY EFFECT ON FV/FM AT THE 95% CONFIDENCE LEVEL (** $p<0.001$; *** $p<0.0001$). THIS TABLE SHOWS THE MEAN AND STANDARD ERROR (STD. ERROR) FV/FM FOR THE WATER AND SPECIES LEVEL

Main Effects	**A : Water (dl=1)**		**B : Species (dl=2)**			**C : Time (dl=3)**
p-Value	***		***			***
	with (n=42)	without (n=42)	Ep (n=28)	Ps (n=28)	Sp (n=28)	
Fv/Fm Mean	0.53	0.59	0.53	0.63	0.52	
Std. error	0.01	0.01	0.01	0.01	0.01	

Interactions	**AxB (dl=2)**	**AxC (dl=3)**	**BxC (dl=6)**	**AxBxC (dl=6)**
p-Value	**0.376**	**	***	**0.153**

3.2.Stock-Flux conversion experiment (ICP-MS)

3.2.1. Moss results

The MANOVA analysis allows to clarify the "moss plates" efficiency as a biomonitoring tool (Table II).

The "Species" effect is significant for Pb, Sb, Ti ($p<0.001$), and V ($p<0.05$). These results show that Ps accumulate more all elements of concern than Sp, for all stations and periods of exposure. Some studies have shown a higher Pb, Fe, Ti, and V content of Ps versus Sp for

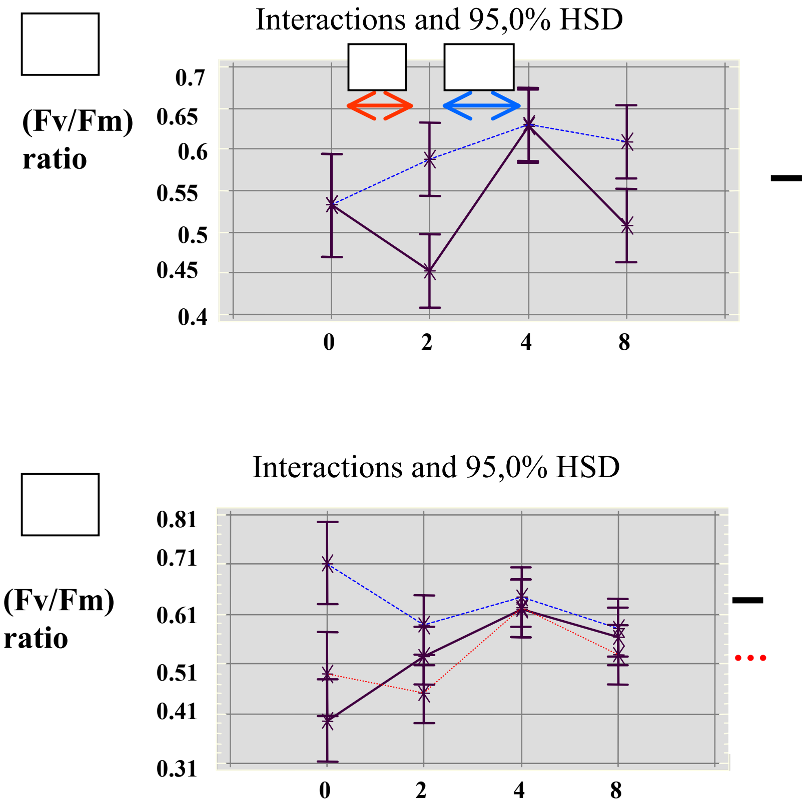


FIG. 2. Controlled factor interactions after MANOVA for the Fv/Fm ratio (Chlorophyll Fluorescence Induction parameter) (with). The significant interactions are: A- (Water×Time) with watered and unwatered "moss plates", "D" and "R" indicate the drought and rainy time periods ; time (t = 0, 1, 2, 4 and 8 weeks); B- (Species×Time) with the three moss species tested Pleurozium schreberi (Pl. schr.), Pseudoscleropodium purum (Scl. Pur.) and Eurhynchium praelongum (Eur. Pr.); time (t = 0, 1, 2, 4 and 8 weeks).

passive biomonitoring campaign [21]. Environmental pathways for these elements often involve particulate matter emitted from natural (Ti, V soil dusts) or anthropogenic (Pb, Sb) sources like car exhausts [22,23,24]. The better capture efficiency of Ps versus Sp could be explained by a wider surface contact area due to a more open leaf shape, leading to an efficient dust trap. The species content difference is of several µg.g^{-1} for Pb, Ti and ng.g^{-1} for Sb and V, with a concentration doubled for Sb. Ps seems to be more efficient in capturing these elements and could be preferred to Sp in a case of choice.

The “Time” effect shows that a cumulative two-month exposure is necessary for significant enrichment of mosses compared to t = 0 for Ba, Cd, Pb, Sb, Ti, U, and V **(**Table II**)**. One month is sufficient for Sb and U. The time required for the “moss Plate” is similar for “moss bags” with one to several month exposition required [10,13]. Some authors recommend more than one month exposure for Pb atmospheric deposition with “moss bag” [25] and agree with the efficiency of “moss bags” mainly for dry deposition [10,12,13]. But, the exact relation between the “moss bag” content and real atmospheric deposition is still not known even if some studies put in evidence possible quantitative conversion with unsedimentable dry deposition (<0.8 µm) [26].

The relative comparisons of "Stations" are possible for Cs, Sb, Sr, U ($p<0.001$), and Ba ($p<0.05$) (Table. II). The two-month exposure is not enough to see significant differences for Pb even if some trends are obvious. For other elements, the two polluted sites "CE" and "SV" are significantly higher than the background "D" and "R" for Cs, Sb, Sr, U. "SV" is significantly higher than "D" for Ba. The mean concentrations of "moss plates" are of the same order of previous passive biomonitoring campaigns with the same moss species [27]. For Sb, stations "D" and "R" are closer to norvegian and german median concentrations (0.09 and 0.171 $\mu g.g^{-1}$ versus 0.29 $\mu g.g^{-1}$) whereas "SV" and "CE" are closer to the maxima indexed for these mosses (0.64 and 0.62 $\mu g.g^{-1}$). For U, the german and norvegian median (0.04 and 0.029 $\mu g.g^{-1}$) are similar to those of stations "D" and "R". Stations "CE" and "SV" are far from indexed maxima (0.45 and 0.89 $\mu g.g^{-1}$) but, transplanted mosses have doubled their content in one month in these two stations. For Cs, the situation is similar to U with "SV" and "CE" stations higher then "D" and "R" content and to classical median encountered (0.18 and 0.22 $\mu g.g^{-1}$). For Sr and Ba, the "moss plate" concentrations are similar to classical moss content (13 and 10.4 $\mu g.g^{-1}$ for Sr ; 24 and 19.4 $\mu g.g^{-1}$ for Ba).

3.2.2. Bulk Collector Results

Treatment reveals significant effect of controlled factors. The "Stations" effect is significant for Ba, Pb, Sb, Ti (Table III). For Ba, the station "CE" (469.7 $\mu g.g^{-1}$) is significantly higher than "D" (349.9 $\mu g.g^{-1}$). The mean concentration of these two stations are similar to concentration maxima found in urban soil (0–2 cm) with 385 $\mu g.g^{-1}$ (median of 72.2 $\mu g.g^{-1}$) [27]. Ba is probably mostly windblown dust with geogenic sources more important than anthropogenic ones. For Pb, "SV" (1728.2 $\mu g.g^{-1}$) is higher than "CE" (761.1 $\mu g.g^{-1}$), and "R"(844.6 $\mu g.g^{-1}$) which are not different but significantly more concentrated than "D" (470.3 $\mu g.g^{-1}$). For the "R" station firstly considered as background, there is a certain evidence of a traffic influence as important as "CE". For "CE", it is surprising that total atmospheric deposition for sedimentable particles is similar to "R" but this could be explained by a real local influence of Pb sedimentable dusts which can distinguish background from heavily polluted sites but less precisely from different medium polluted sites. The Pb particle concentration for all stations makes Pb a major element of the dusts captured by the bulk collectors (% contribution for "SV"). These concentrations are similar to maxima indexed in urban soil (0–2 cm) (976 $\mu g.g^{-1}$) [27]. The car exhausts influence in these stations, especially in "SV"'s for Pb, is clearly put in evidence. Sb is present in Pb-Cu smelters, car exhaust, coal combustion and, for this element, "CE" and "SV" are more concentrated than the two background stations with similar concentrations than maxima in urban soil (18 $\mu g.g^{-1}$) [27]. For Ti , "R" is statistically more concentrated than "D", "CE", and "SV". Ti is one of the major components of sedimentable particles recovered in these passive gauges. Its environmental pathways are mainly soil dusts and median concentrations for urban soils are 1,110 $\mu g.g^{-1}$ and 402 $\mu g.g^{-1}$ for agricultural soils [27] with maxima of 3,170 and 3,010 $\mu g.g^{-1}$. Station Ti concentrations are closer to maxima and put in evidence a main source of dusts captured with these gauges coming from soil after possible atmospheric ressuspension. Comparing "Stations" effect between "moss plates" and "bulk collectors", similar relative station classification is possible for Sb, Ba, and Pb (if only Pb trends are considered for mosses).

The "Size class" effect is significant for Ba, Pb, Sb, and Ti, showing differential element repartition with the four sizes >1 mm (class 1); 1000–200 µm (class 2); 200–40 µm (class 3), and 40–20 µm (class4) (Table III). Ti is more concentrated in class 3 than classes 1, 2 and 4 (lowest concentration in the >1 mm). Ba, Sb, and Pb have a more ubiquitous presence in all the size classes 2, 3, 4 with a higher trend for class 3 (lowest concentration for class 1). These total atmospheric deposition gauges seem to be mainly influenced by ressuspended particles with natural and anthropogenic origins. Dust size classes show different elemental concentrations and are probably of importance for the stock-flux models as independent explicative variables of moss content.

3.2.3. Stock-Flux conversion for Ba, Pb, Sb, Ti, V

After a stepwise method for selecting the minimum number of significant variables ($p<0.05$) and fitting the best explicative models, stock-flux models were built for Ba, Pb, Sb, Ti with quantitative variables (different size class fluxes; time) and qualitative variables (stations, moss species) (Table IV). These models have a relatively strong R^2 (>70% for Ba, Pb, Ti) and very strong R^2=94% for Sb. For Ba, 7 significant variables are involved in the moss stock content explanation (R^2=70%) (size classes 1, 3, 4 for dust fluxes; stations; time). "SV" station estimate can be deduced from the other stations (sum of estimated coefficients=0). There is a significant contribution of the qualitative variable "Stations" in this model, leading to a local and qualitative influence of the transplantation site on the moss content for Ba. There is no species variable in the model and this could be related to the no "species" effect for Ba in "moss plate" data (Table IV). The Time variable is significant for the model as it was for the "Time" effect in the moss plates accumulation. For Pb, R^2 (72%) shows a strong relationship between the dependant variable of moss content and the other 6 variables. The quantitative variables, size classes 1 and 2 are the only significant leading to a more important contribution of coarse particles (1000–200 µm) to the moss content for Pb than the smaller ones. The Time and Species variables are significant reinforcing previous "moss plate" data analysis (Table IV). The station qualitative variable is included in the model showing the importance of Pb local atmospheric deposition to the moss content. The "D" station does not appear for Pb and only the "R", "CE", and "SV", deduced from the other two stations are significant.

This could be interpreted as a necessary enough contaminated level deposition for a significant contribution to the moss content. Perhaps for Pb, two months are not enough for the less polluted site. Sb presents the best fitted model with R^2=94.1% and with 7 significant variables. All qualitative variables "Species" and "Stations" are involved confirming previous significant "specie" effect with "moss plate" (Table IV) and again, the local qualitative influence of atmospheric coarse deposition on the moss content. For Sb, the coarse fractions fitted the model are the three classes 2, 3, 4 (particles from 1000 to 20 µm). For Ti, the model shows 7 significant variables (R^2=82.5%) with the qualitative variables Species and Stations. For Ti, the dust size class involved in the model are class 1, 4 (>1 mm; 40–20 µm). Studies on atmospheric capture of dusts by mosses (28, 29) have demonstrated their better efficiency for coarse particles (20–175 µm) and, that the standardised active biomonitoring method of "moss bags" had better capture efficiency on particles over a 20 µm diameter less influenced by abiotic conditions like wind speed. The stock-flux conversion is possible between the "moss plates" content and coarse sedimentable particles for Ba, Pb, Sb and Ti. But, the models built are dependant on qualitative variables such as "Species" and "Stations" leading to a preliminary moss specie choice before exposure and a necessary in site calibration of the biological tool before any stock-flux conversion.

3.3. In vivo distribution of element enrichment (SXRF)

The sensitivity of SXRF analysis allows to identify 15 elements with a 14 keV photon beam energy (S, K, Cl, Ca, Cr, Mn, Fe, Cu, Zn, Ni, Se, Ti, As, Ba, and Pb). All MANOVA results are detailed in another work [30] and, this paper focuses on the (Segment×Time) (Se×T) interaction factor in order to locate the moss annual segment enrichment with this short time exposure.

This significant interaction (Se×T) effect for Cl, Se, Ti, and Pb **(Table V, Fig. 3)** shows different short time behaviour between elements. Cl increases with time in all the shoot, but after one month it is more concentrated in the basal part (SII, two-years old segment vs SI, one year old segment). For Ti, one month of exposure reverses the segment repartition compared to t=0 with the basal part enriched versus the upper one. This enrichment continues a bit during the second month. At t=0 for Se, the upper part is richer in Se, after one month the increase of the SII leads to a homogeneous repartition along the shoot, and finally, a SII enriched to SI after two months. For Pb, the t=0 homogeneous situation is followed by a significant enrichment of SII versus SI which also captured Pb but not as much as basal part. These results are in accordance with a simple particular capture of dust from a geogenic (Ti) or anthropogenic (Pb) sources. Aerial dispersion of heavy metals often involves particular matter as industrial emissions or carbonaceous material released by vehicular exhaust like in the Station "SV" exposition. Previous studies have found a homogeneous repartition of Cl between a wide range of coarse particle diameters (0,08 to 16 µm) [31], leading to a possible Cl particular enrichment of mosses. For Se, a small fraction may exist in the gaseous state in the atmosphere but, the majority of Se is expected to be present in particular form mainly emitted from coal and oil combustion [32]. Mosses have demonstrated their better efficiency for coarse particle (20–175 µm) [28,29] and previous results with the stock-flux model have shown the significant contribution of the coarse atmospheric dry deposition on the moss content. So, atmospheric particles containing these elements could fall on moss and be more or less retained with gravity for coarse particles or static phenomenons for smaller ones [1]. All these *in vivo* observations reflect integrated input and output of elements falling on a moss shoot (Fig. 3). For active biomonitoring, these results show that old parts are the preferentially sites of recent capture for short-time exposure with mainly dry depositions. Moreover, poorly water soluble elements (Pb, Ti) seem to be under a saturation effect of capture after one month of exposure for Sp specie (Table. V).

Regression analysis (Fig 3) allows a quantitative conversion of *in vivo* SXRF Pb data to concentration results ($\mu g.g^{-1}$). So, with SXRF *in vivo,* quantification of metals and trace elements in mosses are possible on a very small amount of material (1 mm^2 biological surface), without any pre-treatment (dry out, mineralisation), and with consecutive analysis possible due to non destructive method. These results illustrate the hypothesis that particle trapping by bryophytes may be a major source of poorly water-soluble elements and, that moss content can reflect recent environmental conditions for dry and coarse depositions. The use of such biomonitors should be precise in purpose, protocole of pre-treatment, choice of plant's parts, and analysed elements.

TABLE II. SUMMARY OF THE MANOVA FOR “MOSS PLATES” MULTIELEMENTARY CONCENTRATIONS ($\mu g.g^{-1}$) WITH THE CONTROLLED FACTORS A: “SPECIES” (MOSS SPECIES TESTED WITH *PLEUROZIUM SCHREBERI* (Ps) AND *PSEUDOSCLEROPODIUM PURUM* (Sp); B: “TIME” (TIME OF TRANSPLANT EXPOSURE WITH t = 0, 1c (ONE CUMULATIVE MONTH), 2c (TWO CUMULATIVE MONTHS), AND (2c-1c) (2nd MONTH); C: “STATIONS” (D, R: BLANK STATIONS; CE, SV: POLLUTED STATIONS MAINLY INFLUENCED BY TRAFFIC AND CAR EXHAUSTS). SINCE 19 p-VALUES ARE LESS THAN 0.05, THESE FACTORS HAVE A STATISTICALLY EFFECT ON THESE MULTIELEMENTAL CONCENTRATIONS AT THE 95% CONFIDENCE LEVEL (* $p<0.05$; ** $p<0.001$; *** $p<0.0001$; ns: NO SIGNIFICANT; S. of sq.: SUM OF SQUARES). THIS TABLE SHOWS THE MEAN AND STANDARD ERROR (m +- st. e.) ELEMENT CONCENTRATIONS ($\mu g.g^{-1}$) FOR THE SPECIES, TIME, AND STATIONS LEVELS. THE NUMBER OF ICP-MS ANALYSIS IS INDICATED IN BRACKETS.

Main Effects	**Elements**	**Ba138**	**Cd114**	**Cs133**	**La139**	**Pb206**	**Sb121**	**Sr88**	**Ti49**	**U238**	**V51**
A: Species	**S. of sq.**	0.252	0.584	0.003	0.7483	2.5831	20.72	0.038	6.08	0.06	2.02
dl=1	**p-value**	**ns**	**ns**	**ns**	**ns**	***	***	**ns**	***	**ns**	*
Ps (n=120)	m +- st.e.	12.88+-0.71	0.23+-0.01	0.67+-0.04	0.46+-0.04	10.95+-0.51	0.29+-0.01	12.95+-0.87	21.01+-1.11	0.093+-0.005	1.35+-0.05
Sp (n=120)	m +- st.e.	13.42+-0.71	0.26+-0.01	0.64+-0.04	0.42+-0.04	8.65+-0.51	0.16+-0.01	13.42+-0.87	13.87+-1.07	0.081+-0.005	0.98+-0.05
B: Time	**S. of sq.**	5.204	10.63	1.652	6.761	10.462	12.96	8.807	9.38	16.4	10.9
dl=3	**p-value**	**	***	*	**	***	***	***	***	***	***
t=0 (n=22)	m +- st.e.	11.89+-1.43	0.20+-0.02	0.72+-0.08	0.42+-0.09	7.18+-1.03	0.15+-0.02	9.72+-1.74	13.95+-2.45	0.057+-0.010	0.97+-0.09
t=1c (n=108)	m +- st.e.	10.45+-0.65	0.21+-0.01	0.49+-0.03	0.39+-0.04	8.34+-0.47	0.23+-0.01	12.39+-0.79	15.75+-0.97	0.089+-0.005	1.02+-0.04
T=(2c-1c) (n=22)	m +- st.e.	14.59+-1.43	0.25+-0.02	0.75+-0.08	0.44+-0.09	10.52+-1.03	0.20+-0.02	12.94+-1.74	15.75+-2.01	0.078+-0.010	1.21+-0.09
t=2c (n=88)	m +- st.e.	15.68+-0.72	0.30+-0.01	0.68+-0.04	0.51+-0.04	13.18+-0.52	0.31+-0.01	17.7+-0.89	24.31+-1.03	0.123+-0.005	1.46+-0.05
C: Stations	**S. of sq.**	3.024	0.158	5.888	2.736	1.012	27.79	5.365	2.39	5.7	2.19
dl=3	**p-value**	*	**ns**	***	**ns**	**0.07**	***	***	**ns**	**	**ns**
D (n=66)	m +- st.e.	11.65+-0.9	0.24+-0.01	0.58+-0.05	0.44+-0.06	8.91+-0.64	0.16+-0.01	11.34+-1.09	14.74+-1.33	0.066+-0.006	1.05+-0.06
R (n=44)	m +- st.e.	11.98+-1.12	0.25+-0.02	0.49+-0.06	0.40+-0.07	9.55+-0.80	0.16+-0.02	10.04+-1.36	14.17+-1.66	0.068+-0.008	1.03+-0.08
CE (n=66)	m +- st.e.	14.09+-0.9	0.25+-0.01	0.85+-0.05	0.44+-0.06	10.56+-0.64	0.24+-0.01	16.81+-1.1	19.95+-1.36	0.105+-0.006	1.23+-0.06
SV (n=64)	m +- st.e.	14.88+-0.9	0.22+-0.01	0.71+-0.05	0.47+-0.06	10.19+-0.65	0.35+-0.01	14.56+-1.1	20.9+-1.4	0.108+-0.006	1.34+-0.06

TABLE III. SUMMARY OF THE MANOVA FOR “OWEN” GAUGES SEDIMENTABLE ATMOSPHERIC PARTICLES ($\mu g.g^{-1}$) WITH THE CONTROLLED FACTORS A: “TIME” (t=1, 1^{ST} MONTH; T=2, 2ND MONTH); B: “SIZE CLASSES” (4 SIZE CLASSES OF SEDIMENTABLE PARTICLES); C: “STATIONS” (D, R: BLANK STATIONS; CE, SV: POLLUTED STATIONS MAINLY INFLUENCED BY TRAFFIC AND CAR EXHAUSTS). SINCE 10 p-VALUES ARE LESS THAN 0.05, THESE FACTORS HAVE A STATISTICALLY EFFECT ON THESE MULTIELEMENTAL CONCENTRATIONS AT THE 95% CONFIDENCE LEVEL (**p<0.001; *** p<0.0001; ns: NO SIGNIFICANT; S. of sq.: SUM OF SQUARES). THIS TABLE SHOWS THE MEAN AND STANDARD ERROR (m +- st. e.) ELEMENT CONCENTRATIONS ($\mu g.g^{-1}$) FOR THE TIME, SIZE CLASSES, AND STATIONS LEVELS. THE NUMBER OF ICP-MS ANALYSIS IS INDICATED IN BRACKETS.

Main Effects	**Elements**	**Ba**	**Pb**	**Sb**	**Ti**
A: Time	**S. of sq.**	3.45	10.3	6.17	1.16
dl=1	**p-value**	**ns**	**0.001**	**ns**	**ns**
t=1 (n=96)	m +- st.e.	419.7+-21.4	1093.9+-41.3	10.2+-3.5	2968.4+-117.4
t=2 (n=96)	m +- st.e.	395.4+–43.4	808.2+-83.8	9.8+–4.1	2736.1+-237.9
B: Size Classes	**S. of sq.**	93.45	49.46	152.9	199.4
dl=3	**p-value**	***	***	**	***
>1mm (n=48)	m +- st.e.	153.9+-36.6	803.8+-70.8	5.7+-3.3	1003.2+-200.9
1000-200µm (n=48)	m +- st.e.	538.2+-36.6	862.8+-70.9	8.8+-2.5	3193.3+-200.9
200–40µm (n=48)	m +- st.e.	394.9+-36.4	1117.6+-70.9	12.5+-3.6	3976.5+-200.9
40-20µm (n=48)	m +- st.e.	543.2+–49.9	1020.1+-96.4	5.1+-3.2	3235.8+-273.6
C: Stations	**S. of sq.**	11.75	120.7	210	24.42
dl=3	**p-value**	***	***	***	***
D (n=48)	m +- st.e.	349.9+-36.6	470.3+-70.6	4.3+-4.1	2712.4+-202.1
R (n=48)	m +- st.e.	409.2+-49.9	844.6+-96.4	4.2+-4.1	3816.1+-273.6
CE (n=48)	m +- st.e.	469.7+-36.5	761.1+-70.6	13.9+-5.1	2927.2+-200.9
SV (n=48)	m +- st.e.	401.3+-36.6	1728.2+-70.9	19.8+-5.9	1953.3+-200.9

TABLE IV. RESULTS OF THE FITTED MULTIPLE LINEAR REGRESSION MODELS TO DESCRIBE THE RELATIONSHIPS BETWEEN THE "MOSS PLATES" CONTENT (EXPRESSED AS LOGARITHMIC $\mu g.g^{-1}$) AND INDEPENDANT VARIABLES. QUANTITATIVE VARIABLES ARE "TIME" AND THE FOUR SIZE CLASSES OF SEDIMENTABLE PARTICLES (EXPRESSED AS LOGARITHM OF FLUXES TERM, $\mu g.m^{-2}.t^{-1}$). THE QUALITATIVE VARIABLES ARE "SPECIES" (-1 FOR *PSEUDOSCLEROPODIUM PURUM* AND +1 FOR *PLEUROZIUM SCHREBERI*), STATIONS (ST1 FOR "D", ST2 FOR R, ST3 FOR CE AND THE SV ESTIMATE IS DEDUCED FROM THE OTHERS WITH ESTIMATE SUM=0). SINCE p-VALUE ARE LESS THAN 0.05, THERE IS A STATISTICALLY SIGNIFICANT RELATIONSHIP BETWEEN THE VARIABLES AT THE 95% CONFIDENCE LEVEL (*p<0.05; ** p<0.001; *** p<0.0001; ns: NO SIGNIFICANT). THE R-SQUARED STATISTIC INDICATES THE% VARIABILITY EXPLAINED BY THE MODEL AS FITTED.

Multiple regression analysis

Dependant Variables (Moss Plates)	**log(Ba138) R^2=70%**			**log(Pb206) R^2=72%**			**log(Sb121) R^2=94.1%**			**log(Ti48) R^2=82.4%**		
Parameter	Estimate	Std. er.	p	Estimate	Std. er.	p	Estimate	Std. er.	p	Estimate	Std. er.	p
Constant	3.698	0.4322	***	1.2572	0.1124	***	-1.3274	0.0749	***	3.5896	0.3809	***
species			**ns**	0.0628	0.0117	***	0.2425	0.0127	***	0.0499	0.0144	**
log(flux s.c.p. 1)	0.1622	0.0555	*	0.0491	0.018	*			**ns**	0.1037	0.0218	***
log(flux s.c.p. 2)	-0.1557	0.0724	*	0.1555	0.045	**	-0.2718	0.1328	*			**ns**
log(flux s.c.p. 3)			**ns**			**ns**	0.6826	0.1532	***			**ns**
log(flux s.c.p. 4)	-0.2696	0.0985	*			**ns**	0.1479	0.0428	**	-0.1288	0.0766	*
ST1	-0.3132	0.072	**			**ns**			**ns**	-0.4023	0.1195	*
ST2	-0.23	0.0732	*	-0.1075	0.0252	***	-0.4813	0.1709	*	-0.3062	0.0713	***
ST3	0.2845	0.1153	*	0.2008	0.0418	***	0.2248	0.1186	*	0.4489	0.0648	***
Time	0.5186	0.1423	**	-0.09	0.0309	*	-0.4738	0.1049	***	0.1706	0.0799	*

TABLE V. SUMMARY OF THE MULTIFACTOR ANALYSIS OF VARIANCE FOR SXRF DATA WITH THE (SEGMENTXTIME) INTERACTION SIGNIFICANT FOR Cl, Ti, Se AND Pb ANALYSED ON FIRST ANNUAL SEGMENT (I) AND SECOND ANNUAL SEGMENT (II) OF *PSEUDOSCLEROPODIUM PURUM* AFTER t=0, 1 AND 2 MONTH OF EXPOSURE IN "MOSS PLATES". MEAN AND STANDARD ERROR VALUES (M +-Std.Er.) ARE EXPRESSED AFTER ($) LOGARITHMIC AND (£) SQUARE ROOT TRANSFORMATIONS OF VARIABLES (ELEMENT COUNTING/DIFFUSIVE COUNTING FOR 600 SECONDS TIME ACQUISITION, PHOTON MACROBEAM OF 14 keV). HOMOGENEOUS GROUPS ARE IDENTIFIED BY A COLUMN OF X's. WITHIN EACH COLUMN, THE LEVELS CONTAINING X's HAVE GROUPS OF MEANS WITH OR WITHOUT STATISTICALLY SIGNIFICANT DIFFERENCES (95% CONFIDENCE INTERVALS).

		Cl$^{\$}$			**Ti$^{£}$**		
Segment x Time	Time (month)	0 (n=18)	1 (n=40)	2 (n=33)	0 (n=18)	1 (n=40)	2 (n=33)
M $^{+}$- Std.Er	**I (n=55)**	1.94+0.08	2.03+0.06	1.95+0.06	1.21+0.24	1.63+0.17	1.59+0.2
	II (n=36)	1.85+0.11	2.21+0.07	2.3+0.08	0.49+0.34	2.0+0.2	2.14+0.23
Level: Segment	**I**	**X**	**X**	**X**	**X**	**X**	**X**
95.0% conf. level	**II**	**X**	**X**	**X**	**X**	**X**	**X**

		Se$^{\$}$			**Pb$^{\$}$**		
Segment x Time	Time (month)	0 (n=18)	1 (n=40)	2 (n=33)	0 (n=18)	1 (n=40)	2 (n=33)
M $^{+}$- Std.Er	**I (n=55)**	0.8+0.05	0.96+0.04	0.94+0.04	1.85+0.07	2.14+0.05	2.23+0.06
	II (n=36)	0.55+0.07	0.99+0.04	1.05+0.05	1.67+0.2	2.27+0.06	2.43+0.07
Level: Segment	**I**	**X**	**X**	**X**	**X**	**X**	**X**
95.0% conf. level	**II**	**X**	**X**	**X**	**X**	**X**	**X**

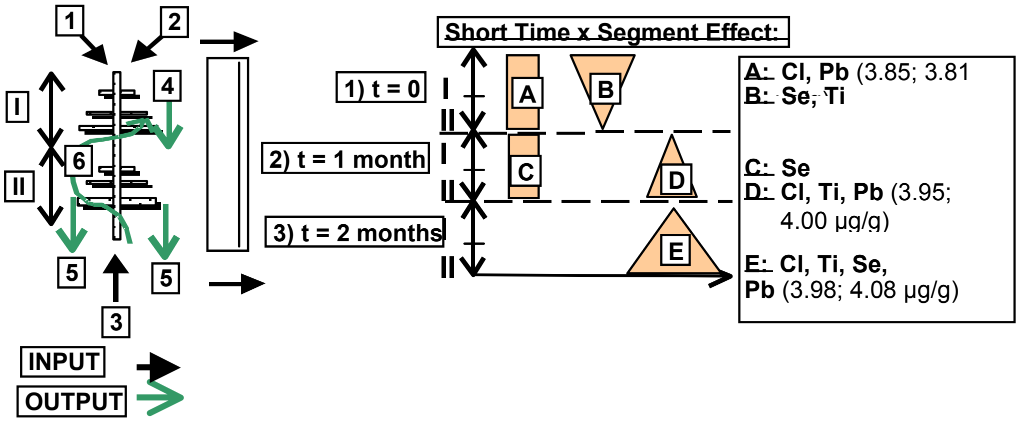


FIG. 3. Scheme of principal factors influencing trace elements Input and Output on a pluriannual shoot of moss, 1) wet, dry atmospheric depositions, 2) leachate from upper vegetation, 3) soil contamination, 4) young to older parts movements, 5) material losses (decomposition), 6) elements remobilization from old to younger parts. In vivo elements distribution by SXRF technique gives integrated observation of all these factors. The in vivo short time enrichment (Table. 5) effects through the elements distribution shows homogeneous, pyramidal or reversed pyramidal segments repartitions on I (first annual segment) and II (second annual segment). SXRF to ICP-MS conversion for Pb in brackets (R^2=98.1% with Pb_{icp}=3.565+0.046Pb_{sxrf}).

4. CONCLUSION

The chlorophyll fluorescence induction has demonstrated the biological activity stability of *Pleurozium schreberi* (Ps), *Pseudoscleropodium purum* (Sp) in this active biomonitoring tool. The necessary water presence has been shown to buffer the drought period effects on the Fv/Fm measurement as well as the rapid moss biological activity recovering with rainy period. The "moss plate" allows significant Sb, U, Ba, Cd, Pb, Ti, V accumulation in one and two months exposure from initial moss content. With this time and conditions exposure, relative sites comparisons are possible for Ba, Cs, Sb, Sr and U as species effect with a systematic more concentrated Ps species versus Sp for Pb, Sb, Ti, and V. The Stock-Flux conversion is possible for Ba, Pb, Sb, and Ti (R^2>70%) demonstrated the significant influence of coarse particles (1000–20 μm) in the moss content. But this also put in evidence the significant influence of qualitative variables such as the moss species and the site of exposure itself, leading to the evidence of a local qualitative influence of atmospheric coarse deposition on the moss content. In parallel, the SXRF experiment illustrates the possibility to locate the *in vivo* enrichment along the moss shoot exposed to dry deposition in their natural and vertical position. These *in vivo* microanalysis and multielemental SXRF technique allows to study and quantify very small amount of intact biological and, as a few work has been done on the process of particles entrapment by bryophyte surface, perspectives of future SXRF macro- or microbeam analysis are very encourageous for such environmental studies.

This "Moss Plate" has to be improved (time of exposure, water dependancy) but, as it allows a better understanding of moss-dry deposition relationships with a moss biological activity stability, the next step could be to assess some bioavailability aspects.

ACKNOWLEDGEMENTS

We are grateful to P. Chevallier and M. Bonnin-Mosbah for providing important assistant for the SXRF analysis, to S. Boiziau and S. Doublet for the concrete realisation of the "Moss Plate" support material and, to C. Elichegaray from the ADEME Agency for financial support. We are indebted to G. Meyer for active discussions and support. This work was carried out with the financial support of ADEME (Energy and Environmental Mastery Agency, France).

REFERENCES

[1] MARKERT, B., Plants as Biomonitors: indicators for heavy metals in the terrestrial environment, (MARKERT, B., Ed.), VCH, Weinheim, (1993) 643 pp.

[2] CEBURNIS, D., VALIULIS, D., Investigation of absolute metal uptake efficiency from precipitation in moss, Science of the Total Environment **226** (1999) 247–253.

[3] RÜHLING, A., TYLER, G., An ecological approach to the lead problem, Bot. Notiser **121** (1968) 321–342.

[4] BRÜNING, F., KREEB, K. H., Mosses as Biomonitors of Heavy Metal Contamination within Urban Area, Plants as Biomonitors: indicators for heavy metals in the terrestrial environment, (MARKERT, B.,Ed.), VCH, Weinheim (1993) 395–401.

[5] BROWN, D. H., Physiological and biochemical assessment of environmental stress in bryophytes and lichens, Bioindiactors of Environmental Health, (MUNAVAR, M., HÄNNINEN, O., ROY, S., MUNAVAR, L., KÄRENLAMPI, L., BROWN, D.H., Eds.), Ecovision World Monograph Series, SPB Academic Publishing, Amsterdam, The Netherlands, (1995) 203–206.

[6] PUCKETT, K. J., Bryophytes and lichens as monitors of metal deposition, Lichens, Bryophytes and Air Quality, (NASH, T. H., WIRTH, V., Eds.), Cramer, J., Berlin, Stuttgart, **30** (1988) 231–267.

[7] HERRMANN, R., Biomonitoring of organic and inorganic trace pollutants by means of mosses, Bryophytes their chemistry and chemical taxonomy, (ZINSMEISTER, H. D., MUES, R., Eds.), Oxford Science Publications (1990) 319–335.

[8] CLOUGH, W.S., The deposition of particles on moss and grass surface, Atmospheric Environment **9** (1975) 1113–1119.

[9] TAYLOR, F.G., WITHERSPOON, J.P., Retention of simulated fallout particles by lichens and mosses, Health Physics **23** (1972) 867–869.

[10] LODENIUS, M., TULISALO, E., Environmental Mercury Contamination Around A Chlor-Alkali Plant, Bull. Environ. Contam. Toxicol. **32** (1984) 439–444.

[11] MARTIN, M.H., COUGHTREY, P.J., Biological Monitoring of Heavy Metal Pollution, London, Applied Science Publishers (1982) 475 pp.

[12] HYNNINEN, V., Monitoring of airborne metal pollution with moss bags near an industrial source at Harjavalta, southwest Finland, Ann. Bot. Fennici **23** (1986) 83–90.

[13] CAMERON, A.J., NICKLESS, G., Mosses as collectors of airborne heavy metals, Water, Air and Soil Pollution **7** (1977) 117–125.

[14] DUCRUET, J.M., Les herbicides inhibiteurs du Photosystème II, Les Herbicides, mode d'action et principes d'utilisation, sous la direction de René Scalla, INRA, Paris (1991) 79–114.

[15] BOLHÀR-NORDENKAMPF, H.R., ÖQUIST, G., Chapter 12, Photosynthesis and production in a changing environment, a field and laboratory manual, (HALL, D.O., SCURLOCK, H.R., BOLHÀR-NORDENKAMPF, H.R., LEEGOOD, R.C., LONG, S.P., Eds.), Chapman & Hall, (1993) 464 pp.

[16] STEINNES, E., et al., Reference materials for large-scale metal deposition surveys, Accred. Qual. Assur. **2** (1997) 243–249.

[17] AYRAULT, S., et al., Multi-analysis of trace elements in mosses with inductively coupled plasma mass spectrometry, Biological Research Elements (2000) accepted.

[18] WANG, J. X., et al., VIRESYME: Logiciel d'exploitation des spectres de fluorescence, Note CEA-N-2756, (1993) 231–235.

[19] SASS, L., et al., Thermoluminescence studies on the function of Photosystem II in the desiccation tolerant lichen *Cladonia convoluta*, Photosynthesis Research (1996) 205–212.

[20] SCHOFIELD, W.B., Introduction to Bryology, Macmillan Publishing Company, New York (1985) 431pp.

SIMULATION OF FALLOUT FOR TRANSLOCATION STUDIES OF ^{137}Cs AND ^{90}Sr IN BEAN PLANTS (*PHASEOLUS VULGARIS*)

J.F. MACACINI[1], E.A. DE NADAI FERNANDES[2], M.H.T. TADDEI[1]

[1] Comissão Nacional de Energia Nuclear — Laboratório de Poços de Caldas — CNEN/COLAB, Poços de Caldas MG, Brazil
[2] Centro de Energia Nuclear na Agricultura — Universidade de São Paulo — CENA/USP, Piracicaba SP Brazil

Abstract

In case of an accident at a nuclear power plant with liberation of radioactive material into the atmosphere, knowledge on the behaviour of plant species when in contact with radionuclides is indispensable for safety reasons. The radioactive contamination of crops, due to fallout, should be carefully assessed since the consumption of contaminated food is the main route of population exposure. An important route through which agricultural products are contaminated by radionuclides is leaf-fruit translocation. In order to quantify the leaf-fruit translocation coefficients for Cs and Sr in the common bean (*Phaseolus vulgaris*), variety black diamond, an experiment was carried out in a greenhouse with completely randomised block design. The bean plants were contaminated inside a device especially designed to avoid environmental contamination. Cs activity was determined by gamma ray spectrometry, while chemical separation followed by beta counting of Y was used for Sr determination. The model used for translocation indicated functional dependence between the moment of tracer application and the level of physiological development of the bean plant.

1. INTRODUCTION

Brazil has at present two nuclear plants — Angra I, in commercial operation since 1982 [1], and Angra II, in pre-operational tests, located at the beach of Itaorna, municipal district of Angra dos Reis, Rio de Janeiro state. They are 130 km from Rio de Janeiro, 220 km from São Paulo, and 350 km from Belo Horizonte [2], three capital state cities in the Southeast area, with about 70 million inhabitants. This growing use of nuclear fission for electric power generation in the country requires a critical appraisal of safety aspects at nuclear plants. In the eventuality of an accident with the liberation of radioactive material into the atmosphere, knowledge of the behaviour of vegetable species cultivated in the fallout area is indispensable.

An important route of contamination of agricultural products with radionuclides from fallout is the leaf-fruit translocation. The translocation process is quantified by the translocation factor, which is defined as the fraction of the activity deposited on the foliage which is transferred to the edible parts of the plants until the moment of harvest [3]. It is dependent on the element, the plant type and variety (e.g. early versus late), the time of deposition and the growth rate and mineral status of the plants. Considering the sensitivity to the time of deposition, it is obvious that even small deviations in development can cause differences in the observed translocation factor [4].

Although the translocation studies were initiated in the fifties [5], data are still scarce for vegetable species characteristically produced in the geographical conditions of Brazil — soybean, sugar cane, cassava, bean, rice, etc. Several artificial radionuclides have been used in experiments with hazel-nut, chestnut, walnut [6], winter and spring wheat, barley, rye, potato, carrot, and green bean [7], grapes [8], and bean [9], however, executed in climatic conditions different from Brazil.

The dietary habits of Brazilians are very peculiar and, in spite of the continental dimensions of the country, the rice and bean pair is the basic representative of all the main meals in the whole country. Beans (*Phaseolus vulgaris*) are produced in commercial scale from very near to thousands of kilometers away from the nuclear plants. The preference in the state of Rio de Janeiro is the consumption of the variety black diamond. In 1998 the Brazilian production was 3.1 million tons, with 0.7 million tons in the Southeast area [10].

Dynamic radioecological models have been presented for evaluation of the consequences of accidental releases of radionuclides, with emphasis on rural environments, focusing at routes in food chain, like the ECOSYS-87 developed for the German rural environment [3]. The existing model in Brazil, PARATI — Program for the Assessment of Radiological Consequences in a Town and of Intervention after a Radioactive Contamination [11], is only applicable to urban areas.

CORAL — Concentration of Radionuclides in Food — is a project in development conceived by IRD (Instituto de Radioproteção e Dosimetria), Rio of Janeiro — RJ, to establish a model to estimate the doses due to ionising radiation, according to the ECOSYS-87, addressed to the Brazilian rural atmosphere. Thereby, the CORAL project will need data relative to agricultural activities, plant species more commonly cultivated and planting times, besides leaf-fruit translocation coefficients. In this context, the present work is the first contribution with the objective of evaluating Sr and Cs translocation coefficients for bean plants.

2. MATERIAL AND METHODS

This experiment was conducted at the CNEN/COLAB, located in Poços de Caldas, MG, which is participating in the CORAL project.

Special care was taken with soil preparation for planting, because the bean plant is very demanding in nutrients [12]. The soil (oxissol) originated from the Poços de Caldas plateau. To the volume of 1000L of soil, 60L of coarse sand, 150L of tanned cattle manure, and 0.8kg of fertilizer NPK (4:14:8%) were added. This homogenised soil mixture presented a pH of 7.1, 34.1% of organic matter, 0.075% of phosphorus, 0.12% of potassium, 0.90% of calcium, and 0.056% of magnesium. The soil was placed in plastic vases, with a volume of 5.0L, distributed inside the greenhouse, and watered in abundance one week before sowing. The temperature was controlled between 17 ^{o}C and 24^{o}C during the experiment.

Standard solutions of Sr and Cs in hydrochloric acid, supplied by IRD, were used to prepare working solutions with activities of 100 Bq mL^{-1} for Sr and Cs. A final aspersion activity of 600 Bq m^{2} for both radionuclides was obtained, similar to the average activity per unit area deposited in Finland for Sr after the accident at Chernobyl [13].

To delimit the aspersion area a campanula was built measuring 1.20 m high and a base diameter of 0.66 m base (Figure 1). To simulate the deposition by fallout, an atomic absorption spectrometer atomiser was fitted at the highest point, permitting nebulization of the working solution onto the bean plants in a vase — during this process the soil was covered with a protective plastic film.

The planting was carried out in the summer of 1999 (February). Four pre-selected seeds were individually sowed per vase. After planting, to avoid loss of moisture, a fine layer of coarse sand was dispensed on the surface of each vase. Thinning of the bean plants was performed 30 days after planting, leaving two vigorous plants. During watering, special care was taken to not wet the leaves.

FIG. 1. Campanula.

Randomised block design composed by four treatments and four blocks was used [14]. The treatments (t_1 t_2 t_3 t_4) correspond to four aspersions, made at 30, 45, 60, and 75 days after the planting. The experimental unit comprised six vases each with two bean plants, from which four vases were aleatorily withdrawn and individually sprinkled inside the campanula with 2.0 mL of the working solution. Of these, one was taken to estimate the retained activity and the three remaining were used for the bean activity determinations. Of the two vases not sprinkled, one was used for foliar area measurement while the other was kept as a blank for the analyses. Regression analysis was carried out in function of the time elapsed between planting and deposition instead of the time before harvesting, because in practice the crop is not harvested immediately after drying of the bean-pods.

The mass of leaf samples, for determination of the retained activity, was recorded after the deposited mist had dried, and the beans, for determination of the activity in the fruit, were recorded and dried at 60° C. Samples of leaves and beans were ground in an orbital mill and stored in polyethylene vials, 61mm in diameter and 3 mm in height. After this, the determination of ^{137}Cs was carried out by gamma spectrometry with a germanium detector (Canberra, GX 4519). The standard used for ^{137}Cs determination was prepared by uniform application of 2 mL of the working solution onto 50 g of leaves freshly picked from the bean plants cultivated in the field and following the same procedures of drying, grinding, and packaging for the experimental leaf samples.

^{90}Sr determination was carried out using chemical separation [15] after ^{137}Cs had been determined for the same sample due to the destructive nature of the ^{90}Sr method. Sr activity was determined by beta activity of its daughter ^{90}Y in proportional detectors, with geometry 2π (5 cm diameter). Leaf samples with higher activities were submitted to normal beta detector (Eberline, BC-4), while bean samples with low activity needed an anti-coincidence system detector (Eberline multi-low-level counter FHT 770T). Samples of leaves and fruits from the same treatment were counted sequentially to avoid half-life corrections.

To evaluate the foliar area of bean plant in cultivation under controlled conditions, all the leaves were picked, including the smaller ones or recently opened leaves, scanned and their areas calculated using the computer program Kontron Elektronik Imaging System version KS 300. The foliar area was used to estimate the leaf area index (LAI). The LAI was determined considering 20 bean plants per unit of ground, which provides total covering of the area at the flowering period when highest efficiencies for light utilization and biomass production are obtained [16].

3. RESULTS

Beans were harvested 90 days after planting, and when natural drying all the bean plants was complete.

The percent value estimated for the retained activity (n=4) in each treatment (Table I) was obtained by comparison of the sprayed activities with the activities in the bean leaves, taken as an estimate of the initial deposition. The dried mass of beans and leaves, the foliar area, and the LAI, are shown in Table II.

The mean translocation coefficients (and standard deviation) for ^{137}Cs for the bean plant were taken from each treatment in the four blocks (Table III).

Although the equipment employed in the determination of the ^{90}Y activity for the beans possessed an anti-coincidence system, only 3 samples presented activities above the detection limit (5.0 Bq kg^{-1}), making the statistical analysis for the translocation of ^{90}Sr unfeasible. In Table IV, the results are presented as translocation coefficients for the three samples.

F tests were applied for the evaluation of the temporal dependence on the translocation of ^{137}Cs, and quadratic regression had the null hypothesis rejected (Table V).

TABLE I. MEAN (N=4) RETAINED ACTIVITY BY TREATMENT, IN%

Treatment	^{90}Sr	^{137}Cs
t1	7.9±0.4	7.7±0.6
t2	16±3.8	15±2.8
t3	22±5.3	20±2.4
t4	8.6±3.8	7.3±3.4

TABLE II. MEAN (N=4) AND STANDARD DEVIATION FOR THE 4 BLOCKS

Treatment	Aspersion (days after planting)	Beans, *d.w.* g	Leaves, *d.w.* g	Foliar area m^2	LAI (m^2 m^{-2})
t1	30	50±8	3.3±0.7	0.11±0.01	1.1±0.1
t2	45	53±6	11.5±1.9	0.36±0.07	3.6±0.7
t3	60	55±3	10.3±1.8	0.47±0.02	4.7±0.2
t4	75	62±5	6.0±3.5	0.22±0.13	2.2±1.3

TABLE III. MEAN SPECIFIC ACTIVITY OF ^{137}Cs IN THE LEAVES AND BEANS AND THE TRANSLOCATION COEFFICIENTS

Treatment	Leaf activity Bq kg^{-1}	Bean activity Bq kg^{-1}	Tr
t1	5100± 1100	82±17	0.08±0.01
t2	2800± 900	290±97	0.16±0.02
t3	4100±900	290±91	0.13±0.02
t4	2800± 900	37±23	0.05±0.01

TABLE IV. SPECIFIC ACTIVITY OF ^{90}Sr IN THE LEAVES AND BEANS AND THE TRANSLOCATION COEFFICIENTS.

Sample code	Aspersion (days after planting)	Leaf activity (± sd) Bq kg^{-1}	Bean activity (±unc.) Bq kg^{-1}	**Tr** (±unc.)
t2b3	45	2900±1100	6.1±1.4	0.003±0.001
t2b4	45	2900±1100	7.7±1.6	0.004±0.001
t3b1	60	4400±1000	6.1±1.4	0.002±0.001

TABLE V. VARIANCE ANALYSIS WITH F TEST FOR THE TRANSLOCATION AND POLYNOMIAL REGRESSION FOR ^{137}Cs.

Causes of variation	Treatments	Blocks	Quadratic regression	Cubic regression
Degrees of freedom	3	3	1	1
p>F	0.0001	0.24	0.0001	0.106

The analysis of the polynomial regression resulted in a second-degree polynomial (equation 1).

$$Tr(t) = -0.000171\ t2 + 0.0171\ t - 0.270 \qquad (1)$$

From this equation, the maximum translocation, observed 50 days after planting (40 days before harvest), was 0.16 (Figure 2).

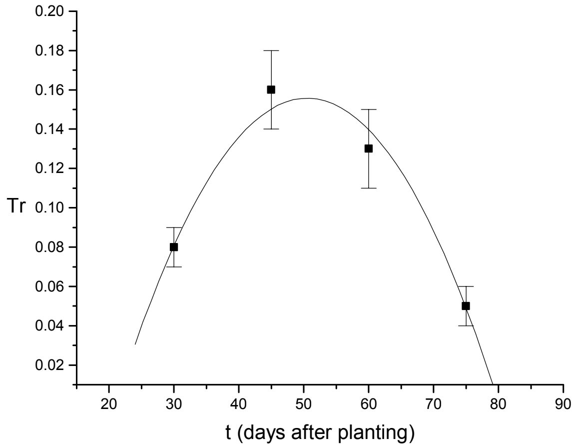


FIG. 2. Translocation of ^{137}Cs in bean plants.

4. DISCUSSION

^{90}Sr is classified as having very low mobility inside plants, with ^{137}Cs having a greater mobility, thereby being more translocated [5]. Our results with ^{90}Sr and ^{137}Cs corroborate this information.

Oestling et al. [9], in an experiment with six vases and a bean plant per vase, present translocation coefficients for ^{137}Cs between 0.11 and 0.32, before formation of the edible parts. In the present experiment, values between 0.14 and 0.20 were obtained for the same stage of development.

The foliar activity standard deviation in each treatment varied between 22% and 32%. This can be attributed to the fact that plants, taken for estimation of retained activity in the leaves, are not the same ones used for the estimation of fruit activity. Uncertainties of this magnitude can be admitted for this kind of agronomical experiment, with values of approximately 25% indicated by [7].

5. CONCLUSIONS

Functional dependence was observed between the coefficients for leaf-fruit translocation and the time elapsed between planting and moment of spray or physiological state of development of the bean plant. This dependence was also observed for the leaves weight, foliar area, and LAI.

The maximum production of dry material, foliar area, and LAI, occurred between 45 and 60 days after planting (Table II), when the maximum translocation (0.16) was verified for ^{137}Cs, coinciding with peak flowering.

The coefficients for the translocation of ^{90}Sr were between 0.002 and 0.004, two orders less than those obtained for ^{137}Cs.

REFERENCES

[1] LEITE, A.D., A energia do Brasil, Rio de Janeiro: Editora Nova Fronteira, (1997), 528 pp.

[2] SANTINA, M.D., Determinação das zonas de segurança da Central Nuclear de Angra dos Reis conforme o modelo de difusão de radiação na atmosfera. São Paulo, (1978), 161 pp. Dissertação (Mestrado) — Escola Politécnica, Universidade de São Paulo.

[3] MÜLLER, H., PRÖHL, G., ECOSYS-87, a dynamic model for assessing radiological consequences of nuclear accidents. Health Physics, v.64, (1993) pp.232–252.

[4] INTERNATIONAL ATOMIC ENERGY AGENCY. Technical Report Series N$^{\underline{o}}$ 364, Vienna, (1994), 75pp.

[5] MIDDLETON, L. J., Absorption and translocation of strontium and caesium by plants from foliar sprays. Nature, 1958.

[6] MONTE, L., QUAGGIA, S., POMPEI, F., FRATARCANGELI, S. The behavior of Cs in some edible fruits. Journal of Environmental Radioactivity, v.11, (1990) pp.207–214.

[7] VOIGT, G., PRÖHL, G., MÜLLER, H., Experiments on the seasonality of the cesium translocation in cereals, potatoes and vegetables. Radiation and Environmental Biophysics, 30, (1991), 295–303.

[8] CARINI, F., SCOTTI, I.A., MONTRUCCOLI, M., SILVA, S. Cs foliar contamination of vine: translocation to grapes and transfer to wine. In: INTERNATIONAL SYMPOSIUM ON RADIOECOLOGY, Vienna, 1996. Vienna: IAEA, (1996). pp.163–169.

[9] OESTLING, O., KOPP, P., BURKART, W., Foliar uptake of cesium, iodine and strontium and their transfer to the edible parts of beans, potatoes and radishes. Radiation Physics and Chemistry, v.33, (1988) pp.551–554.

[10] INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. Produção agrícola de culturas não perenes. http://www.ibge.org/ — IBGE, 2000.

[11] ROCHEDO, E.R.R., CONTI, L.F.C., PARETZKE, H.G. PARATY — A dynamic model for radiological assessments in urban areas. Radiation and Environmental Biophysics, v.35, (1996) pp.243–261.

[12] ROSOLEM, C.A., MARUBAYASHI, O.M. Seja o doutor do seu feijoeiro. Informações Agronômicas, n.68, (1994) pp.1–16.

[13] PAASICALLIO, A.; RANTAVAARA, A.; SIPPOLA, J., The transfer of cesium-137 and strontium-90 from soil to food crops after the Chernobyl accident**.** The Science of the Total Environment, v.155, pp.109–124, 1994. ROSOLEM, C.A.; MARUBAYASHI, O.M. Seja o doutor do seu feijoeiro. Informações Agronômicas, n.68**, (**1994) pp.1–16.

[14] GOMES, F.P., Curso de estatística experimental**.** 13.ed. Piracicaba: Nobel, (1990), 403 pp.

[15] INSTITUTO DE RADIOPROTEÇÃO E DOSIMETRIA -. Determinação radioquímica do Sr**.** Rio de Janeiro: IRD, (1994),12 pp.

[16] PIZAN, N. R., ROSTON, A. J. Manual técnico das culturas: Feijão (*Phaseolus vulgaris L.*) CATI**.** 2.ed.rev.atual. Campinas: CECOR, (1997), Tomo 1. pp. 289–331.

A CHEMICAL SPECIATION STUDY IN AEROSOL SAMPLES

C.S. HIPÓLITO, M.C. FREITAS

ITN, EN10 Sacavém, Sacavém, Portugal

Abstract

The aim of this paper is to present the methodology and relevant results referent to toxic elements for a sequential leaching procedure applied to aerosol samples. Samples were obtained from an atmospheric monitoring carried out at a simultaneously industrial and urban region near Lisbon. Neutron activation technique was used in this chemical speciation study. In order to have the best performance for the analysis experiments with two different materials were made, for supporting the extract solution, a polyethylene box and pellets done with Watman 40 filter paper.

1. INTRODUCTION

Chemical speciation is of interest in analytical chemistry because it is realised that the environmental behaviour of trace elements depends not only on the amounts present but also on their form [1]. The determination of total or pseudo-total metal content gives no indication of the mobility or biovailability of heavy metals in atmosphere. Chemical speciation is of increasing interest in analytical chemistry. It can be defined as either:

(a) the process of identifying and quantifying the different species, forms or phases in which an element is present in a material; or
(b) the description of the amounts and kinds of these species, forms or phases in which an element is present

The species, forms or phases can be defined (a) functionally (according to their role), (b) operationally (according to the procedure used in their isolation), or (c) classically (as specific chemical compounds or oxidation states) ([2], and references there in). The determination of 'plant available' forms is an example of functionally defined speciation, as are terms such as ' biologically active' or 'mobile'.

In operational speciation, the fraction isolated is defined by the physical or chemical process used to obtain it: examples include 'exchangeable', 'reducible' or '*aqua regia* sobuble' forms. One of the most popular methods of operational speciation is sequential extraction, in which the sample is treated with a succession of reagents, of increasing harshness, designed to release metals associated with different phases. A large number of different sequential extraction schemes have been used, many of which are based on the classical, Tessier procedure [3]. The third type of speciation is that in which precise chemical forms are identified and quantified e.g. Cr III and Cr VI in waters, or arsenocholine and arsenobetaine in fish.

Although classical speciation is preferred, it is rarely possible with solid samples such as soil sediment or aerosol samples, and operational methods are commonly applied for this kind of samples.

TABLE I. BCR SEQUENTIAL EXTRACTION PROCEDURE

Step	Reagent	Nominal target phase(s)
1	0.11 mol l^{-1} CH_3COOH	Exchangeable, water and acid-soluble
2	0.10 mol l^{-1} NH_2OH, adjusted to pH 2 ith HNO_3	Reducible (e.g. iron /manganese oxides)
3	8.8 mol l^{-1} hydrogen peroxide, followed by 1.0 mol l^{-1} CH_3COONH_4 adjusted to pH 2 with HNO_3	oxidizable (e.g. organic matter and sulphides)

In attempt to harmonise methodology throughout the EU, and to improve comparability between laboratories, the Commission of the European Communities, Community Bureau of Reference (BCR) recently devised a simple, three stage sequential extraction protocol for operational speciation of trace metals ([2], and references there in). This procedure is shown in Table I.

For aerosol collection, the air sampler used was a Gent PM10 developed at the Gent University in Belgium. It is equipped with NILU (Norwegian Institute for Air Research) stacked filter unit (SFU) that can carry 47 mm filters. Costar-Nuclepore® polycarbonate filters with 8 μm and 0.4 μm pore size were used in each of stages. The air was sampled at 16–18 litters/minute rate that allowed to collect coarse particles with equivalent aerodynamic diameter between 10 and 2.5μm in the first stage and fine particles with EAD < 2.5 μm in the second stage. The filter loads were measured by gravimetry using a balance with 10 μg sensitivity.

2. EXPERIMENTAL

In order to start studies in trace element speciation with atmospheric samples it was followed a leaching procedure [4] (a first step of BCR sequential extraction procedure recommended for solid samples) that consists of putting the aerosol samples (charges between 300 and 500 μg) in contact with 20 ml of 0.11 M acetic acid and shaking overnight in a mechanical shaker at room temperature (150 rpm). The extract was separated from the residue by centrifugation. With the purpose of making INAA the solution extracted was impregnated in a Watman 40 filter paper. The solution was dried with a infrared lamp. Then pellets were done and irradiated with a neutron flux of 5×10^{12} cm^{-2} s^{-1}. A blank pellet was submitted to similar procedure to take into account the contamination due to both the acetic acid and the filter paper.

3. RESULTS AND DISCUSSION

Figures 1 and 2 show the percentage of extraction in fine and coarse filters.

We must emphasise that these are very preliminary results on air particulate matter (APM) on speciation. We are aware that the dissolution of the soluble part of APM was probably not complete, more experiments must be done. Nevertheless a few elements were visible in the extraction fraction and some results could be obtained. A larger amount of elements were soluble in the coarse filter than in the fine one. It can be because there are more soluble compounds in the coarse fraction but it can also mean that the large particles were more easily separated from the filter into the acid acetic solution.

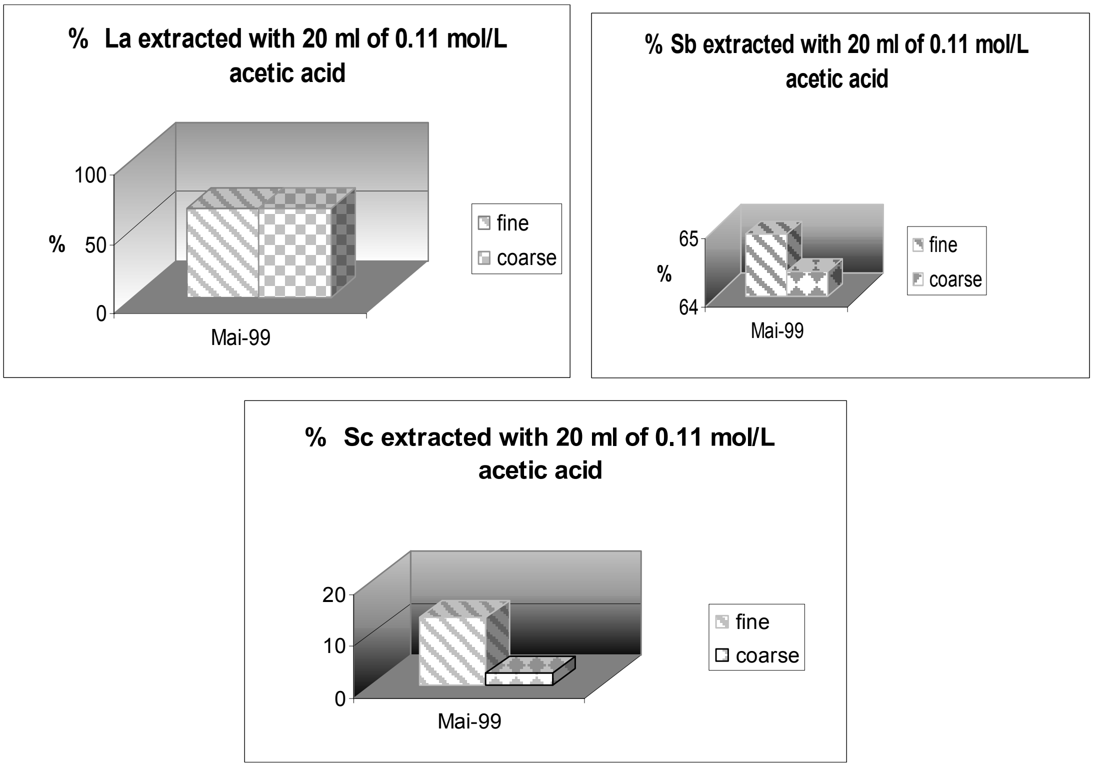


FIG. 1. La, Sb, Sc extracted in fine and coarse filters.

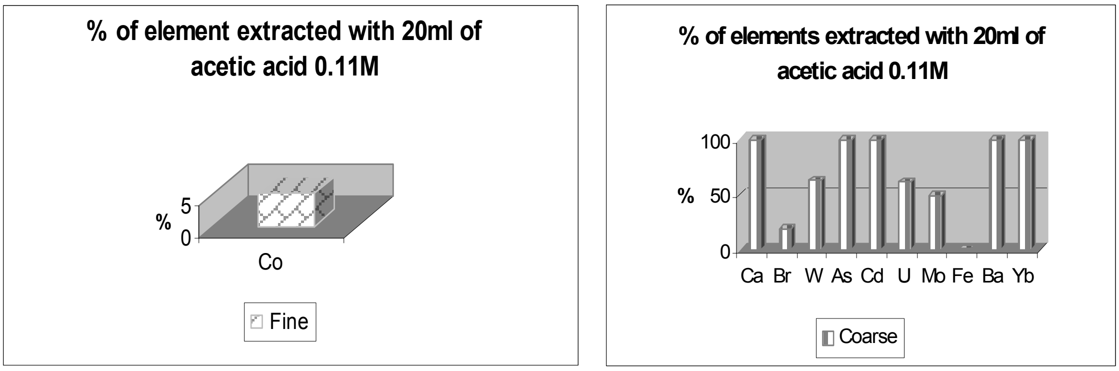


FIG. 2. Co extracted in fine filters, elements extracted in coarse filters.

La and Sc, extracted in both fine and coarse modes, are not pointed as toxic in the legislation for hazardous substances in the air at the work place [5]. For Sb the extraction was larger in the coarse than in the fine fraction. Literature points a single Sb compound soluble in acid [6], which is also a hazardous substance: Sb_2O_3 (suspect of carcinogenic potential for man). Since other Sb is also soluble in acid (Sb III sulphate) we can not be sure if Sb_2O_3 is present. Co was slightly extracted from the fine filter. Many Co compounds are soluble in acid among them Co metal which is hazardous substance suspect of carcinogenic potential for man. For elements completely extracted from the coarse fraction, some acid soluble compounds of Ca and Cd are also hazardous substances, and the soluble compounds of Ba are all toxic. Fe soluble compounds are toxic but our results point out that most of the Fe compounds in the coarse fraction are insoluble. U compounds are hazardous because they are radioactive; in our results over 50% of the U, in the coarse fraction, was put available by solubility. WO_2 is the only acid soluble compound and it is a hazardous substance; then over 50% of the W of our coarse filter is this compound. Mo acid soluble compounds are toxic and almost 50% were available by solubility. As_2O_5 is the only As compound soluble in acid; therefore all the As in the coarse fraction is this compound since it was 100% extracted.

4. CONCLUSIONS

This is a very preliminary study. As expected more conclusions could be taken from coarse fraction. Some important conclusions could be taken:

- Over 50% of the U was extracted in the coarse fraction and all U compounds are hazardous;
- The acid soluble (over 50%) W compound could be identified in the coarse fraction and it is also hazardous;
- All the As in the coarse fraction was extracted and could be identified as the hazardous As_2O_5 which is human carcinogen;
- All Mo acid compounds are hazardous and almost 50% were easily extracted.

REFERENCES

[1] URE, AM., DAVIDSON, CM., “Chemical Speciation in the Environment”, Blackie Academic and Professional, Glasgow, 1995.

[2] DAVIDSON, C.M., FERREIRA, P.C.S., URE, A.M., Some sources of variability in application of the three-stage sequential extraction producer recommended by BCR to industrially contaminated soil. Fresenius J Anal Chem. **363** (1999) 446–452.

[3] TESSIER, A., CAMPBELL, P.G.C., BISSON, M., Sequential extraction procedure for the speciation of particulate trace metals, Anal. Chem. **51** (7) (1979) 844–851.

[4] BÓDOG, I., POLYÁK, K., CSIKÓS-HARTYÁNYI, Z., AND, HLAVAY, J., Sequential extraction procedure for the speciation of elements in fly ash samples, Microchemical Journal **54** (1996) 320–330.

[5] SEILER, H.G., SIGEL, A., SIGEL, H., “Handbook on Metals in Clinical and Analytical Chemistry”, Ed. Marcel Dekker, Inc., N.Y., 1994.

[6] LIDE, D.R., CRC Handbook of Chemistry and Physics, Ed. CRC Press, Boca Raton, 1994.

COMPARISON OF K$_0$-NAA IN LICHENS USING ISOTOPES WITH SHORT AND LONG HALF-LIVES, RESPECTIVELY

P. POMMÉ[1], P. ROBOUCH[2], M.C. FREITAS[3], A.P. MARQUES[3],
M.I. MAGUREGUI[2], M. EGUSKIZA [2]

[1] SCK•CEN, Belgian Nuclear Research Centre, Mol, Belgium
[2] European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium
[3] ITN — Reactor, Sacavém, Portugal

Abstract

Lichens, acting as monitors for environmental pollution, are analysed by instrumental neutron activation analysis. At SCK•CEN, six samples were analysed after a long irradiation of 7 hours and six other samples were analysed after a short irradiation of 2 minutes. Relatively long-lived activation products are used in the first case and short-lived isotopes in the second. Applying k$_0$-standardisation, a comparison of concentration results was done for elements detected by different isotopes in both types of measurements. In addition, all obtained elemental concentrations are compared with measurements performed at ITN.

1. INTRODUCTION

Neutron activation analysis (NAA) of lichens is a useful tool for large scale monitoring of atmospheric pollution (see e.g. ref. [1] and references therein). Lichens are efficient replacements of air samplers for the accumulation of air pollution. The multi-element capability of NAA combined with the k$_0$-standardisation method (see e.g. ref. [2,3]) allow the absolute determination of a wide range of elements. The calibration is based on k$_0$-factors associated with individual y ray lines of decaying activation products. Other calibration constants are obtained from coirradiated Au and Zr standards for neutron flux characterisation and from calculated or measured values of the detection efficiency of the y ray spectrometer at the considered y ray energy.

In recent work [3,4], Van Lierde et al. has determined new k$_0$-factors for 10 different elements, associated with short-lived activation products. Some of the considered activation products are ^{205}Hg (5.2 min.), ^{80m}Br-^{80}Br (4,4205h, 17.68 min.), and ^{124m2}Sb-^{124m1}Sb (20.2 min., 93.0 s). These isotopes can be used as alternatives for the corresponding long-lived isotopes, which are traditionally used for NAA of environmental samples. In this work, we intended to make a comparison of the analytical results obtained with k$_0$-NAA for elements like Br, Hg, and Sb, in lichen samples once measured after a long irradiation (7 hours) using the long-lived isotopes, and on the other hand, measured after a short irradiation (2 min.) using the short-lived isotopes with the recently published k$_0$-factors.

2. EXPERIMENTAL

For this exercise, about 6 gram of dried lichen material was retrieved from an ongoing environmental study in Portugal. They were sampled in vials containing about 0.4 gram. Six were irradiated in channel Y4 of the BR1 reactor (SCK•CEN) for an irradiation of 7 hours, at a typical (thermal) flux of 3×10^{11} s^{-1}cm^{-2}. In Portugal six pellets were irradiated for 5 hours at a typical (thermal) flux of 10^{13} s^{-1}cm^{-2} Measurements were made for 2 h after 3–4 days of decay and 7 h after 3 weeks decay. Six other samples were irradiated during 2 minutes in the S84 channel of the BR1 and measured on a detector on top of the reactor. Hence, the measurements were done on different detectors, calibrated independently following the k_0-standardisation method [1]. The detector on top of the BR1 reactor is also independent from the one used for the determination of the k_0-values for short-lived isotopes in ref. [3,4], but the irradiation channel (S84) is the same.

3. RESULTS AND DISCUSSION

The first minutes after the irradiation, the samples are quite active because of the presence of ^{28}Al (1779 keV). After its decay, the most important activity in the spectra (after a 2 minute irradiation) originates from the decay of ^{56}Mn, ^{38}Cl, and ^{24}Na. Also the decay lines of ^{80}Br are clearly present in the spectra, allowing an accurate determination of the Br concentration. However due to the non-negligible activity in the sample, the abundance of Sb and Hg turns out to be insufficient for the gamma-lines of their corresponding short-lived isotopes to rise above the Compton background.

In Table I the measured concentrations of Br, Sb, and Hg in the lichens are presented. The three elements are observed in the spectra of samples with long irradiation and decay time. Comparing the BR1 data with the "ITN reactor", there seems to be a good agreement. The variation between both sets of data stays within two times sigma. In the case of Br, the value obtained from the short-lived isotope ^{80}Br is in excellent agreement with the concentration value derived from the long-lived ^{82}Br. This confirms the validity of the k_0-values and other corresponding k_0-related parameters associated with this activation reaction and subsequent decay, when applied on the S84 channel of BR1. Unfortunately the other new k_0-factors in ref. [3,4] could not be tested on these samples.

TABLE I. MEASURED CONCENTRATIONS OF Br, Sb AND Hg IN LICHEN, APPLYING A LONG-LIVED AND A SHORT-LIVED ACTIVATION PRODUCT, RESPECTIVELY. THE STATISTICAL UNCERTAINTY CORRESPONDS TO ONE SIGMA (INTERNAL VARIATION AMONG SIX SAMPLES).

Analyte	Portuguese reactor 5 h irradiation	BR1, Y4 7 h irradiation	BR1, S84 2 min irradiation
Br	19.4 ± 1.4 ppm	22.7 ± 0.4 ppm	22.1 ± 0.6 ppm
Sb	180 ± 15 ppb	213 ± 21 ppb	< 15 ppm
Hg	197 ± 13 ppb	160 ± 50 ppb	< 500 ppm

In Table II an overview is shown of most elements identified and quantified in the lichens by INAA. The measured quantities range from the% level down to ppb. Short-cycle measurements yield additional information on the concentration of a few elements, such as Al, Cl, Cu, Dy, I, Mg, Mn, Ti, U, and V. There is a fair agreement amongst the results of the different irradiations, setting aside some bias between the data of both reactors.

TABLE II. MEASURED CONCENTRATIONS OF VARIOUS ELEMENTS IN LICHEN, APPLYING A LONG-LIVED AND A SHORT-LIVED ACTIVATION PRODUCT, RESPECTIVELY. THE STATISTICAL UNCERTAINTY CORRESPONDS TO ONE SIGMA (INTERNAL VARIATION AMONG SIX SAMPLES).

	Portuguese reactor 5 h irradiation	BR1, Y4 7 h irradiation	BR1, S84 2 min irradiation	
Al			0.37 ± 0.01	%
As	1.212 ± 0.025	1.266 ± 0.015		ppm
Ba	15.5 ± 1.0	25 ± 3	23 ± 2	ppm
Br	19.4 ± 1.4	22.7 ± 0.4	22.1 ± 0.6	ppm
Ca	0.72 ± 0.02	0.64 ± 0.13		%
Cl			948 ± 18	ppb
Co	710 ± 30	818 ± 16		ppb
Cr	4.8 ± 0.2	7.0 ± 0.3		ppm
Cs	622 ± 23	663 ± 8		ppb
Cu			93 ± 32	ppm
Dy			424 ± 99	ppb
Fe	0.179 ± 0.005	0.194 ± 0.004		%
Hf	317 ± 9	371 ± 21		ppb
Hg	197 ± 13	160 ± 50		ppb
I			7.4 ± 0.2	ppb
K	0.332 ± 0.016	0.362 ± 0.009	0.338 ± 0.026	%
La	2.48 ± 0.12	2.52 ± 0.05		ppm
Mg			0.102 ± 0.007	%
Mn			33.0 ± 0.8	ppb
Na	212 ± 12	232 ± 11	218 ± 8	ppm
Nd	2.4 ± 0.3	4 ± 2		ppm
Rb	10.0 ± 0.5	11.7 ± 0.3		ppm
Sb	180 ± 15	213 ± 21		ppb
Sm	401 ± 20	390 ± 70		ppb
Sc	545 ± 21	638 ± 10		ppb
Se	396 ± 43	710 ± 220		ppb
Sr		23 ± 2		ppm
Ta	56 ± 2	63 ± 3		ppb
Tb	50 ± 4	57 ± 2		ppb
Th	586 ± 31	609 ± 19		ppb
Ti			255 ± 23	ppm
U	138 ± 28	180 ± 20	300 ± 30	ppb
V			9.7 ± 0.2	ppm
Yb		220 ± 30		ppb
Zn	45 ± 1	51.1 ± 1.5		ppm
Zr		27 ± 5		ppm

4. CONCLUSIONS

Instrumental neutron activation analysis was applied to analyse lichens of Portuguese origin. A wide range of trace elements could be identified and quantified by means of the k_0-method. The results obtained at the BR1 (Mol, Belgium) are in fair agreement with those obtained at the ITN reactor (Sacavém, Portugal). The recently published k_0-values [3,4] for the short-lived ^{80}Br yields concentration values in good agreement with the results derived from the long-lived ^{82}Br.

REFERENCES

[1] FREITAS, M.C., REIS, M.A., MARQUES, A.P., WOLTERBEEK, H.TH., Dispersion of chemical elements in an industrial environment studied by biomonitoring using *Parmelia sulcata*, J. Radioanal. Nucl. Chem. **244** (2000) 109–113.

[2] CORTE, F. DE, "The k_0-standardization Method; a move to the optimization of neutron activation analysis", Proefschrift Aggregaat voor het Hoger Onderwijs, Ghent University, 1987.

[3] POMMÉ, S., "On the applicability of k_0-NAA in extreme conditions", PhD thesis, Ghent University, Analytical Chemistry Lab, 1999.

[4] LIERDE, S. van, CORTE, F. de,.BOSSUS, D., SLUIJS, R. van, POMMÉ, S., Determination of k_0 and related nuclear data for short-lived radionuclides to be used in KAYZERO-NAA at DSM research, Nucl. Instr. and Meth. **A422** (1999) 874–879.

BIO-MONITORING OF TRACE ELEMENT AIR POLLUTION IN THE SADO ESTUARY

A.P. MARQUES[1], M.C. FREITAS[1], M.A. REIS[1],
O. de OLIVEIRA[1], H.Th. WOLTERBEEK[2]

[1]ITN — Instituto Tecnológico e Nuclear, Sacavém, Portugal
[2]IRI — TuDelft, Delft, Netherlands

Abstract

Transplants of the lichen *Parmelia sulcata* were suspended in nylon bags in a region within a rectangle of 15 km wide and 25 km long on a grid 2.5 km × 2.5 km, centered in the oil power station of Setúbal. In each of the 47 places two sets of four transplants each were hanged. Care was taken i) in covering the sets with a polyethylene roof to prevent leaching of elements in the lichen, ii) in building a hanging system which could rotate according to the wind direction, iii) in orienting one set towards the wind (F set) and the other set against the wind (T set). For a 9-month period and every three months, one transplant of each set was collected. Analysis were performed by INAA and PIXE, two nuclear analytical techniques which complementarity has been emphasized. A comparison for some common elements determined by both techniques is presented and discussed. Results of Pearson correlations for the three campaigns are also presented and discussed.

1. INTRODUCTION

Studies have shown that the survey of a large territory with lichens makes identification of atmospheric pollution sources possible [1–6]. One of the regions pointed out in the national survey with high levels of pollutants was Sado region [1]. A more detailed study was performed taken into account the position of the lichen transplants according to the wind direction [6]. The main objective was the identification and differentiation of local and distant sources. On the other hand it was also important a good agreement between the two nuclear techniques used for analysis, as a quality control and to guarantee the complementarity of the two techniques. Once observed some discrepancy between common elements a special attention was given to the last sampling campaign (September 1998) reducing the lichen transplant to a more fine powder. PIXE is essentially a surface technique and therefore sample homogeneity is important. Homogeneity of a sample is a very important property especially when a minimum sample of mass is used. PIXE analysis strongly depends upon particle size and sample homogeneity since it is a surface technique. It is well known that if the particle size is variable, which implies that the surface topography is very non-uniform, it can affect the absorption of X rays [7]. The range of 2.5 MeV proton beam in this type of samples (lichen material samples) is about 20 μm while lichen powder samples that we use, have a grain size approximately up to 200 μm. A very non-uniform surface can affect the absorption of X rays and as a consequence PIXE analysis. Preparations of samples for analysis is very important in this case, especially grinding and homogenization, in order to achieve a good agreement between common elements. However the production of homogeneous samples for analysis is difficult, particularly if the grain size is variable. INAA is a technique that uses a larger sample weight and it is a bulk analytical method so it is not much affected.

2. METHODS

Lichen transplants were suspended in September 1997 and collected in March 1998 (3 months exposure), June 1998 (6 months exposure), and September 1998 (9 months exposure). In the laboratory they were washed for 30 sec. in de-ionized water, freeze-dried, and ground (for about 5 min. at 1500 rpm) in a Teflon mill [1,2]˙ The 3rd campaign transplants were submitted to a more carefully grinding (for about 10 min. at 1500 rpm). For INAA analysis pellets of 500 mg were irradiated at the Portuguese Nuclear Reactor (RPI) and measured with an high-purity germanium detector. Concentrations were obtain by k_0 factor method. For PIXE analysis [8], use was made of a pellet of a thin layer of lichen powder in a boric acid support. Samples were irradiated in the Van de Graaff accelerator. The X ray spectra were obtained with a Si(Li) detector and analyzed by AXIL program. Concentrations were obtained by DATTPIXE program. Quality control was pursued by analyzing the IAEA-336 lichen [9], BCR-482 [10], and CTA-OTL-1 tobacco leaves [11] reference materials. We also participated in the NAT–5 intercomparison [12]. Some of these results were presented at Berm-8 [13].

3. RESULTS AND DISCUSSION

In Fig. 1 it is shown PIXE versus INAA comparison for Fe for both F and T sets (March and June 1998). Iron is determined by both techniques with good accuracy. PIXE technique (a surface technique) is very sensitive to particle size and sample homogeneity especially when elements associated with soil are in the matrix. It was observed that PIXE results were systematically larger than INAA values. After being aware of this problem a more careful grinding was performed for the third campaign (September 1998) and the results are shown in Fig. 2. A substantial improvement was reached, demonstrating the importance of particle size in PIXE analysis. It can be observed in Fig. 1 that the deviation grows when concentrations of Fe increase. A possible explanation is that, as the amount of Fe increases, the heterogeneity of this element in the sample increases as well. The result is a larger discrepancy between INAA and PIXE results. Also, it seems that the agreement between the two techniques is better for F transplants. These transplants are subjected to more wind turbulence than the T transplants and so they may carry less dust particles. Some of this dust is removed in the washing procedure but some still remains and may affect the grinding efficiency. Similar results were obtained for bromine. There is a good INAA/PIXE agreement for the third campaign (Fig. 4) that it is not observed for the two first campaigns (Fig. 3).

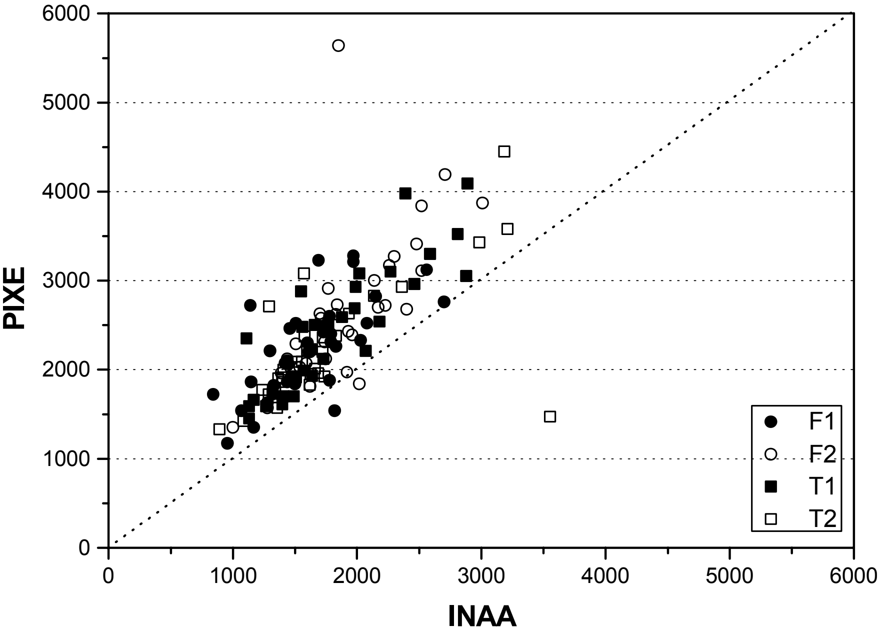


FIG. 1. PIXE versus INAA comparison for Fe for both F and T sets (March: F1,T1, and June: F2, T2, 1998).

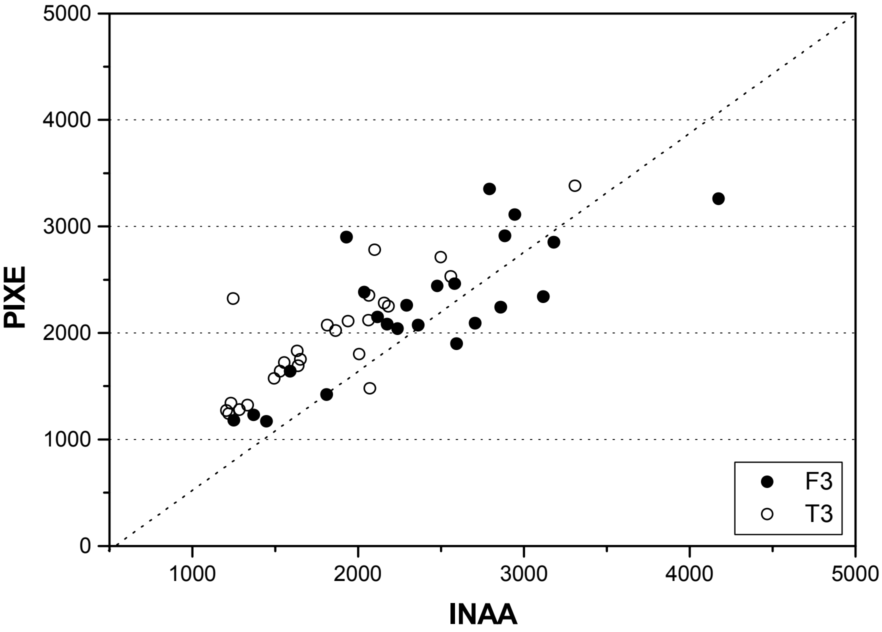


FIG. 2. PIXE versus INAA for Fe for F and T sets collected on September 1998.

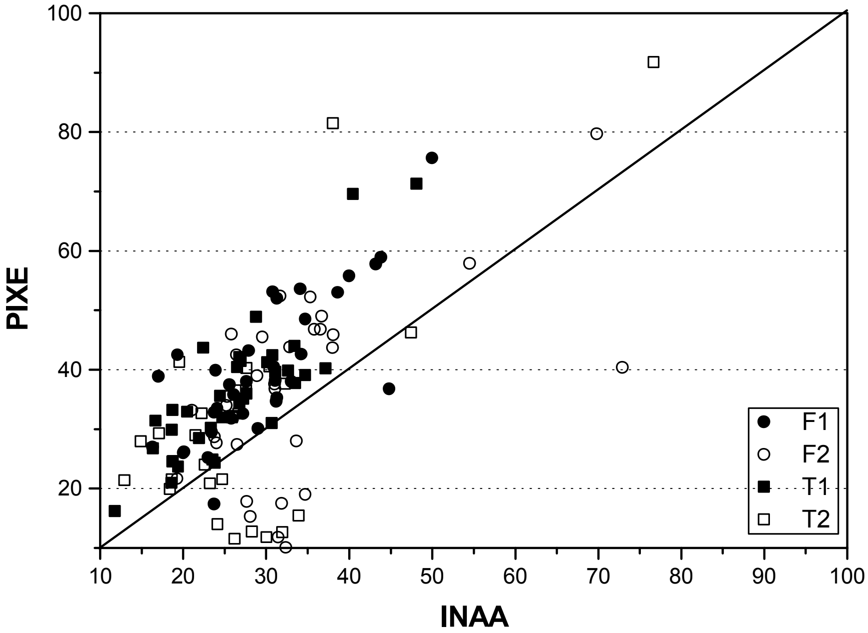


FIG. 3. PIXE versus INAA comparison for Br for both F and T sets (F1, T1: March, and F2, T2: June, 1998).

Pearson correlation matrices, calculated using STATISTICA, were applied to the F and T data sets of the 3rd campaign. Results for the 1st and 2nd campaigns were presented and discussed elsewhere [14].

The influence of the sea is observed in both transplants for all campaigns. It spreads through the south, crossing Tróia peninsula and the Sado estuary covering all sampling grid [14]. Table I report the correlations for elements associated with sea salt spray [15]. It can be observed that they are high and very similar for both transplants, especially for Na, Cl, and Br, the most important elements associated with this source.

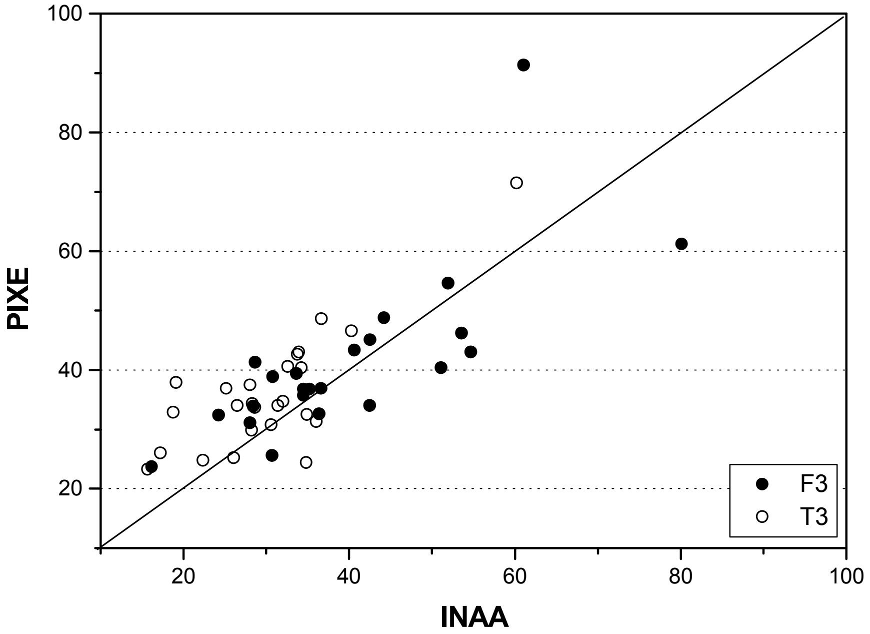


FIG. 4. PIXE versus INAA for Br for F and T sets collected on September 1998.

Table II presents the results for elements associated with oil combustion. The main vanadium source is the oil power station situated more or less in the center of the grid. The correlation between elements associated with oil combustion [15] is more intense in the F transplants. In the 3rd campaign V and Ni show higher correlation values. On the first and second campaigns, correlation was not so obvious (F1: 0.52, F2: 0.65, T1: 0.42, T2: 0.59). But since the source is so intense, after a 9-months period, wind oriented lichens (F-transplants) show visible correlation. In previous publications [14] elemental mapping contents were presented for some elements. For the mapping of V the influence of the local oil power station did not reveal relevant differentiation for F and T transplants. However with Pearson correlations values it is possible to observe that the F transplants are more affected by this source.

Strong correlation values for Ni, Cu, Pb, and Sr for all campaigns for both transplants were observed, as shown in Table III. It is also observable a miscellaneous of emissions from sources of industrial origin.

TABLE I. RESULTS FROM PEARSON CORRELATION MATRICES FOR F AND T SETS FOR SEA SALT SPRAY ASSOCIATED ELEMENTS FOR THE 1st, 2nd, AND 3rd CAMPAIGNS

ELEMENTS	CORRELATION FACTOR F TRANSPLANTS	CORRELATION FACTOR T TRANSPLANTS
Na/Cl	0.97 0.79 0.85	0.96 0.90 0.91
Na/S	0.75 -0.10 0.79	0.75 0.34 0.74
Na/Br	0.63 0.76 0.81	0.76 0.53 0.76
Cl/S	0.74 0.08 0.72	0.81 0.42 0.79
Cl/Br	0.59 0.29 0.55	0.74 0.30 0.66
S/Br	0.37 -0.31 0.64	0.69 0.21 0.62

TABLE II. RESULTS FROM PEARSON CORRELATION MATRICES FOR F AND T SETS FOR OIL COMBUSTION ASSOCIATED ELEMENTS FOR THE 1st, 2nd, AND 3rd CAMPAIGNS

ELEMENTS	CORRELATION FACTOR F TRANSPLANTS	CORRELATION FACTOR T TRANSPLANTS
V/Ni	0.52 0.65 0.86	0.42 0.59 0.61
V/Pb	0.28 0.82 0.78	0.44 0.62 0.46
V/Cu	0.36 0.83 0.93	0.36 0.73 0.43
V/Zn	0.45 0.36 0.86	0.15 0.34 0.71
V/Cr	0.18 0.27 -0.25	0.26 0.56 0.37
V/Mn	0.09 -0.05 0.32	0.11 0.05 0.19

TABLE III. RESULTS FROM PEARSON CORRELATION MATRICES FOR F AND T SETS FOR SOME ASSOCIATED ELEMENTS FOR THE 1st, 2nd, AND 3rd CAMPAIGNS

ELEMENTS	CORRELATION FACTOR F TRANSPLANTS	CORRELATION FACTOR T TRANSPLANTS
Ni/Mn	0.47 0.31 0.54	0.57 0.59 0.49
Ni/Zn	0.45 0.72 0.88	0.18 0.56 0.71
Ni/Pb	0.61 0.59 0.93	0.83 0.88 0.72
Ni/Cu	0.67 0.59 0.93	0.83 0.83 0.73
Ni/Sr	0.61 0.70 0.92	0.60 0.85 0.93
Cu/Pb	0.88 0.87 0.92	0.83 0.87 0.82
Cu/Sr	0.38 0.85 0.79	0.39 0.76 0.76
Pb/Sr	0.33 0.89 0.89	0.60 0.86 0.72

4. CONCLUSIONS

As expected it was concluded that particle size is very important especially for PIXE technique. Iron and bromine in the third campaign, which has a more fine powder, present a much better correlation for the two techniques. Also it seems to be observed some differentiation between the two sets (F and T) on this matter. The agreement between the two techniques is better for the F transplants. The T transplants appear to be more affected by wind dust and so T transplants material may be less homogeneous. As referred above, PIXE analysis is very sensible to material homogenization.

Elements associated with sea salt spray show visible correlation for F and T data sets for the 3 campaigns. Oil combustion is identified as a local source, with visible correlation after 9 months especially for the F data set. Also there are some elements correlated that may have an industrial origin but it is not possible a correct identification yet. It is possible to distinguish 3 different effects when applying Pearson correlations to lichen wind oriented transplants; a source effect (high correlation values source associated elements), a time effect (stronger correlations for transplants subjected to longer periods of exposure), and a position effect (it is possible to determine if it is a local or distant source).

REFERENCES

[1] FREITAS, M. C., REIS, M. A., ALVES, M. A., WOLTERBEEK, H. TH., VERBURG, T., GOUVEIA, M. A., Bio-monitoring of trace-element air pollution in Portugal: Qualitative survey, J. Radioanal. Nucl. Chem., Articles **217 (1)** (1997) 21–30.

[2] FREITAS, M. C., and NOBRE, A. S., Bioaccumulation of heavy metals using *Parmelia sulcata* and *Parmelia caperata* for air pollution studies, J. Radioanal. Nucl. Chem., Articles **217 (1)** (1997) 17–20.

[3] FREITAS, M. C., REIS, M. A., ALVES, L. C., MARQUES, A. P., COSTA C., Environmental assessment in an industrial area of Portugal, Biol. Trace Elem. Res., **71/72** (1999) 471–479.

[4] FREITAS, M. C., REIS, M. A., ALVES, L. C., WOLTERBEEK, H. TH., "Distribution in Portugal of some pollutants in the lichen *Parmelia sulcata*, Environmental Pollution **106** (1999) 229–235.

[5] REIS, M. A., ALVES, L.A., FREITAS, M. C., VAN Os B, WOLTERBEEK, H. TH., Lichens (*Parmelia sulcata*) time response model to environmental availability, Sci. Total Env. **232** (1999) 105–115.

[6] FREITAS, M. C., REIS, M. A., MARQUES, A. P., WOLTERBEEK, H. TH., Dispersion of chemical elements in an industrial environment studied by biomonitoring using *Parmelia sulcata*, J. Radioanal. Nucl. Chem. **244 (1)** (2000) 109–113.

[7] RANDLE K., ALJUNDI J., MAMAS C. J. V., SOKHI R. S., EARWAKER L. G., A comparative-study of Neutron-Activation Analysis and Proton-Induced X-Ray-emission analysis for the determination of heavy-metals in estuarine sediments, Nucl. Instr. and Meth. in Phys. Res. B **79 (1–4)** (1993) 568–570.

[8] REIS, M. A., ALVES, L. C., JESUS, A. P., Matrix effects correction for quantitative TTPIXE analysis, Nucl. Instr. and Meth. in Phys. Res. B **109/110** (1996) 134–138.

[9] STONES, F., FREITAS, M. C., PARR, R. M., ZEISLER, R., Elemental characterization of a candidate lichen research material-IAEA 336, Fresenius J. Anal Chem. **352** (1995) 227–231.

[10] QUEVAUVILLIER, PH., HERZIG, R., MUNTAU, H., The certification of the contents (mass fractions) of Al, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in lichen — CRM 482, Report EUR 16841 EN, Brussels (1996).

[11] DYBCZYNSKI, R., POLKOWSKA- MOTRENCO, H., SAMCZYNSKI, Z., SZOPA, Z., Preparation and Certification of the Polish Reference Material "Oriental Tobacco Leaves" for Inorganic Analysis, Raporty IchTJ. SERIA A nr 1/96, Inst. Nucl. Chem. and Techn., Warsaw (1996).

[12] BLEISE, A., SMODIS, B., Report on the intercomparison run NAT-5 for the determination of trace and minor elements in two lichen samples, IAEA, Vienna (1999).

[13] MARQUES, A. P., FREITAS, M. C., REIS, M. A., OLIVEIRA, O. DE, WOLTERBEEK, H. TH., Lichen material analysed by PIXE and INAA: a comparative study, Presented at the Eighth International Symposium on Biological Environmental Reference Materials (BERM-8), Maryland, USA, September 17–22, 2000.

[14] FREITAS, M. C., REIS, M. A., MARQUES, A. P., WOLTERBEEK, H.TH., Use of lichen transplants in heavy metal atmospheric deposition studies, J. Radioanal. Nucl. Chem., Articles (in press).

[15] Nriagu, J. O., Control and fate of atmospheric trace metals, Natural versus antropogenic emissions of trace metals to the atmosphere, Mathematical and Physical Sciences, Series C — vol.268, pp. 3–13, 1989.

BIOMONITORING TRACE ELEMENT ATMOSPHERIC DEPOSITION USING LICHENS IN CHINA

ZH.H. ZHANG[1], Z.F. CHAI[1], X.Y. MAO[1], J.B. CHEN[2]

[1] Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing , China
[2] Systematical Mycology and Licheonology Lab, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

Abstract

Concentrations of 34 elements, Ag, As, Au, Ba, Ca, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Mo, Na, Nd, Ni, Rb, Ru, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, Tm, U, W, Yb, and Zn were determined by instrumental neutron activation analysis (INAA) in the early preserved epiphytic lichens (*Parmotrema recticulata*) from the remote Southwestern China area in 1960s, 1980s, and 1990s. The concentrations of Ag, As, Ce, Cr, Cs, Eu, Fe, Hf, La, Nd, Ni, Sc, Se, Sm, Tb, U, Yb, and Zn were dropped down by the temporal prolonation. The elemental concentration levels obtained from the organisms indicate that the extent of heavy metal atmospheric deposition among the sampling sites was declining during the past decades.

1. INTRODUCTION

Lichen is one of the biomonitors with good accumulation property for determining the heavy metal deposition in terrestrial ecosystems. This well-established technique has been widely used in European countries for evaluating atmospheric deposition status since late 60s [1]. This work is the first attempt in China to apply this technique to retrospective study of the atmospheric heavy metal deposition trend level for the period of nearly thirty years (1960s-1990s) in a remote Southwestern China area (see Fig. 1) by using the early preserved lichen samples. The purpose of this study is two-folded:

(1) Assessment of atmospheric background deposition;
(2) Evaluation of the long-term anthropogenic emission impact on this area.

Species of large size epiphytic foliose lichens (*Parmotrema recticulata*, *Parmotrema Austrosinensis, Parmotrema tinctorum*, *Parmotrema cristifera,* and *Parmelia Subtinctoria*) were offered by the State Lichen Sample Bank in Institute of Microbiology, Chinese Academy of Sciences. All the selected lichen samples have been well reserved in a especially ultra-clean specimen bank. A total of 34 elements were determined by INAA including rare-earth elements (REEs), thorium, and uranium.

2. MATERIALS AND METHODS

The selected 23 epiphytic lichen samples (11 samples were *P. recticulata*) and 13 of the bark substrates were prepared as the experimental materials. About 100 to 300 mg samples dried at the room-temperature were ashed by ceramic fiber muffle furnace for 40 minutes at 540°C. Each ashed powder sample was packed with two layers of aluminium foils. Samples were irradiated for 8 hours in a heavy water nuclear reactor at the Chinese Institute of Atomic Energy (CIAE) at a thermal neutron flux of 3.35×10^{13} n cm^{-2} s^{-1}. After decay for 5 and 20 days, the samples were counted twice for medium and long-lived nuclides by an HPGe detector. Analytical quality control was assured by using a series of standard reference materials (SRMs): IAEA-336 Portuguese Lichen, GBW08505 Chinese Tea, GBW08501 Chinese Peach Leaf, and GBW 07605 Chinese Tea (GSC-4).

FIG. 1. Sampling sites of the lichens and their substrates.

3. RESULTS AND DISCUSSION

The mean values and ranges for 34 elemental concentrations in *P. recticulata* across nearly 30 years collected from the Yungui Plateau is presented in table I.

The accumulation capacity for trace elements in *P. recticulata* during the past decades shows that the more than half of the surveying values were lowered along with the time at the sampling place. Comparing the data with some literature values for different lichen species in European countries, it can be concluded that the reported values of most elements are within the range of concentrations in the European lichens, which should be regarded as a characteristics of lichen from relatively clean areas [2,3].

The REEs concentration distribution patterns in *P. recticulata* at various sampling times were proximately consistent with each other (see Fig.2).

The uptake of widely considered industrial pollution elements, chromium, iron, and zinc, in the lichen *P. recticulata* and its bark substrate was compared at the same condition of sampling sites and ambient environment. It indicates the higher elemental burdens of the three elements in *P. recticulata*. In terms of the airborne heavy metal accumulation concentrations, *P. recticulata* seems to be a very efficient biomonitor (see Fig.3).

TABLE I. ELEMENT CONCENTRATIONS (μ g/g) IN *P. RECTICULATA* FROM THE YUNGUI PLATEAU, SOUTHWESTERN CHINA COLLECTED FROM 1964 TO 1994

1.Element	1960s	(n = 2)	1980s	(n = 5)	1990s	(n = 4)
	Range	Mean	Range	Mean	Range	Mean
Ag	0.177–0.307	0.242	0.0843-0.174	0.116	0.0593–0.107	0.0728
As	1.10–11.3	6.20	0.479–2.63	1.24	0.213–0.904	0.468
Au	0.00096–0.0010	0.0010	0.00084–0.0021	0.0015	0.00037–0.00076	0.00049
Ba	90–114	102	39–111	66	16–237	96
Ca	3478–5062	4270	2223–11579	6640	3775–6288	5533
Ce	11.0–11.0	11.0	1.6–7.8	5.5	1.5–4.6	2.9
Co	1.04–16.6	8.82	0.477–2.29	1.14	0.339–4.08	1.30
Cr	6.33–50.8	28.6	2.65–12.3	6.05	1.45–1.92	1.68
Cs	1.24–2.28	1.76	0.11–1.55	0.83	0.190–0.618	0.493
Eu	0.150–0.164	0.158	0.0484–0.256	0.118	0.00529–0.0424	0.0285
Fe	3290–10432	6861	1111–4935	2523	664–885	805
Hf	0.383–0.601	0.492	0.175–1.08	0.453	0.0906–0.148	0.123
K	3773–4289	4031	2458–6787	4055	2478-3642	3058
La	3.50–4.39	3.94	1.66–9.21	3.82	0.889–2.23	1.32
Lu	0.0268–0.128	0.0774	0.0261–0.138	0.0559	0.00441–0.0107	0.0620
Mo	0.475–0.829	0.652	0.486–2.56	1.14	0.179–0359	0.235
Na	297–350	324	124–889	453	52.7–95.7	72.7
Nd	3.21–3.84	3.52	1.79–7.54	3.23	0.673–1.28	0.952
Ni	3.00–24.2	13.6	1.77–4.50	2.92	0.892–3.75	2.06
Rb	12.5–17.5	15.0	7.27–20.5	11.7	12.6–15.5	13.8
Ru	1.30–1.48	1.39	0.527–1.39	0.929	0.235–4.36	1.73
Sb	0.195–0.918	0.556	0.216–24.0	5.05	0.0658–0.499	0.283
Sc	1.17–5.57	3.37	0.428–2.45	1.12	0.232–0.314	0.277
Se	0.388–0.423	0.406	0.148–0.386	0.254	0.136–0.254	0.178
Sm	0.683–0.695	0.689	0.253–1.24	0.544	0.173–0.262	0.212
Sr	19.6–25.8	22.7	11.9–38.2	23.8	16.3–47.1	29.6
Ta	0.0564–0.0863	0.0714	0.0219–0.170	0.0745	0.0158–0.026	0.0205
Tb	0.123–0.124	0.124	0.0391–0.186	0.0736	0.0152–0.0261	0.0207
Th	1.50–1.59	1.54	0.364–2.51	1.02	0.214–0.293	0.256
Tm	0.0298–0.0440	0.0369	0.0172–0.0469	0.0266	0.0136–0.017	0.0156
U	0.151–0.230	0.190	0.0635–0.176	0.104	0.0374–0.0502	0.0450
W	0.221–0.299	0.260	0.204–0.432	0.300	0.0998–0.190	0.128
Yb	0.312–0.314	0.313	0.0885–0.554	0.213	0.0435–0.0696	0.0600
Zn	35.3–39.0	37.2	17.7–58.8	33.1	10.8–27.2	18.0

The concentrations of Ag, As, Ce, Cr, Cs, Eu, Fe, Hf, La, Nd, Ni, Sc, Se, Sm, Tb, U, Yb, and Zn in lichen *P. recticulata* shows that these values definitely follow the pattern of falling down along with the time (see Fig. 4). How to explain this phenomenon? Tracing back the Chinese modern history, there seems to find out the answer. There once was the Great Leap Forward movement spreading out every corner of China in late 1950s. One of the main duty was the promotion of indigenous method for steel-making. Then broke out the large-scale ecological damage by the so-called "Red Guard" youngsters during 1960s to 1970s at the time of Culture Revolution. The series movements also influenced the sampling area. So it was lichen as biomonitor which recorded the ecosystem destroy and rehabilitation by anthropogenic activity.

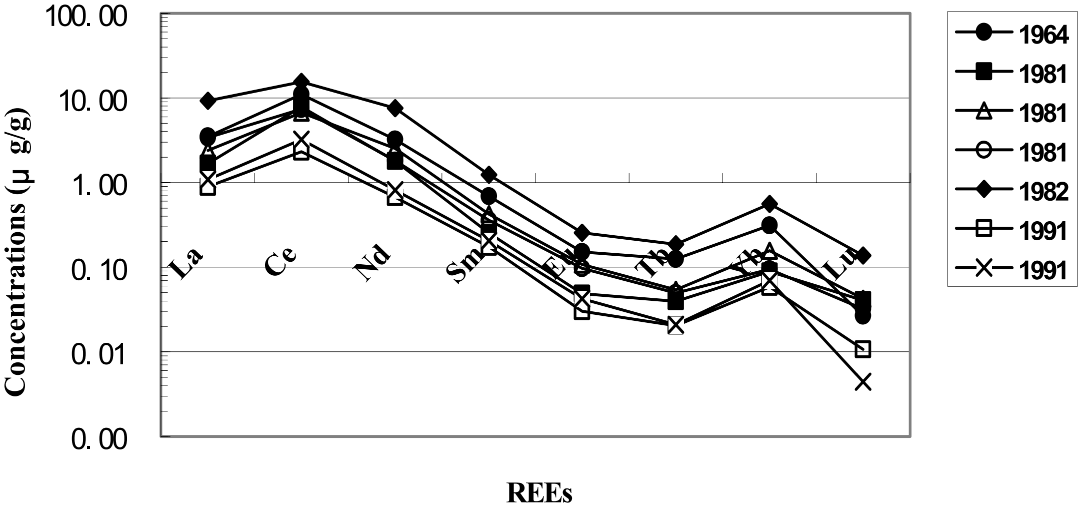


FIG.2. REEs concentrations in P. recticulata from 1960s to 1990s.

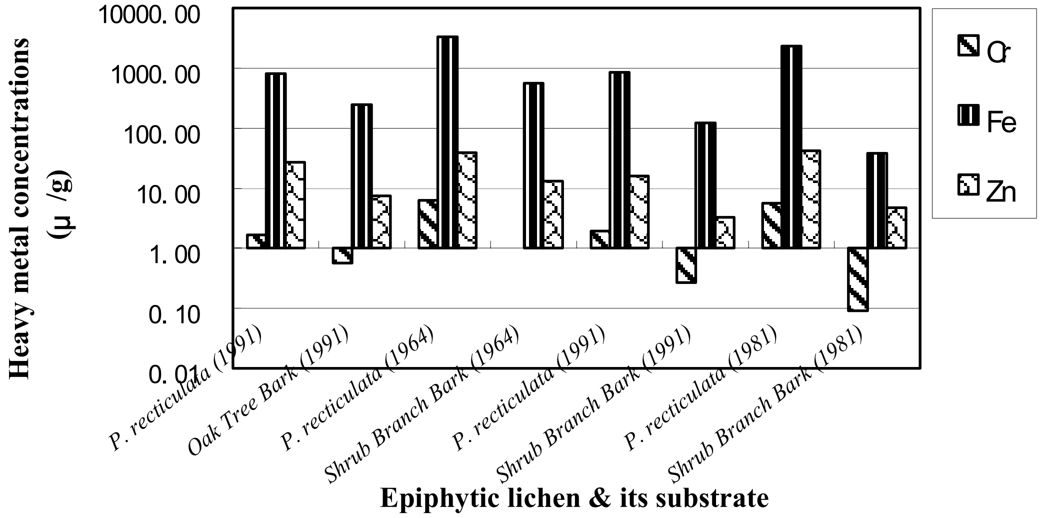


FIG.3. Elemental enrichment capacity in P. recticulata vs. bark substrate.

A significant positive correlation between thorium and uranium was found in the 23 epiphytic lichen samples and 13 bark substrate samples (see Fig. 5). Th and U are the naturally occurring elements in the earth's crust. Higher plants possess the ability to absorb the soluble Th and U via roots [4]. The corresponding enrichment in epiphytic lichens favors the mechanism that vascular plants first absorb U and Th from soil, which are subsequently transferred to lichen by leaching from living or dead plant materials [5].

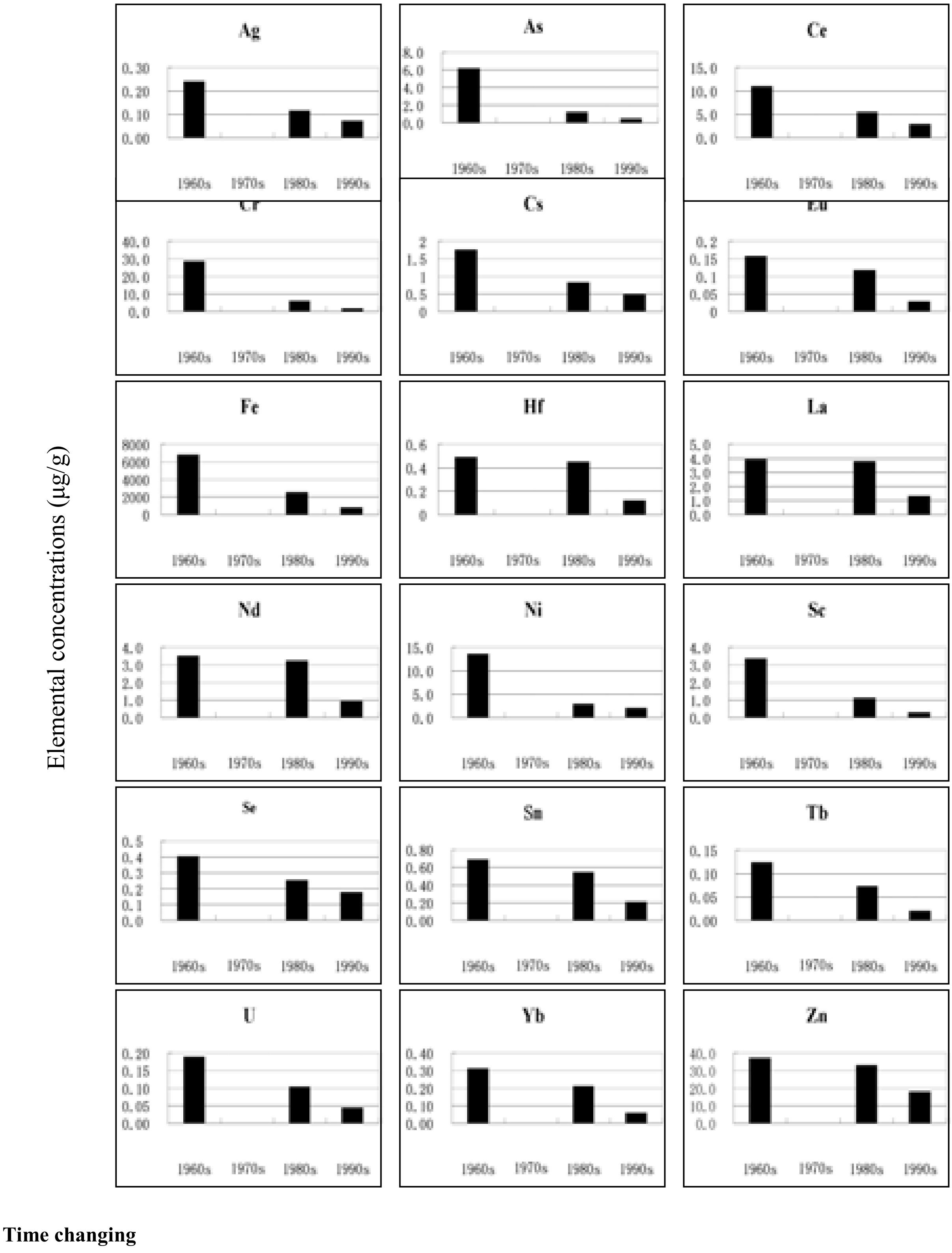


FIG. 4. Variation for elemental concentrations in P. recticulata vs. time changing.

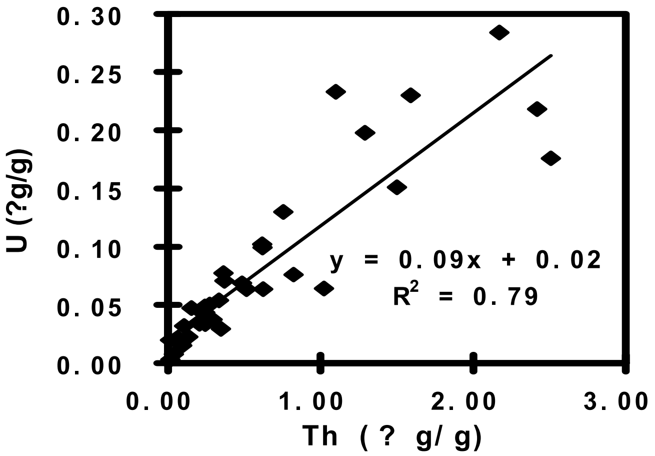


FIG. 5. Concentration correlation of thorium vs. uranium.

REFERENCES

[1] MARKET, B., WAPPELHORST, O., WECKERT, V., SIEWERS, U., FRIESE, K.,BREULMANN, G., The use of bioindicators for monitoring the heavy-metal status of the environment, J. Radioanal. Nucl. Chem. **240** (1990) 425–429.

[2] JERAN, Z., JACIMOVIC, R., BATIC, F., SMODIS, B., WOLTERBEEK, H. TH., Atmospheric heavy metal pollution in Slovenia derived from results for epiphytic lichens Atmospheric heavy metal pollution in Slovenia derived from results for epiphytic lichens, Fresenius J. Anal. Chem. **354** (1996) 681–687.

[3] STONE, S. F., FREITAS, M. C., PARR, R. M., ZEISLER, R., Elemental characterization of a candidate lichen research material — IAEA- 336, Fresenius J. Anal. Chem. **352** (1995) 227–231

[4] K-PENDIAS, A., PENDIAS, H., (1984), Trace Elements in Soil and Plants. Boca Raton, CRC Press.

[5] BERG, T., RYSET, O., STEINNES, E., VADSET, M., Atmospheric trace elements disposition: principal component analysis of ICP-MS data from samples, Environ. Pollut. **88** (1995) 67–77.

INTERCALIBRATION OF HEAVY METAL ACCUMULATION IN SOME INDICATOR MOSS SPECIES AS A BASIS FOR MAPPING ATMOSPHERIC HEAVY METAL DEPOSITION IN UKRAINE

O. BLUM, A. GLEBOV
National Botanical Garden, National Academy of Science of Ukraine, Kiev, Ukraine

Abstract

The moss interspecies comparisons of elemental concentrations (*Cd*, *Cu*, *Cr*, *Fe*, *Hg*, *Ni*, *Pb*, *V* and *Zn*) were carried out. The moss species considered were both pleurocarpous — *Pleurozium schreberi* (**Ps**), *Hylocomium splendens* (**Hs**), *Brachythecium curtum* (**Bc**), *Rhytidiadelphus squarrosus* (**Rs**), and acrocarpous moss — *Dicranum polysetum* (**Dp**). Samples of mosses were collected at selected lowland and mountainous locations in Ukrainian coniferous forests. Relative levels of the efficiency of heavy metal accumulation by these intercalibrated moss species (8–19 pairs) were compared. Each pair of mosses included *Pleurozium schreberi* as basic indicator species and one from the above-mentioned alternative species. The linear regression and coefficient of correlation equations have been calculated. The evaluation of adequacy of the obtained models was made using Fisher criterion. The significant correlations (P=0.05) using Fisher criterion was obtained for *Zn* and *Hg* (**Ps** vs. **Hs**, n=8), for *Cd*, *Cu,* and *Hg* (**Ps** vs. **Bc**, n=8) and for *Cd*, *Cr*, *Cu*, *V*, *Zn,* and *Hg* (**Ps** vs. **Dp**, n=19). Reliable correlation between **Ps** and **Rs** for the above mentioned 9 elements was not found.

1. INTRODUCTION

The territory Ukraine is rather large (603,700km$^{2)}$ and its eco-geographical conditions are very different. The forests occupy about 15.6% of the total area of Ukraine but this percentage ranges from 40.2% in Carpathians to 3.8% in steppe regions of southern and eastern Ukraine [2].

In 1995 Ukraine was involved in the project “Atmospheric Heavy Metal Deposition in Europe — estimations based on moss analysis”. It is impossible to organize the heavy metal biomonitoring in a territory with such a big variation of eco-geographical parameters as there exist in Ukraine using only single monitor moss species within the survey. One of the principal solutions of this problem may be the use of several moss species. In this case, the survey should be accompanied by full calibration of interspecies variations in response to variable element concentrations.

Only a few studies have been carried out on interspecies moss calibration so far [1,3–6, 9–11,13–14]. Some authors [13] came to the conclusion that the use of calibrations in extrapolation modes is greatly restricted by the necessary reservations in geographically larger-scaled applications.

In our biomonitoring study, various moss species were sampled, including pleurocarpous mosses — *Pleurozium schreberi (Brid.) Mitt., Hylocomium splendens (Hedw.) B.S.G., Hypnum cupressiforme Hedw., Pseudoscleropodium purum (Hedw.) Fleisch. ex Broth, Rhytidiadelphus squarrosus (Hedw.) Warnst., Brachythecium curtum Lindb.,* and acrocarpous mosses — *Dicranum polysetum Sw., Polytrichum commune Hedw.* and *Polytrichum formosum Hedw.,* At some locations it was possible to sample, besides *Pleurozium schreberi* as main indicator species, also the alternative indicator moss *Hylocomium splendens* and two other alternative indicator mosses: pleurocarpous: *Brachythecium curtum, Rhytidiadelphus squarrosus,* and acrocarpous moss: *Dicranum polysetum,* which are characteristic of the native participation of various botanical and geographical country zones. The primary purpose of this paper is to present a preliminary comparison of accumulated concentrations between these interspecies pairs of mosses (from 8–19 sampling sites).

The relative levels of the heavy metal accumulation efficiency of the moss species were compared with the basic indicator species *Pleurozium schreberi.* Equations for the linear regression and the coefficient of correlation were calculated. The adequacy of the obtained models was evaluated by the Fisher criterion.

2. MATERIAL AND METHODS

2.1. Sampling of mosses

Samples of mosses were collected during the summer-autumn period (1995–1999) from selected lowland (Polissia area — Rivne, Zhytomyr, Kyiv, Chernigiv regions) and mountainous (Ukrainian Carpathians — L'viv, Ivano-Frankivs'k, Chernivtsi, Zakarpattia regions) forested areas of Ukraine. The sampling sites were located at least 300 m from main roads and at least 100 m from any road. At each sampling site 7–10 subsamples were collected and mixed in the same bag. The volume of each sample was 1 to 2 dm^3. The following pairs (n=8–19) of moss species for intercalibration were taken: **Ps** vs. **Hs**, **Ps** vs. **Bc**, **Ps** vs. **Rs,** and **Ps** vs. **Dp**.

2.2. Sample preparation

The samples were cleaned and only 2–3 cm of the upper parts of moss branches or shoots were taken for analyses. The moss samples were not washed. After cleaning the moss samples they were randomized and two, samples approximately 1 g each were taken and dried at 40°C during 24 hours. Then one sample was used for wet ashing and another was dried at 105°C. All metal concentrations are expressed as µg/g at this temperature.

For mercury the moss samples were not dried at 40°C and were analyzed in air dry weight condition.

During wet digestion the moss samples (1g) were dissolved in 10 ml of ultra-pure concentrated nitric acid and bidistilled water (1:1). Then they were mineralized in Kjeldal flasks, first at room temperature (12 hours) and then subsequently at the temperature 150–170°C using a special sand bath plate. Successive addition of ultra-pure reagents (HNO_3, H_2O_2,,and H_2O) were used to induce digestion of samples. Solutions were filtered after completing the mineralization process, and then diluted with bidistilled water. Each series of 17 digested and analysed samples included the measuring of a blank and a standard interlaboratory material M/95 (*Pleurozium schreberi* of HM Europe Survey — 1995 international programme).

2.3. Sample analysis

The majority of elements (*Cd, Cr, Cu, Ni, Pb,* and *V*) in the samples were determined with the aid of a Saturn 3P-1 atomic absorption spectrophotometer (Ukraine), fitted with Graphite-2 electrothermic atomizer, which can measure element concentrations up to $10^{-6} \div 10^{-8}$%. The macroelements (*Fe* and *Zn*) were analyzed with the aid of a S-115 PKS atomic absorption spectrophotometer (Ukraine) by using a propane-air flame. The mercury was determined by cold vapour method with the aid of a Saturn 3P-1 of atomic absorption spectrometer. The precision of measurements was ±5–15% for the above-mentioned elements.

3. RESULTS AND DISCUSSION

The preliminary results on four intercalibrated moss pair species sampled in Ukraine are summarized in Tables I-IV.

TABLE I. COMPARISON OF ELEMENTAL CONCENTRATION (μg/g d.w.) IN *PLEUROZIUM SCHREBERI* (X VARIABLE) AND *HYLOCOMIUM SPLENDENS* (Y VARIABLE) COLLECTED AT THE SAME SITES

Element	n	Concentration range (x)	Concentration range (a)	Median ratio y/x	Mean ratio coefficient	Correlation coefficient
Cd	8	0.12–0.84	0.12–1.00	1.19	1.36	0.41
Cr	8	1.20–3.57	1.28–2.34	0.75	0.79	0.51
Cu	8	4.20–8.65	4.07–10.33	1.06	1.09	0.72
Fe	8	389–1192	361–1412	0.91	0.90	0.84
Hg	8	0.04–0.071	0.047–0.079	1.17	1.13	0.92
Ni	8	1.52–4.18	1.45–3.67	0.89	0.91	0.56
Pb	8	3.32–8.65	2.96–10.19	1.05	1.06	0.85
V	8	2.38–4.75	2.02–5.58	0.86	0.98	0.70
Zn	8	19.00–46.58	18.4–45.5	1.12	1.07	0.95

TABLE II. COMPARISON OF ELEMENTAL CONCENTRATION (μg/g d.w.) IN *PLEUROZIUM SCHREBERI* (X VARIABLE) AND *BRACHYTHECIUM CURTUM* (Y VARIABLE) COLLECTED AT THE SAME SITES

Element	n	Concentration range (x)	Concentration range (y)	Median ratio y/x	Mean ratio coefficient	Correlation coefficient
Cd	8	0.11–0.39	0.16–0.44	1.54	1.43	0.91
Cr	8	0.88–4.56	0.40–2.83	1.06	0.99	0.59
Cu	8	2.83–15.58	2.28–10.83	1.02	0.92	0.95
Fe	8	264–1029	251–1459	1.08	1.18	0.45
Hg	5	0.05–0.194	0.08–0.19	1.26	1.18	1.00
Ni	8	0.96–3.33	1.24–4.60	1.53	1.43	0.30
Pb	8	2.79–6.09	1.22–8.42	1.31	1.30	0.08
V	8	1.01–3.20	0.69–4.28	1.06	1.20	0.61
Zn	8	19.00–67.68	42.4–81.6	1.69	1.63	0.76

TABLE III. COMPARISON OF ELEMENTAL CONCENTRATION (µg/g d.w.) IN *PLEUROZIUM SCHREBERI* (X VARIABLE) AND *RHYTIDIADELPHUS SQUARROSUS* (Y VARIABLE) COLLECTED AT THE SAME SITES

Element	n	Concentration range (x)	Concentration range (y)	Median ratio y/x	Mean ratio coefficient	Correlation coefficient
Cd	8	0.16–0.62	0.13–0.87	1.03	1.09	0.59
Cr	8	0.37–4.45	1.45–3.40	1.09	0.97	0.54
Cu	8	4.28–12.00	5.98–19.9	1.10	1.27	0.78
Fe	8	112–3295	305–903	0.98	0.67	0.44
Hg	5	0.047–0.093	0.031–0.069	0.91	1.02	0.78
Ni	8	0.70–4.18	0.55–4.86	1.33	1.13	0.45
Pb	8	2.27–8.97	3.01–6.80	0.88	0.88	0.12
V	8	0.39–4.68	0.79–3.85	0.51	0.78	0.41
Zn	8	27.90–46.58	29.1–52.9	1.15	1.14	0.53

TABLE IV. COMPARISON OF ELEMENTAL CONCENTRATION (µg/g d.w.) IN *PLEUROZIUM SCHREBERI* (X VARIABLE) AND *DICRANUM POLYSETUM* (Y VARIABLE) COLLECTED AT THE SAME SITES

Element	n	Concentration range (x)	Concentration range (y)	Median ratio y/x	Mean ratio coefficient	Correlation coefficient
Cd	19	0.10–0.36	0.14–0.60	1.43	1.22	0.79
Cr	19	0.63–5.41	0.74–9.21	1.73	1.23	0.86
Cu	19	2.83–18.37	2.37–22.8	1.04	0.86	0.91
Fe	19	112–657	310–1485	1.49	1.87	0.54
Hg	16	0.047–0.194	0.07–0.16	1.41	1.41	0.34
Ni	19	0.91–4.64	1.12–3.71	1.12	0.84	0.79
Pb	19	2.24–6.68	3.62–9.18	1.43	1.16	0.48
V	19	0.51–3.84	1.00–6.15	1.89	2.61	0.90
Zn	19	14.60–54.30	14.9–42.7	1.07	0.84	0.78

Figures 1–2 show the obtained models of comparison of element concentrations in intercalibrated mosses employing Fisher's criterion for evaluation of models adequacy.

Concerning moss species **Ps** and **Hs,** in previously intercalibration studies, some authors concluded that these two species do not give significant differences in element concentrations, and therefore can be used for mapping of atmospheric heavy metal deposition directly without calibration [5,11]. But other authors have found differences between metal concentrations depending on the element studied. According Ref. [6], concentrations of *Cu*, *Fe*, *Mn*, *Pb*, *Ni,* and *V* were 14 to 24% higher in **Hs** than in **Ps** while *Cd*, *Mn*, *Zn,* and *Cr* concentrations were similar in both mosses. Similar data were reported for these moss species sampled in Germany (11 locations): **Hs** gave significantly higher (16–28%) values for *Fe*, *Ni*, *Pb,* and *V* as compared to **Ps** [13]. This is not surprising because the uptake efficiency for a given element differs between different moss species [8]. **Ps** and **Hc** were significantly correlated (P=0.05) for *Hg* and *Zn* with the correlation coefficient 0.92 and 0.95, respectively (Fig. 1). Lower correlation at the range 0.70–0.85 was found for *Cu*, *Fe*, *Pb,* and *V*.

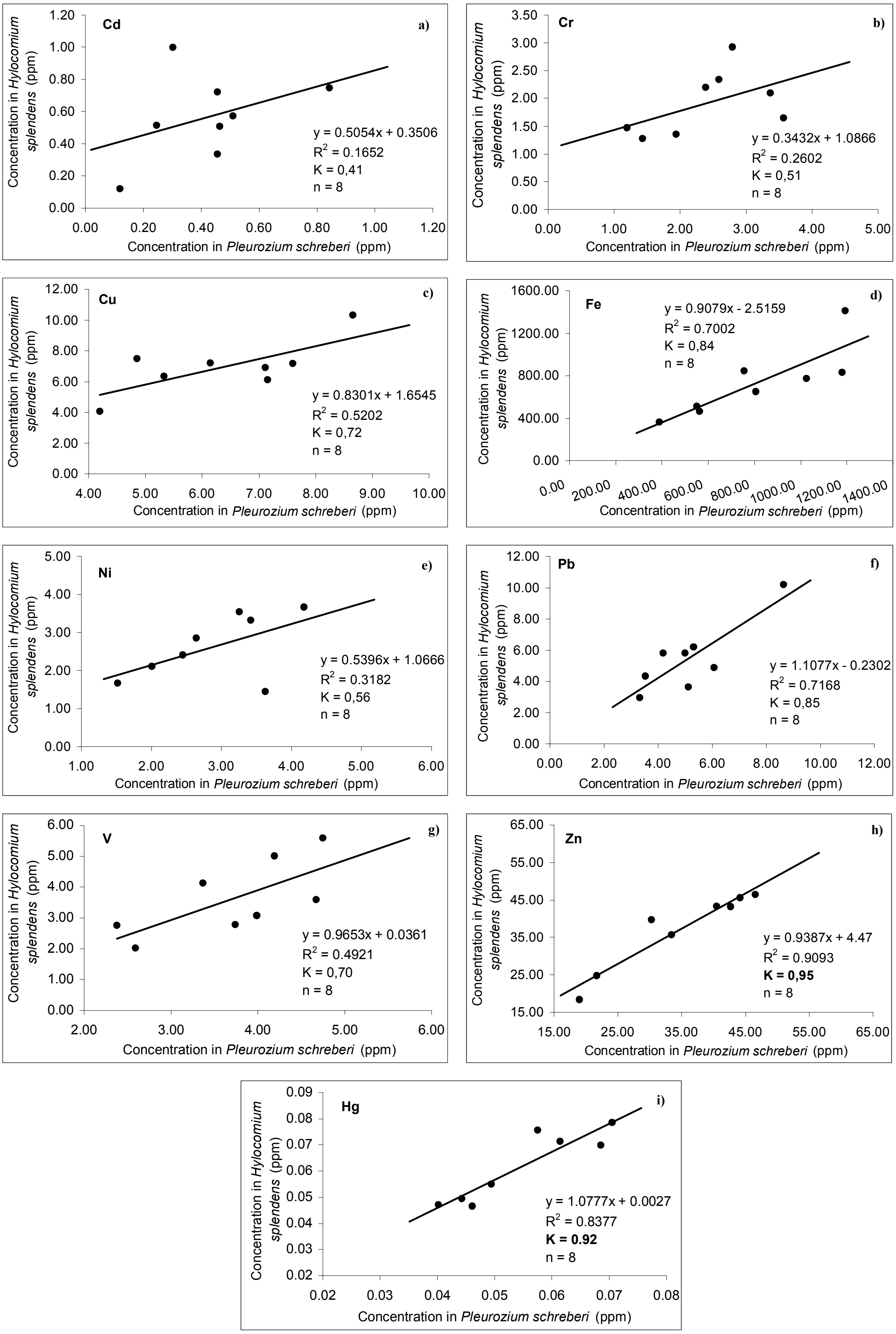


FIG. 1. Comparisons of concentrations (ppm=µg/g, dry weight basis) of heavy metals in Pleurozium schreberi and Hylocomium splendens.

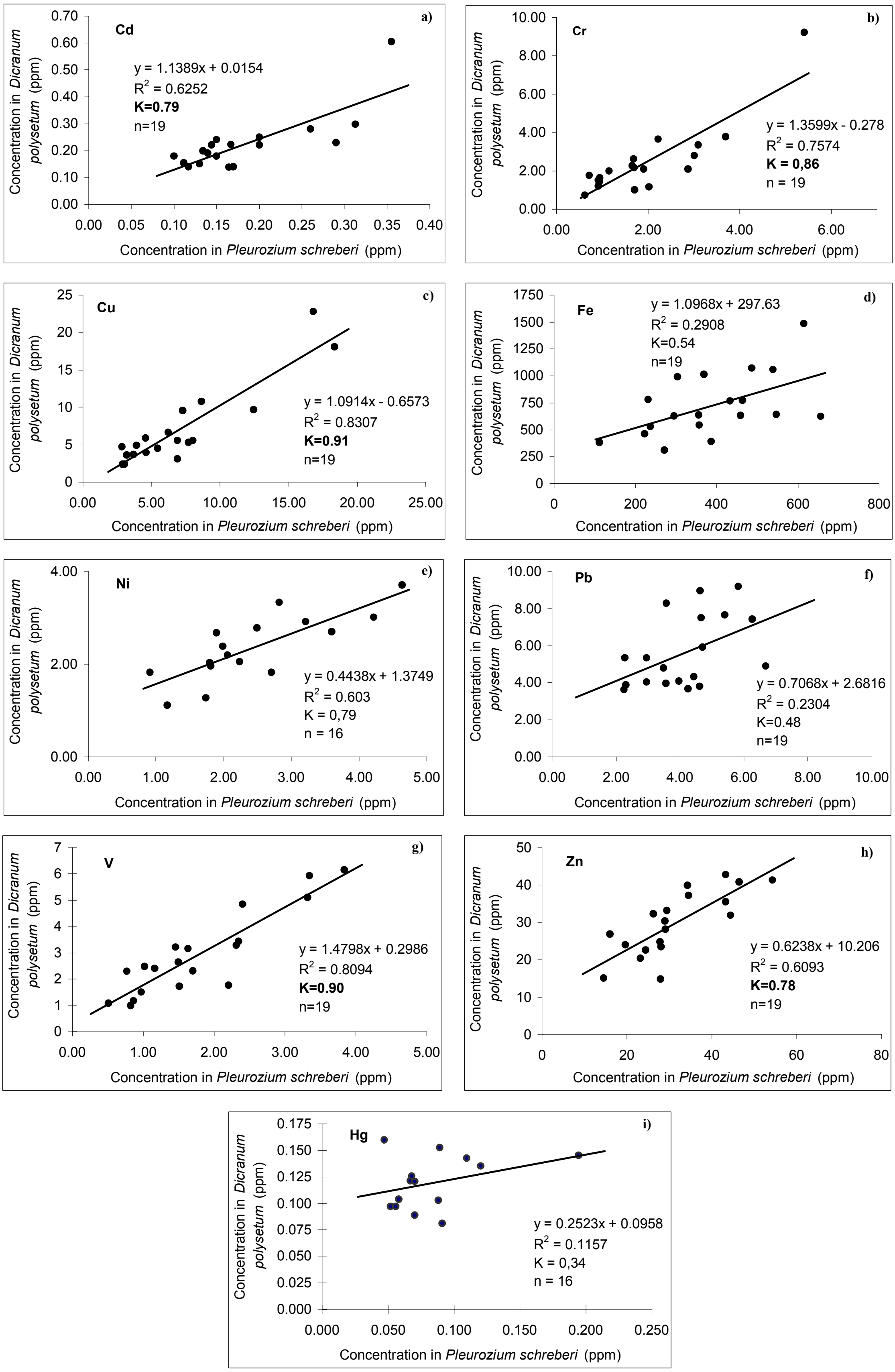


FIG. 2. Comparisons of concentrations (ppm=µg/g, dry weight basis) of heavy metals in Pleurozium schreberi and Dicranum polysetum.

Ps and **Bc** showed significant correlation for *Cd* (0.91), *Cu* (0.95), and *Hg* (1.0). It is worth to notice that the concentrations of all elements were higher in **Bc** than in **Ps**. There was no significant correlation for **Ps** versus **Rs**. The highest correlation coefficients (0.78) were found both for *Cu* and *Hg*. Nobody before has compared element concentrations in pleurocarpous species with acrocarpous moss species because the last are not recommended as bioindicator. Therefore it was very surprising to find significant correlation (P=0.05) between **Ps** and **Dp** for *Cd*, *Cr*, *V,* and *Zn* (Table IV, Fig. 2).

As the interspecies correlation may depend on the range of element concentrations and chemogeographical conditions, these aspects should be of similar scaling for both interspecies calibration [12]. In the values obtained during the interspecies calibration process it could be comprised also uncertainties arising from various aspects of sampling, local variations, and methods of element analysis [7]. Therefore, the calibration of elemental concentrations in various moss species may not simply lead to a controlled use of more than one single species within any heavy metal deposition survey [13]. At the same time it is evident that the preliminary results available for some interspecies calibration in Ukraine are similar to the results obtained in France [4]; then they should be able in principle to be used on national HM deposition. maps using a calibration factor. However, it is necessary to improve the method of calculation of reliable interspecies calibration coefficient. The data set should be enough representative and the possible above-mentioned method-associated uncertainties should be minimized.

ACKNOWLEDGEMENT

The authors are thankful to Aleksey Chekhovich for computer statistical processing of the moss intercalibration data and for figure drawings.

REFERENCES

[1] BOILEAU, L.J.R., BECKETT, P.J., LAVOIE, P., RICHARDSON, D.H.S., Lichens and mosses as monitors of industrial activity associated with uranium minning in Northern Ontario, Canada. Part I: Field procedures, chemical analysis and interspecies comparisons. Environ. Pollution. Ser. B **4** (1982) 69–84.

[2] BUKSHA, I.F., MESHKOVA, V.L., RADCHENKO, O.M., SIDOROV, A.S., Air pollution and Climate change effects on health of the Ukrainian forests: monitoring and evaluation. In: BYTNEROWICZ A., ARBAUGH M.J., SCHILLING S.L. (technical coordinators). 1998. Proceedings of the international symposium on air pollution and climate change effects on forest ecosystems; 1996 February 5–9; Riverside, CA. Gen. Tech. Rep. PSW-GTR-166. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture (1998) 332 pp.

[3] FOLKESON, L., Interspecies calibration of heavy metal concentrations in nine mosses and lichens: applicability to deposition measurement. Water, Air and Soil Pollution **11** (1979) 253–260.

[4] GALSOMIÈS, L., LETROUIT, M.A., DESCHAMPS, C., SAVANNE, D., AVNAIM, M., Atmospheric metal deposition in France: initial results on moss calibration from the 1996 biomonitoring. The Science of the Total Environment **232** (1999) 39–47.

[5] HALLERAKER, J.H., REIMANN, C. DE CARITAT, P., FINNE, T.E., KASHULINA, G., NISKAAVAARA, H., BOGATYREV, I., Reliability of moss (*Hylocomium splendens* and *Pleurozium schreberi*) as a bioindicator of atmospheric chemistry in the Barents region: Interspecies and field duplicate variability. The Science of the Total Environment **218** (1998) 123–139.

[6] ROSS, H., On the use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) for estimating atmospheric trace metal deposition. Water, Air and Soil Pollution **50** (1990) 63–76.

[7] RÜHLING, Å. (ed.) Atmospheric heavy metal deposition in Europe — estimations based on moss analysis. Report Nord 1994:**9**, Nordic Council of Ministers, Copenhagen (1994) 53 pp.

[8] STEINNES, E., Use of mosses as biomonitors of atmospheric deposition of trace elements. Biomonitoring of atmospheric pollution (with emphasis on trace elements) — BioMAP. Vienna, IAEA (2000) 100–107.

[9] SUCHAROVÁ, J., SUCHARA, I., Atmospheric deposition levels of chosen elements in the Czech Republic determined in the framework of the international bryomonitoring program 1995. Sci. Total. Environ. **223** (1998) 37–52.

[10 TAYLOR, F.G., and WINTHETRSPOON, J.P., Retention of simulated fallout particles by lichens and mosses. Health Physics **23** (1972) 867–869.

[11] THÖNI, L., Vergleich von Elementenkonzentrationen in drei Biomonitoringmoosen untereinander und mit Depositionsfrachten im Bulksammler nach Bergerhoff. BUWAL-FUB, Egg (1996) 89 S.

[12] WOLTERBEEK, H.TH., BODE, P., Strategies in sampling and sample handling in the context of large scaled plant biomonitoring surveys of trace element air pollution. Sci. Total Environm. **176** (1995) 33–43.

[13] WOLTERBEEK, H.TH., KUIK, P., VERBURG, U., HERPIN, U., THÖNI, L., Moss interspecies comparison in trace element concentrations. Environ. Monit. Assess. **35** (1995) 263–286.

[14] ZECHMEISTER, H.G., Annual growth of four pleurocarpous moss species and their applicability for biomonitoring heavey metals. Environ. Monit. Assess. **53** (1998) 441–445.

AIR POLLUTION STUDIES BY PLANTS GROWING NEAR SOME INDUSTRIAL OBJECTS OF UZBEKISTAN

M. KADIROVA[1], N.M. MUKHAMEDSHINA[1]
A.A. MIRSAGATOVA[1], N. NORBOEV[2], M. AMANOV[2]

[1] Institute of Nuclear Physics, Uzbekistan Academy of Sciences,
Ulugbek, Tashkent, Uzbekistan
[2] Tashkent State Agrarian University, Uzbekistan,
Kibray Region, Tashkent District, Uzbekistan

Abstract

Leaves and seeds of many popular kinds of plants in Uzbekistan was used to study atmospheric pollution near such industrial objects as Tadjik aluminum factory (TadAF), Chirchik works of heatproof and refractory metals, Asaka automobile works and Tashkent institute of nuclear physics (INP). Leaves of fruit plants: vineyard, tomato and apple, apricot, quince, peach, persimmon, pomegranate, mulberry trees as well as leaves of technical crops: cotton, corn, mint and clover, which grow near above specified objects and also seeds of water-melon, tomato, aubergine, bulgarian pepper, pumpkin, grapes, cherry and persimmon from areas near aluminum factory were sampled. The purpose of choice of so much investigated vegetation was to select plants which can be used as biomonitors and which best accumulate in themselves the most harmful pollution from soil and air in order to subsequent destruction of these collector plants will be made. Investigations were conducted by nuclear techniques and by physical and agrotechnical methods. Tashkent State Agrarian University has used the alternative methods. Multielement instrumental neutron activation analysis (INAA) techniques for determination of 27 elements in plant leaves and seeds have been developed.

1. INTRODUCTION

In recent years lower plants such as mosses [1,2] or lichens [3] have been used as biomonitors together with aerosol filters and impactors [4,5] for atmospheric pollution studies. For dry weather countries in the last years bark and leaves of trees [6,7] have been used. Due to dry climatic conditions it is difficult to find moss or lichen in Uzbekistan too. To study air pollution and aluminum factory harmful ejection influences on plants INP in cooperation with Tashkent State Agrarian University were gathering two years in summer season many kinds of plant leaves and seeds widely popular over the country. These year samples were collected near the works of refractory metals, automobile works and in the INP in order to compare the environmental conditions of these regions.

In result of serious errors admitted for the choice of place of a gigantic aluminum factory building in Tursunzade city of Tadjikistan in 7–8 km from border with Uzbekistan, the subtropical valley of Uzbekistan with high density of population and advanced agricultural manufacture has appeared under effect of the factory harmful ejection on environment, person, animals, silkworms, and plants. The specific natural-climatic conditions of the factory accommodation were not taken into account on stage its designing. For this region, it is typical dry and almost windless weather, often calm situations and inversion of temperature.

Here valley-mountainous circulation of air masses dominates, which promotes carrying and dispersion of harmful emissions in ground bed on far distances. The distribution of emissions occurs by rather narrow band on Gissar valley, mainly aside Sariassiya region of Uzbekistan [8]. It is known, that aluminum factories throw out in environment fluorine hydride, solid fluorides, and nitrogen dioxide, sulphur dioxide, hydrocarbons, ions of heavy metals and others. Moreover Sariassiya and Uzun regions are under the effect of objects of producing phosphorus fertilization and porcelain. Since 1990 the conditions of region atmospheric pollution were checked by means of three stationary observing posts. These posts are placed practically along the valley on a southwest direction in different distances from the factory: in Sariassiya region two stations — in (10–12km) and (16–18km), in Denau region one station in (30–35km). From the data obtained in stationary observing posts for example, the highest average monthly concentration of fluorine hydride in summer time took place in Tursunzade and Sariassiya regions. It reached 3 and 8 MPC (maximum permissible concentration) respectively, ordinary in summer months concentration of fluorine hydride exceeded in 1.5–2 times the MPC value and the contents of sulphurous gas and nitrogen dioxide in Sariassiya region at dry season exceed 1.5- 2 times the MPC values. It is useful to have information on heavy metals and trace elements contents in the environment of these regions.

2. EXPERIMENTAL

Samples from 4 sites near aluminum factory, 2 sites near automobile works, 1 site near works of refractory metals and 1 site in the institute of nuclear physics were collected. The site in Djarcurgan region on the distance of 150–200 km from aluminum factory is accepted as control region. On each site 5 subsamples within 100 × 100 m area from the height of 2 to 3 m of trees were taken, washed by distilled water and then they were dried to constant weight at room temperature. The dried samples were crushed in agate roller and analyzed by INAA comparative technique using the water — water reactor (WWR-SM) of Tashkent INP. Characteristics of developed techniques for determination of 27 elements in plants are shown in table I. The determination of this large number of elements was achieved using complex conditions of irradiation, including varying neutron energy and choosing optimum time parameters which are given in the table. Samples and standards (IAEA 0393 algae) were irradiated, for short — lived nuclides (Na, K, Mn, Cu, Sr) by thermal neutrons (7.5×10^{11} cm$^{-2}\cdot$s^{-1} neutron fluence) during 16 h; and for long lived nuclides (Ti, As, Se, Br, Rb, Cd, Sb, Ba, La, U, Sc, Cr, Fe, Co, Ni, Zn, Zr, Sb, Ce, Cs, Hg, Th) by fission flux (10^{14} cm^{-2}s^{-1} neutron fluence) during 20 h. Nuclear characteristics of the determined elements are given in table II.

TABLE I. CHARACTERISTICS OF DEVELOPED INAA TECHNIQUES

Determined elements	**Sample mass, g**	**Neutron flux and spectra, n·cm^{-2} s^{-1}**	**Time**		
			Irradiation	**Cooling**	**measuring**
Na, K, Mn, Cu, Sr	0.2	7.5×10^{11}, thermal	16 h	2–3 h	5 m
Ti, As, Se, Br, Rb, Cd, Sb, Ba, La, U	0.2–0.5	10^{14} fission spectra	20 h	10 d	5 m
Sc, Cr, Fe, Co, Ni, Zn, Zr, Sb, Ce, Cs, Hg, Th				20 d	10 m

TABLE II. NUCLEAR CHARACTERISTICS OF DETERMINED ELEMENTS

nuclide	reaction	radio-nuclide	cross section, barn	half-life	Representative gamma rays energy,(absolute intensity) MeV
^{23}Na	n,γ	^{24}Na	0.530±0.005	15.03h	1.37(1.0), 2.75(1.0)
^{41}K	n,γ	^{42}K	1.46±0.03	12.36h	1.52(0.18)
^{45}Sc	n,γ	^{46}Sc	26.5±0.1	83.9d	0.889(1.0), 1.12(1.0)
^{47}Ti	n,p	^{47}Sc	0.026±0.003	3.44d	159.4(0.73)
^{50}Cr	n,γ	^{51}Cr	15.9±0.2	27.7d	0.320(0.2)
^{55}Mn	n,γ	^{56}Mn	13.3±0.2	2.58h	0.845(0.99), 1.81(0.3), 2.16(0.16)
^{58}Fe	n,γ	^{59}Fe	1.15±0.02	45d	1.099(0.57), 1.29(0.43)
^{59}Co	n,γ	^{60}Co	37.2±0.2	5.27y	1.17(0.999), 1.33(0.999)
^{58}Ni	n,p	^{58}Co	0.102±0.005	71.3d	810.8(0.99), 511.0(0.3)
^{63}Cu	n,γ	^{64}Cu	4.5±0.1	12.74h	0.511(0.37), 1.35(0.005)
^{64}Zn	n,γ	^{65}Zn	0.78±0.02	244d	1.115(0.508)
^{75}As	n,γ	^{76}As	4.3±0.1	26.4h	0.559(0.45), 0.657(0.064)
^{74}Se	n,γ	^{75}Se	51.8±1.2	120d	0.136(0.54), 0.265(0.58), 0.279(0.25), 0.400(0.116)
^{81}Br	n,γ	^{82}Br	2.69±0.09	35.3h	0.554(0.71), 0.619(0.43), 0.698(0.29), 0.776(0.84)
^{85}Rb	n,γ	^{86}Rb	0.46±0.02	18.7d	1.077(0.088)
^{86}Sr	n,γ	^{87}Sr	0.84±0.06	2.81h	0.388(0.83)
^{94}Zr	n,γ	^{95}Zr	0.056±0.004	65.5d	0.724(0.44), 0.757(0.55)
^{98}Mo	n,γ	^{99}Mo	0.130±0.006	66.02h	0.14(0.85,^{99m}Tc), 0.74(0.14)
^{114}Cd	n,γ	^{115}Cd	0.300±0.015	2.22d	0.492(0.081), 0.524(0.275)
^{123}Sb	n,γ	^{124}Sb	4.33±0.16	60.3d	0.603(0.98), 0.722(0.110), 1.691(0.49)
^{133}Cs	n,γ	^{134}Cs	29.0±1.5	2.06y	0.569(0.154), 0.605(0.976), 0.796(0.854)
^{130}Ba	n,γ	^{131}Ba	13.5±3	11.6d	0.124(0.32), 0.216(0.217), 0.3732(0.13), 0.496(0.421)
^{139}La	n,γ	^{140}La	9.03±0.33	40.23h	0.33(0.185), 0.487(0.430), 0.816(0.223), 1.596(0.955)
^{140}Ce	n,γ	^{141}Ce	0.57±0.04	32.5d	0.145(0.48)
^{202}Hg	n,γ	^{203}Hg	5.04±0.38	47d	0.279(0.815)
^{233}Th	n,γ	^{233}Th ↓ ^{233}Pa	7.4±0.1	22.2m ↓ 27d	0.300(0.058), 0.312(0.337)
^{238}U	n,γ	^{239}U ↓ ^{239}Np	2.7±0.02	23.5m ↓ 2.35d.	0.106(0.227), 0.278(0.141)

3. RESULTS AND DISCUSSION

Experimental results of leaves and seeds analysis from 4 sites near TadAF for two years in summer season have been obtained.

Comparison results of leaves and seeds analysis indicated that content of all elements in seeds of tomato and grapes are less than in leaves of tomato and vineyard except Cu and Rb in tomato. Elemental content variation in seeds from 4 sites of TadAF as well as in leaves from the same regions indicated that mild (Denau), average (Dashnabad — Sariassiya 1) and highly (Sariassiya) polluted areas are observed near TadAF. These results are in agreement with the data of other organizations who investigated fluoride contamination in these regions and with results of Tashkent State Agrarian University studies on the stability decree of specific plants to harmful ejection depending on their location from the object and dependencies of stability of the contents endogenous protective substances (routine and others) in plants depending on effect of harmful ejection of the object.

Comparative assessment of fruit trees and technical culture investigated leaves indicated that the best collectors of air pollution may be considered mulberry and mint. In less degree quince, vineyard and tomato, clover respectively We think that mint (less tomato, clover), mulberry (less quince, vineyard, pomegranate) can be used as biomonitors in Uzbekistan. Our studies are preliminary, not regular, carried out one and two-time only, so our conclusions can not be final. This work must be continued.

Due to the limited number of pages imposed, we present only the data of investigated leaves in Sariassiya region, highly polluted with fluorine and some other compounds, and on mulberry leaves from other studied objects.

In table III the elemental contents of 27 determined elements in different kinds of plant leaves from 2 sites in Sariassiya region near TadAF are given.

In table IV the elemental contents of mulberry leaves from some investigated industrial objects in Uzbekistan are given.

Obtained data are compared with the generalized table of typical chemical elements spreading in soil, plants and human blood [9]. Preliminary comparative analysis of these data will show that contents of K, Cr, Zn, Cs, Ba, and Th are on the same level as in the typical elemental contents of vegetation, the contents of Na, Mn, Se, Rb, Sc, and La elements are less than in the typical elemental contents of plants and the contents of Sc, Ti, Cr, Fe, Co, Ni, Cu, As, Br, Zr, Mo, Cd, Sb, Ce, Hg, and U elements are higher than in the typical plants. The reason of these element contents variations will be find in future works.

In general we can take the followed conclusions: 1) mulberry and mint leaves can be used as biomonitors in summer season; 2) such technical culture as clover can be used as a collector of harmful substances from soil and air in order to subsequent destruction of this collector plant; 3) all investigated regions are polluted; 4) the Sariassiya region is polluted heavily; 5) the plant, which can be used as biomonitor for yearly monitoring, must be found.

TABLE III. RESULTS OF INAA DETERMINATION OF ELEMENTAL CONTENT IN DIFFERENT PLANTS OF SARIASSIYA REGION IN UZBEKISTAN (CONCENTRATION IN ppm)

Element	mint	clover	cotton	corn	tomato	mulberry	apple	apricot	quince	Vine yard	peach	pomegranate
Na	380	160	700	310	1700	230	140	350	310	200	180	220
K	19000	20000	13000	29000	14000	15000	17000	32000	18000	7000	17000	20000
Sc	0.32	0.061	0.077	0.12	0.23	0.15	0.055	0.092	0.052	0.17	0.061	0.070
Ti	270	180	270	450		210		180		210	200	
Cr	5.2	8.5	0.93	1.8	9.0	2.3	1.3	1.9	1.5	2.5	1.7	1.2
Mn	66	20	25	32	96	46	25	33	820	49	94	40
Fe	1400	340	400	670	3600	760	290	480	240	840	340	320
Co	0.78	0.44	0.25	0.49	1.1	0.58	0.27	0.33	0.55	0.57	0.31	0.33
Ni	25	13	18	17	17	21	22	21	29	26	21	14
Cu	16	9.1	7.9	6.9	27	23	9.2	5.4	9.7	9.8	9.2	29
Zn	39	42	61	14	55	33	12	24	16	2	28	<6.0
As	5.3	3.2	1.9	6.2		5.6	1.7	2.7	7.2	1.5	2.2	2.9
Se	0.0098	0.0073	0.0090	0.00385	0.0085	0.0043	0.0099	0.0056	0.0065	0.0056	0.0053	0.0057
Br	7.5	16	6.5	29		12		1.7	4.0	1.9	2.8	
Rb	2.6	3.7	2.5	6.0	0.69	1.2	2.0	5.1	0.95	3.3	5.8	0.60
Sr	8.6		5,2			6.3						
Zr	30	44	28	82	70	62	12	57	15	29	48	18
Mo	0.70	0.79	0.67	1.0	4.5	0.90	0.94	054	0.80	0.75	0.68	1.5
Cd	3.6	2.7	3.3	8.0	95	1.1	92	6.5	30	1.7	2.0	59
Sb	0.39	0.16	0.13	0.27	0.52	0.29	0.12	0.25	0.074	0.25	0.25	0.11
Cs	0.065	0.017	0.043	0.11	0.21	0.042	0.026	0.062	0.030	0.026	0.069	0.0075
Ba	9.5	5.0	7.2	14	7.1	6.3	6.0	7.8	6.5	5.8	11	4.3
La	0.12	0.036	0.040	0.040	0.065	0.046	0.047	0.076	0.022	5.6	0.050	0.0094
Ce	0.47	0.37	<0.25	0.42		<0.2		<0.25		<0.25	<0.25	
Hg	2.8	1.8	0.92	3.8	0.47	3.7	0.48	1.1	0.84	3.0	0.23	1.1
Th	0.052	0.0013	0.022	0.071	0.077	0.042	0.011	0.032	0.0067	0.073	0.019	0.0089
U	2.3	0.28	0.30	0.82		0.56		0.74		1.2	0.41	

TABLE IV. RESULTS OF INAA DETERMINATION OF ELEMENTAL CONTENT IN MULBERRY LEAVES FROM SOME INDUSTRIAL OBJECTS OF UZBEKISTAN (CONCENTRATION in ppm)

	Sariassiya	Djarkurgan	Asaka district	Asaka works	Tashkent INP
Na	230±30	67±8	74±8	77±8	36±4
K	15000±1000	16000±1000	17000±1000	18000±1000	17000±1000
Sc	0.15±0.02	0.016±0.002	0.027±0.003	0.033±0.004	0.015±0.002
Ti	210±20	230±20	190±20	200±20	150±20
Cr	2.3±0.2	0.71±0.08	0.98±0.1	1.2±0.2	0.63±0.07
Mn	46±5	73±8	35±4	41±4	57±6
Fe	400±40	130±20	200±20	300±30	150±20
Co	0.25±0.03	0.13±0.02	0.25±0.03	0.23±0.03	0.12±0.02
Ni	18±2	15±2	25±3	12±2	13±2
Cu	23±3	33±4	12±2	11±2	7.3±0.8
Zn	33±3	25±3	28±3	23±3	23±3
As	5.6±0.6	1.8±0.2	0.88±0.1	2.2±0.2	1.0±0.1
Se	0.0043±0.0006	0.0089±0.001	0.0046±0.0006	0.0069±0.0008	<0.0018
Br	12±2	2.8±0.3	5.5±0.7	6.9±0.8	2.0±0.3
Rb	1.2±0.2	2.5±0.3	3.0±0.3	2.4±0.3	4.0±0.4
Sr	6.3±0.7	6.6±0.7	4.8±0.5	4.3±0.5	2.5±0.3
Zr	62±8	40±6	40±5	28±3	25±3
Mo	0.90±0.10	0.53±0.07	0.46±0.06	0.31±0.04	0.42±0.06
Cd	1.1±0.1	4.7±0.5	1.3±0.2	4.1±0.5	0.83±0.09
Sb	0.29±0.03	0.24±0.03	0.28±0.03	0.18±0.03	0.096±0.01
Cs	0.042±0.005	0.015±0.002	0.014±0.002	0.033±0.004	<0.02
Ba	6.3±0.7	11±1	2.7±0.3	1.3±0.2	3.5±0.4
La	0.046±0.005	0.017±0.002	0.025±0.003	0.022±0.003	0.013±0.002
Ce	<0.2	<0.2	1.4±0.2	1.1±0.2	<0.2
Hg	0.92±0.1	0.79±0.08	1.4±0.2	1.2±0.2	0.43±0.05
Th	0.022±0.002	0.028±0.003	0.0086±0.001	0.0062±0.0007	0.0054±0.0006
U	0.56±0.06	0.22±0.03	0.17±0.02	0.23±0.03	0.31±0.03

REFERENCES

[1] STEINNES, E, "Use of neutron activation analysis in environmental research: examples from Norway", Applications of isotopes and radiation in conservation of the environment (Proc. Int. Symp. Karlsruhe, 1992), IAEA-SM-325/174, Vienna (1992) 387–396.

[2] FRONTASYEVA, M.V., STEINNES, E., Epithermal neutron activation analysis of mosses used to monitor heavy metal deposition around an iron smelter complex, Analyst **120** (1995) 1437–1440.

[3] FREITAS, M.C., REIS, M.A., ALVES, L.C., WOLTERBEEK, H.Th., VERBURG, T., Biomonitoring of trace-element air pollution in Portugal: Qualitative survey, J Radioanal. Nucl. Chem. **217** (1997) 21–30.

[4] AMUNDSEN, C.E., HANSSEN, J.E., RAMBAEK, J.P., SEMB, A., STEINNES, E., Long-range transport of trace elements to Southern Norway studied by INAA of air filters, J. Radioanal. Nucl. Chem. **114** (1987) 5–12.

[5] WANG XINFU, Determination of concentrations of elements in the atmospheric Aerosol of the urban and rural area of Beijing in winter, Int. Conf. on Nuclear analytical methods in the life sciences (NAMLS), Abstracts, Beijing (1998) 60.

[6] FREITAS, M.C., MATOS, V., MARQUES, A.P., Olive tree bark as biomonitor, Int. Conf. on Nuclear Analytical Methods in the Life Sciences (NAMLS), Abstracts, Beijing (1998) 62.

[7] NI BANGFA, TIAN WEIZHI, NIE HUINING, WANG PINGSHENG, HE GAOKUL, Study on air pollution in Beijing's major industrial areas using multielements in biomonitors and neutron activation analysis techniques, Int. Conf. on Nuclear analytical methods in the life sciences (NAMLS), Abstracts, Beijing (1998) 85.

[8] KONYUKHOV, V.G., "Assessment of influence of aluminum factory on environment" in the Book of RECOMMENDATION of scientifically-practical conference on consideration of condition of execution results "Objective research program of works on reducing of emissions of polluting substances in environment by Tadjik aluminum factory up to (MPL) maximum permissible levels" Tursunzade (1991) 7–13. (in Russian).

[9] KIST, A.A., "Phenomenology of biogeochemistry and bioinorganic chemistry", Tashkent (1987) 236 pp. (in Russian)

POSTERS

SIXTY YEARS OF ANTHROPOGENIC ACTIVITIES IN LAGOA OLHO D'ÁGUA, BRAZIL

E. VALENTIM[1], E.A.N. FERNANDES[2], C.A. HAZIN[1],
C. MEDEIROS[3], R.A. LIMA[1], R.S. AMARAL[1]

[1] Departamento de Energia Nuclear, UFPE, Recife, PE Brazil
[2] Centro de Energia Nuclear na Agricultura — SP, Piracicaba SP, Brazil
[3] Departamento de Oceanografia, UFPE, Recife PE, Brazil

Abstract

The Lagoa Olho D'Água is a shallow tropical lagoon located in NE-Brazil. The urban and industrial development of the area around the lagoon that began around 40 years ago caused an increasing population inflow into border communities around the lagoon, but especially in its northern and eastern portions. There has been increasing concern on the impact of uncontrolled land occupation, as infrastructure developments have not accompanied the rate of population growth. Since then, the lagoon has become a sink for domestic and industrial wastes, for 95% of the spontaneous settlements in this region occur on its margins and only 15% of these are connected to the sewer network. In order to implement a program for restoring the characteristics of this estuary, it is necessary to assess the ecological impact of human activities in the aquatic environment. To accomplish this goal we analysed sediment samples in order to determine both its rate of deposition and trace metals content. Simultaneously, samples of the fish species "tilapia" were collected for analysis of trace metals by INAA. Sediment cores were taken with a core sampler at the northern, central and southern sections of the lagoon. Measured sedimentation rates varied from 0.54 to 3.23 $\text{cm}\cdot\text{year}^{-1}$, with larger rates corresponding to the northwestern lagoon shores. The causes for the 2–5 fold overall decrease in sedimentation rates in the last 30–40 years are probably related to the highly eutrophic conditions, to the increase in organic matter inputs to the system, and to the circulation regime in the lagoon. This would be consistent with the trace metals content found in the lagoon, as well as, with the industrial history of the area.

1. INTRODUCTION

The Lagoa Olho D'Água is a 3.75 km^2 shallow tropical lagoon located in NE-Brazil, 17 km to the south of Recife, the Capital of Pernambuco State, and 2 km from the Atlantic Ocean, being one of the largest water reservoirs in Brazil.

The area around the lagoon was sparsely populated up to the fifties, mainly by squatters who lived basically on fishing. The urban and industrial development of the area that began around 40 years ago caused an increasing population inflow into border communities around the lagoon, but especially in its northern and eastern portions. According to information obtained from the county Administration Office, a low-income population estimated in 100,000 inhabitants lives on its margins. Around 1,200 persons make their living by catching fish and clams in the lagoon.

There has been a lot of concern about the uncontrolled land occupation and its impact on the quality of the ecosystem. The main cause of preoccupation is the fact that infrastructure developments have not accompanied the rate of population growth. An example of this is the lack of appropriate sewage network and treatment facilities. As a consequence, the lagoon has become a sink for domestic and industrial wastes, for 95% of spontaneous settlements in this region occurred on its margins and only 15% of these are connected to the sewer network.

The discharge of untreated sewage has already caused the contamination of water and sediments, affecting the population of aquatic species, which has been decreasing in the same proportion as the pollution of the water increases. In addition to the sewage discharge, chemical pollutants may be also contributing to the deterioration of water quality, since many industries in the area can be discharging their effluents into the lagoon. The species “tilapia” *(Tilapia sp)* e “carapeba” (*Diapterus sp)* are the most resistant species found in the lagoon [1].

The objectives of the present work were to provide a preliminary assessment of the content of Trace metals in the Lagoa Olho D’Água and to estimate the impact of the presence of such pollutants on the local population.

2. STUDY AREA

The Lagoa Olho D'Água is a well-mixed shallow environment that receives a large discharge of raw domestic sewage. It is estimated that an annual solid load of 16 tons enters the lagoon, mostly through the Canal Olho D’Água. This artificial channel was built in 1940, connecting the lagoon with the Jaboatão River estuary (Figure 1). The remainder load enters the lagoon in a dispersed manner, being generated in the low incoming settlements located in the lagoon shores.

The water level in the lagoon is governed by the ocean waters that flow inwards and outwards through the Canal Olho D’Água according to the high and low tides, and by the amount of rainfall and water evaporation. The average water depth in the lagoon ranges from 0.40m in the dry season to 1.62m in the wet season, causing some flooding in the houses that were built on its margins. The lagoon is a lenthic environment, with instantaneous velocities always below to 0.4 $cm\cdot s^{-1}$. The circulation in the lagoon is mainly dictated by the SE trades, that prevail in the area from February to November, with a mean velocity of 3.2 $m\cdot s^{-1}$ and, in a smaller scale and more restricted to its southern portion, by the tidal regime. Salinity levels vary seasonally in response to precipitation/evaporation regimes being ca. 1.5% during the rainy season and increasing to 22% during the dry season [2].

3. MATERIALS AND METHODS

In order to accomplish the proposed objectives several analysis of the bottom sediments from the lagoon were carried out. Sampling covered areas of the direct influence of industrial and urban releases and those under the preferential domestic discharge zones. A total of six sampling stations were selected for this study. The locations of the sampling stations are shown in Figure 1.

Sediment cores were collected at the northern, central and southern sections of the lagoon through the use of a core sampler with a 100 cm long-, 7.5 cm internal diameter- PVC tube. After collection, the tubes containing the cores were frozen and stored in the vertical position at 4 ^{0}C [3,4]. Each core was sectioned in 3cm increments with a stainless steel sediment sampler. Aliquots of 5 g of dry sediments for each layer were then dissolved with HNO_3–HF for ^{210}Pb analysis. A small fraction of each sample was reserved for activation analysis [5,6].

The ^{210}Pb content was determined through its decay product ^{210}Bi [7] whereas ^{226}Ra was determined by the gross alpha counting method [8,9]. In both cases a low background proportional counter was used. The age of individual sediment layers was determined by applying the so-called constant rate of supply method [10,11]. Sedimentation rates were computed by a linear least square fitting of the sediment layer depth *versus* age relationship. The trace metals profile in sediments and in samples of the fish species "tilapia" were determined through instrumental neutron activation analysis (INAA).

4. RESULTS AND DISCUSSION

The results obtained for the sedimentation rates are shown in Figure 2. The highest sedimentation rates were found at sampling stations S4 and S5, close to the area where most settlements are concentrated and at S1, to the south of the Olho D'Água Canal.

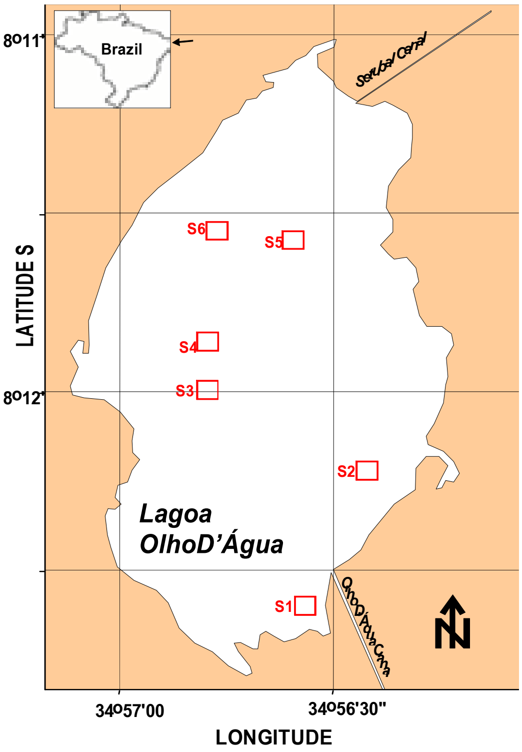


FIG. 1. Area map and location of sediment (S1-S6) sampling stations

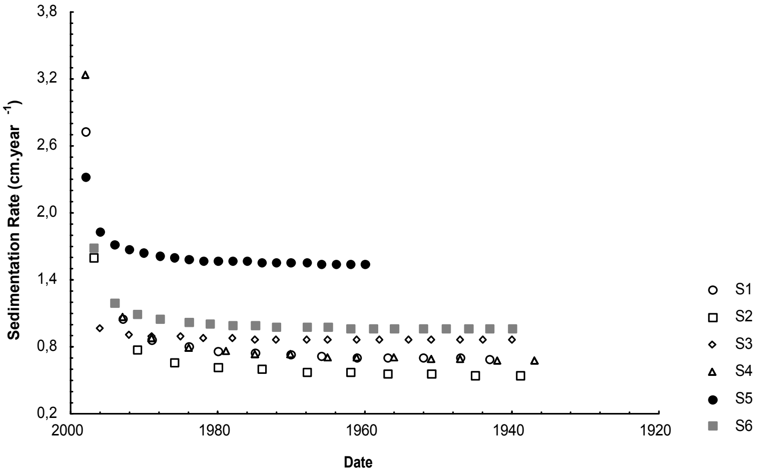


FIG. 2. Evolution of the sedimentation rate over the last years at six distinct regions of the Lagoa Olho D'Água.

The pronounced increase observed in sedimentation rates in the last 20 years is probably related to the high eutrophic conditions, to the increase in organic matter inputs to the system, and to the circulation regime at the lagoon, mainly after 1980, when the number of settlements along its northwestern shore increased abruptly. This increase is consistent with the trace metals profile obtained in the preferential domestic and industrial discharges zones, near the Olho D'Água Canal (Figures 3–4)

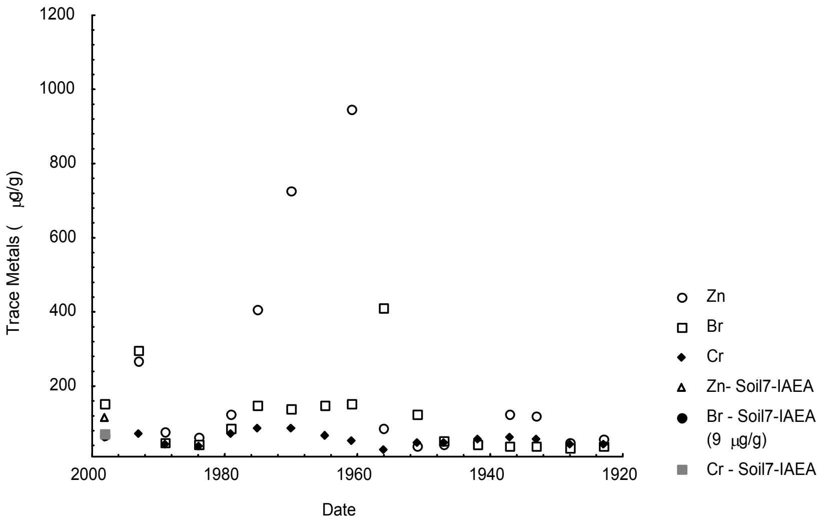


FIG. 3. Concentrations of Zn, Br and Cr in the preferential discharge zones, near the Olho D'Água Canal.

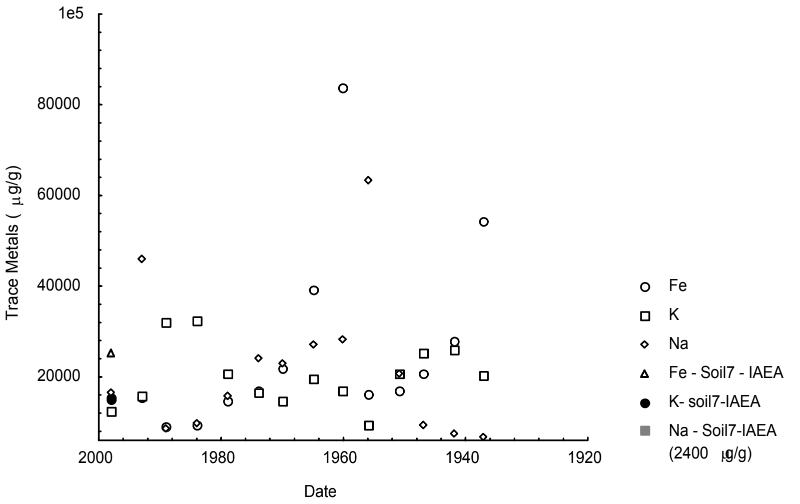


FIG. 4. Concentrations of Fe, K and Na in the preferential discharge zones, near the Olho D'Água Canal.

The elevated concentrations of Zn and Fe observed in the 60's and 70's match the industrial history of the region, since many industries were established in the area during that period. These industries manufacture fertilizers, paints, steel plates, etc. The increase in the concentrations of Na, K, and Br, on the other hand, occurred after the construction of the Canal Olho D'Água, in its northern portion, connecting the lagoon to the ocean, through the Jaboatão River.

TABLE I. METAL CONCENTRATION IN THE EDIBLE PARTS OF *TILAPIA* FROM OLHO D'ÁGUA LAGOON, PERNAMBUCO

References	Metal		
	Cr	Fe	Zn
Lagoa Olho D'Água	0.56 ± 0.06	19.45 ± 1.98	32.25 ± 0.21
Fernandes et al. (1994)	0.08 ± 0.02	1.1 ± 0.11	2.2 ± 0.13

[a] Results are in mg/kg wet weight

TABLE II. ACCEPTABLE AND ESTIMATED METAL DOSES FOR JABOATÃO DOS GUARARAPES POPULATION ON A FISH CONSUMPTION BASIS.

Metal	Estimated dose (mg/day)	Acceptable dose (mg/day) (USEPA)
Zn	1.61	14
Cr	0.03	0.35
Fe	0.97	not specified

The trace metals content of the edible portion of tilapia, a detritivorous fish species, are shown in Table I. The concentrations found in this species are higher than those measured by Fernandes et al. [12] in the Jacarepaguá lagoonal system, Rio de Janeiro.

For assessing the rate of ingestion of trace metals by people living around the lagoon, a consumption of 50g of fish per day was assumed. Table II shows a comparison between the calculated rates of ingestion and the accepted values suggested by the USEPA.

Based on these findings, we may conclude that the local population is not exposed to unacceptable metal doses, derived from the consumption of fish from the lagoon.

REFERENCES

[1] AZEVEDO, D. M. P. Eficiência da *eichhornia crassipes* na absorção de Cd, Pb, Cr, Ca, Mg, eK na água da Lagoa Olho D'Água — Jaboatão dos Guararapes, — PE, *private communication*, 1997.

[2] CPRM. Projeto Diagnóstico do Meio Físico da Bacia Lagoa Olho D'água — *Relatório Técnico*, Companhia de Pesquisas em Recursos Minerais, 1997.

[3] JOSHI, S. R. & DURHAM, R. W. Determination of ^{210}Pb, ^{226}Ra and ^{137}Cs in Sediments. Chem. Geol. **18** (1976) 155–160.

[4] JOSHI, L. & KU, T. L. MEASUREMENT of ^{210}Pb from a sediment core off the coast of California. J. Rad. Chem. **52(2)** (1979) 329–334.

[5] KOSTA, L.; RAVNIK V.; BYRNE, A. R.; STIRNE, J.; DERMELI, M.; STEGNAR, P. Some trace elements in the water, marine organisms and sediments of the Adriatic by neutron activation analysis. J. Rad. Chem. **44** (1978) 317–332.

[6] REBELLO, A. L.; HAEKEL W.; MOREIRA, I.; SANTELLI, R.; SHROEDER, F. The fate of heavy metals in an estuarine tropical system. Mar. Chem. **18** (1986) 215–225.

[7] VALENTIM, E.; HAZIN, C. A.; KHOURY, H. J.; LIMA, R. A.; GODOY, J. M. ^{210}Po e ^{210}Pb em águas subterrâneas da região fosfática de Pernambuco. In Anais do III Encontro de Aplicações Nucleares, V. 1, pp. 311–315, Águas de Lindóia, SP, Agosto, 1995.

[8] SUBRAMANIAN, V.; SITASAWARD, R.; JOSHI, L.U. Rate of sedimentation in the Yamuna River around Delhi using the ^{226}Ra — ^{210}Pb method. J. Radioanal. Nuclear Chem. **90 (2)** (1985) 271–276.

[9] GODOY, J. M.; MOREIRA, I.; WANDERLEY, C.; SIMÕES, F. F.; MOZETO, A. A. An Alternative Method for the Determination of Excess ^{210}Pb in Sediments. Radiat. Prot. Dosim. **75** (1998) 111–115.

[10] JOSHI, S. R. & SHUKLA, B. S. AB INITIO derivation of formulations for ^{210}Pb dating of sediments. J. Radioanal. Nuclear Chem. **148 (1)** (1991) 73–79.

[11] GODOY, J. M.; MOREIRA, I.; BRAGANÇA, M. J.; WANDERLEY, C.; MENDES, L. B. A study of Guanabara Bay sedimentation rates. In Anais do III Encontro de Aplicações Nucleares, V. 1, pp. 179–185, Águas de Lindóia, São Paulo, Agosto, 1995.

[12] FERNANDES, H.M.; BIDONE, E.D.; VEIGA, L.H.S.; PATCHINEELAM, S.R. Heavy-metal pollution assessment in the coastal lagoons of Jacarepaguá, Rio de Janeiro, Brazil. Environ. Pollut. **85** (1994) 259–264.9

A COMPARATIVE STUDY FOR RESULTS OBTAINED USING BIOMONITORS AND PM10 COLLECTORES IN SADO ESTUARY

C.J. COSTA, A.P. MARQUES, M.C. FREITAS, M.A. REIS, O. De OLIVEIRA

ITN, EN10 Sacavém, Sacavém, Portugal

Abstract

In 1996 a program was started, financed by the Environmental Ministry of Portugal and International Atomic Energy Agency, aiming to study the inorganic atmospheric pollutant dispersion in Sado estuary. Gent PM10 air samplers were used for air particulate matter sampling. The sampling sites chosen were Palmela, Faralhão and Troia (forming a triangle around the fuel-fired power station of Setúbal). Transplants of *Parmelia sulcata* Taylor were suspended in nylon bags within a rectangle 15 km wide and 25 km long on a grid 2.5 km × 2.5 km, centred in a fuel power station. Two sets of 4 transplants were hanged in each of the 47 locations, one set facing the wind and the other set opposing the wind. Each support avoids heavy rain to prevent eventual elemental leaching. The transplants were suspended in December 1997 for one-year period; every three months, one transplant of each set was collected. The 4th campaign transplants were collected together with the 3rd campaign because just a few were still left. Both lichen transplants and PM10 filters were analysed by INAA, in the Portuguese Research Reactor (RPI), and PIXE in the Van de Graaff accelerator. A comparative study of results obtained for the two sampling procedures is presented in this work.

1. INTRODUCTION

The first national study using lichens as biomonitors, made in Portugal in 1993, showed that the Sado estuary is one of the regions with larger concentrations of heavy metals and other elements in the country [1–3]. These high contents are originated not only by the large number of local industries in the region, but also by the influence of the very industrialised and populated metropolitan area of Lisbon, located north.

In 1996 a program was started aiming to study the inorganic atmospheric pollutant dispersion in this area. The project included two methods: direct measurements of the air contents in heavy metals by the use of air particulate matter samplers, and the use of lichens as biomonitors of the air quality.

Since one of the major industries in the region is the fuel power station of Setúbal, three air particulate matter samplers were installed in is neighbourhood, Fig.1.

Trace element monitoring with lichens has been used as an important tool when large areas are involved [4], for which otherwise an enormous amount of air samplers would be needed. In this study transplants of *Parmelia sulcata* Taylor were suspended in nylon bags within a rectangle 15 km wide and 25 km long on a grid 2.5 km × 2.5 km, centred in a fuel power station, Fig.1. Two sets of 4 transplants were hanged in each of the 47 locations, one set facing the wind and the other set opposing the wind.

2. METHODOLOGY

2.1. Air Particulate matter sampling

Air collection is made with Gent samplers [5]. This kind of sampler is equipped with a SFU (stacked filter unit) which carries two 47 mm diameter Nuclepore® polycarbonate filters.

Filters of 8 and 0.4 μm pore sizes are used in the first and second stages, respectively. Air is sampled at a rate of 15 l/min, which allows the collection of PM2.5 in the second stage. The samplers inlet is a PM10 separator so that the first filter collects only coarse particles (PM10–2.5) having aerodynamic diameter between 2.5 and 10 μm.

The stations of Palmela and Faralhão are working since June 1994 and January 1995, respectively. In those stations the air collector worked 10 min. in each 2 hours, corresponding to 14 hours of sampling per week. The filters were replaced every week by new ones. The station of Troia started to work in April 1998 and the filters were replaced every two weeks because of sampling difficulties, since it is necessary to cross the Sado River. In this case, the collector was operating during two weeks but only 5 minutes out of each two hours were used for collection. This results in the same collection time of 14 hours.

Filter loads are measured by gravimetry using a balance with 0.1 μg sensitivity and elemental analyses are carried out using INAA [6] and PIXE [7] techniques. For elemental analysis the filters are cut into three parts: one half is analysed by INAA, one quarter is analysed by PIXE and the other quarter is kept for other possible measurements of replicates.

2.2. Lichens transplants

The lichens were collected from olive trees at about 1.5 meters above the soil, in areas considered clean from the pollution point of view. Samples of about 2 grams of lichen were put in nylon bags and suspended at about 1.5 meters above the soil. Forty-seven hanging systems were installed, four transplants were hanged in order to be facing the wind direction (called F) and four transplants on the opposite direction (called T). Protection against leaching due to heavy rainfall is also provided by this system. The lichens were suspended in December 1997. During the year of exposure some of the systems were lost mainly due to vandalism.

In the laboratory, the lichens were taken off the nylon bags, cleaned, washed in distilled water and freeze-dried. Then they were ground in a Teflon mill and analysed by INAA and PIXE in pellet form.

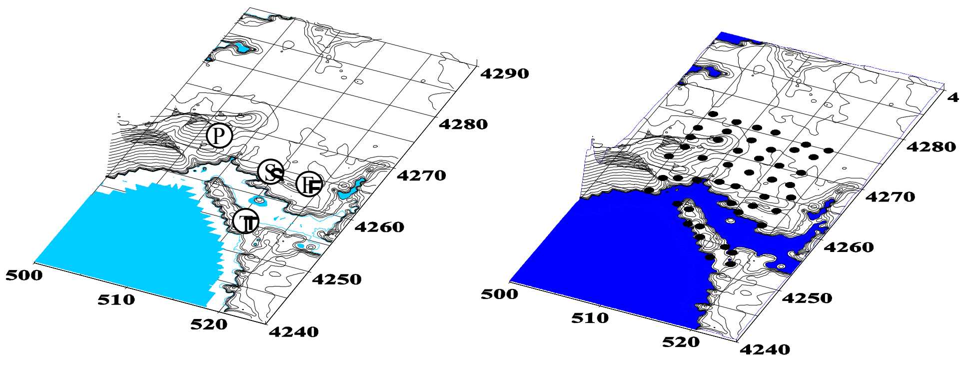


FIG. 1. On left, the location of the three air sampling stations: Palmela (P), Faralhão (F) and Troia (T); and the fuel power station of Setúbal (S). On right, the location of the lichens transplants.

3. RESULTS AND DISCUSSION

3.1. Arsenic

Table I and Fig. 2 show the aerosol results, and Fig. 3 shows the results obtained for the lichen transplants. The dispersion of the points in the Fig. 2 led us to conclude that the source of this element does not seem to be unique. Indeed, this region has some agricultural activities, mainly wine production. The fertilisers used may be the most important source of this element. We can also see that the average values of As in the three stations are not very different.

3.2. Vanadium

Table II and Fig. 4 show the aerosol results, and Fig. 5 shows the results obtained for the lichen transplants. Vanadium is an important tracer of the fuel combustion. Indeed, from Fig. 4 we can see that the concentrations found in both stations seem to be correlated, leading us to conclude that the major source of this element is the same. We can also see that Faralhão reveals higher concentrations, as expected, since it is located closer the fuel power station. The lichen results reinforce these conclusions, pointing out clearly the source. F and T transplants do not show relevant differentiation between them, so the emitting source is so intense that influences the surrounding area, not depending of the exposure direction to the wind.

3.3. Antimony

Table III and Fig. 6 show the aerosol results, and Fig. 7 shows the results obtained for the lichen transplants. From Fig. 6 we can see that the source of Sb seems to be the same in both sampling stations, since for both stations they have a good correlation. Although, it might be observed that Faralhão station detects more intense emissions, which indicate that the source is located closer to this station. The lichen results show that the fuel power station is probably the responsible for these emissions.

3.4. Mercury

Table IV and Fig. 8 show the aerosol results, and Fig. 9 shows the results obtained for the lichen transplants. Hg is an element essentially originated from anthropogenic activities and is normally linked to metallurgic industries and some chemical industries. Let us notice that in Barreiro (north of the region) a very important chemical industry (Quimigal) is placed that may influence the stations of Palmela and Faralhão. When compared to these two stations the levels of Hg in Troia are quite low. The sources of this element seem to be various and have not been yet clearly identified.

TABLE I. As CONCENTRATIONS (ngm^{-3}) IN THE FINE AND COARSE FRACTIONS ON THE THREE SAMPLING STATIONS

		As fine	As coarse
Troia	Average	4.50E-01	2.10E-01
	Std	8.51E-02	1.08E-01
	Min	3.14E-01	9.29E-02
	Max	5.71E-01	3.84E-01
	count	12	12
Palmela	Average	4.18E-01	3.61E-01
	Std	2.20E-01	2.40E-01
	Min	1.02E-01	7.70E-02
	Max	9.82E-01	8.82E-01
	count	20	17
Faralhão	Average	4.35E-01	3.96E-01
	Std	2.87E-01	2.37E-01
	Min	6.90E-02	8.34E-02
	Max	1.36E+00	1.04E+00
	count	23	21

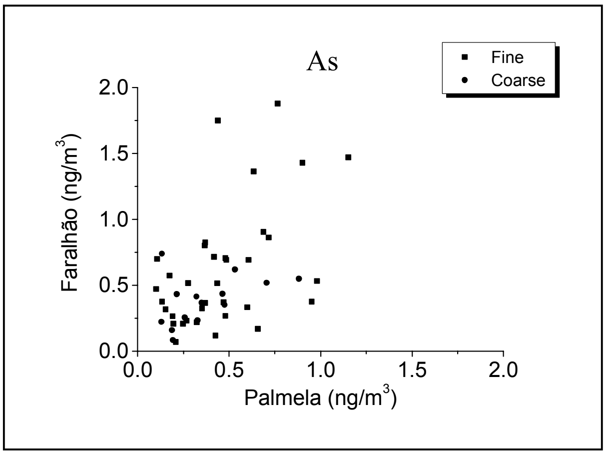


FIG. 2. Faralhão versus Palmela for As concentrations.

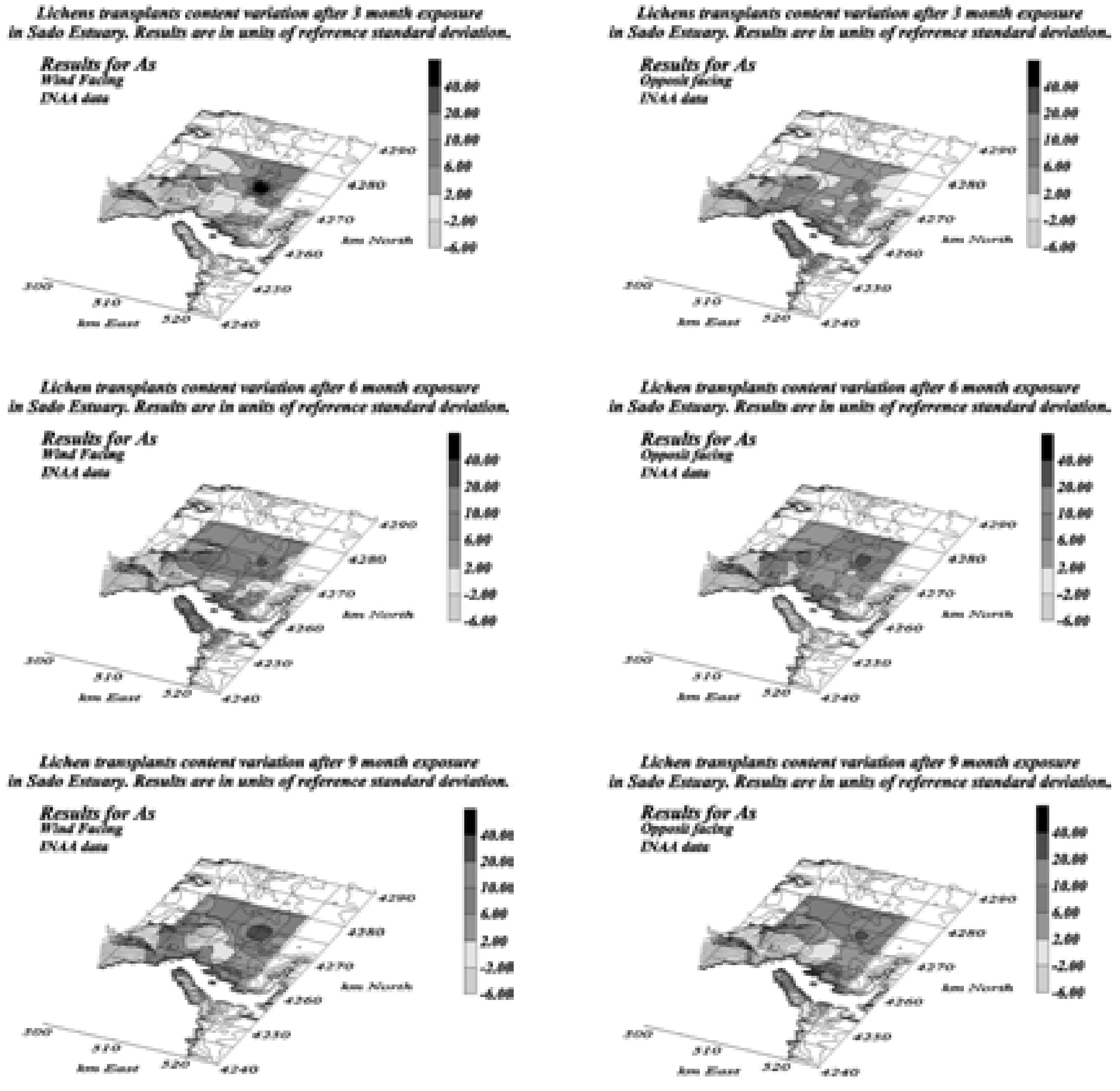


FIG. 3.Variation of As contents in the lichens after 3, 6 and 9 months exposure.

TABLE II. V CONCENTRATIONS (ngm^{-3}) IN THE FINE AND COARSE FRACTIONS ON THE THREE SAMPLING STATIONS.

		V fine	V Coarse
Troia	Average	-	-
	Std	-	-
	Min	-	-
	Max	-	-
	count	-	-
Palmela	Average	6.95E+00	6.55E+00
	Std	4.77E+00	5.98E+00
	Min	2.22E+00	9.55E-01
	Max	2.77E+01	2.12E+01
	count	36	18
Faralhão	Average	7.36E+00	3.56E+00
	Std	4.42E+00	3.16E+00
	Min	1.87E+00	1.16E+00
	Max	2.26E+01	1.25E+01
	count	35	18

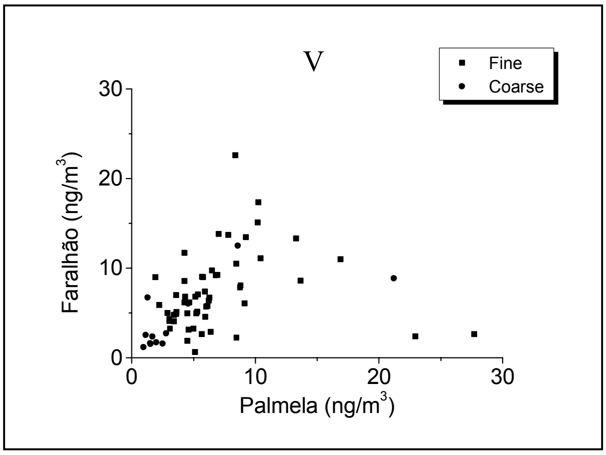


FIG. 4. Faralhão versus Palmela for V concentrations.

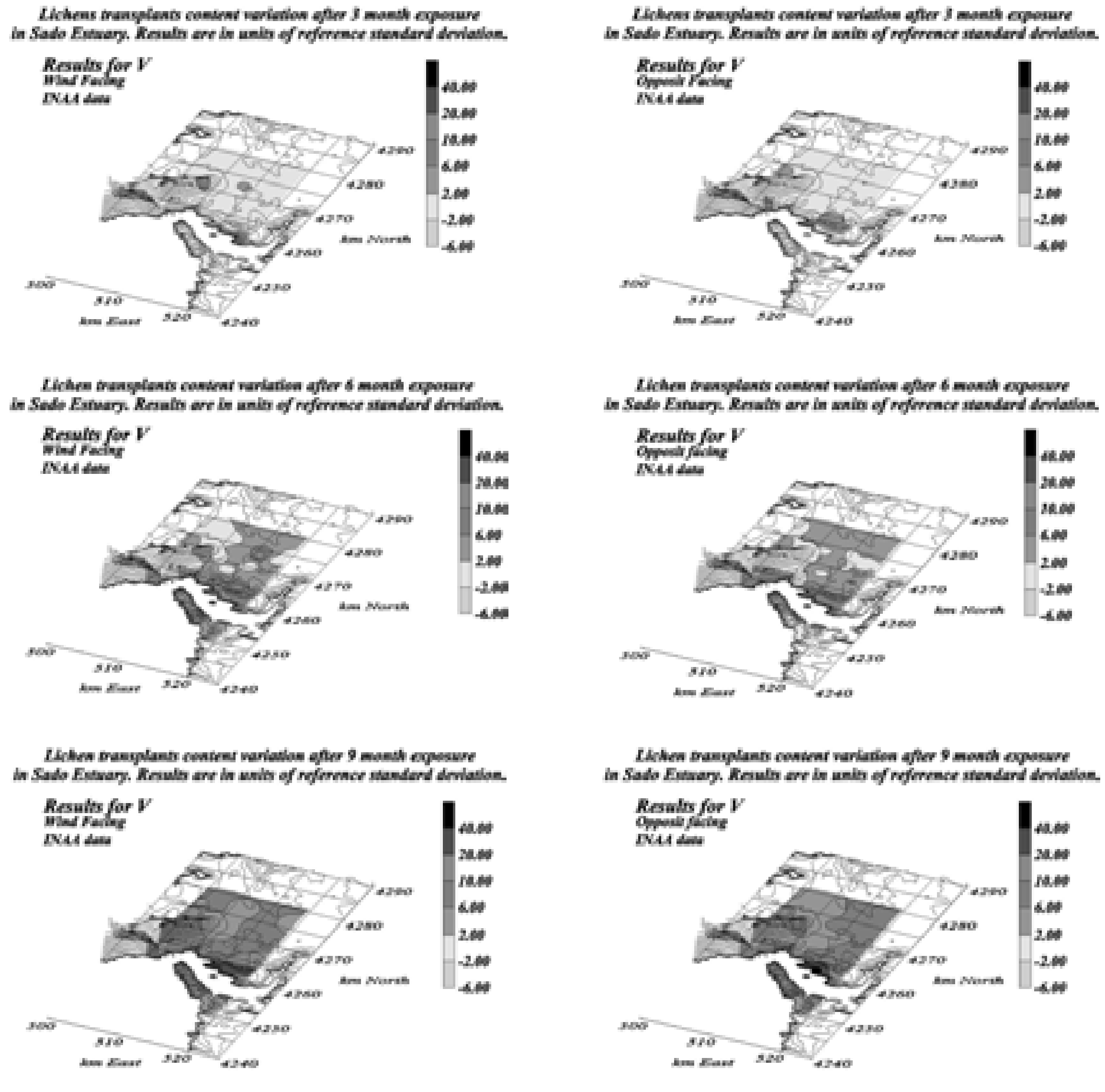


FIG. 5.Variation of V contents in the lichens after 3, 6 and 9 months exposure.

TABLE III. Sb CONCENTRATIONS (ngm^{-3}) IN THE FINE AND COARSE FRACTIONS ON THE THREE SAMPLING STATIONS

		Sb fine	Sb coarse
Troia	Average	5.82E-01	2.31E-01
	Std	1.21E-01	5.85E-02
	Min	4.43E-01	1.21E-01
	Max	7.93E-01	3.10E-01
	count	12	12
Palmela	Average	7.77E-01	4.91E-01
	Std	5.68E-01	7.34E-01
	Min	2.25E-01	1.18E-01
	Max	2.98E+00	3.64E+00
	count	23	21
Faralhão	Average	1.82E+00	4.79E-01
	Std	5.35E+00	7.79E-01
	Min	3.00E-01	7.80E-02
	Max	2.63E+01	3.97E+00
	count	23	23

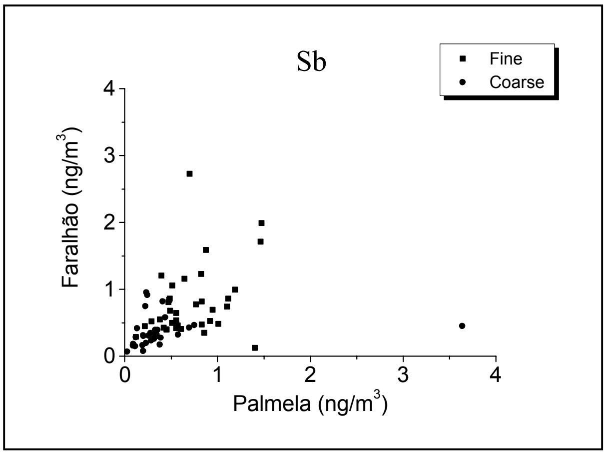


FIG. 6. Faralhão versus Palmela for Sb concentrations.

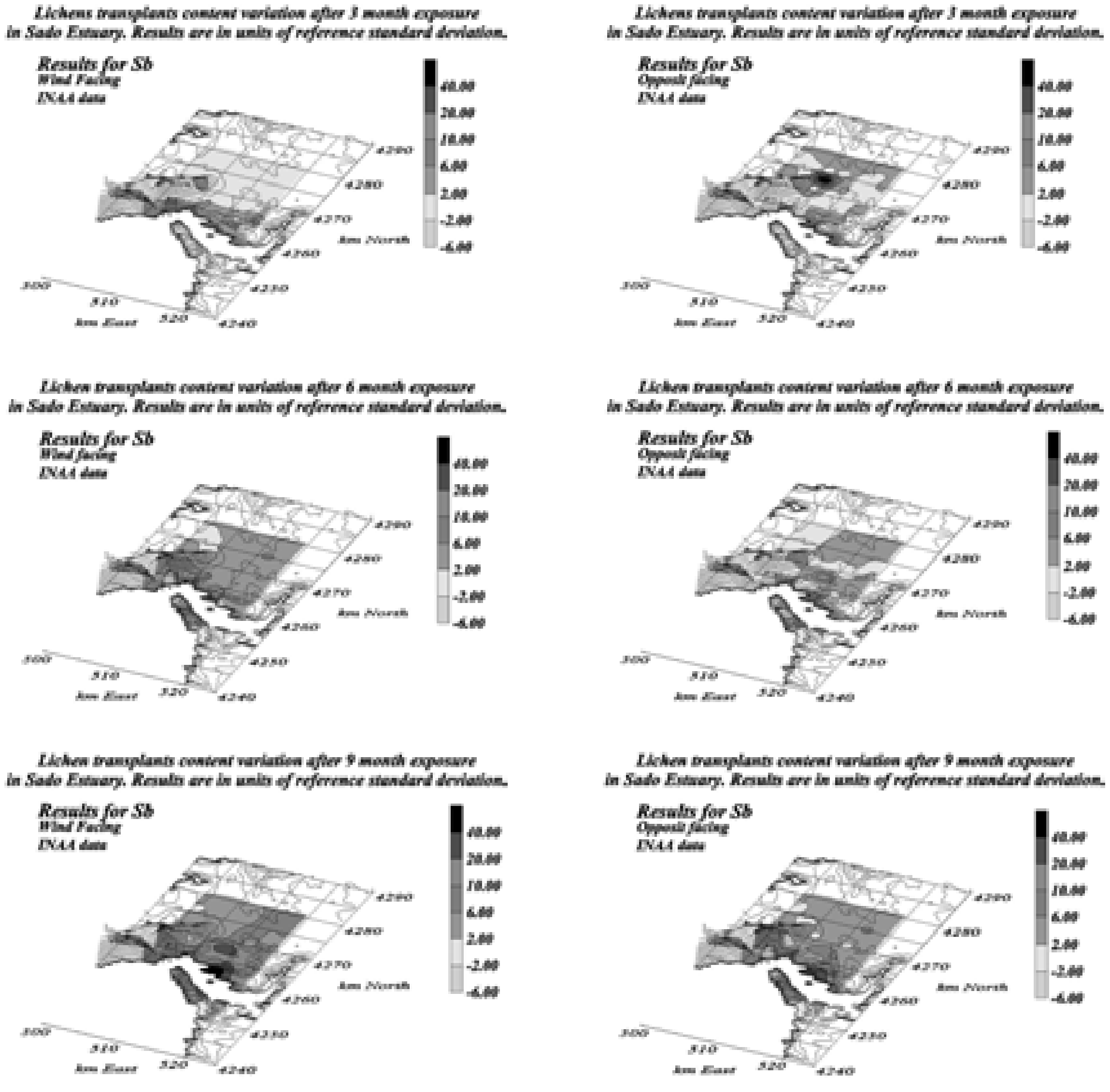


FIG. 7.Variation of Sb contents in the lichens after 3, 6 and 9 months exposure.

TABLE IV. Hg CONCENTRATIONS (ngm^{-3}) IN THE FINE AND COARSE FRACTIONS ON THE THREE SAMPLING STATIONS.

		Hg fine	Hg coarse
Troia	Average	1.52E-01	6.73E-02
	Std	5.57E-02	6.31E-02
	Min	8.69E-02	1.34E-02
	Max	2.25E-01	1.57E-01
	count	7	4
Palmela	Average	5.51E-01	2.13E-01
	Std	2.52E-01	2.37E-02
	Min	1.81E-01	1.87E-01
	Max	1.11E+00	2.33E-01
	count	18	3
Faralhão	Average	5.56E-01	1.80E-01
	Std	1.92E-01	8.94E-02
	Min	2.19E-01	8.62E-02
	Max	1.05E+00	3.75E-01
	count	21	9

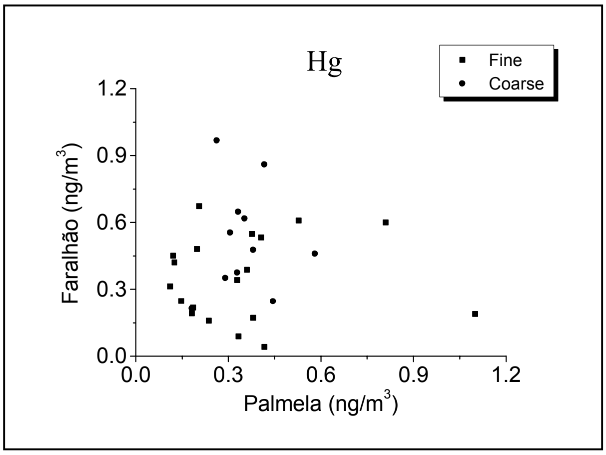


FIG. 8. Faralhão versus Palmela for Hg concentrations.

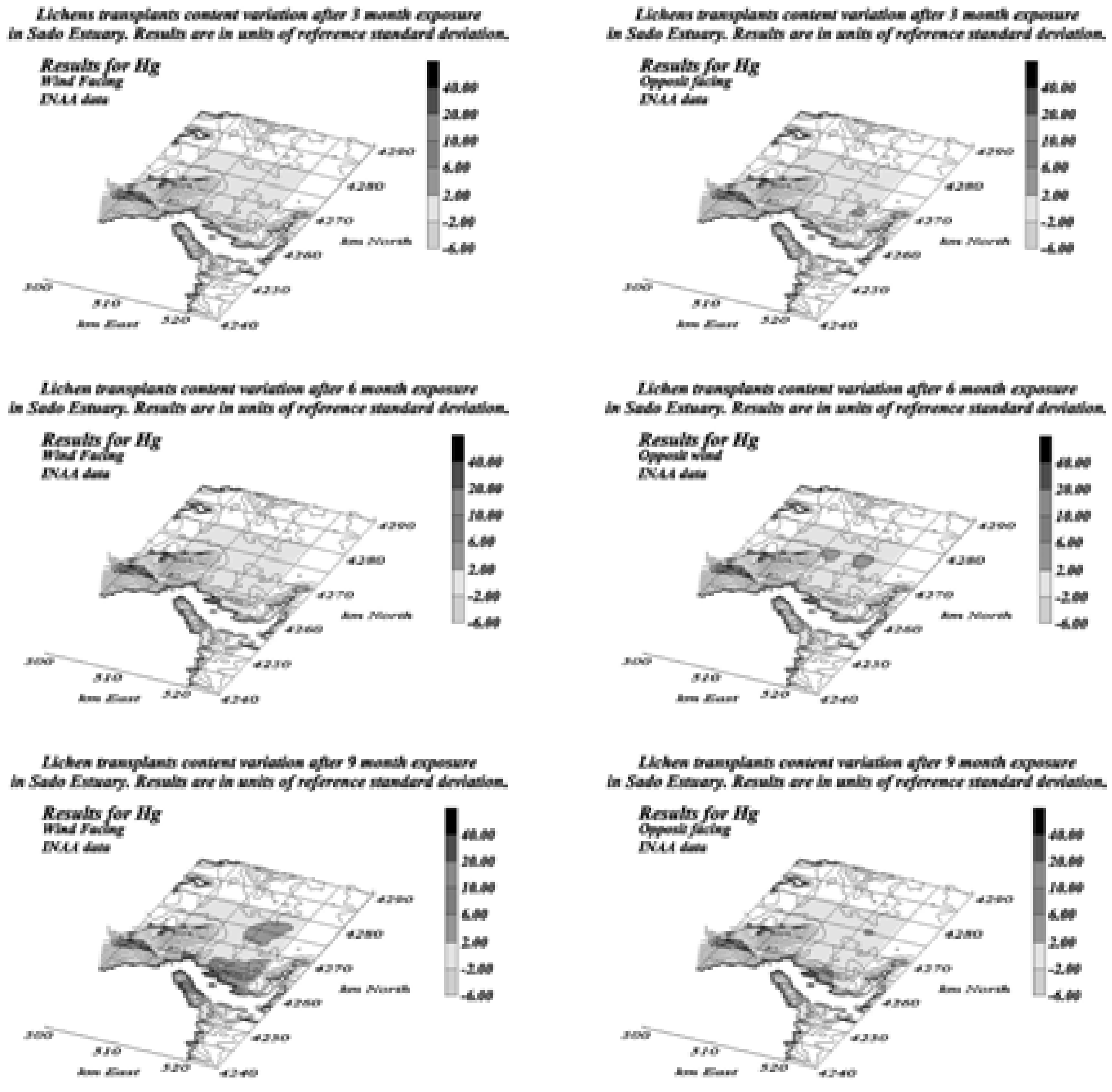


FIG. 9.Variation of Hg contents in the lichens after 3, 6 and 9 months exposure.

REFERENCES

[1] FREITAS, M.C., et al., "Monitoring of Trace-Element Air Pollution in Portugal: Qualitative Survey", J. Radioanal. Nucl. Chem., Articles **217**(1) (1997) 21–30.

[2] FREITAS, M.C., NOBRE, A.S., "Bioaccumulation of Heavy Metals using *Parmelia Sulcata* and *Parmelia Caperata* for Air Pollution Studies, J. Radioanal. Nucl. Chem., Articles **217**(1) (1997) 17–20.

[3] REIS, M.A., et al., "Main Atmospheric Heavy Metal Sources in Portugal by Biomonitor Analysis", Nucl. Instr. and Meth. In Physics Research **B109/110** (1996) 493–497.

[4] FREITAS, M.C., et al., "Elemental Accumulation in Lichens Transplants in the Neighbourhood of Thermal Power Stations, ANST/Nuclear Methods in Environmental Research **74** (1996) 117–118.

[5] HOPKE, P.K., et al., "Characterization of the Gent Stacked Filter Unit PM10 Sampler", Aerosol Science and Technology **27 (**1997**)** 726–735pp.

[6] FREITAS, M.C., MARTINHO, E., "Neutron Activation Analyses of Reference Materials by the k0-standardization and Relative Method" Analytica Chimica Acta, Short communication **219**, (1989), 317–322pp.

[7] REIS, M.A., ALVES, L.C., "A Computer Package for TTPIXE Analysis", Nuclear Instruments and Methods in Physics Research **B68** (1992), 300pp.

MONITORIZATION OF THE WASTE INCINERATOR EFFECTS IN AN INDUSTRIAL AREA IN THE NORTH OF LISBON

M.M. FARINHA, A. MARQUES, M.C. FREITAS, M.A. REIS, S.M. ALMEIDA

ITN, EN10 Sacavém, Sacavém, Portugal

Abstract

This study is a sequence of a heavy metal monitoring contract made with IDAD/ValorSul, who is responsible for an urban waste incinerator placed in an industrial area in the north of Lisbon. The area for monitorization involves a 10 km circle around the incinerator. Two collecting procedures were followed: Gent collectors and biomonitorization. Gent collectors have been used since 25th January 1999, in order to sample air particulate matter in three stations situated in the North of Lisbon — Bobadela, S. João da Talha and Quinta da Piedade. These collectors allow the separation between fine and coarse particles. The sampling is made twice a week: one weekend day and one midweek day. For biomonitorization two sampling sites were chosen: one at 2 km northwest (upstream) from the incinerator and the other close to the incinerator (a few meters down river). The biomonitors (two different species) were chosen from the local vegetation (*in situ*). This procedure was started in April 1999. The filters and the plants are analysed by the multielemental nuclear technique Neutron Activation Analysis (INAA). A comparison between results obtained by both collecting procedures is presented.

1. INTRODUCTION

Air collection is made with Gent collector developed by the University of Gent in Belgium [1]. This collector is equipped with a SFU (stacked filter unit) which carries two Nuclepore® polycarbonate filters with 47mm. Filters of 8 and 0.4 μm pore sizes are used in each of two stages. The air is sampled at a rate of 15 l/min, which allows the collection of coarse particles with $2.5\mu m<EAD<10$ μm in the first stage — PM10, and fine particles with $EAD<2.5$ μm in the second stage — PM2.5. The collector works during a 24 hours period, 40 minutes out of each hour in a total of 16 hours per day [2,3].
The biomonitors and the aerosol filters are analysed by INAA [4].

2. METHODOLOGY

The filter loads are measured by gravimetry and elemental analyses are made by INAA. For elemental analysis the filters are cut into three parts, being each half is analysed by INAA

Biomonitors are collected every six months, one in spring and one in autumn in two zones, up and downstream the Valorsul incinerator. The species selected from the area of study are *Olea Europaea* and *Conyza* [5].

For INAA analysis pellets of 500 mg were irradiated together with 0.1% Au-Al wires as comparators. Two types of short irradiation (neutron flux: $2.8 \times 10^{12} cm^{-2}s^{-1}$) are used: 18 sec for the species collected upstream of the incinerator, and 1 min for the species collected downstream the incinerator. Long irradiations (5 hours, neutron flux: 1×10^{13} $cm^{-2}s^{-1}$) were performed in the same pellets after letting them decay 1 week. Short and long irradiations are performed at the Portuguese Nuclear Reactor. Samples are measured with a high-purity germanium detector (FWHM=1.75 keV at 1.33 MeV of ^{60}Co) for 5 min. after 3 min. waiting time (short irradiation), and 2–4 hours after 4 and 30 days of waiting time (long irradiation). For results treatment k_0 factor method is used. Concentrations are calculated with SOLANG and SINGCOMP programs.

3. RESULTS AND DISCUSSION

In order to make the comparison between the elemental content in the air and in biomonitors, we have calculated the average values for PM10 concentrations in aerosol during specific periods of time. These average values are compared with the results obtained for the biomonitor sample. Since the sampling of aerosol started in January 1999 and the first collection of biomonitor was in April 1999, we are able to compare elemental concentration evolution in the air as well as in biomonitors, since the start up of the incinerator. As we can see in Fig 1., in the period May 1999–July 1999 the incinerator has increased the treatment of domestic urban waste in about 1500 t/day.

In this work, comparison is made for Sb, as an example (see Tables I and II).

TABLE I. TOTAL ANTIMONY CONCENTRATIONS OF PARTICULATE MATTER (ng/m^3) IN AIR

Date	Total Sb conc. in air (ng/m^3)
Jan 99 to April 99	2.65
Jan 99 to Oct 99	3.39
Jan 99 to April 00	3.45
Jan 99 a Out 00	3.32

TABLE II. ANTIMONY CONCENTRATIONS (ng/g) FOR *OLEA EUROPAEA* (A) AND *CONYZA (*C) IN THE SAMPLING MADE SINCE 1999.

Date of sampling	Sb conc. in species A (ng/g)	Sb conc in species C (ng/g)
April 1999	28	90
October 1999	74	170
April 2000	71	143
October 2000	70	258

Results for the correlation between concentrations of antimony in air and in biomonitors are presented in Fig. 2.

Both species show a significant increase in concentration after the beginning of waste treatment in the incinerator. However, it is unclear that this increase is due to the incinerator alone, since in this area other polluting industries can be found.

The increase of antimony is more relevant from the first to the second campaign as show in Fig 2. For the *Olea Europaea* the antimony concentration has stabilised after the second sampling. *Conyza* species appears to have a seasonal behaviour, as shown in Table II.

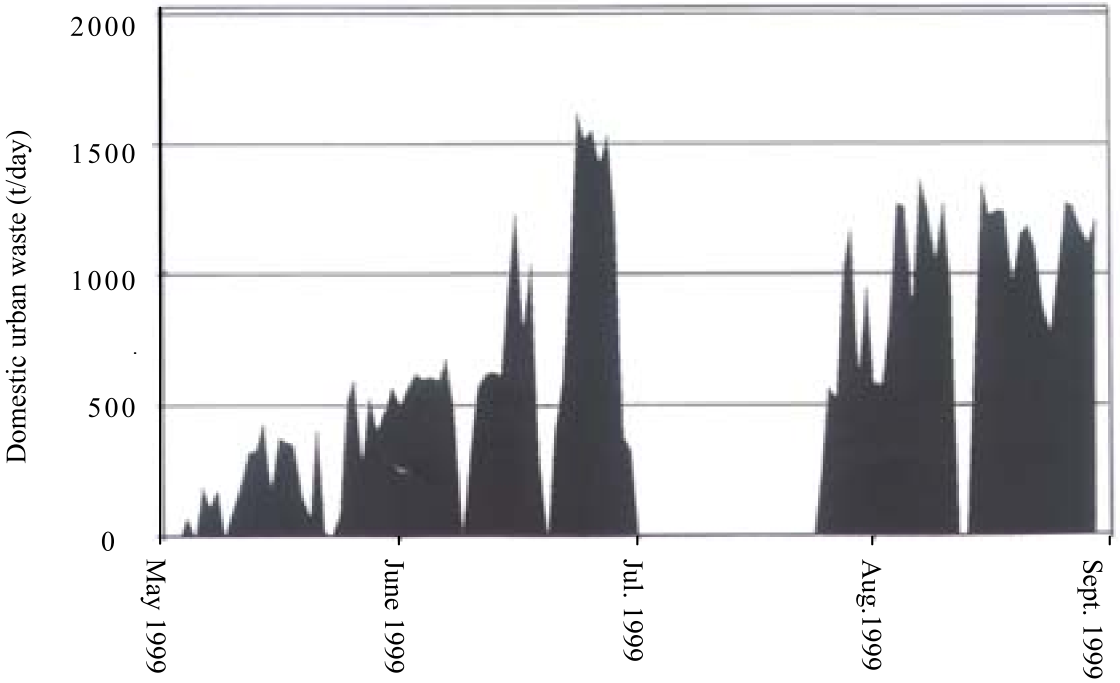


FIG. 1. Domestic urban waste treatment evolution (t/day) [7].

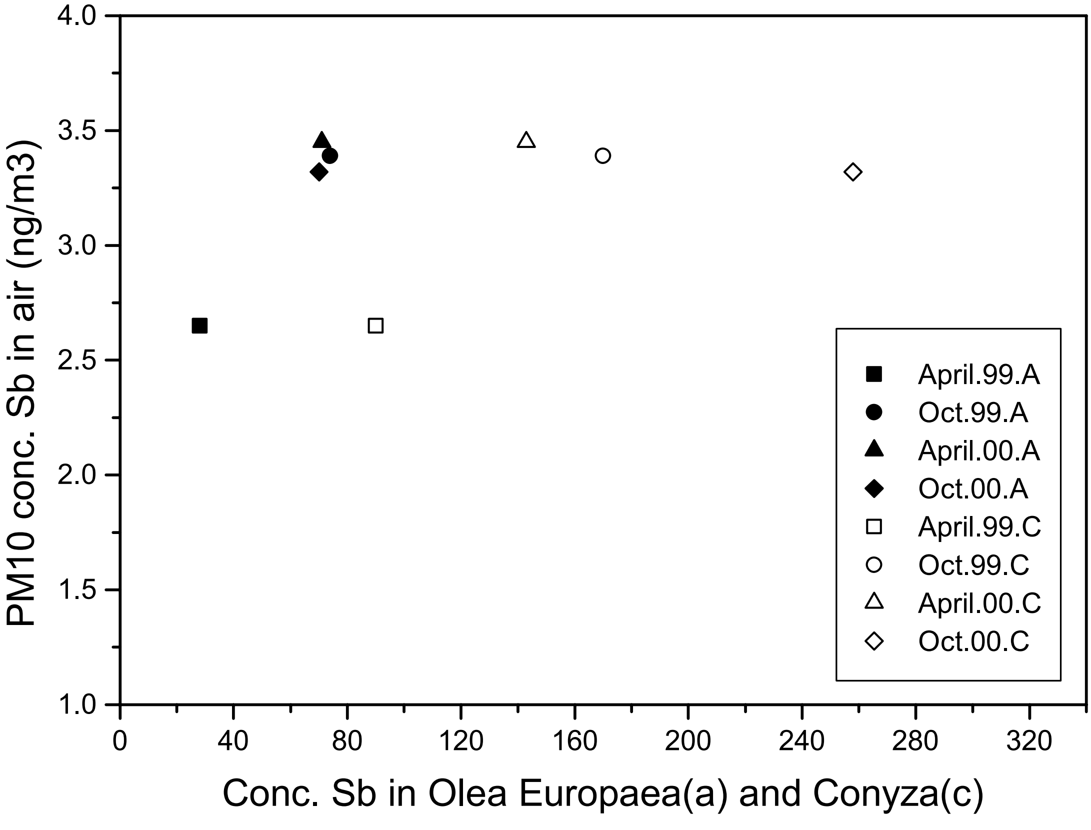


FIG. 2. Correlation between antimony concentrations in air and in two biomononitors (Olea Europaea (a) and Conyza (c)) in the area of study.

REFERENCES

[1] HOPKE, P.K., XIE, Y., RAUNEMAA, T., BIEGALSKI, S., LANDSBERGER, S., MAENHAUT, W., ARTAXO, P., COHEN, D., Characterisation of Gent stacked filter unit PM10 sampler, Aerosol Science and Technology **27** (1997) 726–735.

[2] ALVES, L.C., REIS, M.A., FREITAS, M.C., Air particulate matter characterisation of a rural area in Portugal, Nuclear Instruments and Methods in Physics Research **B136/138** (1998) 941–947.

[3] ALVES, L.C., REIS, M.A., FREITAS, M.C., GOUVEIA, M.A., Elemental analysis of particulate matter and source identification in Lisboa, X-Ray Spectrometry **27** (1998) 313–320.

[4] FREITAS, M.C., MARTINHO, E. Neutron activation analyses of reference materials by the k0-standarzation and relative method, Analytica Chimica Acta, Short communication **219** (1989) 317–322.

[5] VALDÉS, B.,TALAVERA, S., FERNÁNDEZ-GALIANO, E., Flora vascular de Andalicía Occidental, (1987), 11–12pp.

[6] Valorsul, Rede de vigilância da qualidade do ar, private communication (1999), 4pp.

SUBSTRATE INFLUENCE ON ELEMENTAL COMPOSITION OF *CANOPARMELIA TEXANA* LICHENIZED FUNGI

M. SAIKI[1], L.K. HORIMOTO[2], M.B.A. VASCONCELLOS[1], M.P. MARCELLI[2]

[1] Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP,
Supervisão de Radioquímica, São Paulo, SP, Brazil
[2] Instituto de Botânica, Seção de Micologia e Liquenologia, São Paulo, SP, Brazil

Abstract

In order to investigate the contribution of substrate derived elements to the elements present in lichens, *Canoparmelia texana* species and their respective tree bark substrates and xylem tissues (sapwoods) were analysed by instrumental neutron activation analysis. Concentrations of the elements Al, As, Br, Ca, Cl, Co, Fe, K, La, Mn, Mo, Na, Sb Se, and Zn were determined in these samples. Concentrations for most elements obtained in lichen were generally similar to or higher than those results found for bark substrates and xylem tissues. The exceptions were Mo and Br. The lowest concentrations of Mo were found in all lichens samples and concentrations of Br were the lowest in lichens sampled on bark substrates of the palm-tree and eucalyptus. These results indicated that the tree bark contribution for lichen elemental concentrations depends on the element and varies among the substrate types of trees but barks are not a significant source of metals to *C. texana* species. Also, the concentrations of most of elements present in xylem samples were of the same levels or lower than those obtained for bark substrates. *C. texana* is a foliose species that occurs well fixed on the surfaces of tree barks however the substrate influence on elemental composition of lichens was very small or absent for various elements, indicating that this species could be used in biomonitoring studies.

1. INTRODUCTION

Epiphytic lichens are able to concentrate various pollutants and thus the analyses of their metal contents have been studied to be used in the environmental monitoring. In these studies it is generally assumed that lichen species accumulate metals from air and precipitation, and only to a minor extent from the substrate.

However this assumption is questionable mainly for lichens growing on substrates rich in minerals or when the lichens are well fixed on the substrates. Besides the experimental data have indicated that element present in lichens can be originated from tree bark substrates. De Bruin and Hackenitz [1] obtained concentrations of Ba, Ca, Cd, Mn, and Zn in *Lecanora conizaeoides* similar to those found in barks and they concluded that there is a possibility of the influence of the substrate on the lichen element content. Sloof and Wolterbeek [2] analysed lichens, barks, and rings of the supporting trees and concluded that Cd, Mn, and Zn levels in lichens might have been originated from both wet and dry deposition and the bark substrate. On the other hand, lately bark materials have also been analysed as biomonitoring tools to indicate and characterise deposition of inorganic pollutants [3,5].

In this paper, in order to evaluate the possibility of lichen element uptake from bark substrates of supporting tree, comparisons were made between the elemental concentrations present in epiphytic lichens with those obtained in the bark substrates and in xylem tissues (sapwoods).

The biomonitor *Canoparmelia texana* chosen for this investigation is one of the most widely spread lichenized fungi species in open places of natural primary and secondary vegetal formation as well as inside cities all over the Brazilian territory except on the coastal cities. It is an epiphytic foliose lichen tolerant to pollution. In non polluted ecosystems, this species is limited to twigs and branches in well–lit woods or on the trunks of exposed trees and in polluted or urban areas it occurs frequently covering almost the whole tree trunks.

The analytical method utilised was instrumental neutron activation analysis and during each series of analysis the quality of the results was checked by simultaneous analysis of standard reference materials. In the previous papers [6,7], results of the analysis of reference materials were presented and their accuracy was generally found to be within 10%.

2. MATERIAL AND METHODS

2.1. Sample collection and preparation

In the present work for each lichen sample, their respective bark substrate and xylem were analysed. The samples were collected at two considered low pollution sites situated at the Campus of São Paulo University, São Paulo, SP and on Vila Velha Park, Ponta Grossa, PR. Samples of *Canoparmelia texana* (Tuck.) Elix & Hale were collected together with the wood branches or trunks at a height of about 1.5 m above the ground and from different types of trees: palm tree (Sample 1), eucalyptus(Sample 2), rubber-tree (Sample 3), and the twigs of a wild bush (Sample 4). In this sample collection, the trunk or branch of trees were cut diametrically and wrapped up in a clean paper foil to bring to the laboratory. For the analyses the lichen samples were removed from the bark substrate using a titanium knife. The lichens were, firstly, cleaned using a pair of tweezers with teflon points and by examining them under an Olympus zoom stereoscopic microscope model SZ 4045 to remove eventual bark substrates or extraneous materials. Then, they remained immersed in distilled water for one minute to remove dust and sand. Next, lichens were placed on filter paper, freeze-dried, and ground manually in a small agate mortar to obtain a fine powder.

The 2–3 mm thick dark brown external layers of branches or trunks were defined as bark substrates. The barks were not separated in their outer and inner layers. After removing the substrates, a next thin layer of about 5 mm of xylem tissue was obtained for the analyses. Xylem tissue is the internal part or the sapwood of the tree, where the mobility of the elements from the roots to the leaves occurs. The bark substrates and the xylem tissues were obtained in small chips that were washed using distilled water. Next, they were also freeze dried for about 30 hours.

2.2. Preparation of elemental standards

Stock solutions of elements were provided from Spex Chemical or they were prepared by dissolving high purity metals or salts in pure reagents or distilled water. Single or multielement solutions were prepared by using appropriate amounts of these stock solutions and they were pipetted onto sheets of Whatman 42 filter paper. After drying these sheets in a desicator, they were placed in polyethylene envelopes that were heat sealed for irradiation with the samples.

2.3. Procedure used for neutron activation analysis

The samples, ranging in mass from 80–150 mg weighed in polyethylene envelopes were used for instrumental neutron activation analysis. Irradiation of 5 minutes were carried out using a pneumatic transfer system of the IEA-R1m nuclear reactor and under a thermal neutron flux of 4×10^{11} n cm^{-2} s^{-1} for the determination of Al, Cl, K, Mn, and Na. Longer irradiations of 16 h under thermal neutron flux of about 10^{12} n cm^{-2} s^{-1} were performed for the determinations of As, Br, Ca, Co, Cr, Fe, K, La, Mo, Na, Sb, Se, and Zn. Samples and standards were measured at least twice after adequate decay times using a Canberra GX2020 hyperpure Ge detector which was coupled to Model 1510 Integrated Signal Processor and System 100MCA Card also from Canberra. The detector used had a resolution (FWHM) of 0.90 keV for 122 keV gamma rays of ^{57}Co and 1.78 keV for 1332 keV gamma rays of ^{60}Co. The gamma ray spectra were processed using VISPECT software[8] that evaluates peak areas (counting rates) and gamma ray energies of the photo peaks. The radioisotopes were identified by gamma ray energies and half lives and the concentrations of the elements were calculated by comparative method.

3. RESULTS AND DISCUSSION

Table I and II show the results obtained in the analysis of Al, As, Br, Ca, Cl, Co, Fe, K, La, Mn, Mo, Na, Sb, Se, and Zn in *C. texana* and their respective tree bark substrates and xylem tissues. These results were normalized in relation to the concentrations obtained in bark substrate and are presented in Fig1.To verify the substrate contribution to the element content in lichen, the elemental concentrations of barks were compared to those obtained in lichens. Lichens sampled from four different trees presented concentrations of As, Cl, Fe, K, La, and Mn higher than the data obtained for their respective tree bark substrates. For Al, Ca, Na, Sb, Se, and Zn , concentrations of these elements in lichens were the same levels or higher than those presented by bark substrates depending on the type of tree substrate. These results indicate that for this set of elements the bark substrates were not significant source of metal to *C. texana*. However for Mo, the concentrations of this element in lichens were of the same magnitude or lower than those found in barks and/or xylem tissues. Also the lichens collected from palm tree (Sample 1) and from eucalyptus (Sample 2) presented the lowest concentrations of Br. These results indicate that the uptake from the substrate by lichens varies among the substrates and elements.

In conclusion, the results obtained here show the possibility of using *C. texana* for evaluating air quality and as a biomonitor of trace element in the environment since most of elements analysed in lichens presented higher concentrations than those of bark substrates. *C. texana* is a foliose lichen very well fixed on the tree barks, however if this uptake from bark by lichen occurs, its quantity is very low when compared to element accumulations from both wet and dry deposition.

TABLE I. ELEMENTAL CONCENTRATIONS IN *CANOPARMELIA TEXANA* SPECIES AND THEIR RESPECTIVE TREE SUBSTRATES OF PALM-TREE AND EUCALYPTUS

Elements	Sample 1 from palm-tree			Sample 2 from eucalyptus		
	Lichen 1	Bark 1	Xylem 1	Lichen 2	Bark 2	Xylem 2
Al, μg g^{-1}	789 ± 24[a]	437 ± 11	432 ± 10	1398 ± 36	1502 ± 54	8.2 ± 2.6
As, μg kg^{-1}	469 ± 11	59 ± 7	46 ± 5	501 ± 2	95 ± 3	21.7 ± 5.9
Br, μg g^{-1}	23.0 ± 0.1	72.3 ± 0.3	40.0 ± 0.1	2.40 ± 0.03	5.41 ± 0.02	0.324 ± 0.003
Ca, %	0.27 ± 0.02	0.240 ± 0.005	0.114 ± 0.004	2.24 ± 0.03	0.357 ± 0.004	0.055 ± 0.006
Cl, μg g^{-1}	529 ± 21	188 ± 10	284 ± 15	886 ± 16	134 ± 6	5.2 ±2.2
Co, μg kg^{-1}	295 ± 4	404 ± 11	396 ± 12	307 ± 9	358 ± 5	8.7 ± 0.5
Fe, μg g^{-1}	540 ± 3	290 ± 7	149 ± 4	845 ± 9	365 ± 4	2.8 ± 0.2
K, μg g^{-1}	2516 ± 87	512 ± 9	233 ± 6	1076 ± 74	483 ±4	242 ± 2
La, μg kg^{-1}	1311 ± 6	1189 ± 5	1050 ± 4	1985 ± 2	720 ± 6	39.7 ± 0.6
Mn, μg g^{-1}	138 ± 2	106 ± 5	129± 2	743 ± 7	70.8 ± 0.9	14.9 ± 0.2
Mo, ,μg kg^{-1}	1250 ± 32	1337 ± 57	1112 ± 30	501.6 ± 0.3	1134.0 ± 0.7	594 ± 7
Na, μg g^{-1}	53.0 ± 0.5	21 ± 3	25 ± 3	63.2 ± 0.3	59.6 ± 1.4	52 ± 2
Sb, μg kg^{-1}	200 ± 2	184 ± 4	125 ± 2	217. ± 2	307 ± 2	7.3 ± 0.2
Se, μg kg^{-1}	141 ± 17	168 ± 21	168 ± 36	264 ± 27	320 ± 16	19 ± 2
Zn, μg g^{-1}	98.4 ± 0.4	95.9 ± 0.6	105 ± 2	122.9 ± 0.9	54.9 ± 0.6	3.1 ± 0.3

(a) - Uncertainties calculated using statistical counting errors of standards and samples.

TABLE II. ELEMENTAL CONCENTRATIONS IN *CANOPARMELIA TEXANA* SPECIES AND THEIR RESPECTIVE TREE SUBSTRATES OF RUBBER - TREE AND WILD BRUSH

Elements	Sample 3 from rubber-tree			Sample 4 from wild bush		
	Lichen 3	Bark 3	Xylem 3	Lichen 4	Bark 4	Xylem 4
Al, µg g^{-1}	1782 ± 45	128 ± 5	90 ± 2	801 ± 25	558 ± 13	19 ± 1
As, µg kg^{-1}	530 ± 2	127 ± 2	151 ± 1	343 ± 6	173 ± 2	13.6 ± 0.7
Br, µg g^{-1}	4.5 ± 0.1	1.14 ± 0.01	0.75 ± 0.01	3.30 ± 0.01	1.78 ± 0.01	0.386 ± 0.002
Ca, %	2.94 ± 0.08	1.55 ± 0.01	1.06 ± 0.02	5.74 ± 0.03	0.457 ± 0.003	0.073 ± 0.001
Cl, µg g^{-1}	612 ± 3	183 ± 10	111 ± 5	665 ± 15	12 ± 4	1.1 ± 0.5
Co, µg kg^{-1}	718 ± 17	152 ± 3	127 ± 3	110 ± 2	272 ± 12	67 ± 4
Fe, µg g^{-1}	1844 ± 16	108 ± 7	40 ± 7	626 ± 4	457 ± 7	14.0 ± 0.9
K, µg g^{-1}	5244 ± 12	790 ± 11	169 ± 10	296 ± 9	195 ± 9	45.1 ± 0.9
La, µg kg^{-1}	3075 ± 3	980 ± 8	1068 ± 7	663 ± 12	304 ± 6	25.3 ± 0.5
Mn, µg g^{-1}	33.5 ± 0.3	29.2 ± 0.1	6.29 ± 0.08	73.0 ± 0.4	59.8 ± 0.7	21.3 ± 0.2
Mo, ,µg kg^{-1}	882 ± 25	1299 ± 20	2746 ± 27	911 ± 24	795 ± 18	1271 ± 13
Na, µg g^{-1}	152.3 ± 0.7	107 ± 4	15.0 ± 0.1	20.6 ± 0.3	21.6 ± 0.4	13.7 ± 0.3
Sb, µg kg^{-1}	497 ± 5	89 ± 1	71 ± 1	67 ± 2	51 ± 1	26.2 ± 0.5
Se, µg kg^{-1}	335 ± 65	41 ± 5	53 ± 7	98 ± 8	92 ± 12	44 ± 2
Zn, µg g^{-1}	175 ± 2	28.4 ± 0.3	29.9 ± 0.8	32.2 ± 0.25	39.8 ± 0.2	9.1 ± 0.1

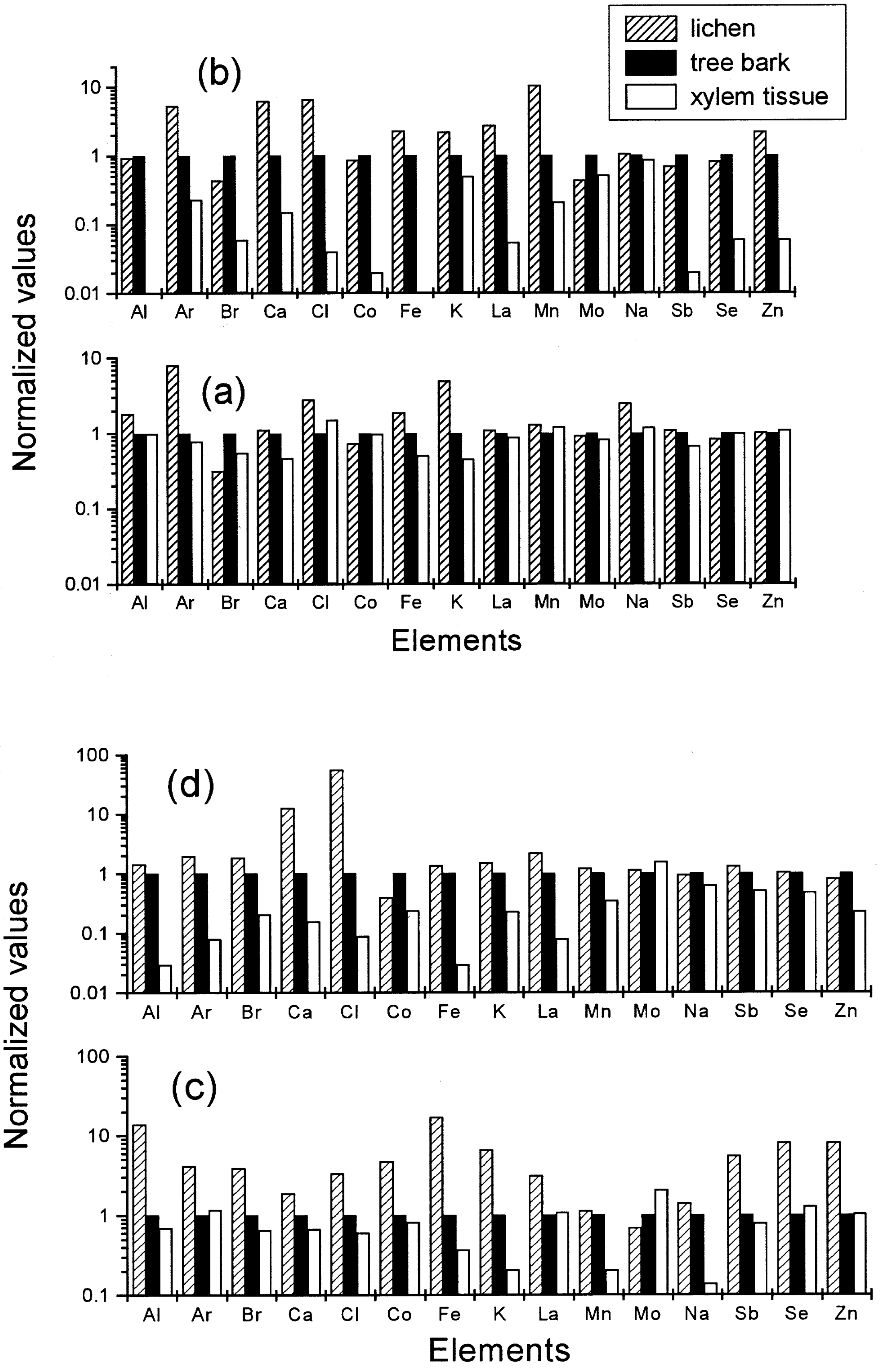


FIG. 1. Normalized values of elemental concentrations obtained for lichen, bark substrate and xylem tissue. (a): palm tree; (b): eucalyptus; (c): rubber-tree; (d): wild bush.

ACKNOWLEDGEMENTS

The authors acknowledge to FAPESP and CNPq from Brazil and also the IAEA

REFERENCES

[1] DE BRUIN, M., HACKENITZ, E., Trace element concentrations in epiphytic lichens and bark substrate, Environ. Pollut. (Series B), **11** (1986) 153–160.

[2] SLOOF, J. E., WOLTERBEEK, H., Substrate influence on epiphytic lichens, Environ. Monit. Assess., **25** (1993) 225–234.

[3] NAREWSKI, U., WERNER, G., SCHULZ, H., VOGT, C., Application of laser ablation inductively coupled mass spectrometry (LA-ICP-MS) for the determination of major, minor, and trace elements in bark samples, Fresenius J. Anal, Chem., **366** (2000) 167–170.

[4] SCHULTZ, H., POPP, P., HUHN, G., STARK, H. J., SCHUURMANN, G., Biomonitoring of airborne inorganic and organic pollutants by means of pine tree barks. I. Temporal and spatial variations, Sci Tot. Environ., **232** (1–2) (1999) 49–58.

[5] BOHN, P.; WOLTERBEEK, H, VERBURG, T., MUSILEK, J., The use of tree bark for environmental pollution monitoring in the Czech Republic, Environ. Pollut., **102** (1998) 243–250.

[6] SAIKI, M., CHAPARRO, C. G., VASCONCELLOS, M. B. A., MARCELLI, M. P., Determination of trace elements in lichens by instrumental neutron activation analysis, J. Radioanal. Nucl. Chem**.**, **217**, (1) (1997) 111–115.

[7] COCCARO, D.M B. B., SAIKI, M., VASCONCELLOS, M.B.A., MARCELLI, M. P., Analysis of *Canoparmelia texana* lichens collected in Brazil by neutron activation analysis, Proceedings, Biomonitoring of atmospheric pollution (with emphasis on trace elements)- BioMap, IAEA-TECDOC–1152, IAEA, Vienna (2000), 143–148.

[8] PICCOT, D., Laboratoire Pierre Sue, CEA-CNRS, Centre d'Etudes de Saclay, France, personal communication, 1989.

ATMOSPHERIC QUALITY IN ARGENTINA EMPLOYING TILLANDSIA CAPILLARIS AS BIOMONITOR

M.L. PIGNATA[1], C. ALBARRÁN[1], E. ELS[1], R.R. PLÁ[2],
E.D. WANNAZ[1], G.L. GUDIÑO[1], L. ORELLANA[3]

[1] Cátedra de Química General. Facultad de Ciencias Exactas,
Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina
[2] Comisión Nacional de Energía Atómica (CNEA), Unidad Radioquímica,
Sector Técnicas Analíticas Nucleares, Buenos Aires, Argentina
[3] Instituto de Cálculo, Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires, Buenos Aires, Argentina

Abstract

The objective of this research was to evaluate the biondicator capacity of *Tillandsia capillaris* f. *incana* (Mez.), widely distributed in Argentina, in relation to the accumulation of heavy metals and to its physiologic response to air pollutants. A sampling area with a surface of 50,000 km^2 was selected in the central region of the Republic Argentina. The area was subdivided in grids of 25 × 25 km being collected pools at *T. capillaris* in each one of the intersection points when this was present. Of each pool three subsamples were analyzed independently. Besides, for 20% of the points, quintupled samples were collected in order to analyze the variability inside the site. The content of Co, Cu, Fe, Ni, Mn, Pb, and Zn was determined by Atomic Absorption Spectrometry. The concentration of chemical-physiological parameters was also determined with the objective of detecting symptoms of foliar damage. Chlorophylls, phaeophytins, hydroperoxy conjugated dienes, malondialdehyde, and sulfur were quantified in *T. capillaris*. Some of these parameters were used for calculating a pollution index. Data sets were evaluated by one-way ANOVA, correlation analysis, principal component analysis, and mapping. Different patterns of geographical distribution were obtained for the different metals that allow to reflect the so much contribution of natural and anthropogenic emission sources. According to our results it can be inferred that Fe, Mn, and Co probably originated from the soil. For Pb, the highest values were in the mountainous area, which can be attributed to the presence of minerals that close contains Pb in granitic rocks. Ni showed origin mainly anthropogenic, with values a risen in places close to industrial centers. For Zn the highest values were in areas of agricultural development, as well for Cu, whose presence could be related to the pesticides employment.

1. INTRODUCTION

Due to the low population density, the total air pollutant emissions of Argentina are probably still low in comparison with highly industrialized countries. However, and like in some other South American countries, they are rapidly increasing and, even though the detailed emission inventories of urban and industrial centers are being compiled by state environmental agencies, they are scarce and inefficient [1].

Although a significant deterioration of ambient air quality, caused by the locally and regionally high air pollutant emissions from different kinds of sources, has been observed for a long time, the establishment of environmental agencies and the installation of air-monitoring networks did not take place until the 90's and only in a few cities. Major air pollution problems are occurring at urban and industrial centers, increasing pollution levels, however, they can also be observed at remote sites as a consequence of agricultural practices and mineral mining and processing [2].

Thus, the use of air pollution biomonitors represents an important contribution to Argentina where measurements of particulate matter or other types of pollutants in big areas would require expensive technical equipment, not available in the country at this moment.

The *Bromeliaceae* constitutes a group of plants with exclusive distribution in the neotropic [3]. The *Tillandsioideae* subfamily include mainly epiphytic species with a slow growth and an extraordinary capacity to obtain water and nutrients from the atmosphere, atmospheric pollutants included [4]. *Tillandsia usneoides* (Linneaus) proved to be an efficient accumulator of atmospheric Hg in the surrounding at a chlor-alkali plant in Rio de Janeiro (Brazil) [5]. It had been previously used for the evaluation of fluoride in rain water [6] and Hg in urban areas [7]. *T. aeranthos* and *T. recurvata* were employed in Porto Alegre (Brazil) in relation to sulfur and heavy metal contents in industrialized and residential areas [8]. In Colombia, the airborne heavy metals deposition in the highly industrialized Cauca Valley was examined using *T. recurvata* as accumulative indicator [9]. *Tillandsia* genus, which is highly spread in South America, shows a large number of species, characterized because of their high tolerance to hydric stress. However, there are few works reporting on their physiological response to pollutants, and the species growing in Argentina have not been studied considering their biomonitoring ability.

Although several studies have shown that trace element concentrations in biomonitors show average concentration of particulate matter in air and both wet or dry depositions of pollutants over a certain time [10,11], this kind of research has not been carried out so far on a large scale in Argentina. In this way, we set to assess the behaviour of *Tillandsia capillaris* as bioindicator of air quality, considering both their accumulative properties and their physiological response.

2. METHODS

2.1. Study area and sampling

The study area was selected with a surface of 50,000 km^2 in the central region of the Republic Argentine, defined by a quadrilateral whose extreme points had the following co-ordinates: to the West, 31º 25’ 21” S, 65º 24’ W; to the East, 31º 41’ 15” S, 62º 38’ 34” W; to the North, 30º 36’ S, 63º 15’ W; to the South, 32º 52’ S, 64º 10’ 12” W. Land morphology is highly variable, ranging from a mean altitude of about 250 m in the south-east to more than 2,500 m to the mid-west. There are cities (high and medium sized) and many small villages in the area; industrial plants, mainly metallurgical, petrochemical, chemical, food, vegetable oil, and cement, are mostly located in the centre and south where the highest population density is recorded.

For sampling purposes, the chosen area was divided according to a square pattern, each square of 25 by 25 km (80 sampling points in the area). Samples of *Tillandsia capillaris* f. *incana* (Mez.) at each intersection point were collected when present. The total of sampling points was 27 and the collection sites were located at least 500 m from major routes and highly populated areas, and at least 300 m from streets with lower traffic density (Figure 1).

Each pool consisted of 15–20 individuals, randomly taken along the four cardinal directions within an area of 100 × 100 m, no more than 100 m from the geographically referenced point. Extraneous material was removed from each sample and they were put in paper bags. The collection was done using plastic gloves to avoid any risk of sample contamination [12]. In

order to analyze intra-site variability, five-replicate samples were collected at 25% of the sites. At these sites, five pools were collected, from the same substrate if possible.

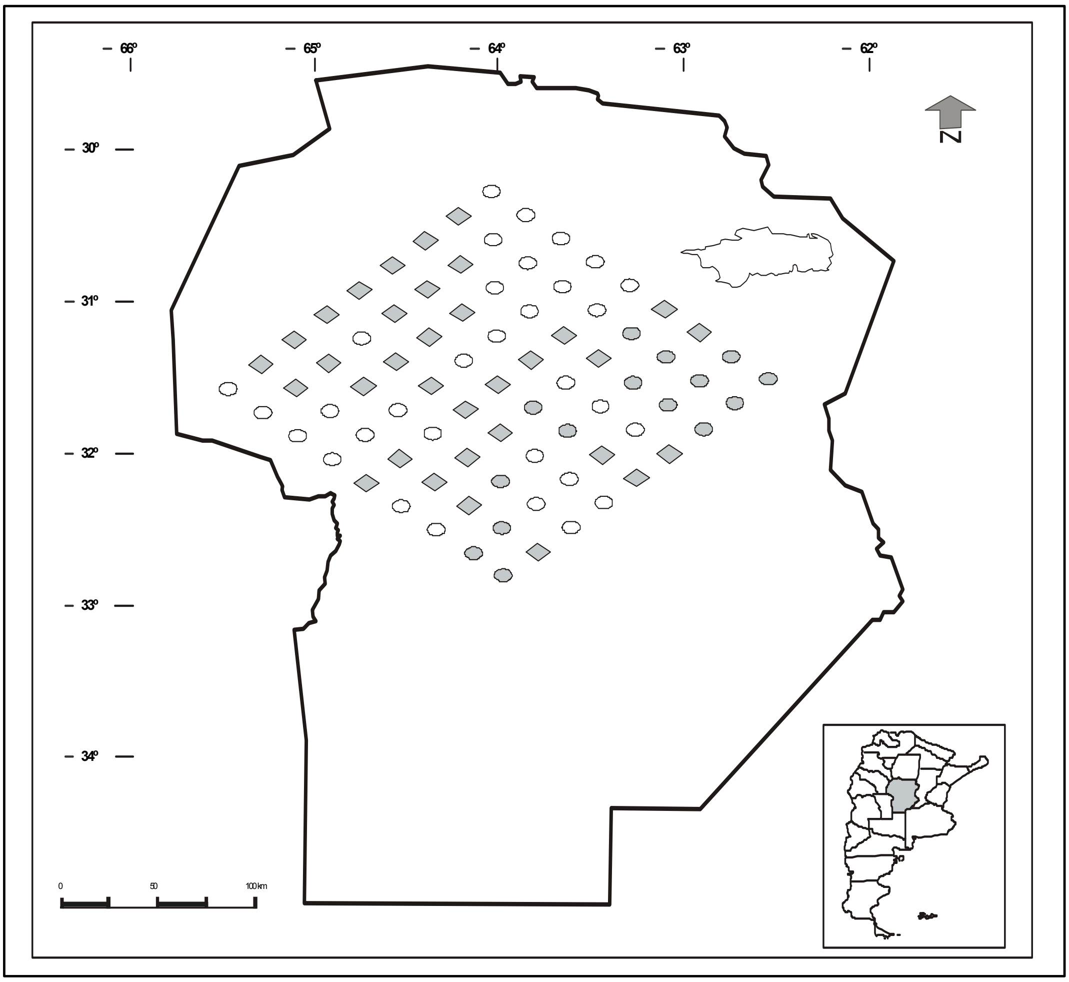


FIG 1. Location of the study area and individual sampling points in the survey made in Córdoba, Argentina. ◊ Sampled points for T. capillaris. ●Sampled points where T. capillaris was not found. ○Not sampled points in this survey.

Sampling was conducted only if a five-days-without-rain condition was fulfilled. Once at the laboratory, part of the plant material was separated to determine water content (DW/FW ratio). The rest of the material was stored in plastic vials at –15 ºC in darkness for chemical physiological determinations and prepared for metal determinations. Chemical determinations were done in triplicate, from independent sub-samples in each sample corresponding to each pool.

2.2. Atomic Absorption Spectrometry determinations

The samples were washed with cold (4 °C) bi-distilled water in a relation 1:50 W/V. Once washed, the samples were put in a Petri capsule and dried in oven at 50 ± 2 °C for 72 hours.

Masses of about 0.5 g of dry material were ground and reduced to ashes at 650ºC for 4 hours. The ashes were digested with HCl (18%): HNO_3 (3:1) at mild heat and the solid residue was separated by centrifugation. Finally, the volume was adjusted to 50 ml with Milli Q water and analysed by AAS using a Buck Spectrophotometer Model 210-VGP in order to determine the concentration of Co, Cu, Fe, Mn, Ni, Pb, and, Zn. Likewise, blanks of the digest were prepared and analysed [13]. The results were expressed in $\mu g.g^{-1}$DW.

2.3. Chemical-physiological parameter quantification

2.3.1. Dry weight / fresh weight ratio

The dry weight / fresh weight (DW/FW) ratio of the samples was determined by drying 1 g of fresh material at 60 ± 2 °C until constant weight. The results were expressed in g DW. g^{-1} FW.

2.3.2. Chlorophylls

A portion of 200 mg of plant material was homogenised in 10 ml EtOH 96% v/v with an Ultra Turrax homogeniser and the supernatant separated. Afterwards, HCl 0.06 M was added to clear chlorophyll extract (1 ml HCl and 5 ml chlorophyll extract) in order to produce phaeophytin formation. Absorption of chlorophylls and phaeophytins, and phaeophytins alone (after addition of HCl) was measured with a spectrophotometer Beckman DU 7000. Concentrations of chlorophylls and phaeophytins were calculated on a fresh weight basis [14] and were expressed in mg . g^{-1} FW.

The ratios chlorophyll b/chlorophyll a (Chl-b /Chl-a) and phaeophytin a /chlorophyll a (Phaeoph-a /Chl-a) were also calculated.

2.3.3. Sulfur content

Five millilitres of $Mg(NO_3)_2$ saturated solution were added to 0.5 g of plant and dried in an electric heater. Subsequently, the sample was heated in an oven for 30 min at 500 °C. Ashes were then suspended in HCl 6 M, filtered and the resulting solution, boiled for 3 minutes. The solution was brought to 50 ml with distilled water. The amount of SO_4^{2-} in the solution was determined by the acidic suspension method with $BaCl_2$ which subsequently allowed for the calculation of sulphur contents in each sample [15]. The concentration was expressed in mg of total sulphur g^{-1} FW.

2.3.4. Peroxidation product estimation

Malondialdehyde (MDA) was measured by a colorimetric method. The amount of MDA present was calculated from the extinction coefficient of 155 mM^{-1} cm^{-1} [16].

Hydroperoxy conjugated dienes (HPCD) were extracted by homogenisation of the plant material in 96% v/v EtOH at a ratio of 1:50 FW/V. The absorption was measured in the supernatant at 234 nm and its concentration was calculated by means of $\varepsilon = 2.65 \times 10^4$ M^{-1} cm^{-1} [17]. Results were expressed as μmol g^{-1} FW.

2.4. Data Analysis

Statistical analysis was based on the mean value of the determinations performed in the three sub-samples arising from each sampling point. Pearson's correlation coefficients were calculated for studying the relationships among chemical elements and physiological variables. Principal components were obtained based on the correlation matrix of the measured chemical variables or calculated from them. Hierarchical analysis of variance was used to evaluate the different sources of variability in the study. All results from eight samples points with five replicates were subjected to either simple or straight-forward statistical treatment to estimate means and components of variances. In order to evaluate the distribution of metals in the area, mapping was used.

3. RESULTS AND DISCUSSION

3.1. Sources of Variability Analysis

The determination of the local variance implies that all aspects of the survey are taken into consideration: selection of biomonitor, definition of sampling sites, sampling, sample handling, and elemental analysis, in agreement to Wolterbeek *et al.* [18]. Taking both the local variance and the total survey variance into consideration, the local variation may be seen as the survey noise whereas the survey variance may be used to express the survey signal. According to Wolterbeek *et al.*[18], combining both signal in a signal-to-noise ratio, the survey has been given a measurable expression of quality. This approach means that all survey aspects like biomonitor species, sampling site or local variance become part at the signal-to-noise ratio properties of the survey. In the biomonitoring literature, generally all information is about the signal, hardly a survey report contains information about the noise. Since the survey noise is as essential as the survey signal, the inevitable conclusion is therefore that, hither to, hardly a survey report contains information on quality.

Table I shows the estimates of variance components obtained from a hierarchical analysis of variance, in which three random effects or sources of variability were considered:

TABLE I. CHEMICAL-PHYSIOLOGICAL PARAMETERS (a) AND HEAVY METALS (b) MEASURED IN *T. CAPILLARIS*. SURVEY VARIANCE, LOCAL VARIANCE AND SIGNAL-TO-NOISE RATIOS.

(a)

	Chl-a	Chl-b	Phaeoph-a	Phaeoph-b	HPCD	MDA	Sulfur	DW/F
Survey variance	0.0123	0.0026	0.0166	0.0070	0.0001	0.00003	0.00007	0.0021
Local variance	0.0030	0.0005	0.0047	0.0010	0.1272	0.00006	0.00006	0.0007
Signal–to-noise ratio	4.0682	5.3899	3.5429	7.0598	0.0001	0.45807	1.05469	3.1652

(b)

	Cu	Pb	Zn	Co	Ni	Mn	Fe
Survey variance	0.0930	0.0141	19.1732	0.0002	0.1079	71.8710	585.8700
Local variance	0.0763	0.0235	7.4667	0.0004	0.0601	19.5305	234.5043
Signal-to-noise ratio	1.2196	0.5981	2.5678	0.6147	1.7958	3.6799	2.4983

(a) The variability among sample points, called survey variance, was calculated according to:

$$\text{Survey variance} = \text{Survey variance of all the sampling points} + \text{Local variance}/k + \text{experimental variance}/(k \times n)$$

where k = number of local replicates and n = number of sub-samples.

(b) The variability among localization's within a sample point (five replicates in each sample point), which is called "local variance".

Local variance = Local variance + experimental variance/n

where the experimental variance is the variability among different sub-samples and chemical determinations, and n is the number of sub-samples.

The signal-to-noise ratio proposed by Wolterbeek *et al.* [18], was obtained as the ratio between the survey and local variance. Thus, the denominator of the signal-to-noise ratio should contain all sources of variability other than the variability among sampling points that are far enough to represent different environmental conditions.

The results for signal-to-noise ratio shows that pigments, sulfur and DW/FW ratio may be recognized as the parameters that show the highest quality survey. Concerning metals, Mn, Fe, Zn, Ni, and Cu show the highest survey variability.

TABLE II. EIGENVECTORS OBTAINED IN PRINCIPAL COMPONENTS ANALYSIS OF CHEMICAL-PHYSIOLOGICAL PARAMETERS AND HEAVY METALS MEASURED IN *T. CAPILLARIS*.

	Component			
	1	2	3	4
Chl-a	-0.9703	0.0415	0.1138	-0.0028
Chl-b	-0.9404	0.1316	0.0706	-0.0163
Phaeoph-a	-0.9302	0.0490	0.1754	-0.0795
Phaeoph-b	-0.7467	0.2097	-0.0321	0.0074
Chl-b/Chl-a	0.7048	0.2451	-0.1107	0.0555
Phaeoph-a/Chl-a	0.6829	0.0554	0.1387	-0.1711
HPCD	0.1018	-0.0673	0.8279	0.0819
MDA	0.0474	0.7279	0.1405	-0.2415
Sulfur	-0.6872	-0.2812	-0.1370	-0.0382
DW/FW	0.0127	0.4018	0.7126	0.0004
Cu	0.0856	-0.1684	0.3305	0.4564
Pb	0.0889	0.7542	-0.0625	-0.0351
Zn	-0.4046	-0.1332	0.7176	0.0717
Co	-0.2639	0.6845	-0.0699	0.4736
Ni	0.0520	0.0415	-0.0164	0.9319
Mn	-0.5070	-0.3554	0.2371	0.4930
Fe	-0.6570	-0.0152	0.0096	0.4518
Total variance (%)	34.15	12.51	12.12	9.55
Eigenvalues	5.81	2.13	2.06	1.62

3.2. Principal Component Analysis and Pollution Index

In order to reduce the initial size of the problem, a principal component analysis was carried out which allowed us to find the relationships between the variables that better synthesize total variability of data. The eingenvalues of four components for data on chemical variables are given in Table II. The first component accounts for 34.15% of the variance. The score for this component is greatly affected by concentrations of Chl-a, Chl-b, Phaeoph-a, Phaeoph-b, and Chl-b/Chl-a ratio. The second principal component accounting for 12.51% of the total variance describes MDA and Pb content. The third and fourth component explained 12.12% and 9.55% of the variance respectively, and shows HPCD, DW/FW, Zn and Ni, as important variables.

This analysis allowed the selection of those parameters, that for their incidence in the data variability, could be included in a pollution index (PI_T) which can be used to compare different sampling sites in terms of physiological damage on the bioindicator. Thus, PI_T values are related to different air quality levels reflected by metals content and gaseous air pollutant effects:

$$PI_T = [(\text{Chl-b}/\text{Chl-a}) + (Pb/Pb_m) + (Zn/Zn_m) + (Ni/Ni_m)] \times (MDA/MDA_m) \times (DW/FW)$$

where Chl-b is the chlorophyll b concentration in $mg.g^{-1}FW$; Chl-a is the chlorophyll a concentration in $mg.g^{-1}FW$; Pb, Zn, and Ni content in $\mu g.g^{-1}DW$; MDA is the malondialdehyde concentration in $\mu mol.g^{-1}FW$. The parameter with subindex m in the denominator represents the arithmetic mean values calculated from all the sampling points.

3.3. Correlation analysis among chemical variables

Table III shows the correlations between the quantified chemical variables in *T. capillaris* leaves. Pigments showed a positive correlation with Fe. On the other hand, Chl-a, Chl-b, and Phaeoph-a showed a positive correlation with sulfur content and Mn; Chl-b showed a positive correlation with Co.

Membrane lipid peroxidation caused by atmospheric pollutants [19], was estimated by MDA and HPCD determinations. MDA shows positive correlation with Pb. This is an interesting result because the increase in MDA concentration has been noted as an indicator of leaf damage produced by air pollution in tree leaves [20]. On the other hand HPCD showed a positive correlation with Zn and the DW/FW ratio. These results suggest an important oxidative effect, probably caused by Zn and Pb, as peroxidation of unsaturated fatty acids is most probably found only during high oxidation pressure, as cited by Heath and Castillo [21].

For this species, high levels of Zn could be related to signs of foliar damage, which is evidentiated by an increase in HPCD and a loss of water content. Sulphur content showed a negative correlation with DW/FW and a positive correlation with Fe. Among metals only Fe showed a positive correlation with Mn.

TABLE III. PEARSON'S CORRELATION COEFFICIENT OF CHEMICAL VARIABLES MEASURED IN *T. CAPILLARIS*. BOLD COEFFICIENTS INDICATE THAT THE CORRELATION WAS SIGNIFICANTLY DIFFERENT FROM ZERO (* $p \leq 0.05$; **$p \leq 0.001$; ***$p \leq 0.0001$)

	Chl a	**Chl b**	**Phaeoph-a**	**Phaeoph-b**	**Chl-b/Chl-a**	**Phaeoph-a / Chl-a**	**HPCD**	**MDA**	**Sulfur**
Chl-b	**0.97*****								
Phaeoph-a	**0.98*****	**0.97*****							
Phaeoph-b	**0.67*****	**0.71*****	**0.63****						
Chl-b/Chl-a	**-0.70*****	**-0.54***	**-0.67*****	-0.37					
Phaeoph-a/Chl-a	**-0.62****	**-0.56***	**-0.48***	**-0.48***	**0.52***				
HPCD	0.04	0.03	0.06	-0.18	0.07	0.02			
MDA	0.02	0.06	0.04	0.03	0.18	0.16	0.08		
Sulfur	**0.63****	**0.56***	**0.58***	0.23	**-0.53***	**-0.46***	-0.05	-0.15	
DW/FW	0.05	0.08	0.10	0.13	0.10	0.08	**0.43***	0.29	**-0.41***
PI$_T$	0.04	0.07	0.07	0.07	0.14	0.11	**0.44***	**0.76*****	-0.22
Cu	0.02	-0.05	0.02	-0.03	-0.18	0.14	0.25	0.01	-0.09
Pb	-0.12	-0.10	-0.12	-0.06	0.11	0.05	-0.09	**0.46***	-0.11
Zn	**0.43***	0.32	**0.43***	0.25	**-0.51***	-0.23	**0.46***	-0.08	0.27
Co	0.29	**0.45***	0.26	0.30	-0.04	-0.15	-0.11	0.19	-0.01
Ni	-0.01	-0.04	-0.08	-0.13	-0.01	-0.21	0.13	-0.12	0.04
Mn	**0.48***	**0.47***	**0.47***	0.34	-0.31	-0.31	0.20	-0.37	0.38
Fe	**0.56***	**0.58***	**0.50***	**0.62****	-0.21	**-0.48***	-0.01	-0.14	**0.45***

	DW/FW	**PI$_T$**	**Cu**	**Pb**	**Zn**	**Co**	**Ni**	**Mn**
PI$_T$	**0.74*****							
Cu	-0.04	0.05						
Pb	0.03	**0.48***	-0.09					
Zn	0.30	0.38	0.20	-0.03				
Co	0.08	0.33	0.04	0.37	0.05			
Ni	-0.01	0.06	0.31	-0.17	0.08	0.33		
Mn	0.11	-0.11	0.18	-0.27	0.28	-0.01	0.19	
Fe	0.01	0.04	-0.10	-0.07	0.32	0.29	0.23	**0.64*****

3.4. Mapping

Figures 2–10 show maps of the geographical distribution of metal content sulphur and the PI_T values in *T. capillaries*. The distribution of Fe and Mn was similar, the highest levels were found in samples taken in the central and southern zones of the study area. According to the correlation analysis, these metals have probably the same origin. Thus, soil particles could be considered their main source, as mentioned by Steinnes *et al.* [22].

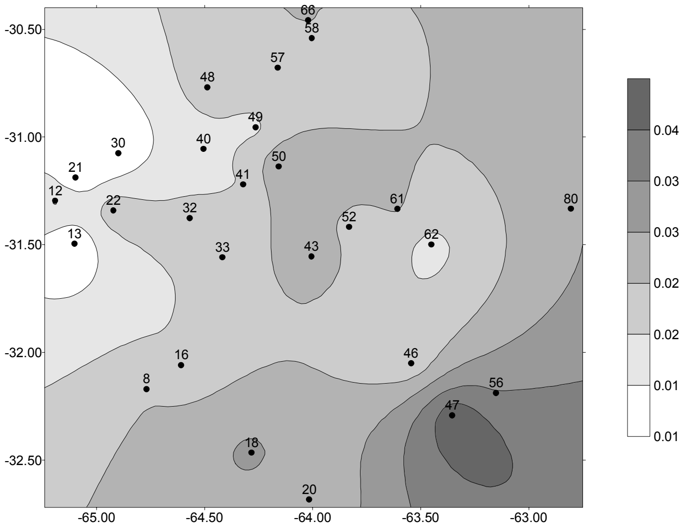


FIG. 2. Distribution map of sulphur content in T. capillaris (mg/gFW).

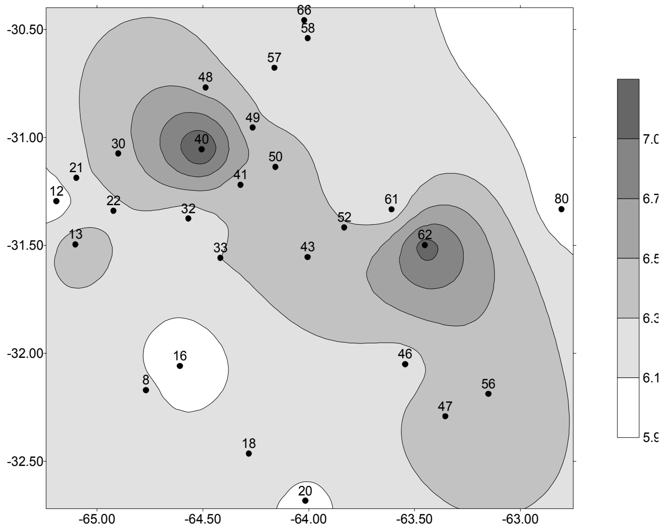


FIG. 3. Distribution map of Cu content in T. capillaris (μg/gDW).

The distribution map of Zn shows high contents of this element in the central and southeast zones, which reveals anthropogenic sources. As regard Pb distribution map, high values can be seen in mountain areas, therefore its presence in the air could be related to the granitic rocks, characteristic of Córdoba. The high levels found in the centre and southeast, could be related to the presence of anthropogenic emission sources. Meanwhile, Cu probably has a mining origin and some particles come from long-range-transport. The highest contents were detected in the northwest zone.

In the PI_T distribution map, it can be seen that the central, west, and southeast zones were the ones where the major damage of the bioindicator was found. High index values were also found in the northeast, perhaps related to focused agricultural and mining activities, characteristic of this area. The central zone coincides with the city of Córdoba, what could explain the high values obtained here. The southeast area is strictly agricultural, so the high PI_T values found there could be related to the use of pesticides.

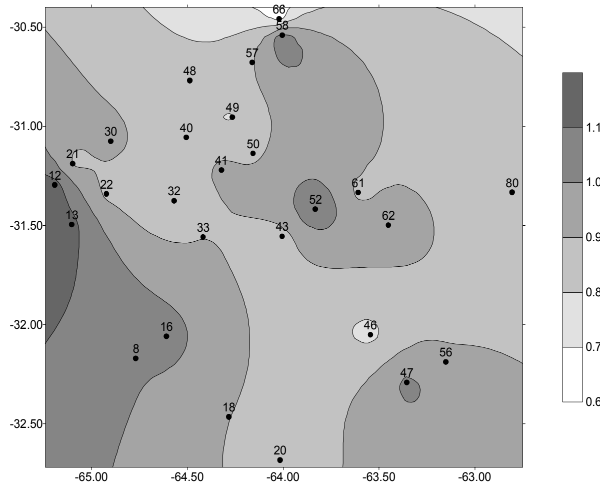


FIG. 4. Distribution map of Pb content in T. capillaris (µg/gDW).

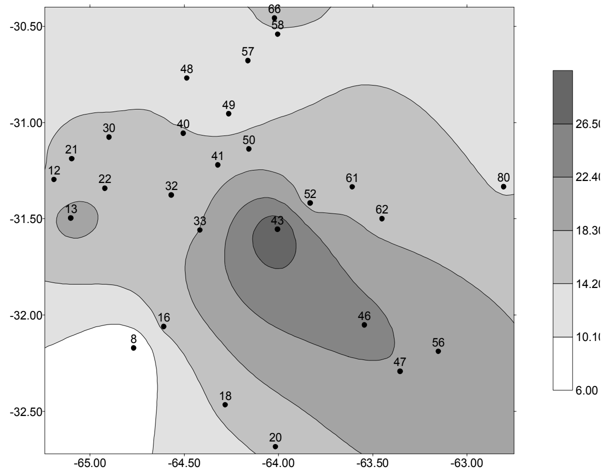


FIG. 5. Distribution map of Zn content in T. capillaris (µg/gDW).

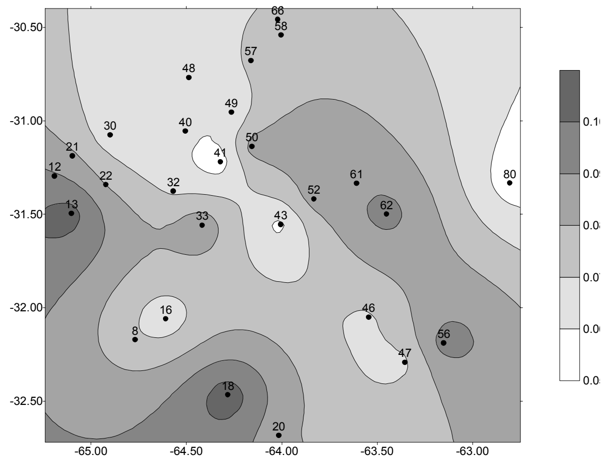


FIG. 6. Distribution map of Co content in T. capillaris (µg/gDW).

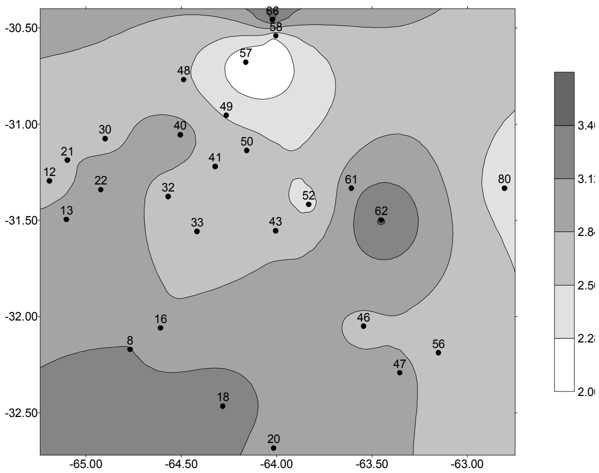


FIG. 7. Distribution map of Ni content in T. capillaris (µg/gDW).

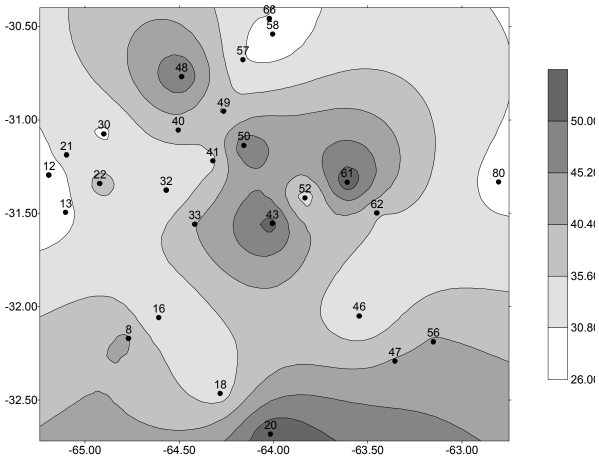


FIG. 8. Distribution map of Mn content in T. capillaris (µg/gDW).

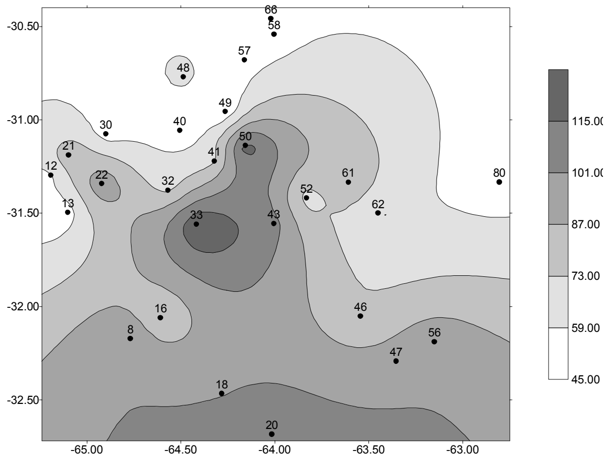


FIG. 9. Distribution map of Fe content in T. capillaris (µg/gDW).

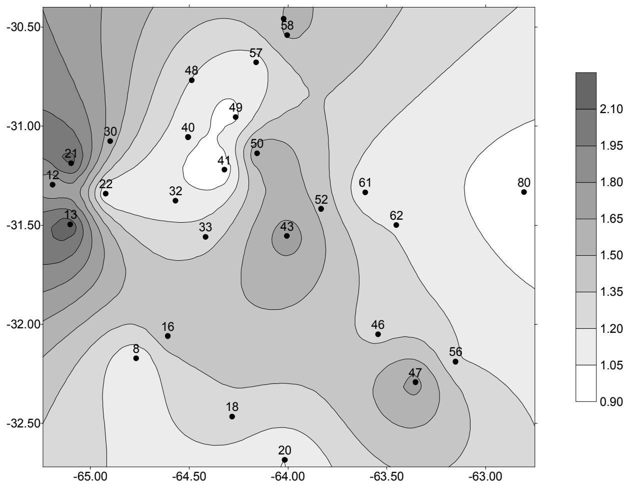


FIG. 10. Distribution map of PI_T in T. capillaris.

ACKNOWLEDGEMENTS

This work was partially supported by the International Atomic Energy Agency, Consejo de Investigaciones Científicas y Tecnológicas de la provincia de Córdoba (CONICOR) and by Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba (SECyT).

REFERENCES

[1] WORLD RESEARCH INSTITUTE. International Institute for Environment and Development, Internationaler Umveltatlas: Jahrbuch der Welt-Ressourcen, Vol. 1, Ecomed, Landsberg, Germany (1988).

[2] MORETTON, J., GUASCHINO, H., AMICONE, C., BELETZKY, V., SÁNCHEZ, M., SANTORO, V., NOTO, B., "Contaminación del Aire en Argentina: aspectos generales, legislación y situación en Capital Federal y provincia de Buenos Aires", Ediciones Universo, Buenos Aires (1996) 1–127.

[3] SMITH, L., Flora Neotrópica, Monographia Nº 14 parte 2. Haffner Press. New York (1977) 894–897.

[4] BENZING, D., BERMUDES, D., Epiphytic bromeliads as air quality monitors in south Florida, Selbyana **12** (1991) 46–53.

[5] CALASANS, C., MALM, O., Elemental mercury contamination survey in a chor-alcaly plant by the use of transplanted Spanish moss, *Tillandsia usneoides* (L.) Sci. Total Environ. **208** (1997) 165–177.

[6] STREHL, T., ARNDT, U., Alterações apresentadas por *Tillandsia aeranthos* e *T. recurvata* (Bromeliaceae) expostas ao HF e SO_2, Iheringia Ser. Bot. **39** (1989) 3–17.

[7] MALM, O., FRESITAS FONSECA, M., HISSNAUER MIGUEL, P., RODRIGUES BASTOS, W., NEVES PINTO, F., Use of epiphyte plants as biomonitors to map atmospheric mercury in a gold tgrade center city, Amazon, Brazil. Sci. Total Environ. **213** (1998) 57–64.

[8] FLORES, F.E.V., O uso de plantas como bioindicadores de poluiçao no ambiente urbano-industrial: experiências en Porto Alegre, RS Brasil, Tübinger Gor. Stud., (1987) 96–97.

[9] SCHRIMPF, E., Air pollution patterns in two cities in Colombia S.A. according to trace substance content of an epiphyti (*Tillandsia recurvata* L.) Water, Air and Soil Pollut. **21** (1984) 279–315.

[10 STEINNES, E., Biomonitors of air pollution by heavy metals. In "Control and fate of Atmospheric Trace Metals", (PACYNA, J.M, OTTAR, B. Eds.), Kluwer Academic Publishers, Dordrecht (1989) 321–338.

[11] MARKERT, B., Instrumental Analysis of Plants. In "Plants as Biomonitors — Indicators for Heavy Metals in the Terrestrial Environment", (MARKERT, B., Ed.), VCH-Publisher, Weinheim, New York (1993) 65–103.

[12] SLOOF, J. E., Environmental Lichenology: Biomonitoring Trace-element Air Pollution. Thesis, gelft University of Technology, Delft, The Netherlands (1993).

[13] PFEIFFER, H.N., BARCLAY-ESTRUP, P., The use of a single lichen species, *Hypogimnia physodes*, as an indicator of air quality in Northwestern Ontario. Bryologist **95** (1992) 3–41.

[14] WINTERMANS, J.F.G.M., DE MOTS, A., Spectrophotometric characteristics of chlorophylls a and b and their pheophytins in ethanol. Biochim. Biophys. Acta **169** (1965) 448–453.

[15] GONZÁLEZ, C.M., PIGNATA, M.L., The influence of air pollution on soluble proteins, chlorophyll degradation, MDA, sulphur and heavy metals in a transplanted lichen, Chem. and Ecol. **9** (1994) 105–113.

[16] KOSUGI, H., JOJIMA, T., KIKUGAWA, K., Thiobarbituric acid-reactive substances from peroxidized lipids. Lipids **24** (1989) 873–881.

[17] LEVIN, A.G., PIGNATA, M.L., *Ramalina ecklonii* (Spreng.) Mey. and Flot. as bioindicator of atmospheric pollution in Argentina, Can. J. Bot. **73** (1995) 1196–1202.

[18] WOLTERBEEK, H.TH., BODE, P., VERBURG, T.G., Assessing the quality of biomonitoring via signal-to-noise ratio analysis, Sci. Total Environ. **180** (1996) 107–116.

[19] SAKAKI, T., KONDO, N., SUGAHARA, K., Breakdown of photosynthetic pigments and lipids in spinach leaves with ozone fumigation, Physiol. Plant. **59** (1983) 28–34.

[20] CAÑAS, M. S., CARRERAS, H. A., ORELLANA, L., PIGNATA, M. L., Correlation between enviromental conditions and foliar chemical parameters in *Ligustrum lucidum* Ait. exposed to urban air pollutants, J. Environ. Manage **49** (1997) 167–181.

[21] HEATH, R. L., CASTILLO, F. J., Membrane disturbances in response to air pollutants. Air Pollution and Plant Metabolism (S. Shulte-Hostede, N. M. D. Darral, L. W. Blank and A. R. Wellburn, eds) London, New York: Elsevier Applied Science. (1988) 55–75.

[22] STEINNES, E., RAMBAEK, J. P., HANSSEN, J. E., Large Scale Multi-element Survey of Atmospheric Deposition Using Naturally Growing Moss as Biomonitor, Chemosphere **25** (1992) 735–752.

[23] RANKAMA, K., SAHAMA, TH. G., Geoquímica. Ed. Aguilar. Barcelona (1949) pp. 862.

TRACE-ELEMENT DETERMINATION IN LICHENS OF CÓRDOBA (ARGENTINA) USING NEUTRON ACTIVATION ANALYSIS AND ATOMIC ABSORPTION SPECTROMETRY

R.R. PLÁ[1], R.C. JASAN[1], M.L. PIGNATA[2], R.F. KOPTA[2]

[1] Técnicas Analíticas Nucleares, Comisión Nacional de Energía Atómica,
Buenos Aires, Argentina
[2] Facultad de Ciencias Exactas, Físicas y Naturales,
Universidad Nacional de Córdoba, Córdoba, Argentina

Abstract

Ramalina ecklonii (Spreng.) Mey. & Flot. is a lichen widely distributed in Córdoba, a central province of Argentina. The ability of this lichen as an atmospheric pollution bioindicator has already been studied in relation to its physiological response to air pollutants. This work has to do with the study of *R. ecklonii* in terms of its capacity to accumulate heavy metals and other trace elements. The sampled area, located in the province of Córdoba, covered 50,000 km^2 and was divided following a grid of 25 × 25 km. Lichens were collected at the intersecting points, no least than 500 m from main routes or highly populated centres and individuals were randomly gathered following the four cardinal directions and no more than 100 m from the geographically settled point. From each pool, three sub-samples were taken for independent analysis using atomic absorption spectrometry (AAS) and neutron activation analysis (NAA), for the determination of twenty nine elements (Cu, Ni, Mn, and Pb (AAS) and As, Ba, Br, Ca, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Na, Rb, Sb, Sc, Sm, Ta, Tb, Th, U, Yb, and Zn (NAA). The quality of the determinations was checked using standard reference material and data sets were evaluated using descriptive statistics, correlation analysis, and factor analysis. The highest variation coefficients correspond to Ca, Cr, and Zn. The studied elements were identified as of physiological importance and as emitted by natural (soil and rocks) and anthropogenic sources (non-ferrous metallurgy, coal combustion, oil-fired plants, fossil fuel combustion and, other industries).

1. INTRODUCTION

Due to different reasons, among which low population density may be counted, the total air pollutant emissions in Argentina are probably still low if compared with other countries. However, as it occurs in some other countries of the region, these emissions are rapidly increasing and, in spite of national and provincial environmental agencies' interest, data are still scarce [1].

Increasing pollution levels are mainly observed at urban and industrial centres but they can also occur at remote sites as a consequence of agriculture and mining. Although a significant deterioration of ambient air quality has been observed for a long time, air-monitoring networks were not settled till the 90's and only in a few cities [2]. Thus, the use of air pollution biomonitors represents an important contribution to Argentina, where direct measurements of particulate matter or other types of pollutants, in big areas, would require expensive technical equipment and manpower.

The province of Córdoba, in central Argentina, has within its limits a wide variety of land morphology, vegetation, land use, and population density. Its capital is the second largest city of the country and faces serious atmospheric pollution problems. A wide variety of industries (metallurgy, chemical, petrochemical, food, vegetable oil, and cement) and other anthropogenic activities (agriculture, cattle raising, mining) are located in the province.

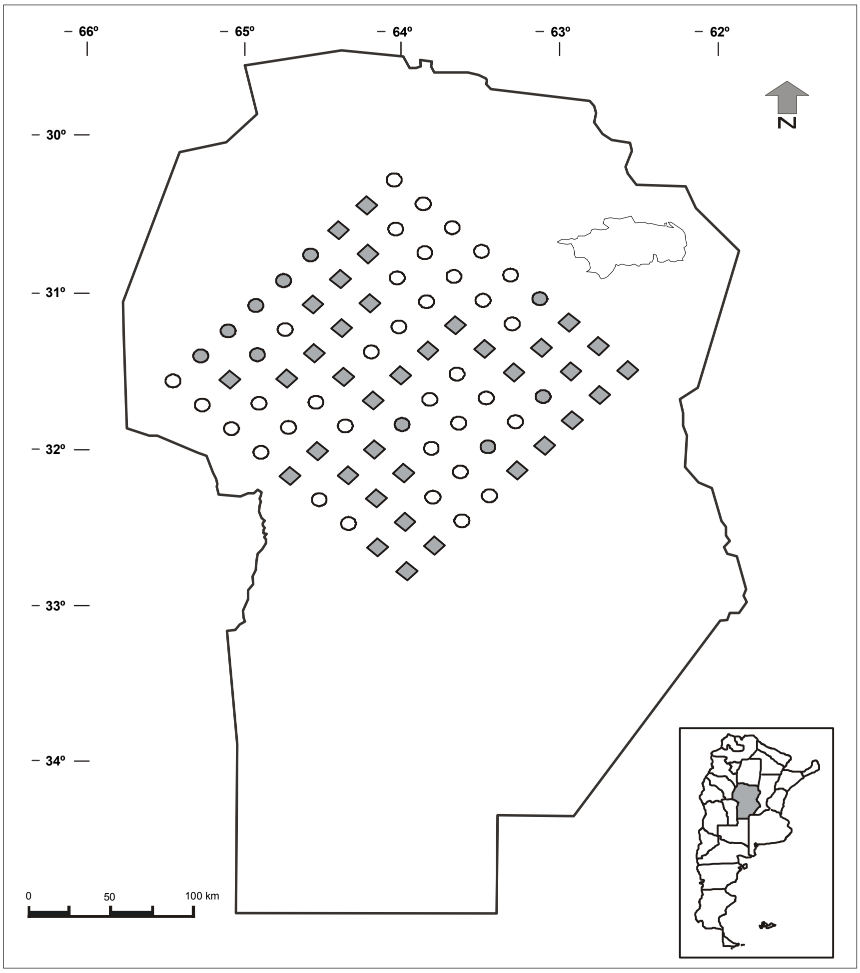


FIG.1. Location of study area and individual sampling points in the survey made in Córdoba, Argentina. Sampled points for R. ecklonii. Sampled points where R. eckonii was not found. Not sampled points in this survey

The lichen *Ramalina ecklonii* (Spreng.) Mey. & Flot. is widely distributed in Córdoba and its ability as an atmospheric pollution bioindicator has already been studied in relation to its physiological response to air pollutants [3–5]. The possibility of systematically assessing air quality from lichens physiological response was described about a decade ago [6] and has been studied for some species in Argentina [7]. In this work *R. ecklonii* is studied in terms of its capacity to accumulate heavy metals and other trace elements in order to gather information about air quality for an area of Córdoba province.

2. METHODS

2.1. Study area and sample collection

The study area covered 50,000 km^2 (Figure 1) and for sampling purposes it was divided according to a square pattern grid, each square of 25 by 25 km. The samples were collected at each intersecting point and the collection sites were located at least 500 m from major routes and highly populated areas and at least 300 m from streets with lower traffic density. At each site, a pool of 40–50 individuals randomly taken along the four cardinal directions within an area of 100 × 100 m was collected.

2.2. Sample preparation and analysis

Multielemental analysis was carried out using instrumental NAA and AAS was used for some metal determination. Previous to AAS, the samples (kept at room temperature) were washed with cold bi-distilled water. For washing purposes, each sample was put into a white nylon mesh bag (previously washed with bi-distilled water) and submerged in a glass of bi-distilled water for about 5 seconds while rotating it 180°, repeating the procedure three times. Then, the samples were put in a Petri capsule and dried in oven at 50 ± 2 °C for 72 hours, ground in a porcelain mortar, homogenised and dried in oven till constant weight. From this material, 0.5 g (dry weight) was taken for metal quantification by AAS. For NAA, the samples were washed in the same way and let dry at room temperature in a clean area. The material was then ground in an agate mortar with the aid of liquid nitrogen addition and then freeze-dried for 24 hours.

For AAS determinations, masses of about 0.5 g of dry material were reduced to ashes at 650°C for 4 hours. The ashes were digested with HCl (18%): HNO_3 (3:1) at mild heat and the solid residue was separated by centrifugation. Finally the volume was adjusted to 50 ml with Milli Q water and analysed using a Buck Spectrophotometer Model 210-VGP in order to determine the concentration of Cu, Mn, Ni and Pb. Blanks of the digest were prepared and analysed in the same way [8].

For NAA determinations, masses of about 200 mg of freeze-dried material were sealed in high purity quartz ampoules for their irradiation, together with two certified reference materials, NIST 1633b Coal Fly Ash and IAEA V10 Hay Powder. The irradiations were done at the RA-3 reactor of the Ezeiza Atomic Centre (Argentine Atomic Energy Commission), of thermal flux 3.10^{13} $cm^{-2}.s^{-1}$ and 4.5 MW, during 8 hours. Instrumental NAA was performed measuring twice, after 6 and 30 day decay, for medium and long-lived nuclides. The measurements were carried out using GeHP detectors (30% efficiency, 1.8 keV resolution for the 1332.5 keV ^{60}Co peak) coupled to an Ortec 919 buffer multichannel module plus Gamma Vision software for data acquisition. Concentrations for the determined elements (As, Ba, Br, Ca, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Na, Rb, Sb, Sc, Sm, Ta, Tb, Th, U, Yb, and Zn) were calculated using software developed at the NAA laboratory. The precision on 12 sets of three replicates was estimated. All the determined elements had a precision better than 12%, except Ba (14%), Gd (17%), and U (14%). Control charts (z-values) [9] for inspection of the normalised concentrations of all elements in one control sample, for a series of measurements, were used. None of the values was outside the $|z| > 3$ range. As control samples, NIST SRM 1547 Peach Leaves, 679 Brick Clay, and 2709 San Joaquín Soil were used.

3. RESULTS AND DISCUSSION

Ramalina ecklonii pools from 38 sites were analysed using AAS and NAA as described previously. Table I shows mean, standard deviation, maximum and minimum, and coefficient of variation values for each element. Coefficient of variation values lay between approximately 33 and 50% for more than 48% of the analysed elements while for 29%, the values are between 50 and 67%. The highest coefficients of variation correspond to Ca, Cr, and Zn.

TABLE I. DESCRIPTIVE STATISTICS FOR *R. ECKLONII* (ALL VALUES IN ppm EXCEPT COEFFICIENT OF VARIATION IN%).

Element	**N**	**Minimum**	**Maximum**	**Mean**	**SD**	**Coeff. of variation**
As	38	0.30	4.95	2.09	0.96	45.9
Ba	37	5.57	44.2	19.0	9.6	50.2
Br	38	0.68	13.9	3.3	2.5	77.0
Ca	29	307	18,035	3600	3400	95.1
Ce	38	0.93	6.82	2.9	1.3	46.3
Co	38	0.24	1.38	0.59	0.28	47.8
Cr	38	0.79	11.94	2.09	1.9	90.5
Cs	38	0.12	0.68	0.32	0.13	39.5
Cu	37	2.50	16.89	6.8	4.1	60.4
Eu	38	0.02	0.12	0.054	0.025	46.0
Fe	38	535	2960	1410	650	46.2
Gd	36	0.03	0.48	0.23	0.11	47.1
Hf	38	0.07	0.49	0.20	0.10	52.8
K	35	400	15,250	3130	2480	79.5
La	38	0.36	3.28	1.74	0.75	43.1
Lu	38	0.0086	0.047	0.020	0.010	48.0
Mn	37	23.3	80.8	54	13	24.7
Na	38	74	1,302	460	240	53.2
Nd	30	0.58	4.39	1.64	0.80	48.9
Ni	37	1.63	3.21	2.21	0.37	16.8
Pb	37	0.77	1.24	0.96	0.13	14.0
Rb	38	2.38	12.51	5.4	2.1	40.0
Sb	38	0.034	0.39	0.10	0.06	64.0
Sc	38	0.19	1.14	0.51	0.23	46.1
Se	36	0.05	0.53	0.23	0.11	48.3
Sm	38	0.08	0.67	0.29	0.15	50.9
Ta	38	0.02	0.08	0.038	0.016	40.9
Tb	30	0.01	0.08	0.036	0.017	47.0
Th	38	0.14	1.02	0.48	0.20	42.3
U	38	0.013	0.34	0.11	0.067	60.9
Yb	38	0.04	0.28	0.113	0.060	53.1
Zn	35	5.07	154	42	38	92.1

Pearson correlation coefficients showed significant correlations at the 0.01 level for those elements characteristic of soil contribution. Correlation significant at 0.01 level was also observed for Ca and K; As with Cu, Pb, Sb, and a few rare earths (RE); Cu with As, Pb, and RE; Sb with As, Se, and some RE; Pb with As and Cu and also Zn with Cr. At 0.05 significance level, negative correlation was found for Pb and Br.

TABLE II. FACTOR ANALYSIS: ROTATED COMPONENT MATRIX

	Component					
	1	2	3	4	5	6
Th	.931		.105		.174	.132
Eu	.931		.133		.194	.157
Co	.926	.162		-.111	.191	
Sc	.912	.154			.178	.239
Cs	.911			.160	.118	.154
Fe	.906	.192	.167		.168	.193
Lu	.904		.170		.299	.162
Hf	.883		.238	.203	.252	.102
Tb	.875					-202
Yb	.874		.269		.287	.118
Se	.854		.192			
Ta	.845	.218	.105	.265		.180
Ba	.794		.249		.310	.200
Gd	.794	.163				
Sm	.743		.385	.191	.356	.177
La	.673		.471	.271	.306	.229
Rb	.649		.105	.123	-.161	-.131
Mn	.610	.236		-.461	-.164	.155
Nd	.602		-.389	.101		.383
U	.527		.522		.259	
Na	.523	.412	.178	.155	.364	.496
K		.941				
Ca	.130	.904				-.165
Sb	.303		.765	.168	-.187	
As	.211		.697	.455		.124
Pb			.301	.839	-.139	-.108
Cu	.263		.179	.771		.254
Br	.108	.494		-.540		.489
Zn	.201		-.150	-.155	.828	
Cr	.422	.191	.144		.724	
Ni	.147	-.140	.101			.864

Results from the application of factor analysis (using SPSS 8.0) are in Table II. Principal Component Analysis was used to generate the factors that were then subjected to Varimax rotation. By keeping those factors with eigenvalues higher than one, six factors were chosen, accounting for aproximately 83% of the total variance, which indicates that the results can be considered as statistically robust. The first factor, with high factor loadings of Th, Eu, Co, Sc, Cs, Fe, Lu, Hf, Tb, Yb, Se, Ta, Ba, Gd, Sm, La, Rb, Mn, Nd, and U, could be assigned to natural soil source while factor 2 (high loadings of Ca and K), to lichen physiology. All the remaining factors [10–12] could be connected to anthropogenic activities: factor 3, with high loadings of Sb, As, to coal combustion; factor 5 (Zn, Cr) to non-ferrous metallurgy, while the activity related to factor 4 (Pb, Cu) will have to be further investigated. Although strong contributions from Pb and Br are in the same factor (factor 4), they have opposite sign thus likely, not representing leaded gas contribution. Ni has a high factor loading in factor 6 which could be explained as representative of fossil-fuel plants.

Distribution maps for certain selected elements were drawn using SURFER 6.04, and Convert 2.1 for the conversion of the coordinates (Figures 2 and 3). As, Cu, Sb, and Pb show a similar distribution pattern for the higher values of concentration. Córdoba, the capital city of the province, is located in the high concentration rounded area, shown in the centre of the maps. Several other cities and villages are located along route 9, a major route running from Córdoba towards the southeast. Towards the west, there is a hilly area above 750 m.a.s.l., with mining and agricultural activities; in the centre and surrounding the capital city there is the so called metropolitan area with different industries located around the capital and some other smaller touristic cities. Towards the east (100–300 m.a.s.l.), the land is mainly used for intensive cattle raising and agriculture and diry farm activities, with some industries and cities. Zn and Cr, highly correlated, have their highest concentration values towards the east and southeast. The highest concentrations of Ni were towards the south, being also observed also high values near the capital city of the province. For those elements related to soil, the pattern of distribution is similar showing an increase of values towards the southeast.

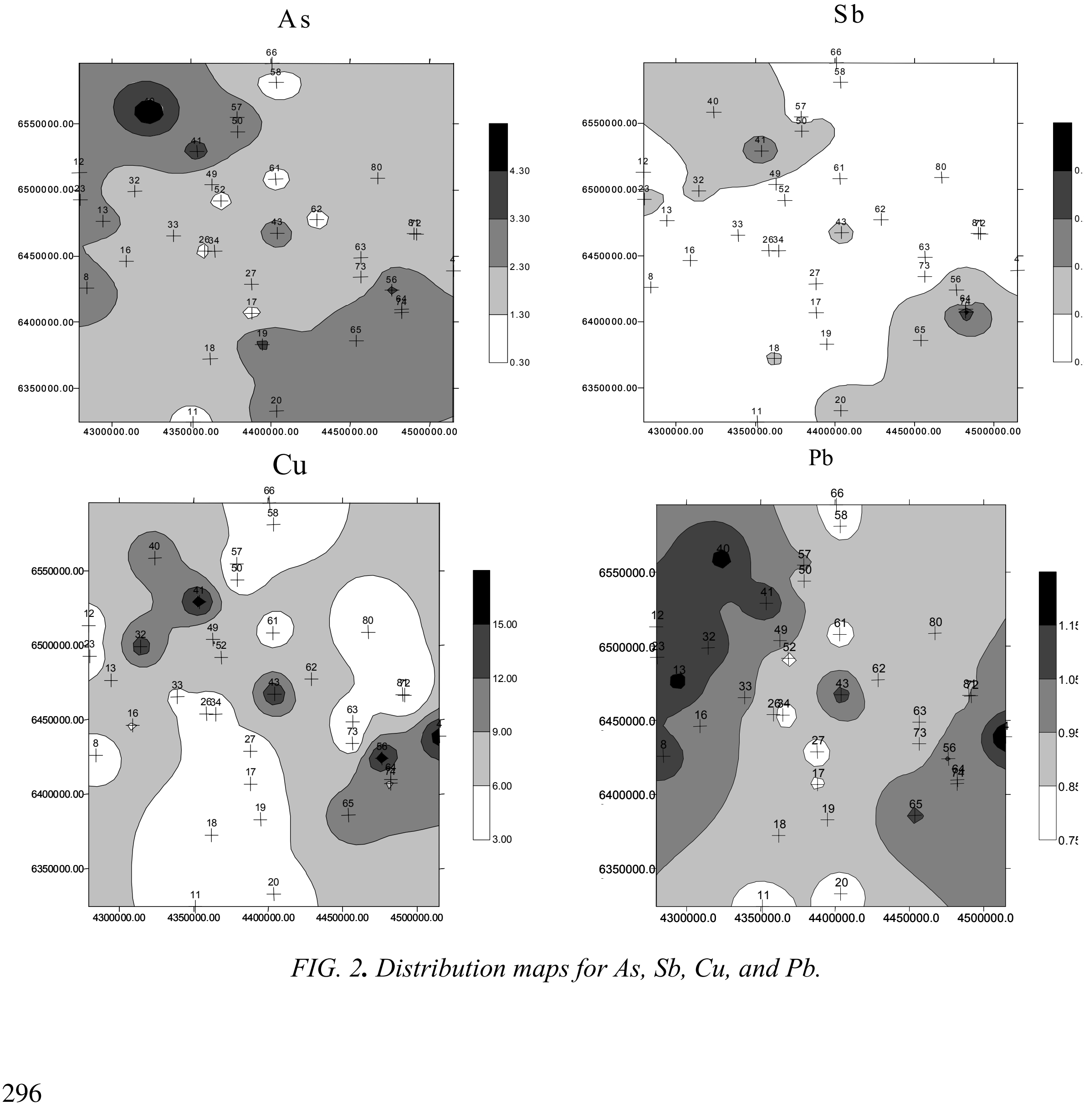


FIG. 2. Distribution maps for As, Sb, Cu, and Pb.

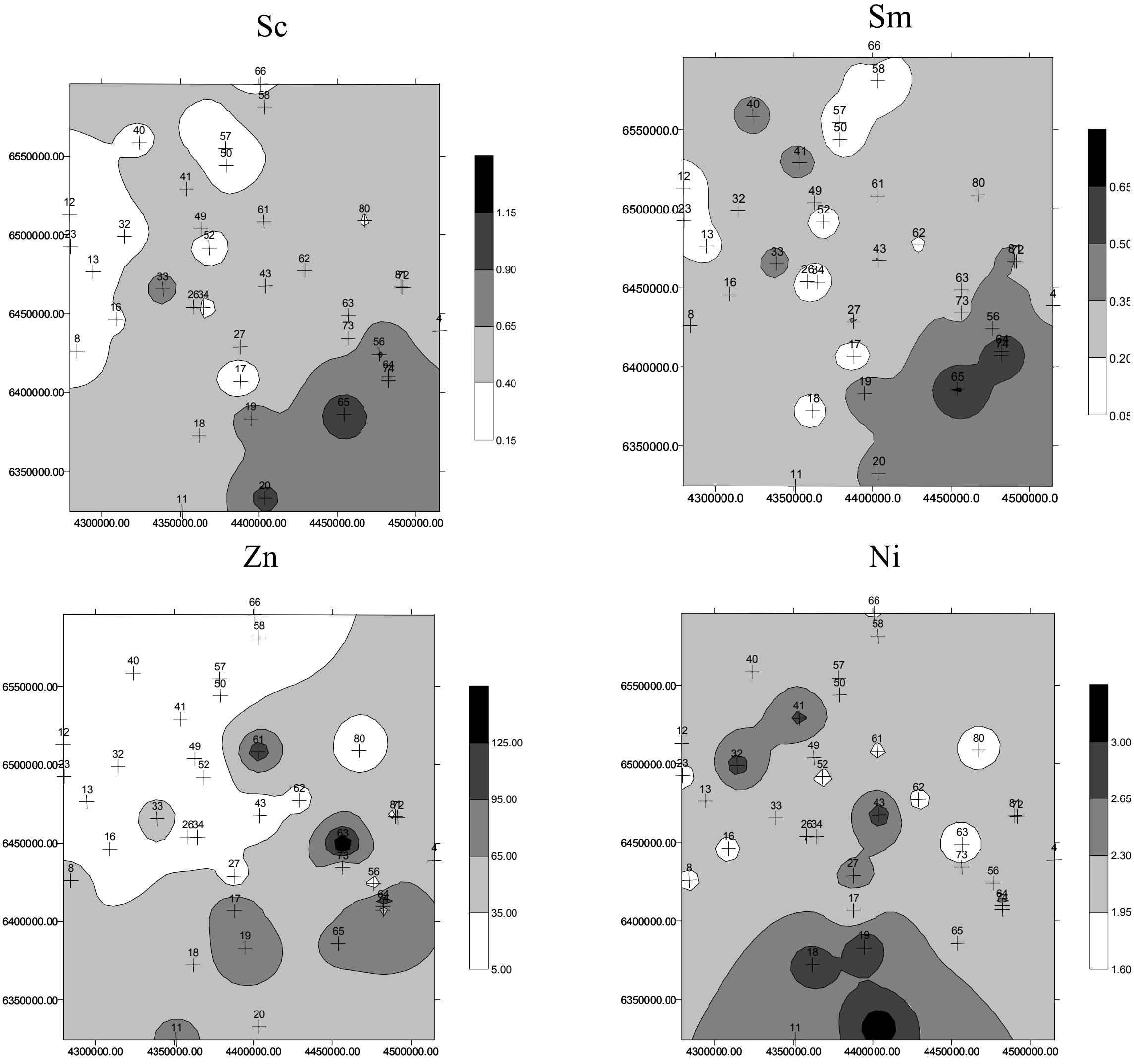


FIG. 3. Distribution maps of Sc, Sm, Zn and Ni.

4. CONCLUSIONS

The data already collected, although the first ones for the study area, have to be considered only as preliminary information for the monitoring of atmospheric deposition in the area. The influence of the capital of the province and of other cities and anthropogenic activities is reflected in the results shown but more studies have to be done. Another sampling campaign, covering the totality of the points, will be conducted in order to obtain new distribution maps and more complete information on source apportionment.

ACKNOWLEDGEMENTS

The authors wish to thank Lic. Oscar Comitto and Luis López of the Geology Unit of the Argentine National Atomic Energy Commission for their collaboration in mapping and also Lic. Ricardo Bianchini (from the same institution) for his help.

REFERENCES

[1] WORLD RESEARCH INSTITUTE. International Institute for Environment and Development, Internationaler Umveltatlas: Jahrbuch der Welt-Ressourcen, Vol 1, Ecomed, Landsberg, Germany (1988).

[2] MORETTON, J., GUASCHINO, H., AMICONE, C., BELETZKY, V., SANCHEZ, M., SANTORO, V., NOTO, B., "Contaminación del Aire en Argentina: aspectos generales, legislación y situación en Capital Federal y provincia de Buenos Aires", Ediciones Universo, Buenos Aires (1996) 1–127.

[3] LEVIN, A.G., PIGNATA, M.L., *Ramalina ecklonii* (Spreng.) Mey. & Flot. as bioindicator of atmospheric pollution in Argentina, Can. J. Bot. 73 (1995) 1196–1202.

[4] GONZÁLEZ, C.M., CASANOVAS, S.S., PIGNATA, M.L., Biomonitoring of air pollution in Córdoba, Argentina, employing *Ramalina ecklonii* (Spreng.) Mey. & Flot., Env. Pollut. 91 (1996) 269–277.

[5] GONZÁLEZ, C.M., PIGNATA, M.L., Effect of pollutants emitted by different urban-industrial sources on the chemical response of a transplanted lichen, *Ramalina ecklonii* (Spreng.) Mey. & Flot.,Toxicol. Env. Chem. (1998).

[6] GARTY, J., KARDISH, N., HAGEMEYER, J., RONEN, R., Correlations between the concentrations of adenosine tri phosphate, chlorophyll degradation and the amounts of airborne heavy metals and sulphur in a transplanted lichen. Arch., Env. Contam. Toxicol. 17 (1988) 601–611.

[7] PIGNATA, M.L., Studies about lichens and the atmospheric pollution in Argentina. In "Lichenology in Latin America: history, current knowledge and applications", (MARCELLI, M.P., SEAWARD, M.R.D., eds.), CETESB, Sao Paulo (1998) 155–164.

[8] PFEIFFER, H.N., BARCLAY-ESTRUP, P., The use of a single lichen species, *Hypogimnia physodes*, as an indicator of air quality in Northwestern Ontario. Bryologist 95 (1992) 3–41.

[9] BODE, P., VAN DIJK, C.P., Operational management of results in INAA utilizing a versatile system of control charts. J. Radioanal. Nucl. Dhem. 215 (1) (1997) 87–94.

[10] RANKAMA, K., SAHAMA, TH.G., "Geoquímica" , Editorial Aguilar (1949), pp. 862.

[11] STEINNES, E., RAMBAEK, J., HANSSEN, J,, Large scale multi-element survey of atmospheric deposition using naturally growing moss as biomonitor. Chemosphere 25 (1992) 735–752.

[12] MARKERT,B., "Instrumental element and multi-element analysis of plant samples. Method and aplications. John Wiley & sons. Chichester (1996) pp. 296.

ATMOSPHERIC METALLIC POLLUTANTS IN THE METROPOLITAN ZONE OF THE MEXICO VALLEY

T. MARTINEZ[1], J. LARTIGUE[1], P. AVILA-PEREZ[2], G. ZARAZUA[2]
L. CABRERA[1], S. TEJEDA[2], A. RAMÍREZ[1]

[1] National University of Mexico. Faculty of Chemistry, Mexico City, Mexico
[2] National Institute of Nuclear Research, Ocoyoacac, Mexico

Abstract

Total suspended particles (TSP) and respirable particles (RP) PM10 were determined by a gravimetric method during the autumn 1999, in samples from seven stations of the manual air quality network (GFD) of the MZMV (Metropolitan Zone of the Mexico Valley). The results show that the TSP fraction is lower than previously reported and such a decrease is related to large particles (natural soil origin) since the PM10 concentration remains constant. Respirable particles PM10 were found to represent an average of 50% of TSP. Metallic elements in air particulate matter (TSP and PM10) were determined by means of X ray fluorescence spectroscopy. The results show that, related to toxicity, Cu, Pb and Zn are the main environmental pollutants in the airborne particulate matter. Their concentrations are 100 to 300 times higher than the average geochemical concentration in the Mexico City's soil and the Earth's crust. The most contaminated zone is the Northeast, where major industries are located. Lead concentration has diminished to date, exception made of Xalostoc station, which frequently reports above the WHO, EPA and Mexican Norm values. Matrix analysis show some correlation between elements allowing us to distinguish natural and anthopogenic elements, these last ones from different sources. It was found that 68% of Pb is bounded to respirable particles PM10. Opposite to this, only 35% of natural elements are fixed to respirable particles.

1. INTRODUCTION

World Health Organization calculates that 70% of world urbane inhabitants did not breath healthy air, at least sometimes. In addition 10% breath air of marginal quality. In spite of that it was estimated in 50 000 to 100 000 the cases of deaths per year in U.S. by air contamination though in general, in that country, levels of contamination are lower than in Developing Countries [1].

In the MZMV the atmospheric pollution has reached significant and worrying levels. The annual emission of pollutants into the atmosphere in 1994 was higher than 4 millions tons of which 75.5% came from vehicles, 12.9% from industry and services and 11.21% from soils and vegetation. The main contaminating factor is fuel consumption which has increased from 16 millions liter per day (mld) en 1989 to 40 mld in 1996 [2].

Thousand of million people around the world suffer health problems caused by industry and atmospheric pollutants (metal and toxic wastes) [3]. Sustainable environmental development in populated areas like the MZMV (20,000,000 of inhabitants) counts for the recipe to improve the health. It has been established a correlation between the contamination level and respiratory sickness. It has also been estimated an increase of 4% in respiratory illness, and 5% in cough cases for an increase of 10 $\mu g\ m^{-3}$ in RP, PM10 (lesser than 10 μm) [4]. PM10 particles could be deposited in the tracheobronchial and alveolar region [4].

The airborne particulate size is considered a major factor in determining toxic effect [4]. Almost all of these correlations have been established based in the total mass of suspended particles (TSP). Nevertheless, it is necessary to take into account the finest particle (coarse, PM10, and fine PM2.5 fractions) to evaluate the inhabitants exposure.

The elemental composition of the aerosol is an important parameter for its characterization; besides, it helps us to locate the sources: natural (soils, vegetation, volcanic eruptions, etc), anthropogenic (fuel consumption in power generation or transport, metallurgical and industrial process, etc.) as well as to define its influence on health.

In the framework of an environmental program [5,6,7], the Faculty of Chemistry of the National University of Mexico, in collaboration with the National Institute of Nuclear Research and the Federal District Government (FDG) performed since 1995, the monitoring of metallic elements in the atmosphere, dry fallout [8], and soils.

The specific goals of this work are to estimate the concentration of TSP and PM10 by means of a gravimetric method. At the same time it has been determined the metallic elements (potentially hazardous to health) concentration in both fractions, by X ray fluorescence spectroscopy. As well, it was intended to establish the origin and correlation between them. Special attention is focused to lead concentration and evolution because it is a well-documented major human toxicant. Some of the health disorders associated with lead exposure affect renal, blood and neurological system.

2. EXPERIMENTAL

Air survey was carried out from September to November 1999 (autumn season) in seven stations of the manual air quality network (GFD) of the MZMV: Xalostoc (XAL), in the Northeast (NE); Tlanepantla (TLA), in the Northwest (NW); these two zones comprise 41% of the national industrial park and an old power plant; Merced (MER), in the Center (CE); Cerro de la Estrella (CES), UAM Iztapalapa (UIZ) and Virgencitas (VIR) in the Southeast (SE); Pedregal (PED) in the Southwest (SW).

Aerosol particles were collected in glass fiber filters for (TSP) and quartz filters for RP/PM10. Filters were exposed during 24 h in high volume samplers (General Metal Gord, GMG-1200) with an average flow rate of 1.5 m^3 min^{-1} in agreement with the Mexican Norm [8], for both TSP and RP/ PM10. Each station was equipped with two kinds of sampling equipment so that the data of both, for the same day, could be compared. The Laboratory of Bacteriology and Physical Chemistry of the Federal District Government (FDG) furnished data on particulate matter weight. Active deposition area was of 7” x 9” and a small section of this was measured in a Total Reflection X ray Fluorescence Spectrometer Model TX-2000 using a traditional 45^o geometry with an IAEA external standard. Analysis conditions were: Voltage, 40 KeV, Current, 30 mA; Counting time, 1000 s; Anode, Mo; Acquisition software, TXRF, acq32 (version 1.0.21); quantification software EDXRF32 acq32 (version 2.0.51).

3. RESULTS AND DISCUSSION

The total average of TSP was 167.63 ± 93.7 μg m^{-3} , lower than those reported from 1990 to 1997 [6,7,10] for the same City, higher than the U.S and Mexican Norms [2, 11] of 75 μg m^{-3} for an annual average, and lower than so-called IMECA 100 (Metropolitan Index Air Quality) [12] of 275 μg m^{-3}. Only Xalostoc (Northeast) and Virgencitas (Southeast) stations show values above the last one. Respirable and highly mutagenic [13] particles represented, in average, 50% of TSP. This is similar to those obtained previously [7,10,14], with a mean concentration of 68.9± 43.9 μg m^{-3} higher than the U.S and Mexican Norms [2, 11] of 50 μg m^{-3} for the annual average, lower also than the IMECA 100 [12], but similar to those reported for Athens in 1990 [15]. This PM10 concentration is similar to that reported previously for the MZMV [7,10]. This means that if TSP have decreased but PM10 not, this decrease is based mainly on large particles (probably because it was the end of the rainy season); so, the most dangerous fraction remains almost constant. Besides, it has been reported [4] that the fine fraction (2.5 μm) represents more than 70% of PM10; it means that around 50 μg m $^{-3}$ could be secondary or photochemical particles.

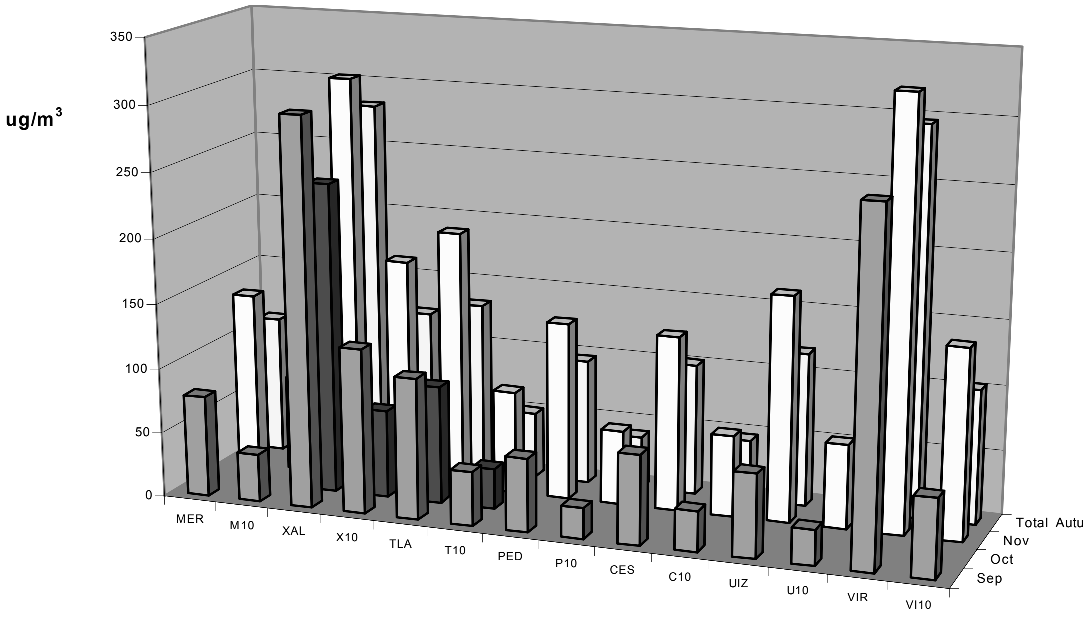


FIG. 1. Monthly concentration of TSP and PM10 for each monitoring station.

Figure 1 shows the monthly TSP and PM10 concentration as well as total average, by monitoring station. As it can be seen the highest concentration of TSP and PM10 were found in the Xalostoc (NE) and Virgencitas (SE) stations.

Tables I and II show the results for metallic elements concentration on TSP and PM10, respectively, for all monitoring stations S, Ga, Rb, Sr and Y, were also detected but not quantified because the external standard did not include them.

Regarding weight abundance, metallic elements can be distributed in three classes: major elements (Ca, Fe, Zn) with a concentration higher than 1 μg m^{3}; minor elements (K, Ti, Cu, Pb) with a concentration between 0.1 and 1 μg m $^{-3}$ (in the case of lead there was an exception for one sample from Northeast (XAL, November, 1999), with a concentration of 6.9 μg m $^{-3}$ in TSP and 5.5 μg m $^{-3}$ in PM10); and trace elements (V, Cr, Mn, Se, Ni), with a concentration between 0.01 and 0.1 μg m $^{-3}$.

Enrichment factors [10,16,17] (taking Si as reference) lower than 10, has been shown by K, Ca, Ti, Fe, Rb, Sr, which are soil related elements and have a significant contribution to the atmospheric pollution. Besides, their concentrations are similar (exception made of some stations in the Northeast and Southest zones) to those reported in 1974 [18], and 1997, for the same City [6,7]. Such values are also similar to previous soil [10], and dry fallout concentrations for the same city at the same sampling period [8]. Fe and Mn concentrations are higher and lower, respectively, to those reported for U. S. cities [19] and lower than prevailing standards for the Threshold Limit Values (TLV), for eight hours occupational exposure [20]. High Ca concentrations have correlation with soil composition and wind direction from Northeast-Southwest, with velocities ranging from 0.5 to 1.5 (m s $^{-1}$), around 10.00 h in the morning. Wind velocity reaches its highest value (4.5 m s$^{-1)}$ from 17:00 to 18:00 h; in the night, wind velocity comes back to the morning values [21]. It seems these natural metallic elements are associated with large TSP since PM10 concentration represents around 35% of TSP. Iron together with Cu, Mn, and Zn is considered as an essential metal, potentially hazardous to health. Ti concentration is similar to that of soil [10].

TABLE I. AUTUMN CONCENTRATION IN TSP DETERMINED BY X ray FLUORESCENCE (μg.m^{-3}).

	Sample		K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu	Zn	Se	Br	Pb	TSP
	zone	Date														
NE	XAL	sep-99	*	7.9	*	*	0.24	0.102	5.77	0.012	0.231	2.71	*	0.027	0.07	298
	XAL	oct-99	*	3.86	*	0.092	*	0.056	4.04	*	0.21	1.87	*	*	0.06	238.8
	XAL	nov-99	*	11	0.71	*	*	0.12	15.05	0.009	*	8.46	*	0.036	6.91	310.28
NW	TLA	sep-99	*	3.17	*	0.16	*	0.48	1.71	*	0.11	*	*	0.007	0.155	108.47
	TLA	oct-99	0.93	3.95	*	0.29	*	*	2.05	*	0.1	2.21	*	0.008	0.15	90.65
	TLA	nov-99	*	9.39	*	0.13	*	0.07	5.72	0.032	0.23	5.19	0.02	0.03	0.31	198.61
CE	MER	sep-99	*	1.93	*	0.18	*	*	0.94	0.005	0.11	1.19	*	*	0.05	77.69
	MER	nov-99	*	4.4	0.72	*	*	0.031	3.26	0.007	0.09	1.46	*	*	0.123	135.79
SE	CES	sep-99	0.22	1.4	*	*	0.017	*	0.83	*	0.08	*	*	*	0.02	68.66
	CES	nov-99	*	5.83	0.09	*	*	0.052	3.41	0.006	0.09	3.75	*	*	0.053	134.03
	UIZ	sep-99	0.22	2.04	*	0.03	0.028	0.033	0.73	0.011	0.12	2.18	*	0.003	0.031	63.94
	UIZ	oct-99	*	8.43	*	*	*	0.07	5.02	0.009	0.18	5.26	0.07	0.022	0.134	172.6
	VIR	sep-99	*	4.18	*	0.14	*	0.03	3.43	*	0.23	*	*	0.003	0.025	265.7
	VIR	oct-99	*	7.25	0.38	0.06	*	0.08	7.06	*	0.47	1.41	0.003	0.017	0.085	326.2
SW	PED	sep-99	*	1.11	*	*	0.03	0.02	0.51	0.004	0.16	0.6	*	0.004	0.038	56.57
	PED	nov-99	*	2.90	*	*	*	*	*	*	0.37	*	*	*	0.053	134.03
	Arithmetic Mean		0.46	4.92	0.48	0.13	0.025	0.095	3.97	0.011	0.19	3.02	0.01	0.015	0.55	167.63
	Standard Deviation		0.41	3.03	0.3	0.08	0.006	0.12	3.7	0.009	0.11	2.27	0.008	0.012	1.76	93.69
	Geometric Mean		0.36	4.02	0.4	0.11	0.024	0.063	2.69	0.009	0.16	2.37	0.008	0.011	0.09	143.08

*under minimum detection level.

V, Ni, Cu, Zn, Br, and Pb, were found in airborne particulate matter in a higher concentration than that in soil. These concentrations range from 5 to 300 times the soil concentration. These metallic elements have been reported with high enrichment factors (above 10) [10,16,17]; and therefore may be considered as anthropogenic from several sources.

V and Zn concentrations are higher than those reported for the same City (from 1974 to 1997) [18,6,7] and for some U.S. cities [19]. They are also higher than the soil and dry fallout concentrations determined in the same sampling period for the MZMV [11,8].

Br and Se have a higher concentration than the average found in some U.S cities. In the case of bromine, it is also higher than that of the dry fallout for the same city and sampling period but lower than values obtained in 1974 and 1990 as well as in 1995 for Cuajimalpa station [5]; and also lower than the TLV limit [20].

Pb total average concentration is lower than those reported from 1990 to 1997 [10,6,7,22]. It is due to the conversion from leaded gasoline to unleaded type in 1996. Additionally, regulations to reduce motorized traffic are in force as a part of stricter environmental law [2]. There was only one exception at the Xalostoc station (Northeast) in November 1999, when lead concentration was 6.9 μg m $^{-3}$ in TSP and 5.5 μg m $^{-3}$ in PM10. Averaging the three months of sampling period, according to the Mexican Norm [23], the resulting concentration for Xalostoc (2.5 µg m $^{-3}$) was higher than the indicative maximum average value of WHO [24], EPA [25], and Mexican Norm (1.5 µg m $^{-3}$) [23]. It is evident that the lead problem has been decreasing to date, yet it is still present. So, Mexico City inhabitants may be significantly exposed to this element as a result of environmental retention (e.g. soil and dust) of previous release, the legacy of past domestic uses (e.g. pipes and paints) and re-emission of soils [17]. Evaluating its concentration, assessment of the relative contribution of different sources and the intake route, are critical because Pb is a major toxic element with claimed effects on intelligence and behavior of young children, antagonist to central nervous system and inhibitor of macrophague alveolar activity [25]. In Mexico City, seven of ten newborn babies before 1994 [1] have had blood levels higher than the WHO and Mexican Norms [26]. Besides, it was found that 68% of lead was bounded to PM10 though other authors mention 90% [17].

TABLE II. AUTUMN CONCENTRATION OF CONTAMINANTS IN PM10 DETERMINED BY X ray FLUORESCENCE (µg.m^{-3}).

Sample			K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu	Zn	Se	Br	Pb	PM10
zone		Date														
NE	X10	sep-99	0.62	3.31	0.17	0.05	*	0.034	1.88	0.004	0.09	0.214	*	0.022	0.048	126.81
	X10	oct-99	0.35	2.16	0.12	0.05	0.006	0.041	1.94	0.008	0.1	0.17	*	0.004	0.037	67.64
	X10	nov-99	*	4.13	0.71	*	*	*	5.06	*	0.31	6.38	*	0.02	5.46	173.15
NW	T10	sep-99	0.24	0.94	0.035	0.05	0.004	0.023	0.47	0.008	0.035	0.32	*	0.005	0.105	41.76
	T10	oct-99	0.19	1.0	0.06	0.08	*	0.025	0.4	0.009	*	0.22	*	0.003	0.074	31.25
	T10	nov-99	*	2.5	*	*	*	0.043	1.42	*	0.073	4.19	0.008	0.005	0.132	78.98
CE	M10	nov-99	*	2.29	0.72	*	*	0.031	1.20	0.008	0.06	3.66	*	0.007	0.095	73.15
	M10	sep-99	0.09	0.23	0.04	0.04	0.005	0.01	0.24	0.006	0.03	0.04	*	0.003	0.027	36.94
SE	C10	sep-99	0.21	0.69	0.04	0.06	0.004	0.012	0.34	0.007	0.04	0.03	*	0.004	0.021	30.93
	C10	nov-99	*	*	1.73	*	*	0.032	1.10	*	0.05	2.26	0.002	0.007	0.061	62.36
	U10	sep-99	*	*	0.02	0.02	*	*	0.16	0.006	0.04	0.014	*	*	0.011	27.13
	U10	nov-99	*	3.11	*	*	*	*	1.26	*	0.1	5.7	*	*	0.053	64.68
	V10	sep-99	0.22	1.0	0.08	0.05	*	0.016	0.8	0.009	0.78	0.04	*	0.004	0.018	60.42
	V10	nov-99	*	1.17	*	*	*	0.093	3.42	*	0.65	5.0	*	0.012	0.063	145.83
SW	P10	sep-99	*	0.24	0.03	0.02	*	0.014	0.19	0.005	0.052	0.031	*	0.004	0.023	23.66
	P10	nov-99	*	1.27	*	*	*	*	0.63	*	0.17	3.85	*	*	0.024	57.22
	Arithmetic Mean		0.27	1.72	0.18	0.05	0.0045	0.03	1.28	0.007	0.17	2.0	0.005	0.008	*	68.87
	Standard Deviation		0.17	1.17	0.26	0.018	0.0007	0.02	1.32	0.002	0.23	2.37	0.004	0.006	1.35	43.92
	Geometric Mean		0.24	1.29	0.08	0.04	0.004	0.026	0.81	0.007	0.095	0.43	0.004	0.006	0.06	58.06

*under minimum detection level.

Correlation coefficients for TSP and PM10 of metallic elements have been obtained. In spite of data, few good correlation coefficients were found between some elements, a fact suggesting grouping to common sources. High correlation between Ca, K, Ti, Mn, and Fe, in TSP and PM10, show a common source: the soil. In spite of Fe showing also a good correlation with automotive labelers source like Pb and Br (r >0.7), we think Fe is essentially of natural origin.

Cu is well correlated with Zn and Br; Pb is well correlated with Br, all of them forming the automotive group [16]. Ni is well correlated with V (PM10) showing an industrial fuel origin [16]; it is also possible to assume a different source for Ni because it is well correlated with Zn, which is not appreciably expelled in fuel industrial combustion. A larger set of samples and the use of complementary methods will allow us to a better understanding of sources and mechanisms.

Figure 2 shows the contaminant concentration averaged by zone for the complete sampling period. As it can be seen in general, the most polluted (metallic elements derived from industrial activities) zones are the Northern ones. Wind transports contaminants to Southeast, which shows high levels. Contaminant concentration, averaged by month, shows that November had the highest levels, because it was almost winter and thermal inversions occurred frequently, beginning at night and remaining to about 10:30; the inversion layer height varies from 200 to 500 m. Maximum mixing depth is about 200 m; similar to that one observed in Japan during summer [27].

Figure 3 shows the pollutants (determined by XRF) evolution in the MZMV. As it can be seen the mean concentration of Cu, Ni, Br, and Pb have diminished, notably the last one from 1997 to date. Zn and Fe concentrations have slightly increased, that could be due to contributions from punctual sources at northern stations. V sometimes comes down and sometimes goes up but remains around the 1990 concentration.

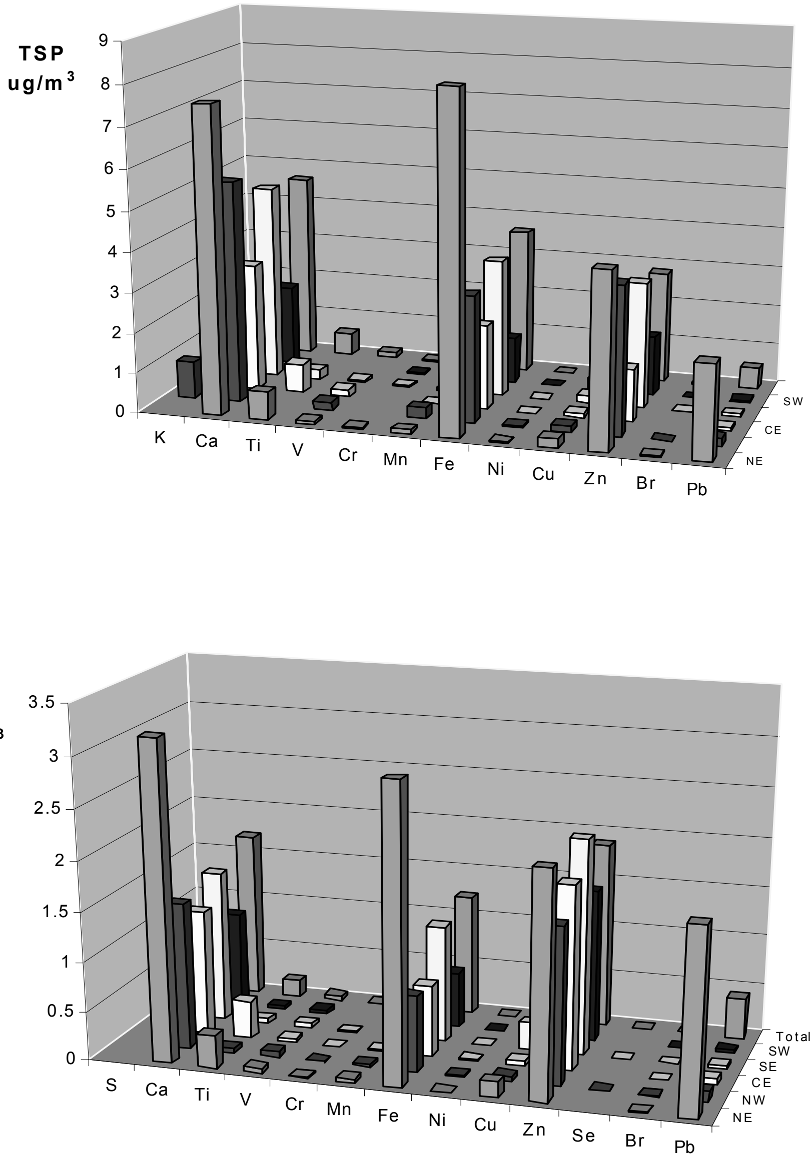


FIG. 2. Monthly contaminants concentration (TSP and PM10)

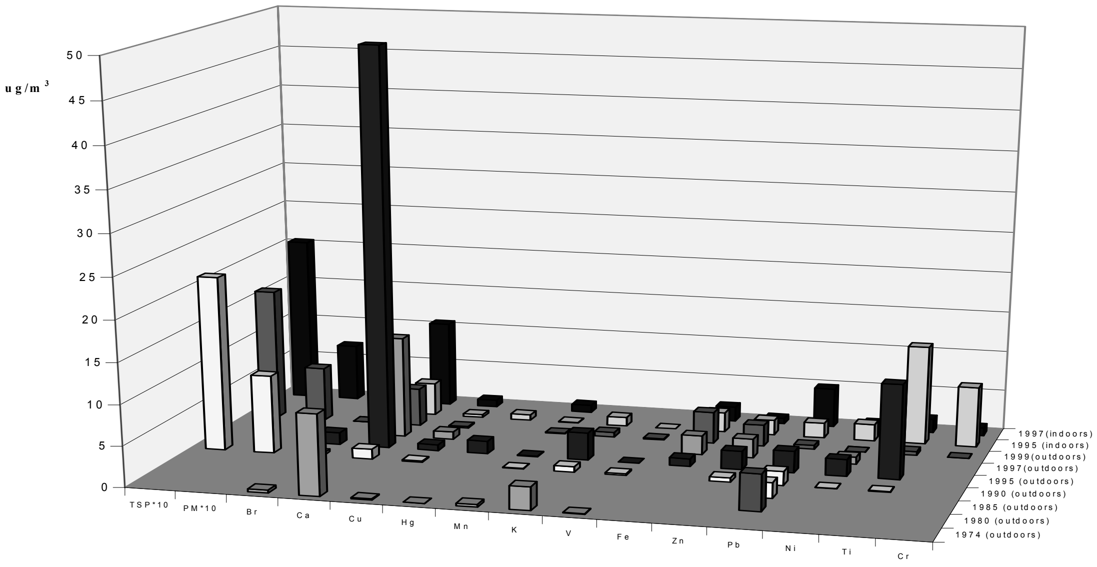


FIG. 3. Pollutants evolution in the Metropolitan Zone of Mexico Valley

4. CONCLUSIONS

Results show that TSP concentration is slightly lower than previously reported. Respirable particles PM10 were found to represent an average of 50% of the TSP and this ratio has not changed significantly in recent years. Toxic anthropogenic metals, such as Pb, were found to be associated in 68% to PM10, opposite to natural metals associated in an average of 35% to large particles TSP.

Pb, Zn, and Cu are the main environmental pollutants in the airborne particulate matter, regarding toxicity. The atmospheric aerosol/soil enrichment in heavy metals is greater in the case of Cu, Zn, and Pb. The most contaminated zones are the northern stations, where major industries are located. Lead concentration has diminished to date, exception in the Northeast station (Xalostoc) where sometimes it is above of WHO, EPA, and Mexican Norm. Elemental analysis of airborne particulate matter and matrix correlation coefficients let us to establish the following groups of elements: those that have an unequivocal soil origin and appear in very similar concentrations (K, Ca, Mn, Fe); elements having a mixed origin (anthropogenic or natural), risky to health (Cr, V, Ni, Br, Se Ni), in concentrations 5 to 100 times the average of the Mexico City soil and Earth's crust values; and others, fitting with the last one group but with concentrations 300 times the soil concentration, such as Zn and Pb. All of them originated in automotive and industrial fuels, though some with a probably different origin.

REFERENCES

[1] MOORE, C., La calidad del aire urbano. Series: Documento Verde 1. Servicio cultural e Informativo de los Estados Unidos, (1994).
[2] INEGI, SEMARNAP. Estadísticas del Medio Ambiente. Informe de la Situación General en Materia de Equilibrio Ecológico y Protección al Ambiente, 1995-1996. Instituto Nacional de Estadística, Geografía e Informática, México, (1998).
[3] NAIR, G., PARR, R.M., CASTELINO J., OIEA Bol. **36 (4)** (1994) 10.

[4] MELGAR, M., RUIZ, M. E. Composición de las partículas suspendidas en la Zona Metropolitana de la Ciudad de Méxic. Tecno-Fórmula Ambiental **1**, No. 2 Abril/Mayo (2000) 10-11.

[5] MARTINEZ T., LARTIGUE, J., NAVARRETE, M., *et al*. Determination of pollutants in dwellings by neutron activation analysis and X ray fluorescenc. J. Radioanal. Nucl. Chem. **216 (1)** (1997) 37-39.

[6] MARTINEZ, T., LARTIGUE, J., AVILA-P, P., *et al*. Analysis of Aerosol Samples in Mexico City J. IAEA TEC DOC **1152** (2000) 209-214.

[7] MARTINEZ, T., LARTIGUE, J., LOPEZ, C., *et al*. INAA of aerosol samples in Mexico City J. Radioanal. Nucl. Chem. **244 (1)** (2000) 127-131.

[8] MARTINEZ, T., LARTIGUE., J., AVILA-PEREZ, P., *et al*. X-ray fluorescence analysis of dry deposit samples in Mexico City Presented in Fith International Conference on Mthods and Applicationns of Radionalitical and Chemistry-MARC V, Hawaii, April, (2000), 9-14.

[9] NOM-CCAM-002-ECOL. Diario Oficial, 18 octobre (1993).

[10] M.BARBIAUX. Characterization of respirable particulate matter in Mexico City. Thesis for Degree. Graduate College of the University of Chicago (1990).

[11] Diario Oficial, 23 Diciembre (1994).

[12] Indice Mexicano de la Calidad del Aire (IMECA). Diario Oficial, 29 noviembre (1982).

[13] VILLALOBOS-PIETRINI, R., BLANCO, S., GOMEZ-ARROYO, S. Mutagenecity assessment of airbone particles in Mexico City. Atmospheric Environment **29 (4)** (1995) 517-523.

[14] BRAVO, H., SOSA R.., TORRES, R., "Particulas suspendidas en la Cidad de México". Memórias. Reunión sobre Salud y Ambiente en la Ciudad de México, México, (1989) 71-79

[15] SCHEFF, P.A.., VALIOZIA, C., Characterization and sources identification of respirable particulate matter in Athens. Atmos. Envrironment. 24 (1990) 203-211.

[16] MIRANDA, J., MORALES, J.R., CAHILL, T.A.., et al.A study of elemental contents in atmospheric aerosols in Mexico City. Atmosfera 5 (1992) 95-108.

[17] PERSON, A, PETITE-COVIAUX, F, LE MOULLEC, Y, et al. Contribution des principales sources en metaux et metalloides a la pollution particulaire dam l'aggloremation parisienne" Pollution Atmosphérique, Juillet-Septembre (1993) 75-108.

[18] NAVARRETE, M., GALVEZ, L., TZONLIMATZIN, E., AGUILAR, C. Determination of particulate air pollutants in Mexico City usin Activation Analysis. Radiochem. Radioanal. Lett., 19 (1974) 163-170

[19] THOMPSON, R. J., Collection and Analysis of Airborne metallic elements. (Risby, T. H. Ed): Ultratrace Metals Analysis in Biologycal Sciences and Environment. American Chemical Society. Washington, D.C. (1979) 54-72.

[20] American Conference of Governmental Industrial Hygienists (ACGIH): Theshold Limit Values (TLV) and Biologycal Exposure Indices for 1985-1986. ACGHI, Cincinnati, Ohio, (1985).

[21] Informe 97, Precipitaciones Acidas en la Zona Metropolitana de la Ciudad de México. Red Automática de Monitoreo Atmosférico. Secretaría del Medio Ambiente del Gobierno del D.F. (1998).

[22] ALBERT, L. A., BADILLO, F. Environmental Lead in Mexico. Review of Environmental Contamination and Toxicology 117 (1991) 1-46

[23] NOM -026-SA 1, Criterios para evaluar la calidad del aire ambiente con respecto al plomo. Diario Oficial, 23 diciembre (1994).

[24] FORTOUL, T., BARRIOS, R., Metales y partículas. Daño al aparato respiratorio. Memorias sobre Salud y Ambiente en la Ciudad de México. Mexico. (1989) 227-231

[25] WHO. Air quality guidelines for Europe. Report 23 Copenhagen (1987) 426.

[26] EPA-600/8-83/028, F. Air Quality Criteria for Lead, 1 (1986).

[26] NOM-EM-OO4-SSA1-1997, Salud Ambiental. Criterios para la determinación de plomo en sangre. Acciones para proteger la salud de la población no expuesta ocupacionalmente. Métodos de prueba.

[27] JICA. The study of Air Pollution Control Plan in the Federal District. Final Report. Mexico City (1998)

MATA DE SANTA GENEBRA, SP, BRAZIL: CAN MINERAL CYCLING IN URBAN FORESTRY FRAGMENT REVEAL ANTHROPOGENIC ACTIVITIES?

E.J. de FRANÇA[1], M.A. BACCHI[1], E.A. de NADAI FERNANDES[1], S. GANDOLFI[2]

[1] Centro de Energia Nuclear na Agricultura, Universidade de São Paulo,
CENA/USP Piracicaba SP Brazil
[2] Escola Superior de Agricultura Luiz de Queiroz,
Universidade de São Paulo, ESALQ/USP, Piracicaba SP Brazil

Abstract

The Mata de Santa Genebra is the largest fragment of semi-deciduous forest in the area of Campinas, second largest industrial region of the State of São Paulo, Brazil. Some research institutions have dedicated efforts to the development of an appropriate model of management, trying to maintain the biodiversity of this reserve. In this context, mineral cycling becomes an important factor to be understood, because it is essential to sustain life within an ecosystem. To assess mineral cycling, 24 elements were determined by INAA in the soil, litter, and leaves compartments of *Esenbeckia leiocarpa* and *Pachystroma longifolium*, the dominant plant species in the central area. The results confirm the recovery of some elements during the senescence process and the plant/soil concentration ratios were clearly different for both species for elements not translocated. Evidence for Br accumulation in the plants was noted, which could be related to an atmospheric contribution.

1. INTRODUCTION

Biodiversity in extensively modified areas is restricted to fragmented landscapes, usually appearing as forestry fragments. Frequently, this situation is a consequence of irrational and intense use of natural resources with the isolated areas being more susceptible to biodiversity loss when compared to the original ecosystem. It is not possible to eliminate human influence from urban forestry fragments, since these areas are historical evidence of human action. However, these forestry fragments must be conserved for as long as possible.

The conservation of a forestry fragment depends mainly on the type and intensity of the impact from human activities and the mechanisms involved in its maintenance must be well understood. In Brazil, federal, state, and municipal laws have supported efforts related to the conservation of such environments.

Mineral cycling can be considered as an indicator of sustainability, since it is essential for the operation and conservation of the components of an ecosystem [1]. Emphasis on mineral cycling studies is usually given to nutrients, however other mineral elements have great scientific and practical application, such as the rare earth elements (REE) and pollution indicators, like As, Br, Cr, Sb, and Zn [2]. To facilitate cycling studies, an ecosystem can be divided into several interrelated compartments [3]. By determining the composition of each compartment, cycling and distribution of the mineral elements can be assessed.

The Mata de Santa Genebra, protected by law since 1983, is located in a heavily industrialized area, subject to many disruptive factors. With effort of six Brazilian research institutions, an appropriate management model has been introduced, complying with reserve conservation and social requirement [4]. About 200 studies have been completed in the area, denoting its scientific importance, but none dealing with mineral cycling.

In this study, the elementary composition of the superficial soil, litter, and leaves compartments was determined, aiming at mineral cycling and distribution studies in the Santa Genebra's area. Data of this kind can contribute to the conservation program being implanted. Determinations were performed by instrumental neutron activation analysis (INAA), which enables multielemental determination without the need for chemical treatment of samples, besides being frequently applied to the study of plants [2–5].

2. MATERIAL AND METHODS

The Mata de Santa Genebra is the largest forestry fragment in the area of Campinas, about 2.5 km^2, being located in the Barão Geraldo district (22°44'45"S, 47°06'33"W). This area is the second industrialized center of São Paulo state, with a seasonal tropical climate and about 1,360 mm of annual precipitation. This forest is surrounded by urbanized areas, highways with intense traffic, and several industries, including a petroleum refinery. The administration of this reserve is under the responsibility of the Fundação José Pedro de Oliveira, its usage being restricted to scientific and cultural activities. Although areas of primary and secondary successions are presented along the borders of the fragment, the forest can be classified as semi-deciduous, since the trees partially or totally shed leaves during the dry and cold season [4].

The central region of the forest, which grows on a clay oxissol, was chosen for this study. Two species are more common in the vegetation, *Esenbeckia leiocarpa* Engl. and *Pachystroma longifolium* (Ness) I.M. Johnstons, from the Rutaceae and Euphorbiaceae families, respectively. These two species account for about 65% of the total number of trees, with a larger contribution of *Pachystroma longifolium*.

Assuming that predominant species absorb larger amount of nutrients, produce and drop more leaves, they will consequently have larger participation in the process of mineral cycling [6]. Therefore, samples were taken from ten *Pachystroma longifolium* trees and four *Esenbeckia leiocarpa*. From each species, about 5 kg of mature leaves were collected, and also 4 kg of the more recently shed leaves that compose the litter sample. The soil sampling was confined to the superficial layer, usually more explored by the roots [3], with material being taken from depths of 0–10 and 10–30 cm. Different samples were taken from the surroundings of each species, allowing the influence of litter on the soil composition to be studied.

The leaves were thoroughly washed with bi-distilled water, and oven-dried at 60°C to constant mass. Soils were dried at 85°C, being all samples subsequently ground. Three portions of 150 mg of material were inserted into polyethylene vials, 9 mm high and 8 mm in diameter, especially manufactured for neutron activation. Together with each vial, a 10 mg Ni-Cr wire was used as flux monitor during irradiation. The activation was accomplished by irradiating with a thermal flux of 10^{13}neutrons cm^{-2} s^{-1} for 8 hours, in the nuclear reactor IEA-R1 of the Instituto de Pesquisas Energéticas e Nucleares (IPEN/SP).

For the gamma radiation measurement, two germanium detectors were used, models GEM 45190 and GMX 50220 from Ortec, with relative efficiencies of 45% and 50%, respectively. The concentrations were determined by the k_0-method [7]. NIST reference materials Apple Leaves (SRM1515), Corn Bran (RM8433), Tomato Leaves (SRM1573a), and Montana Soil (SRM2711), were analyzed for quality control.

3. RESULTS AND DISCUSSION

The concentrations obtained for the reference materials were compared to the respective certified values (Table I), indicating that the analytical procedure was adequate for the determination of 24 elements in plant and soil matrices. For the soil, leaves, and litter compartments of *Esenbeckia leiocarpa* and *Pachystroma longifolium*, these elements were also determined (Table II), although some concentrations are below the detection limit.

For some elements, the concentrations in leaves and litter are higher than reference values found in literature [8,9,10], especially for *Pachystroma longifolium* that presented high Co and REE content. The accumulation of Co for the Euphorbiaceae family has already been discussed in other studies [11,12] and although the REE content in *Pachystroma longifolium* seem to be above normal, similar concentrations were found for *Solanum lycocarpum* growing in "cerrado" ecosystems [13]. Such facts corroborate the idea that environmental influences make it difficult to establish average reference values for elemental concentration in plants [14].

TABLE I. CERTIFIED AND OBTAINED VALUES (mg kg^{-1}) FOR REFERENCE MATERIALS

Element	**SRM 1515 Apple Leaves**		**RM 8433 Corn Bran**		**SRM 1573 Tomato Leaves**		**SRM 2711 Montana Soil**	
	Certified	Obtained	Certified	Obtained	Certified	Obtained	Certified	Obtained
As	0.038	<0.3	0.002*	<0.2	0.112	<0.2	105	105
Ba	49	46	2.4	2.33	63*	62	726	722
Br	1.8*	1.7	2.3	2.35	1300*	1335	5*	5.0
$Ca^{1,2}$	1.526	1.47	420	440	5.05	4.75	2.88	2.84
Ce	3*	3.3	-	-	2*	1.8	69*	73
Co	0.09*	0.09	0.006*	0.006	0.57	0.58	10*	9.8
Cr	0.3*	<1.8	0.11*	<2.5	1.99	<1.8	47*	50
Cs	-	-	-	-	0.053*	0.058	6.1*	6.7
Eu	0.2*	0.24	-	-	-	-	1.1*	1.0
Fe^{2}	83	81	14.8	14.2	368	367	2.89	2.86
Hf	-	-	-	-	0.14*	0.145	7.3*	8.1
$K^{1,2}$	1.61	1.61	566	521	2.7	2.7	2.45	2.47
La	20*	20.81	-	-	-	-	40*	37
Na	24.4	28.3	430	410	136	135	1.14	1.20
Nd	17*	17.8	-	-			31*	34
Rb	10.2	9.7	0.5	0.43	14.89	14.4	110*	120
Sb	0.013*	0.014	-	-	0.063	0.063	19.4	19.1
Sc	0.03*	0.03	-	-	0.1*	0.10	9*	10.1
Sm	3*	3.0	-	-	-	-	5.9*	5.85
Sr	25	24.3	-	-	85*	85	245.3	255.8
Tb	0.4*	0.35	-	-	-	-	-	-
Th	0.03*	0.03	-	-	0.12*	0.126	14*	14
Yb	0.3*	0.16	-	-	-	-	2.7*	3.1
Zn	12.5	12.5	18.6	17.3	30.9	30.8	350.4	350

* non-certified concentration values.

[1] Ca and K given in% for SRM 1515 and SRM 1573.

[2] Ca, Fe, K, and Na, given in% for SRM 2711.

Most of the elements that could indicate human activities presented concentrations below the detection limits. However, the amount of Br in the leaves of both species was found to be high [9], which may point to anthropogenic influence on mineral cycling. For some plant species growing in natural environments, concentrations higher than 40 ppm of Br can originate from atmospheric sources, mainly related to the sea and the burning of fossil fuels [9–15].

Although the high concentrations observed for some elements can be attributed to the plant's accumulative capacity, it may be also considered that the superficial contamination was not efficiently removed during washing. Contamination with resuspended soil is common in plant analysis [16], overestimating some elements due to the higher concentrations found in the soil. Considering that Sc can be used as tracer of soil [17–18], this element was employed to estimate the possible contamination from soil in the leaves and litter compartments.

The concentration of Sc in terrestrial plants is in the order of 8 ppb [8], with no evidence of physiological function, thus levels above this can be attributed to contamination with soil. Supposing that Sc in leaves and litter is due to resuspended soil, the contribution of such contamination in the levels of other elements was estimated (Figure 1). Soil has insignificant influence on these concentrations, except for Fe and Hf. Litter contamination with soil is slightly higher, probably due to the larger soil deposition and to the difficulty in thorough washing, mainly for *Esenbeckia leiocarpa*, whose leaves have a fragile structure.

Since soils are composed of different fractions with distinct elemental concentrations, a certain fraction may predominate in the contamination of leaves [19], so that the soil contribution to the leaves elemental composition could be misestimated. However, for clay soils like that found in the studied area, data [18] demonstrate the similarity in the concentration ratio between the total and fine fractions for Sc, Fe, Th, and REE. Such a behaviour considerably reduces the error in estimating the resuspended soil contribution by Sc contents.

Using Fe as a soil tracer [18] instead of Sc, similar contamination values were found for leaves and litter from *Esenbeckia leiocarpa.* For *Pachystroma longifolium* this change of tracer resulted in smaller contamination values, which may reflect higher Sc uptake by this species compared to other plants.

The ratios between leaf and litter concentrations (Figure 2) indicate that the two species scavenge part of the elements from their leaves before senescence. This strategy is commonly found in oligotrophic ecosystems, where elements recognized as nutrients are completely recycled and retained in the biomass [20–21]. Both species seem to recover elements in a similar proportion, which can be attributed to the environment, since scarce elements restrict plant development.

Potassium is scavenged by the two species, since it is a nutrient at low concentration in the system and of great mobility in plants, thus easily translocated from the senescent leaves [6]. The same behaviour is shown by Rb and Cs, which have chemical characteristics similar to K, and also by Br, Zn, and Sr. There is no reasonable explanation for the apparent recovery of Br, considering that this element does not have clearly identified metabolic functions [9]. Leaching after senescence could explain the lower concentration of Br in the litter, and in some extension the decreases observed for Cs, K, Rb, and Zn.

Several elements, Ca, Co, Na and REE, are not scavenged before leaf fall, which could be due to low translocation, non-essentiality or high concentrations in the soil. Such a situation leads to the movement of elements from soil to litter [3], which tends to enrich the superficial soil. In fact, some degree of enrichment was noticed for Co in the soil under direct influence of *Pachystroma longifolium* and for Na in both soils (Table II).

Although K is largely recovered by the plants, it was also noticed a remarkable enrichment in the superficial layer of both soils. Unlike Na and Co, the plant/soil concentration ratio (CR) is very high for this element (Figure 3), therefore, even after recovery, enough K remains in the litter enriching the soil. Usually, the trees in such an environment are specialized in absorbing mineral elements released from the litter and concentrating into their biomass [20].

Increased concentrations of elements in the superficial soil could explain the clear association observed between *Esenbeckia leiocarpa* and *Pachystroma longifolium* in several areas of Brazil. Some hypotheses suggest a sort of allelopathic effect that impairs the development of other species in such areas. Thus the biodiversity of plant species is affected by this phenomenon, normally understood as a natural process of ecological succession.

TABLE II. ELEMENTAL CONCENTRATIONS (mg kg^{-1} DRY WEIGHT) FOR LEAVES, LITTER, AND SOIL COMPARTMENTS. (a) CONFIDENCE INTERVALS (SIGNIFICANCE LEVEL 0.05). (b) THE VALUES IN BRACKETS ARE PERCENT STANDARD DEVIATION.

Element	**LEAVES** (a)		*LITTER **(a)***		*SOIL **(b)***			
	Esenbeckia	***Pachystroma***	***Esenbeckia***	***Pachystroma***	***Esenbeckia***		***Pachystroma***	
					0–10 cm	10–30 cm	0–10 cm	10–30 cm
As	<0.18	<0.26	<0.11	<0.20	5.90 (7.3)	6.49 (3.9)	6.03 (1.7)	6.83 (2.2)
Ba	17.2 ± 1.1	50.6 ± 2.3	26.1 ± 3.8	44.6 ± 12.7	<440	<440	<440	<440
Br	79.1 ± 4.0	84.1 ± 3.6	53.4 ± 1.8	46.3 ± 2.9	19.3 (8.3)	19.0 (8.3)	23.5 (7.0)	20.6 (6.3)
Ca	16.6 ± 0.8*	22.7 ± 0.8*	21.5 ± 1.3*	22.9 ± 1.1*	<1.2	<1.2	<1.2	<1.2
Ce	0.535 ± 0.011	12.3 ± 0.3	1.54 ± 0.21	19.6 ± 0.6	73.0 (5.1)	76.9 (3.5)	83.8 (11)	84.9 (4.1)
Co	0.293 ± 0.017	30.5 ± 0.7	0.556 ± 0.042	46.3 ± 5.9	37.2 (3.4)	33.5 (5.8)	48.1 (4.9)	38.5 (3.4)
Cr	<2.5	<1.8	<5.3	<4.8	105 (6.7)	109 (3.8)	110 (4.0)	111 (4.8)
Cs	0.448 ± 0.029	0.280 ± 0.011	0.292 ± 0.012	0.150 ± 0.006	<2.6	<2.6	<2.6	<2.6
Eu	0.008 ± 0.0005	0.178 ± 0.008	0.024 ± 0.004	0.429 ± 0.018	0.76 (3.2)	0.81 (3.1)	0.84 (4.2)	0.93 (7.4)
Fe	191 ± 9	106 ± 4	679 ± 89	247 ± 41	172* (6.6)	185* (1.4)	187* (2.4)	186* (1.5)
Hf	0.036 ± 0.002	<0.04	0.107 ± 0.025	0.049 ± 0.012	12.9 (8.2)	14.1 (1.8)	13.0 (2.9)	14.3 (2.8)
K	24.7 ± 1.7*	16.7 ± 0.7*	13.8 ± 0.6*	0.750 ± 0.05*	506 (12)	273 (9.4)	500 (14)	320 (17)
La	0.560 ± 0.045	16.4 ± 0.4	1.65 ± 0.15	21.8 ± 1.3	22.5 (7.8)	21.3 (4.5)	25.3 (8.8)	22.3 (4.3)
Na	40.6 ± 1.8	71.2 ± 1.7	80.2 ± 5.3	95.3 ± 5.3	75.5 (5.0)	51.1 (6.8)	78.2 (9.9)	53.8 (4.9)
Nd	<1.2	8.00 ± 0.10	<0.8	13.0 ± 0.6	<120	<120	<120	<120
Rb	66.9 ± 3.9	41.9 ± 0.8	27.9 ± 1.1	17.1 ± 2.4	<50	<50	<50	<50
Sb	0.044 ± 0.002	<0.03	0.078 ± 0.012	<0.04	0.44 (3.9)	0.43 (13)	0.53 (10)	0.57 (23)
Sc	0.037 ± 0.002	0.072 ± 0.002	0.163 ± 0.005	0.154 ± 0.011	43.9 (7.2)	50.1 (0.8)	46.0 (3.7)	52.5 (3.0)
Sm	0.028 ± 0.005	0.797 ± 0.036	0.104 ± 0.011	1.63 ± 0.10	3.23 (4.2)	3.32 (3.0)	3.47 (4.3)	3.61 (4.6)
Sr	110 ± 8	193 ± 14	111 ± 3	174 ± 10	<660	<660	<660	<660
Tb	<0.01	0.087 ± 0.008	0.011 ± 0.002	0.140 ± 0.009	<1.3	<1.3	<1.3	<1.3
Th	0.041 ± 0.002	<0.04	0.155 ± 0.013	<0.03	10.0 (4.3)	11.0 (1.4)	10.2 (1.7)	11.2 (3.2)
Yb	<0.05	0.140 ± 0.005	0.028 ± 0.002	1.97 ± 0.015	1.79 (3.5)	1.99 (4.7)	1.94 (4.4)	2.17 (11)
Zn	21.8 ± 0.8	43.6 ± 1.8	20.8 ± 2.7	34.5 ± 2.0	181 (6.4)	181 (6.4)	193 (7.1)	173 (5.4)

(*Values given in g kg^{-1})

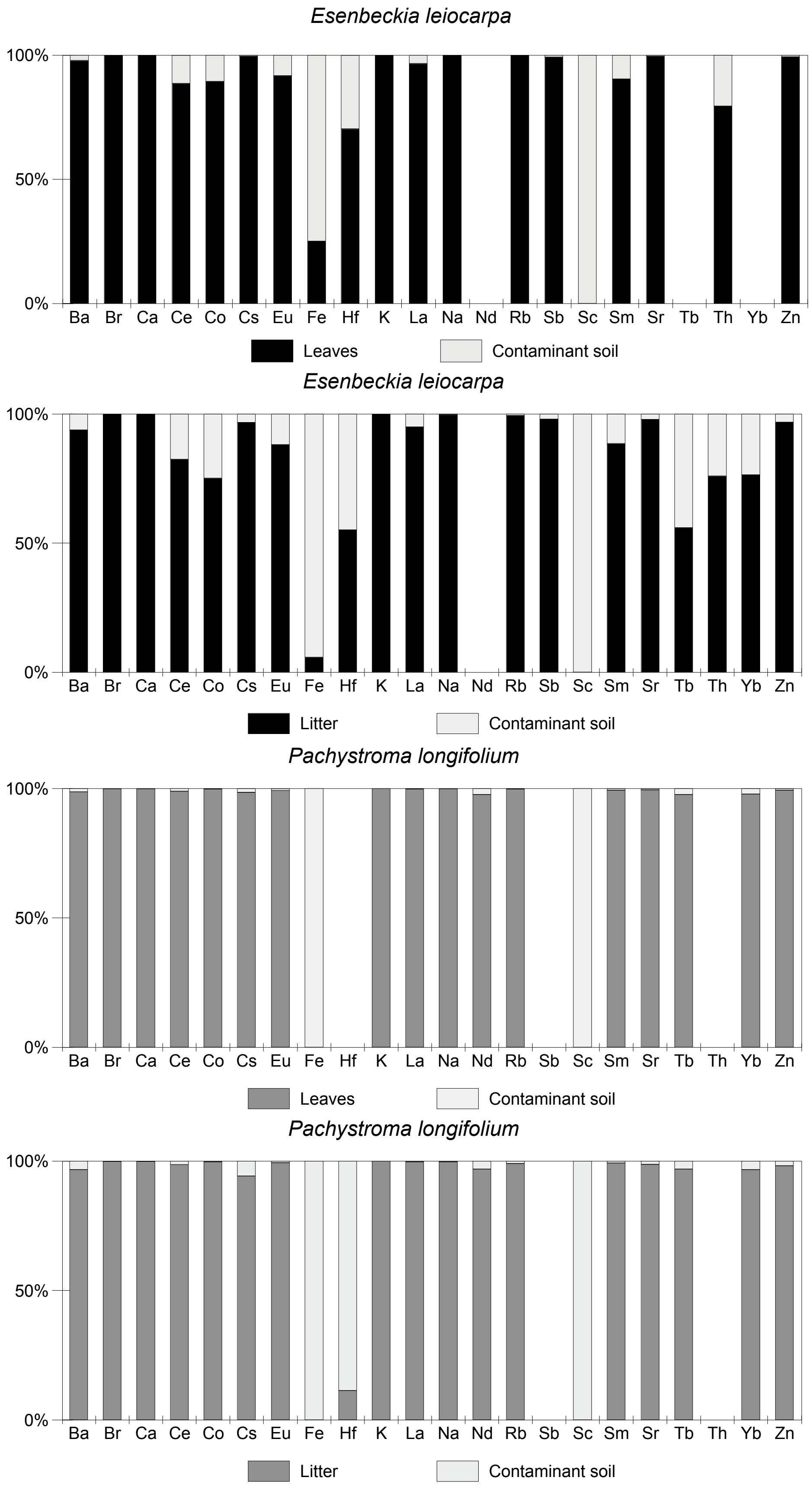


FIG. 1. Soil contamination for leaves and litter compartments

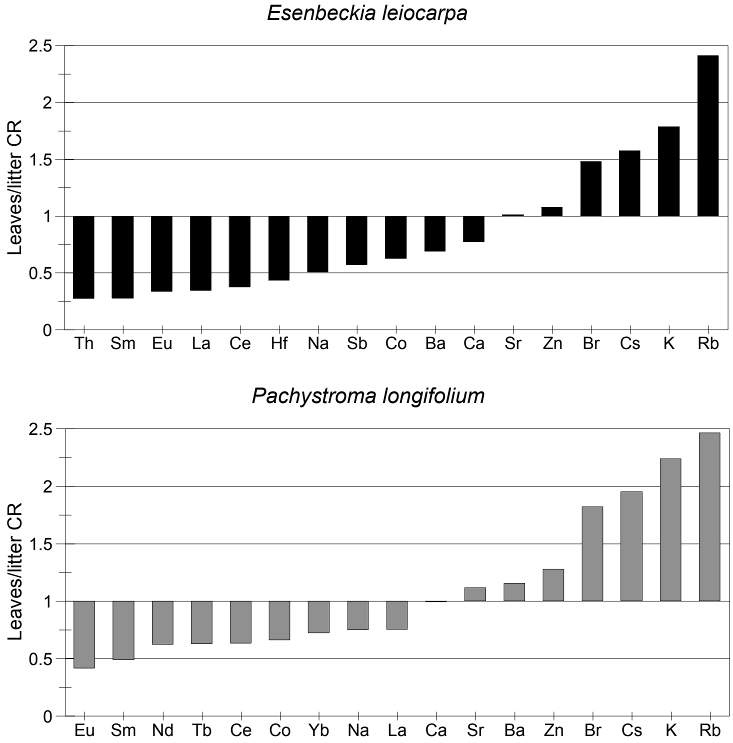


FIG. 2. Leaves/litter concentration ratios (CR) for ***Esenbeckia leiocarpa*** *and* ***Pachystroma longifolium*** *trees*

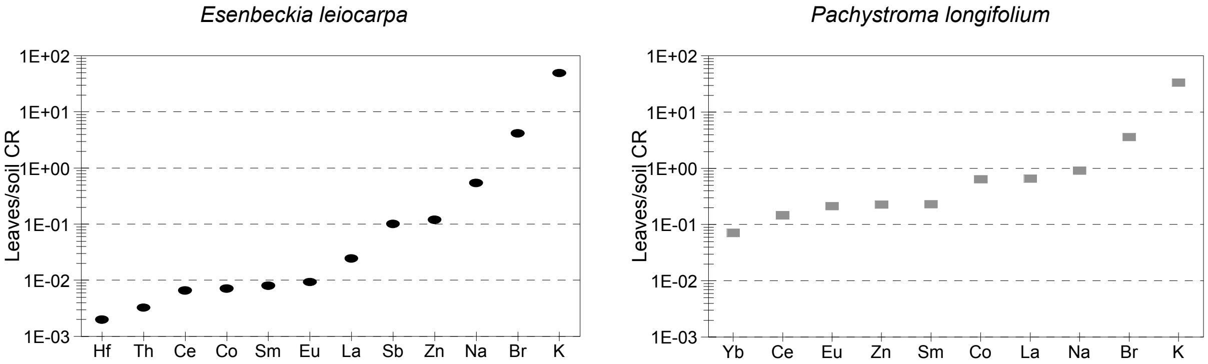


FIG.3. Leaves/soil concentration ratios (CR) for ***Esenbeckia leiocarpa*** *and* ***Pachystroma longifolium*** *trees*

Comparing the plant/soil concentration ratios (CR), one can observe that *Pachystroma longifolium* concentrates some elements, mainly Co and REE, to a higher degree than *Esenbeckia leiocarpa* (Figure 3). Nevertheless, both species presented similar CR values for Br, K, Na, and Zn. Once again, Br deserves a special attention, because its CR values are high, 4.1 and 3.6, respectively for *Esenbeckia leiocarpa* and *Pachystroma longifolium*. The average bromine CR, calculated for 121 samples from several wild species growing in different sampling sites in Japan, was determined as 0.13 [22].

Besides plant species and soil conditions, climate may also have some influence on Br uptake from the soil. For spruce needles, the average CR was 0.048 for 8 sampling sites located in the temperate region [16], while for pine needles, collected from an implanted forest growing under a tropical climate, the CR values ranged between 1.8 and 6.0 [23].

The Br concentration in the leaves of both species suggests the existence of some other source besides soil. Taking into account that the atmospheric contribution for Br concentration in plants is well known and also considering the existence of various possible sources of this element in the area, part of the concentration observed in the leaves may originate from atmosphere. However, further studies are necessary to better understand the behavior of Br in natural environments under a tropical climate, because most of the data found in the literature are obtained from the temperate regions.

ACKNOWLEDGEMENTS

The authors wish to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support granted.

REFERENCES

[1] PUTMAN, R.J., Community Ecology. London: Chapman & Hall, (1994) 178 pp.

[2] AIDID, S.B., Determination of trace elements in leaves of tropical trees in Malaysia by neutron activation analysis. J. Radioanal. Nucl. Chem. **120 (2)** (1988) 335–344.

[3] GOLLEY, F.B., MCGINNIS, J.G., CLEMENTS, R.B., CHILD, G.I., DUEVER, M.J., Ciclagem de Minerais em um Ecossistema de Floresta Tropical Úmida. São Paulo: EDUSP, (1978) 256 pp.

[4] MORELLATO, P.C., LEITÃO FILHO, H.F., (orgs). Ecologia e Preservação de uma Floresta Tropical Urbana. Campinas, SP: UNICAMP, (1995). 136 pp.

[5] NAIDU, G.R.K., DENSCHLAG, H.O., MAUERHOFER, E.; PORTE, N., BALAJI, T. Determination of macro, micro nutrient and trace element concentrations in Indian medicinal and vegetable leaves using instrumental neutron activation analysis. Appl. Radiat. Isot. **50** (1999) 947–953.

[6] MORAES, R.M., DOMINGOS, M., Elementos minerais em folhas de espécies arbóreas de Mata Atlântica e Mata de Restinga, na Ilha do Cardoso, SP. Revista Brazil. Bot. **20 (2)** (1997) 133–138.

[7] BACCHI, M.A., FERNANDES, E.A.N., OLIVEIRA, H. A Brazilian experience on k_0 standardized neutron activation analysis. J. Radioanal. Nucl. Chem. **245 (1)** (2000) 217–222.

[8] BOWEN, H.J.M., Trace Elements in Biochemistry. London: Academic Press, (1966) 241 pp.

[9] KABATA-PENDIAS, A., PENDIAS, H., Trace Elements in Soils and Plants. Florida: Boca Raton, (1984) 315pp.

[10] MARKERT, B., DE LI, Z., Natural background concentrations of rare-earth elements in a forest ecosystem. Sci. Total Environ. **103** (1991) 27–35.

[11] KOYAMA, M., SHIRAKAWA, M., TAKADA, J., KATAYAMA, Y.; MATSUBARA, T., Trace elements in land plants: concentration ranges and accumulators of rare earths, Ba, Ra, Mn, Fe, Co and heavy halogens. J.Radioanal. Nucl. Chem. **112 (2)** (1987) 489–506.

[12] BREULMANN, G., OGINO, K., MARKERT, B., LEFFLER, U.S.; HERPIN, U.; WECKERT, V., KONSCHAK, R., KIKUGAWA, Y., OHKUBO, T. Comparison of chemical elements in Dipterocarpaceae and Euphorbiaceae from a tropical rain forest in Sarawak, Malaysia. Sci. Total Environ. **225 (**1999) 213–240.

[13] MARIA, Sh.P., FIGUEIREDO, A.M.G., CECCANTINI, Gr. Determination of the contents and distribution characteristics of rare earth elements in *Solanum lycocarpum* from tropical ecosystems in Brazil by INAA. J. Radioanal. Nucl. Chem. **244 (2)** (2000) 303–306.

[14] ERNST, W.H.O., Sampling of plant material for chemical analysis. Sci. Total Environ. **176** (1995) 15–24.

[15] GARG, A.N., CHUTKE, N.L., AMBULKAR, M.N., DOGRA, R.K.S., An evaluation of the environmental implications of petroleum refinery emissions by multielemental neutron activation analysis of rumen fluid ash of buffaloes. Appl. Radiat. Isot. **47 (5/6)** (1996) 581–586.

[16] WYTTENBACH, A., TOBLER, L. Effect of surface contamination on results of plant analysis. Commun. Soil Sci. Plant Anal. **29 (7/8)** (1998) 809–823.

[17] FERNANDES, E.A.N., Scandium as tracer in the sugar and alcohol agroindustry. J. Radioanal. Nucl. Chem. **168 (1)** (1993) 41–46.

[18] FERNANDES, E.A.N., Dirt in cane removal influenced by soil characteristics. J. Radioanal. Nucl. Chem. **216 (2)** (1997) 285–288.

[19] HINTON, T.G., KOPP, P., IBRAHIM, S., BUBRYAK, I., SYOMOV, A., TOBLER, L., BELL, C. A comparison of techniques used to estimate the amount of resuspended soil on plant surfaces. Health Phys. **68** (1995) 523–531.

[20] ARNASON, J.T., LAMBERT, J.D., GALE, J. Mineral cycling in a tropical palm forest. Plant and Soil **79** (1984) 211–255.

[21] ODUM, E.P., Ecologia. Rio de Janeiro: Guanabara, (1988) 434pp.

[22] YUITA, K., Overview and dynamics of iodine and bromine in the environment. 1. Dynamics of iodine and bromine in soil-plant system. Japan Agricultural Research Quarterly **28 (2)** (1994) 90–99.

[23] OLIVEIRA, H., FERNANDES, E.A.N., FERRAZ, E.S.B. Determination of trace elements in tree rings of *Pinus* by neutron activation analysis. J. Radioanal. Nucl. Chem. **217 (1)** (1997) 125–129.

TRACE ELEMENTS DETERMINATION IN HIMALAYAN LICHENS

L. BERGAMASCHI[1], E. RIZZIO[1], M.G. VALCUVIA[3], G. VERZA[2],
A. PROFUMO[1], M. GALLORINI[1]

[1] CNR Centro di Radiochimica e Analisi per Attivazione, Pavia, Italy
[2] Ev-K^2-CNR
[3] Dip. di Ecologia del Territorio e degli Ambienti, Università di Pavia, Italy

Abstract

Within the framework of the Ev-K^2-CNR research program of the Italian National Research a specific task project has been initiated for the identification of trace elements in lichens in remote high altitude areas. This is to evaluate the possibility of using lichens as biomonitors of atmospheric pollution trace elements. By Instrumental Neutron Activation Analysis (INAA), more than 30 elements have been determined in some species of lichens collected at different altitudes (from 2000 m to 5000 m) in the region of the Sagarmatha National Park (Nepal). Samples of superficial soils surrounding the lichens were also collected and analysed to calculate the trace element enrichment factors (EF) using scandium as normalizing crust element. Lichens and soils were sampled in 7 sites distributed throughout the Khumbu valley along the way from Katmandu to the CNR Pyramid Laboratory –Observatory at 5,050 m. a.s.l.

1. INTRODUCTION

In 1990, following an agreement with the Royal Nepal Academy of Science, the Italian National Council (CNR) installed a scientific laboratory (Piramide) at 5,050 m of altitude. The laboratory is located in one of the highest inhabited place in the word: the Khumbu valley, at the foot of Mount Everest. From that moment, several multidisciplinary research activities have been initiated in the framework of the CNR scientific program Ev-K^2-CNR.

Among the environmental related researches, the task project AER, supported by CIESSE and Mountain Rquipe sr.l. (Bergamo, Italy), aims to evaluate the possibility of using the lichens as biomonitors of trace element atmospheric pollution [1,2]. The project forecasts the analysis of different species of lichens collected in high altitude remote areas of the world for the determination of the trace elements (TE) concentrations. The results will be useful in assessing a data bank of the baseline values referred to areas that may be considered unpolluted and in evaluating long transport phenomena.

This work presents the first series of data obtained from the analysis of Himalayan lichens collected in different areas at different altitude of the Khumbu valley. More than 25 elements in 15 lichen samples collected in 7 different sites have been determined. In addition, a specific study for the evaluation of the anthropogenic origin of the trace elements investigated, by the calculation of their Enrichment Factors (EF), is carried out. To this purpose, samples of soils, collected in the same areas of the lichens, were also analysed. The EF factors have been calculated normalizing the values versus the concentration of the scandium taken as reference natural element. The EF values obtained in this way were also compared with those calculated from the data of the soils composition reported in literature by Taylor [2].

2. MATERIAL AND METHODS

2.1. Samples

The samples were collected during May 1999 in the Khumbu valley of the Sagarmatha National Park (Nepal), on the way from Katmandu (1,350 m) to the CNR research Pyramid (5,050 m). The highest sample point was at 5,200 m high NE from the Pyramid.

TABLE I. SAMPLING POINTS AND LICHENS SELECTED FOR THE ANALYSIS

Sampling Points	Height (m)	Locality	Sample number	Lichen's genus
1	1350	Katmandu	4	*Parmotrema*
2	2700	Benkar	3 4	*Parmotrema* *Hypotrachyna*
3	3200	Namche	5 4 3 4	*Usnea* *Heterodermia* *Hypotrachyna* *Hypogymnia*
4	3850	Tyangboche	4 5 3	*Hypotrachyna* *Usnea* *Heterodermia*
5	4150	Shomare	4 3	*Usnea* *Hypotrachyna*
6	5090	CNR Pyramid	4 3	*Hypogymnia* *Umbilicaria*
7	5200	Highest point	4	*Alectoria*

In 15 different sites, more than 100 samples of lichens were collected. From the morphological analysis the following 17 different genus were individuated: *Alectoria, Cetraria, Cetrelia, Heterodermia, Hypogymnia, Hypotrachyna, Leptogium, Lobaria, Menegazzia, Ophiparma, Parmelia, Parmotrema, Physcia, Ramalina, Sticta, Umbilicaria and Usnea.* Among the overall lichens collected, pools of selected genus were chosen for the analysis. In dependence of the frequency of their distribution in the different sampling sites, samples of epiphytic lichens of the genus *Parmotrema, Hypotrachyna, Usnea, Heterodermia* and *Hypogymnia* were chosen as biomonitors of 5 areas located from 1,350 m to 4,150 m high. Samples of epilytic lichens *Umbilicaria* and *Alectoria* were collected from the surface of rocks in two points at 5,090 m and 5,200 m of altitude, respectively. The sampling points and the lichens collected and analysed are summarized in Table I.

In the same points samples of superficial soils were collected at about 3 cm depth. All sampling procedures were carried out using titanium knives and plastic tools to avoid possible metals contamination. Lichens and soils samples were stored in filter paper bags and in pre-washed polyethylene containers respectively.

2.2. Sample preparation and analysis

For the classification and the biomorphological investigation, lichens were submitted to stereoscopic microscope. During this procedure the lichens were cleaned from the residues of the bark substrates and other extraneous materials using plastic tweezers and filter paper. Next, lichens were dried in a dessiccator onto P_2O_5. Homogeneous parts of the thallus, corresponding to the last year of growth, were collected under microscope, then sealed in plastic containers for the analysis. Soil samples were dried in oven at 105° C, ground in agate mortar, sieved (50 mesh), and prepared for the analysis, the analysis of lichens were performed onto pools obtained from of at list three individual lichen samples of the same genus collected in each point. The same procedure was used for the analysis of the soil samples.

The major part of the elements has been determined by Instrumental Neutron Activation Analysis [4,5]. The TRIGA MarkII (General Atomic — USA) research nuclear reactor of the University of Pavia was used for the neutron irradiation. Lead and cadmium and, in some cases, nickel measurements were carried out by Electro-Thermal Atomic Absorption Spectroscopy (ETAAS): the samples were dissolved in high pressure Teflon® lined bombs with HNO_3 and HF (3:1).

TABLE II. DETERMINATION OF 24 ELEMENTS IN HIMALAYAN LICHENS COLLECTED IN DIFFERENT AREAS OF KHUMBU VALLEY. CONCENTRATION IN $\mu g \cdot g^{-1}$

	Sampling points and lichens genus analyzed						
	Katmandu (1,350 m)	Benkar (2,700 m)		Namche (3,200 m)			
	Parmotrema	*Parmotrema*	*Hypotrachyna*	*Usnea*	*Etherodermia*	*Hypotrachyna*	*Hypogymnia*
Element							
As	0.13	0.40	0.43	0.78	0.51	0.54	0.37
Br	9.6	6.9	21.5	11.3	6.6	20.0	12.9
Cd	<0.02	n.d.	0.16	0.02	n.d.	<0.02	n.d.
Ce	0.4	1.4	2.3	4.2	2.0	3.2	4.7
Co	0.45	0.50	0.54	0.71	0.52	0.56	0.81
Cr	<1.0	1.8	2.6	3.7	<1.0	1.8	3.7
Cs	0.34	0.29	0.74	0.69	0.82	0.90	0.86
Cu	5.8	n.d.	6.4	5.1	n.d.	6.2	n.d.
Fe	465	832	1166	1843	1101	1180	1659
Hf	0.10	0.09	0.08	0.38	<0.01	0.07	0.28
K	5409	3688	3430	4481	4558	3834	9053
La	0.6	1.2	1.2	2.1	0.7	1.5	1.6
Mg	2279	2203	2231	3552	1946	441	3038
Mn	26.5	43.0	61.2	58.8	31.1	60.8	86.1
Pb	5.5	n.d.	10.7	17.6	n.d.	17.2	n.d.
Rb	29.6	14.0	13.6	17.2	<1.5	8.9	27.6
Sb	2.6	0.9	3.1	0.3	0.2	2.4	0.6
Sc	0.18	0.26	0.37	0.68	0.28	0.46	0.57
Se	0.02	0.52	0.42	0.05	0.27	<0.02	0.85
Sm	0.09	0.24	0.27	0.40	0.15	0.32	0.32
Th	0.34	0.42	0.68	1.08	0.35	0.70	0.91
U	<0.3	<0.3	<0.3	<0.3	<0.3	0.64	<0.3
V	0.7	1.6	2.0	3.1	1.1	3.1	2.8
Zn	45.7	30.1	32.3	31.1	45.2	30.4	45.2

2.3. Quality Control

Quality control of the analytical procedures was carried out by analysing, with the same procedures adopted for the samples, the standard reference materials: SRM Peach Leaves 1547, SRM Apple Leaves 1515 from NIST, and CRM Lichen 482 from BCR.

3. RESULTS AND DISCUSSION

The lichen samples were analysed for the determination of 24 elements. In Tables II and III the concentration as well as the different lichen genus and the sampling points are reported. Unless for the *Alectoria*, collected at 5,200 m height, in which many elements are present at lower concentration, all the other lichens show values that can be considered quite similar without significant variation between the different genus as well as the different sampling points.

TABLE III. DETERMINATION OF 24 ELEMENTS IN HIMALAYAN LICHENS COLLECTED IN DIFFERENT AREAS OF KHUMBU VALLEY.CONCENTRATION IN $\mu g \cdot g^{-1}$

	Sampling points and lichens genus analyzed							
	Tyangboche (3,850 m)			Shomare (4,150 m)		CNR Pyramid (5,090 m)		NE Pyramid (5,200 m)
	Hypotrac.	*Usnea*	*Heteroder.*	*Usnea*	*Hypotrachy.*	*Hypogymn*	*Umbilicari*	*Alectoria*
Element								
As	0.40	0.36	0.32	0.76	0.87	0.43	2.16	0.16
Br	9.2	9.3	4.8	16.8	13.0	12.9	5.5	6.7
Cd	0.04	n.d.	n.d.	n.d.	0.09	0.04	0.05	<0.02
Ce	3.2	2.0	0.8	7.3	6.4	8.0	7.0	0.4
Co	0.54	0.60	0.23	1.45	1.11	1.87	1.05	0.10
Cr	1.3	1.5	<1.0	6.7	3.7	2.6	2.8	<1.0
Cs	0.87	0.31	0.37	2.24	2.08	1.45	2.16	0.44
Cu	5.8	n.d.	n.d.	n.d.	4.9	5.0	17.2	5.3
Fe	980	754	442	3172	2359	1843	2220	183
Hf	0.20	0.09	0.18	0.38	0.18	0.73	0.33	<0.01
K	5113	4082	2494	4031	5880	4228	5844	2291
La	1.5	1.1	0.6	2.5	2.5	3.5	2.9	0.3
Mg	2952	2748	319	5839	4445	3899	3267	382
Mn	83.4	92.1	23.4	86.9	82.9	79.1	27.6	7.18
Pb	16.3	n.d.	n.d.	n.d.	18.9	14.1	22.8	7.3
Rb	18.1	6.1	5.9	14.8	16.5	15.5	15.0	3.6
Sb	0.9	0.7	0.5	2.2	1.0	1.4	0.9	0.9
Sc	0.37	0.30	0.18	1.10	0.85	0.61	0.75	0.42
Se	<0.02	0.65	0.75	0.29	0.79	0.25	<0.02	<0.02
Sm	0.31	0.20	0.11	0.54	0.55	0.83	0.89	0.07
Th	0.77	0.32	0.34	1.74	1.43	1.52	1.52	0.07
U	0.39	<0.3	<0.3	0.70	0.83	0.79	1.33	<0.3
V	1.5	1.7	0.8	5.2	3.5	2.2	4.1	0.4
Zn	27.5	33.5	37.4	26.0	33.9	31.9	69.2	17.1

Table IV reports the trace element concentration found in the soils. Also, in this case, the data refer to pools of four different samples collected in each point.

Using the data obtained in the analyses, the Enrichment Factors (EF) of all the elements have been calculated according the following equation:

$$EF = Cx/Cn \text{ (ambient)}: Cx/Cn \text{ (background)}$$

where Cx is the concentration of the X element whose enrichment is to be determined, and Cn is the concentration of the N normalizing element assumed to be uniquely characteristic of the background. In our case, the ambient consists of samples of lichens while the background consists of samples of the surrounding soils. Elements with EF values significantly higher than 1 can be considered not originated from the background and may be attributed to long transport phenomena from anthropogenic or other natural sources.

In this work scandium was used as normalizing reference crust element. The EF values trends of the considered elements, calculated using the data obtained from the analysis of foliose lichens in each sampling site, are reported in figures 1 and 2. The EF trends can be considered quite similar in all cases. The elements Pb, As, Zn, Sb, Cd, and Br present the highest values in all lichens. These elements may derive not only from the local soils but also, from long distance transport phenomena, from other natural and/or anthropogenic sources. Fig. 3 reports the trend of the EF values obtained from the mean of the concentrations found in the 6 foliose lichens and in the corresponding soils. In Fig.4, it is shown the same EF calculated on the basis of the data of the crust composition given by Taylor [3]. The comparison between the two graphics shows a quite different behavior of some trace elements such as La, Mg, Cs, Se, Rb. These elements, naturally present in the lichens, appear in the Taylor plot with high EF values, probably indicating an apparent anthropogenic or not natural origin. This suggests that the analysis of the soils, surrounding the lichen collection site, gives more precise information for the evaluation of trace metals origin.

TABLE IV. TRACE ELEMENTS DETERMINATION IN SOIL SAMPLES. CONCENTRATION IN $\mu g.g^{-1}$

	Sampling points						
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**
As	0.44	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
Br	1.25	1.25	1.25	1.25	1.25	0.67	0.70
Cd	0.01	0.04	0.01	0.01	0.02	0.01	0.01
Ce	40.8	52.1	47.2	45.1	28.1	24.7	78.9
Co	8.9	8.6	10.1	5.0	10.4	5.1	6.5
Cr	59.81	39.14	52.60	18.69	21.24	18.23	42.91
Cs	12.0	9.6	12.3	14.2	14.2	19.9	17.4
Cu	21.2	13.5	16.3	12.9	21.3	14.1	10.1
Fe	36119	25329	32278	17116	20312	13814	24869
Hf	9.5	4.4	5.8	3.4	3.6	5.2	8.2
K	29504	13270	18295	13328	22956	20922	21995
La	24.1	18.8	20.5	25.1	27.6	12.5	22.8
Mg	39099	37402	31756	20766	20631	38995	33110
Mn	242	221	391	318	621	320	141
Pb	32.3	33.9	29.1	40.6	37.8	24.4	58.3
Rb	334	131	177	103	109	107	318
Sb	0.21	0.12	0.17	0.23	0.12	0.10	0.10
Sc	8.31	6.12	5.82	3.87	5.53	4.32	5.89
Se	1.24	2.13	1.80	1.13	1.66	1.42	0.63
Sm	3.6	2.9	3.2	4.5	4.5	2.8	3.2
Th	11.1	12.4	13.0	11.2	7.2	5.4	21.8
U	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
V	50.8	31.5	54.9	39.9	49.6	42.0	28.0
Zn	28.2	31.5	30.0	39.4	34.1	21.2	26.9

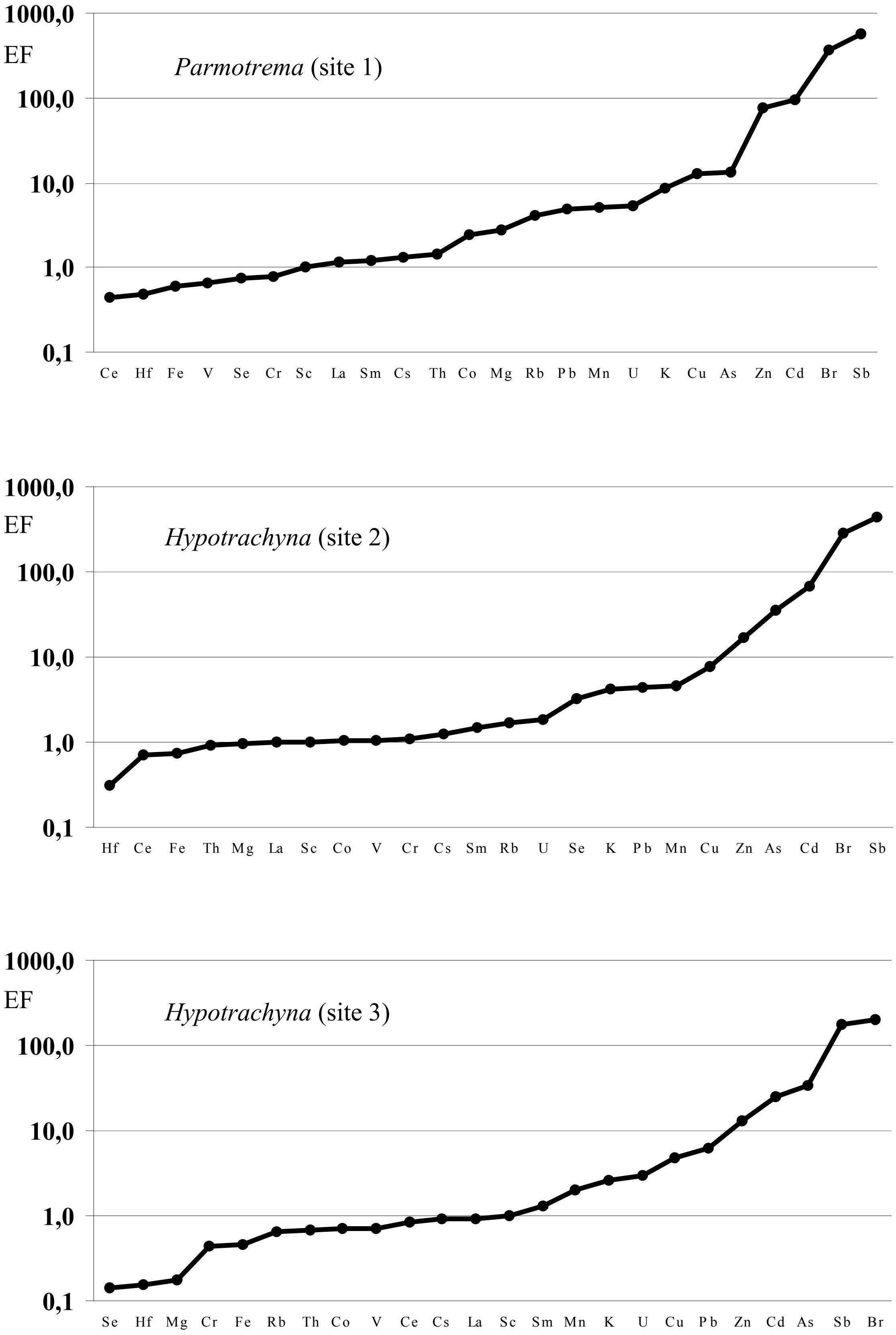
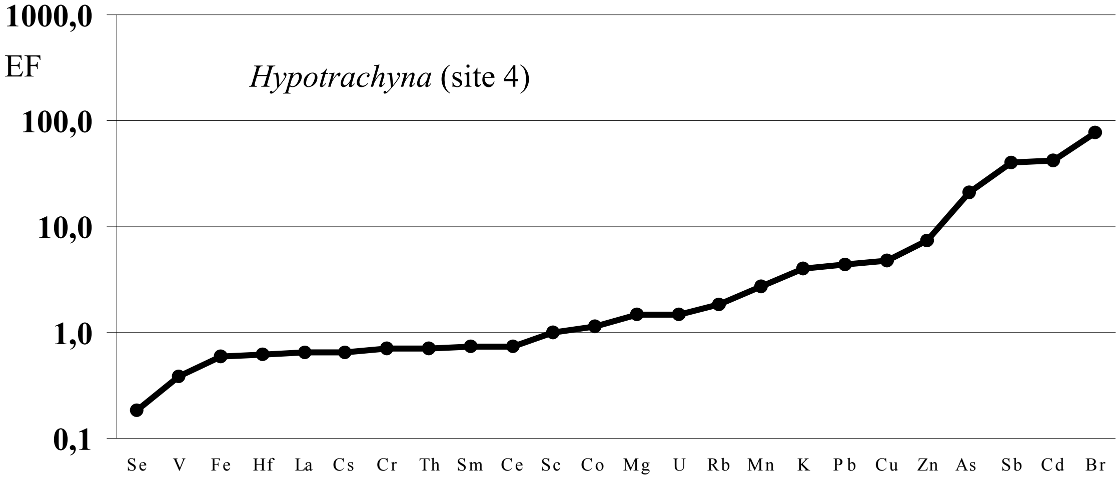
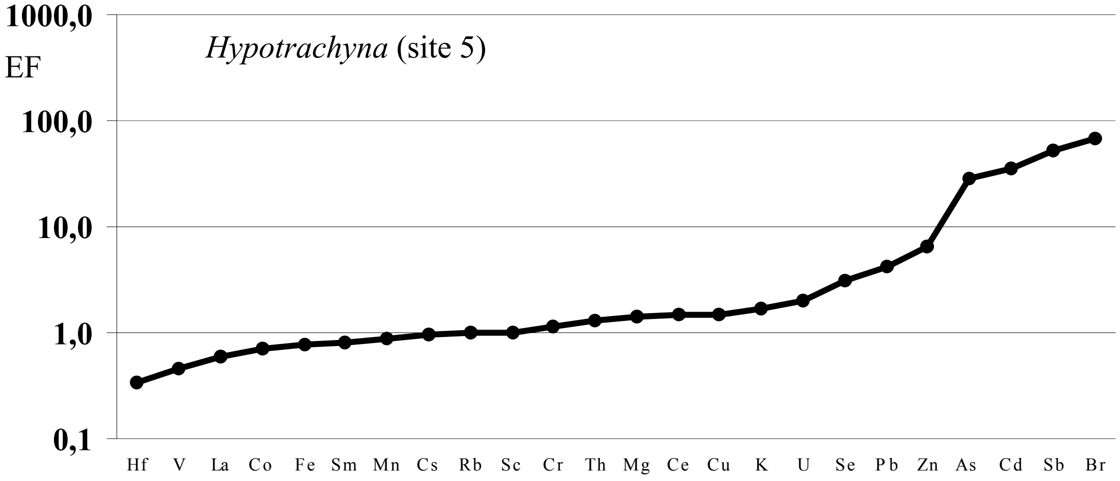


FIG.1. EF trends of trace elements in Hymalaian foliose lichens, calculated vs scandium.





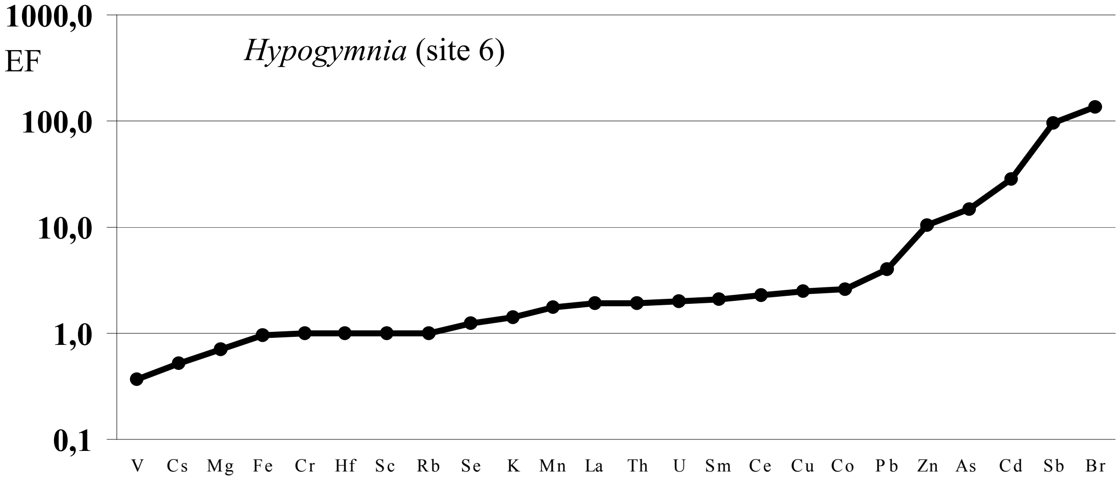


FIG.2. EF trends of trace elements in Hymalaian foliose lichens, calculated vs scandium.

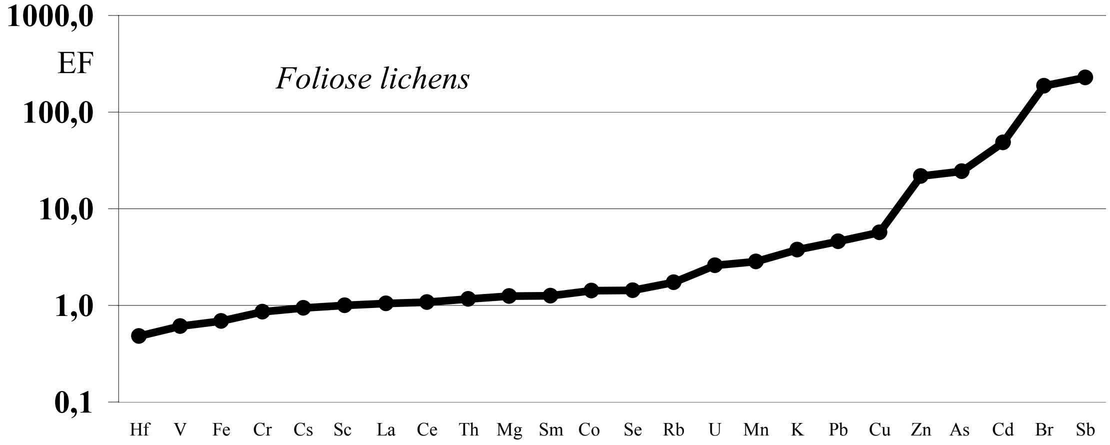


FIG.3. Mean of the EF values vs scandium obtained from the analyses of the foliose lichens and the corresponding soils.

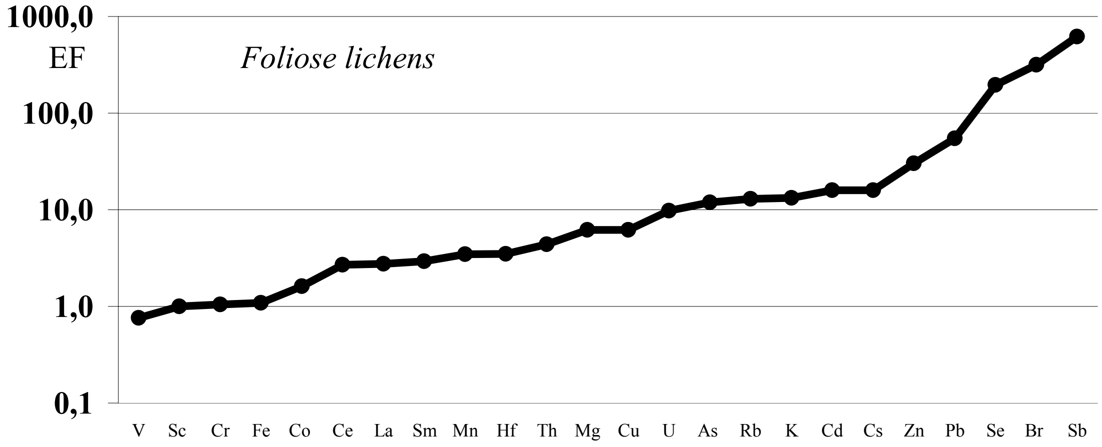


FIG.4. Mean of the EF values vs scandium obtained from the analysis of the foliose lichens and the crustal element concentrations reported by Taylor [3].

ACKNOWLEDGEMENTS

To Mountain Equipe s.r.l. for the sample collection, the logistic support. This work has been partially founded by Mountain Equipe s.r.l.and CIESSE which contributed to Ev-K2-CNR scientific project.

REFERENCES

[1] BARGAGLI, R., Trace Elements in Terrestrial Plants. Springer-Verlag, 1998.

[2] HERZIG, R., LIEBENDORFER, L., URECH, M., AMMAN, K., GUECHEVA, M., AND LANDOLT, W., Passive biomonitoring with lichens as part of an integrated biological measuring system for monitoring air pollution in Switzerland. Int. J. Env. Anal. Chem. **35** (1989) 43–57.

[3] TAYLOR S.R., Abundance of chemical elements in the continental crust: a new table. Geochimica et Cosmochimica Acta **28** (1964) 1273–1285.

[4] FREITAS M.C., REIS M.A., ALVES L.C., WOLTERBEEK H.TH.,VERBURG T., GOUVEIA M.A. Bio-monitoring of trace-element air pollution in Portugal: Qualitative survey. J. Radioanal. Nucl. Chem. **217** (1997) 21–30.

[5] GALLORINI, M., In: Spurny K.R., ed. Aerosol Chemical Processes in the Environment. C.R.C. Press Boca Raton Florida USA: (2000) 432–455.

IAEA QUALITY CONTROL EXERCISES ON BIOINDICATORS

A. BLEISE, B. SMODIŠ
International Atomic Energy Agency,
Vienna

Abstract

Biomonitoring air pollution using plants became an official part of the IAEA projects in 1997. In 1998 the section of Nutritional and Health Related Environmental Studies (NAHRES) organized a quality control exercise on two bioindicators (NAT-5 exercise), comprising two lichen samples. The first lichen was collected at an industrial polluted area in Austria, and the second was reference material IAEA–336, obtained from an unpolluted region in Portugal. 15 laboratories from 15 Member States participated in the NAT-5 exercise, providing 17 sets of results. The following analytical methods were used to obtain values: neutron activation analysis (NAA), inductive coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), and particle induced X ray emission analysis (PIXE). 308 laboratory mean values for 47 elements were reported for the IAEA-336 lichen sample. More than 4 independent laboratory mean values were obtained for 26 elements and these were statistically evaluated. Only about 5% of the results were detected as outliers by the statistical tools. After statistical evaluation, the values were subjected to an assessment according to the recommended procedures for proficiency testing by interlaboratory comparisons (Draft ISO 13528). Reference values for 18 elements were available from the reference sheet of the material IAEA-336. The results presented illustrate the performance of the laboratories and show some discrepancy between non-destructive and destructive analytical techniques with elements of environmental interest.

1. INTRODUCTION

Biomonitoring is an widely accepted tool for assessing the level of air pollution [1] and regularly used in several IAEA Member States. Nuclear and nuclear related techniques have been proved to be particularly appropriate for the analysis of trace element in biomonitors, being multi-elemental, reliable, extremely sensitive for almost all elements of interest, matrix independent and suitable for a large concentration range. Therefore, the section of Nutritional and Health Related Environmental Studies (NAHRES) of the International Atomic Energy Agency (IAEA) started one project on biomonitoring air pollution. The co-ordinated research project (CRP) on “ Validation and application of plants as biomonitors of trace element atmospheric pollution analyzed by nuclear and related techniques” started in 1998.

Quality control exercises were included from the beginning as part of this research project. In every analytical laboratory the use of appropriate quality assurance materials is an essential tool in establishing reliable results. For this purpose the IAEA maintains a database on Natural Matrix Reference Materials (NMRM), which intends to assist laboratories in finding suitable reference materials. This database is now accessible on the Internet through:

http://www.iaea.org/programmes/nahunet/e4/nmrm/index.htm

Within the project on biomonitoring air pollution the IAEA organized two quality control exercises on bioindicators. The first study, NAT-5, started in 1998, the second study, NAT-6, at the end of 1999, and this is not yet completely evaluated.

2. NAT-5 QUALITY CONTROL EXERCISE

The NAT-5 exercise comprised two lichen samples. One sample was the IAEA reference material IAEA-336, the second one a lichen sample from a polluted area in Austria. Each participant received a bottle of each material, along with an information sheet and reporting forms. Participants were asked to determine as many elements as possible from the following list: Al, As, Ca, Cd, Cl, Co, Cr, Cu, Fe, Hg, K, La, Mg, Mn, Na, Ni, Pb, Sb, Sc, Ti, V, Zn. Six independent analytical determinations were requested.

2.1. Data evaluation

In total, 16 laboratories from 16 Member States were invited to participate in this exercise and 15 laboratories reported results. Analytical methods used were: neutron activation analysis (N), inductively coupled plasma emission spectrometry (E), inductively coupled plasma mass spectrometry (M), atomic absorption spectrometry (A), and particle induced X ray emission analysis (P).

Results received from the participants were processed with a computer program called AQCS-PC [2,3]. The program applies four different outlier tests: Grubb's, Dixon's, coefficient of Skewness, and coefficient of Kurtosis. Any laboratory mean thereby identified as an outlier by one of these tests is subsequently excluded from further consideration. The remaining accepted laboratory means are then combined in the usual statistical way, without weighting. Estimates of the overall mean and its associated standard deviation, standard error, and 95% confidence interval are provided. This approach has been used for evaluation of results in numerous IAEA intercomparison studies, and it was adopted, sometimes with certain modifications, by other authors engaged in certification of reference materials based on intercomparison exercises.

In addition, the results provided to the IAEA reference materials were evaluated as a proficiency test according to draft ISO 13528 [4]. In this evaluation all individual results are displayed relatively to the target values using Z-scores on an anonymous basis. The Z-scores are calculated as

$$Z = (x-X)/\sigma,$$

where x is the laboratory result, X the assigned value, and σ the target standard deviation for proficiency assessment.

15 laboratories reported 17 sets of results for both materials. Concerning the IAEA–336 lichen sample 309 values for 47 elements were reported [5]. 26 elements had more than 4 individual average values and were statistically evaluated. The statistical evaluation of the NAT-5 study showed very few outliers (~5%). More than one outlier was obtained for only four measurands. This reveals the good performance of all participants in this study. The relative deviation of the NAT-5 average values from the target values is lower than 10% for all but two measurands (Hg and Sb). The NAT-5 average value for antimony and mercury lies outside the expanded 95% confidence interval of the target values. It was not possible to track the reason for this bias. An thorough evaluation of the analytical techniques applied for these measurands did not reveal any difference. In table I the values for the requested 22 elements are compared with the target values from the reference sheet and figure 1 compares the reference values with the results obtained in the NAT-5 exercise. The results are shown relatively to the reference values, including the expanded confidence intervals from the reference sheet.

TABLE I. COMPARISON OF NAT-5 RESULTS AND REFERENCE VALUES

	No. of results rec.	acc.	Range of accepted results (mg/kg)	Average value (mg/kg)	Target value (mg/kg)	Deviation %	95% CI
Al	12	12	385–802	634	680	6.7	570–790
As	9	9	0.59–0.83	0.68	0.63	8.2	0.55–0.71
Ca	15	13	2122–2820	2489	----		
Cd	4	4	0.10–0.17	0.125	0.117	6.8	0.1–0.134
Cl	7	7	1770–2210	1964	1900	3.4	1600–2200
Co	12	10	0.23–0.34	0.29	0.29	1.4	0.24–0.34
Cr	13	12	0.65–1.39	1.05	1.06	0.8	0.89–1.23
Cu	7	7	2.63–3.85	3.24	3.6	10.0	3.1–4.4
Fe	17	17	360–528	443	430	3.1	380–480
Hg	6	5	0.15–0.18	0.17	0.2	16.5	0.16–0.24
K	15	15	1510–2284	1813	1840	1.4	1640–2040
La	12	10	0.56–0.71	0.63	0.66	5.3	0.56–0.76
Mg	10	10	456–720	590	----		
Mn	13	13	52.0–75.0	64.3	63	2.0	56–70
Na	13	13	266–341	302	320	5.4	280–360
Ni	6	5	0.94–1.15	1	----		
Pb	7	6	4.28–5.00	4.8	4.9	2.7	27.0–33.8
Sb	9	9	0.07–0.11	0.085	0.073	16.4	0.063–0.083
Sc	10	10	0.14–0.19	0.17	0.17	0.6	0.15–0.19
Ti	8	8	6.9–60.9	27.2	----		
V	10	10	1.14–1.80	1.45	1.47	1.2	1.25–1.69
Zn	17	14	29.0–35.9	31.9	30.4	5.0	27.0–33.8

As shown in figure 1 the value for chromium is in very good agreement with the reference value. However, a closer look at the individual results and the analytical technique applied shows a discrepancy between destructive (A, E, M) and non-destructive analytical techniques (N, P) (Fig. 2). Figure 2 shows the values for individual laboratories in the NAT-5 study, the mean value of this study, and the 95% confidence interval calculated from the data of the NAT-5 study. Out of 13 results 7 were determined with non-destructive techniques and one average value determined with NAA was rejected as outlier by the statistical data evaluation. Results obtained with destructive analytical techniques are marked with filled points. The distribution of submitted results shows a discrepancy between the values obtained with destructive and non-destructive methods. However, 12 out of 13 results are inside the 2 Z-score range for accepted values. This difference between values obtained with non-destructive and destructive techniques was also observed in several previous quality control exercises of the IAEA [6].

3. NAT-6 QUALITY CONTROL EXERCISE

Figure 3 shows preliminary results for chromium in the NAT-6 quality control exercise on two moss samples. The distribution of the submitted average values shows a nearly perfect Gaussian distribution, however, the results show again a discrepancy between values obtained by non-destructive and by destructive analytical methods. 17 average values were provided for chromium: 10 participants used destructive analytical techniques, and 7 participants determined the values using non-destructive analytical techniques (NAA). Two results were rejected as extreme outliers having a Z-score of more than +5. It is obvious from figure 3 that three results obtained by destructive analytical techniques are in between the results of the non-destructive techniques. Two laboratories using the same digestion technique, but different analytical methods for determining the measurands submitted these three results. They used HNO_3/HF and pressure digestion for dissolving the sample, and AAS and ICP-AES for the measurement. All participants using sample decomposition without HF provided lower values for chromium. It is known that the chromium recovery is strongly dependent on the analytical technique used, and sample decomposition method applied [7].

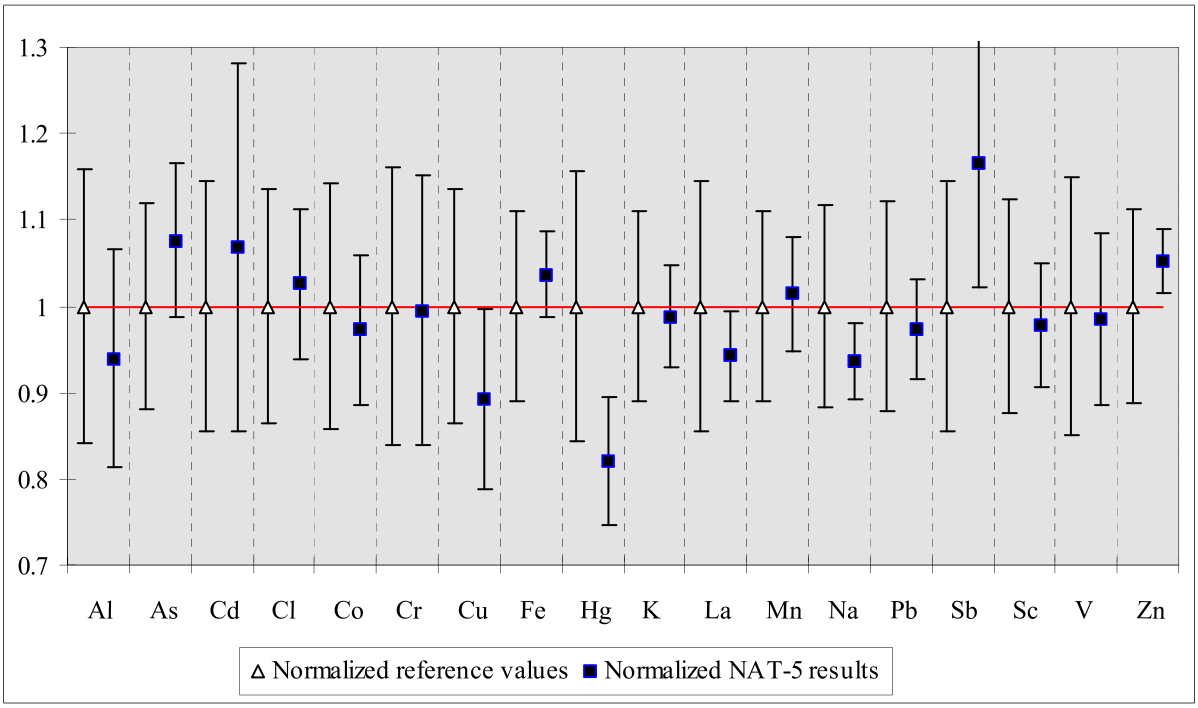


FIG. 1. NAT-5 quality control exercise: comparison of NAT-5 average values with the target value from the reference sheet.

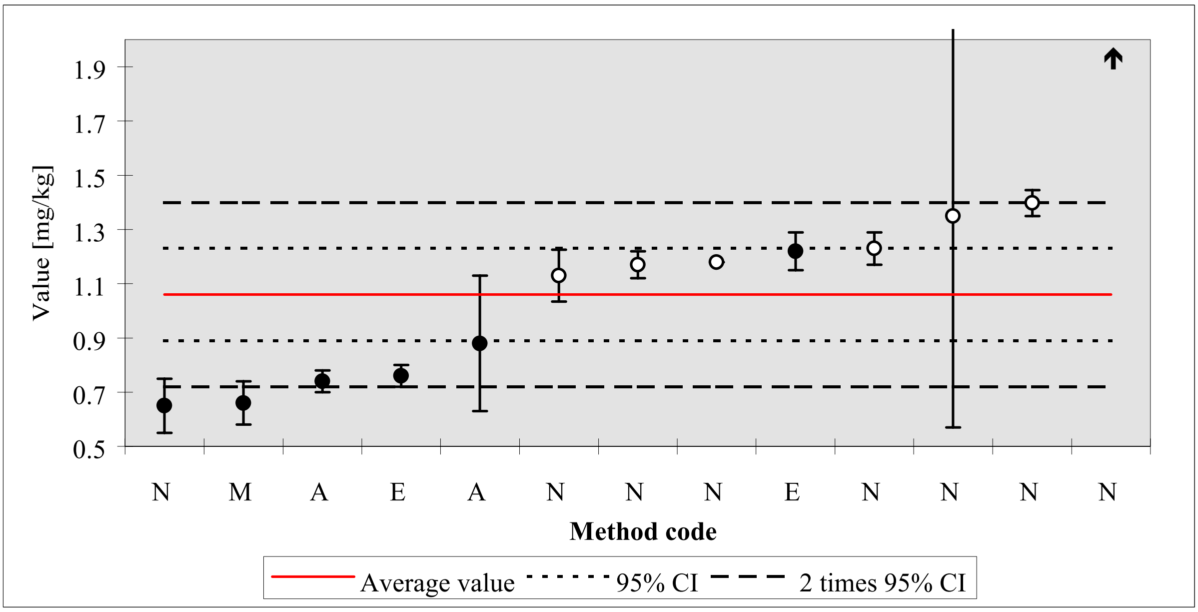


FIG. 2. NAT-5 quality control exercise: laboratory average values for chromium.

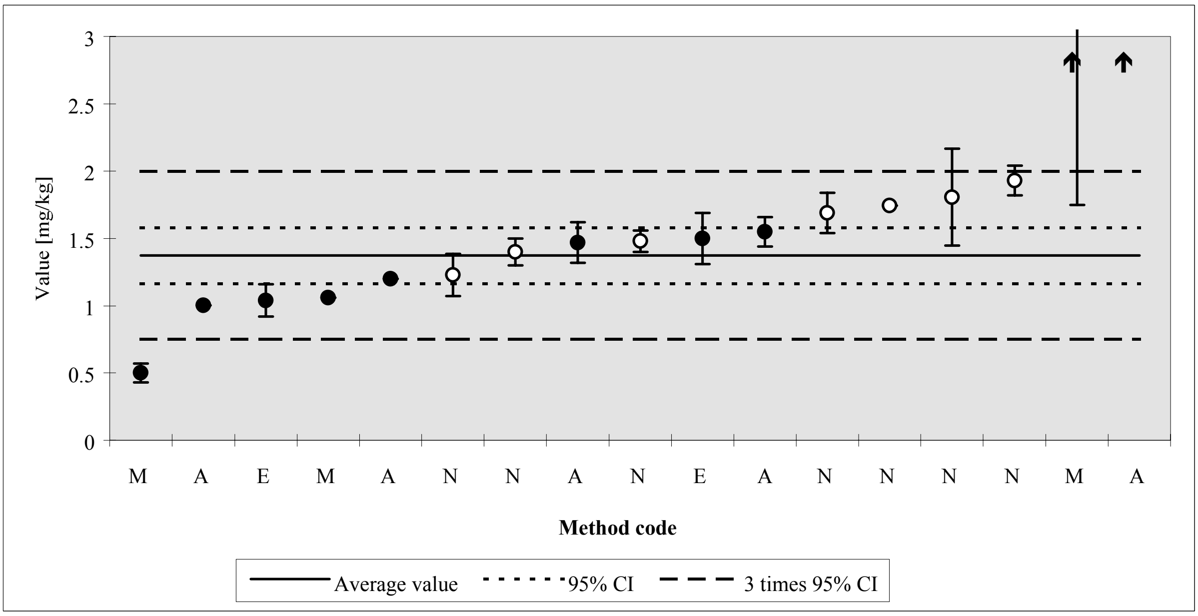


FIG. 3. NAT-6 quality control exercise: laboratory average values for chromium.

4. CONCLUSION

Interlaboratory comparison studies and proficiency tests are useful tools to evaluate the performance of laboratories and to show whether the applied analytical method is suitable for the type of sample. Nuclear analytical techniques, in particular neutron activation analysis (NAA) proved again its capability to analyze a large range of elements of environmental interest. In two quality control studies on bioindicators NAA laboratories showed a good performance. It was revealed that for some measurands of environmental interest a bias may exist in the values derived from non-destructive and destructive analytical techniques. An example is chromium, which revealed the largest bias in the two exercises. The values submitted for both quality control exercises on bioindicators uncover that the total amount of chromium can only be determined with destructive analytical techniques involving a sample digestion with HF. Mineral acids are not sufficient for dissolving the entire amount of several trace elements from biological samples [8]. As a result of there two quality control exercises the use of HF for sample digestion if needed is strongly recommended in addition to an appropriate analytical determination.

Quality control exercises are important tools in obtaining high quality measurement results for bioindicators. Therefore, the IAEA plans to continue organizing quality control exercises.

ACKNOWLEDGEMENTS

The authors wish to thank the 15 participating laboratories for co-operating, checking the values prior to the statistical data evaluation, and providing the results within the given deadline. In addition, we like to thank the Chemistry Unit of the IAEA laboratory in Seibersdorf for preparing and providing the two lichen materials, especially Mr. Z. Radecki, who assisted in arranging the samples for shipment.

REFERENCES

[1] RÜHLING, A., STEINES, E., “Atmospheric Heavy Metal Deposition in Europe 1995–1996”, Nord 1998:15, Nordic Council of Ministers, Copenhagen, 1998.

[2] SZOPA, Z., JASZCZUK, J., DYBCZYNSKI, R.,”A New Multifunctional PC Program for Evaluation of Interlaboratory Comparison Results”, Nukleonika **41 (4)** (1996) 117–127.

[3] SZOPA, Z., DYBCZYNSKI, R., “AQCS — A PC Program for Evaluation of Interlaboratory Comparison Results as Applied to Some New Polish CRMs”, CERM’96, Proceedings, Liptovsky Jan, Slovakia, 30 September–4 October 1996, 72–76.

[4] Draft ISO 13528 “ Statistical methods for use in proficiency testing by interlaboratory comparison”, November 1998.

[5] BLEISE, A., SMODIS, B., “Intercomparison run NAT-5 for the determination of trace and minor elements in two lichen samples”, NAHRES-46, Vienna, December 1999.

[6] HELLER-ZEISLER, S.F., et al., “Report on the intercomparison run for the determination of trace and minor elements in lichen material IAEA-336”, NAHRES-33, IAEA/AL/079, Vienna, June 1999.

[7] KALEMBKIEWICZ, J., FILAR, L., “The influence of soil sample preparation procedure on result of chromium determination by the FAAS method”, Chem. Anal (Warsaw) **42(1)** (1997) 87–93.

[8] Polkowska, H., et al, “Effect of acid digestion method on cobalt determination in plant materials”, Anal. Chimica Acta **408** (2000) 89–95.

CHARACTERIZATION OF DIFFERENT BIOMATERIALS FOR BIOMONITORING THE ATMOSPHERIC POLLUTION

P. AVINO[1], P.L. CARCONI[2], L. LEPORE[1], A. MOAURO[2]

[1] ISPESL — Dipartimento Insediamenti Produttivi ed Interazione con l'Ambiente,
Monte Porzio Catone (Rome), Italy
[2] National Agency of New Technologies, Energy and Environment (ENEA),
Casaccia Center, Rome, Italy,

Abstract

Instrumental Neutron Activation Analysis coupled with the Inductively Coupled Argon Plasma — Atomic Emission Spectroscopy, has been used to evaluate non destructively 25 minor and trace elements in different vegetal materials to be considered as biomaterials useful for atmospheric aerosol sampling. For quantitative evaluations, some reference materials prepared by an Inter-Institutes Committee (CII) and analyzed in an intercomparison campaign have been used. Strong attention has been devoted to the quantification of uncommon elements, important to establish the sensitivity of the employed method and the homogeneity of the sampling.

1. INTRODUCTION

The atmospheric pollution of an urban area is a problem of notable complexity for which it is very difficult to find an appreciable solution. For instance, if we consider, that in the city of Rome, the principal fonts of issue have constituted from the autovehicular traffic [1] and from the fittings of domestic heating it appears clear, as it is difficulty to define a saving plain for a big city like Rome. The increasing interest in the control of environmental pollution by some biochemical and agrochemical investigations often demands analysis of various types of plants, especially lower plants like lichens [2–4].

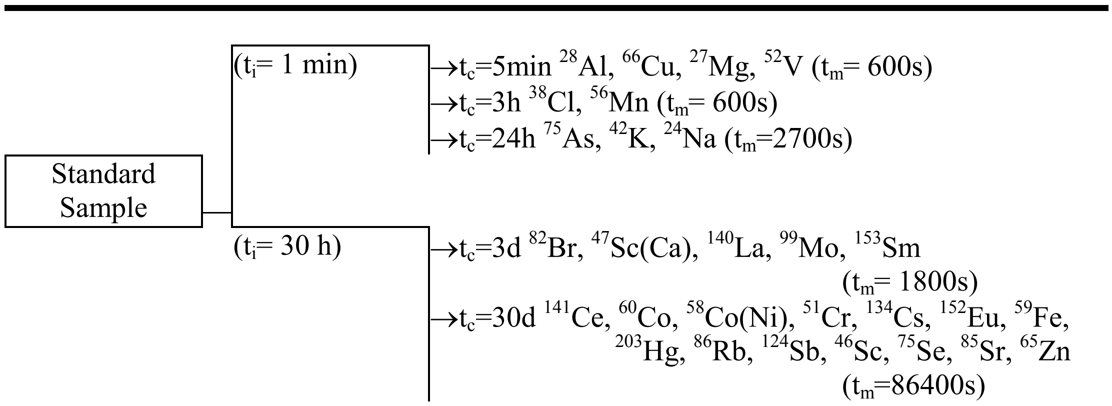
Since long time our group has devoted to understand what is the best plant for that and what is the mechanism to do it. For this reason, an important task of our project is to compare some different kinds of raw materials as biomonitors of the atmospheric pollution to be able to choose the best ones. In particular, the biomaterials involved are algae, bread and durum wheat, potato, apple, pear and tobacco. We would like to present some rapid criteria developed by the authors for choosing the best biomaterials for biomonitoring the atmospheric pollutants, beginning from the analytical procedure to investigate as more possible compounds. In fact, among the available analytical techniques frequently used in such studies, flameless atomic absorption spectrometry and X ray fluorescence, e.g., can be utilized on powdered or pelletized samples. However, in some cases the sample size is small for both techniques to obtain significant results. Instrumental Neutron Activation Analysis (INAA) has no these drawbacks, especially for some ecotoxic metals, so we have analyzed the sample by INAA coupled with the Inductively Coupled Argon Plasma — Atomic Emission Spectroscopy (ICP-AES) to analyze about 25 heavy metals [3,5]. The measurements have been carried out employing reference materials of same matrix prepared by International Atomic Energy Agency (IAEA) and Committee International Institutes (CII) and controlled in different intercomparison campaigns.

2. EXPERIMENTAL

The sampling of the products has been performed in different Italian regions (Emilia, Lazio, Campania and Basilicata, in north, center and south of Italy). In Emilia tomatoes, apples

(Yellow, Spur and Golden types), pears (Conference type), bread and durum wheat were sampled; in Lazio algae (*Spirulina platensis* and *maxima*); in Campania tobacco, tomatoes and apples; in Basilicata tomatoes and durum wheat.

For INAA, the ENEA-TRIGA research reactor has been used as a neutron source for irradiating vegetal samples (0.5–1.0 g in a polyethylene vial) for analytical purposes. Two types of neutron irradiation have been performed: a 30 hours irradiation in the rotating rack at 2×10^{12} $n\cdot cm^{-2}\cdot s^{-1}$ for long-lived nuclides, a 1 minute irradiation in the pneumatic device at 2×10^{13} $n\cdot cm^{-2}\cdot s^{-1}$ for short-lived ones. In Figure 1 the experimental conditions are schematically reported.



t_i= irradiation time
t_c = cooling time
t_m= measurement time

FIG.1. Flow scheme for INAA analysis.

After irradiation, γ ray spectrometry measurements of different duration (see Fig. 1) were carried out using an HPGe Canberra detector (resolution 1.9 keV at 1332 keV) connected to a multichannel equipped with software packages that allows all quantitative peak evaluations. The quantitative determinations are made by comparing the activities of the sample and a Standard Reference Material irradiated and measured in the same conditions.

3. RESULTS AND DISCUSSION

The peculiarities of INAA are: i) the sample is not dissolved or incinerated, avoiding contamination from chemicals and manipulations: in this way a "blank" is not necessary; ii) many elements can be determined simultaneously with high sensitivity [6], as can be seen in Table I.

In Table II, INAA results are reported for to some elements in different Standard Reference Materials (X and CV% refer to the mean value and the coefficient of variation, respectively). For them, the usual analytical methods have hardly an adequate sensitivity, instead their determination is very important and full of useful information. In fact, lanthanides have been considered up to now irrelevant in biological processes: as reported by many authors, the distribution pattern of these elements in plants and soils is not very different [7]. However, in a work [8] it is reported a possible antagonistic role played by these elements in Ca and Mg mechanisms, suggesting useful investigations in this field. Further, lanthanides and rare elements, as Sc, Cs, Rb can also be used in homogeneity tests for candidate reference material [9] or as pollution indicators or in homogeneity tests.

In Table III it is summarized the minimum and maximum levels of the elements determined in the examined agricultural products like apples, algae, pears, bread (B) and durum (D) wheat, tobacco, in different conditions of traffic, pollution, use of fertilizers, etc. The precision of the analysis (RSD) is generally between 3 and 10%

From a nutritional point of view these values are very interesting. In fact, if we make a provision of the weekly intake of some elements as dietary components and we compare our values with the recommended by National Academy of Sciences (NAS) [10] and World Health Organization (WHO) [11] we find that cobalt, chromium, and copper values are exceeded (Table IV).

From an environmental point of view we have chosen these biomaterials for their particular characteristics, i.e. using them in the atmospheric control through their power of entrapping the most common hazardous metals. Of course, in all the studied cases the plant contamination depends widely on the place where they grow. Figure 2 shows a γ-spectrum of neutron irradiated tobacco sample.

TABLE I. ACTIVATION NUCLIDES BY (n, γ) REACTION

Element	**Nuclear reaction**	**$T_{1/2}$**	**Energy (keV)**	**Sensitivity (µg/g)**	**Interference**
Al	$^{27}Al(n,\gamma)^{28}Al$	2.31 m	1778.9	0.8	
As	$^{75}As(n,\gamma)^{76}As$	26.3 h	559.2	0.008	^{122}Sb
Br	$^{81}Br(n,\gamma)^{82}Br$	35.87 h	776.6	0.02	
Ca	$^{46}Ca(n,\gamma,\beta^-)^{47}Sc$	3.43 d	159.8	260	
Ce	$^{140}Ce(n,\gamma)^{141}Ce$	32.5 d	145.4	0.01	
Cl	$^{37}Cl(n,\gamma)^{38}Cl$	37.29 m	1642.0	35	
Co	$^{59}Co(n,\gamma)^{60}Co$	5.4 a	1332.5	0.0008	
Cr	$^{50}Cr(n,\gamma)^{51}Cr$	27.8 d	320.1	0.09	
Cs	$^{133}Cs(n,\gamma)^{134}Cs$	2.07 a	795.8	0.003	
Cu	$^{65}Cu(n,\gamma)^{66}Cu$	5.1 m	1039.0	8.0	^{70}Ga
Eu	$^{151}Eu(n,\gamma)^{152}Eu$	12.2 a	1407.5	0.0002	
Fe	$^{58}Fe(n,\gamma)^{59}Fe$	45.1 d	1098.6	5.0	
Hg	$^{202}Hg(n,\gamma)^{203}Hg$	46.9 d	279.1	0.01	^{75}Se
K	$^{41}K(n,\gamma)^{42}K$	12.52 h	1524.7	260	
La	$^{139}La(n,\gamma)^{140}La$	40.27 h	1595.4	0.0005	
Mn	$^{55}Mn(n,\gamma)^{56}Mn$	2.58 h	1810.7	0.1	
Mo	$^{98}Mo(n,\gamma)^{99}Mo$	2.75 d	739.5		
Rb	$^{85}Rb(n,\gamma)^{86}Rb$	18.66 d	1076.6	0.4	
Sb	$^{123}Sb(n,\gamma)^{124}Sb$	60.2 d	1690.9	0.01	
Sc	$^{45}Sc(n,\gamma)^{46}Sc$	83.9 d	889.4	0.0008	
Se	$^{74}Se(n,\gamma)^{75}Se$	121.0 d	264.6	0.01	^{182}Ta
Sm	$^{152}Sm(n,\gamma)^{153}Sm$	47.1 h	103.2	7.0	
Sr	$^{84}Sr(n,\gamma)^{85}Sr$	64.0 d	514.0	0.08	
V	$^{51}V(n,\gamma)^{52}V$	3.76 m	1434.4	10	
Zn	$^{64}Zn(n,\gamma)^{65}Zn$	245.0 d	1115.4	0.04	^{46}Sc

TABLE II. INAA VALUES (µg/g,%) FOR SOME ELEMENTS HARD TO QUANTIFY, IN DIFFERENT VEGETAL CII SAMPLES.

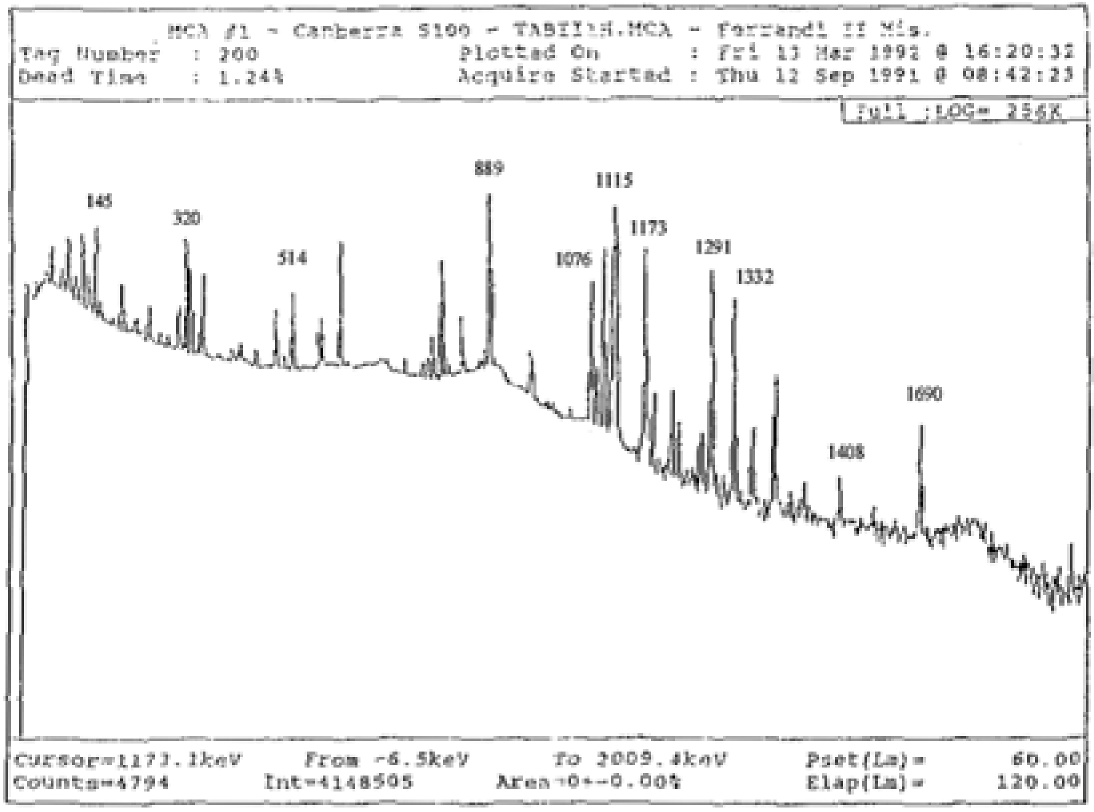
	Th	**Sc**	**Eu**	**Ce**	**Sm**	**La**	**Cs**	**Rb**
Ray-Grass								
X	0.273	0.273	0.046	2.34	0.360	0.884	0.075	15.4
CV%	4.0	4.1	4.2	9.8	3.6	6.2	6.1	8.8
Betterave								
X	0.120	0.062	0.021	0.770	0.123	0.332	0.071	29.0
CV%	11.7	4.1	4.2	9.8	12.5	15.9	6.1	8.8
Salade								
X	0.101	0.071	0.016	0.777	0.162	ab	0.054	17.4
CV%	12.5	9.3	10.8	7.9	14.8		6.0	7.2
Tourble-blonde								
X	0.152	0.212	0.041	2.0	0.373	0.904	0.049	2.1
CV%	14.6	8.4	13.0	18.0	15.3	9.5	17.7	5.1
Ecorce de pin								
X	0.463	0.271	0.117	4.1	0.290	2.5	0.158	12.0
CV%	10.6	6.9	18.6	18.0	5.5	22.1	15.6	12.1
Pomme fruit								
X	0.024	0.007	0.002	0.098	0.027	0.243	0.020	6.4
CV%	14.2	23.9	22.4	24.6	18.6	19.3	10.4	4.3

TABLE III. CONCENTRATION INTERVALS EXPRESSED AS µg/g (MIN-MAX). LOD: LIMIT OF DETECTION; N.D.: NOT DETECTED, *: BY ICP-AES ANALYSIS, **: EXPRESSED AS%.

	Apple	**Alga**	**Tomato**	**D. wheat**	**B. wheat**	**Pear**	**Tobacco**
Al	28.0–33.3	n.d.	18.0–33.0	20.5–51.5	20.8	86.5	3300
As	0.03–0.25	n.d.	0.10	0.01–0.15	0.03	n.d.	0.79
Br	0.21–0.59	13.6–125	1.00–40.0	1.88–3.29	3.29	n.d.	54.0
Ca	269–587	1110–17100	2600–2500	452–800	716	n.d.	28700
Cd*	1.16–170	<LOD	2.21–3.40	1.24–1.35	<LOD	1.65	3.81
Ce	<LOD	N.D.	0.16	<LOD	<LOD	<LOD	4.53
Cl	84.0–92.6	4890–5960	5000–7000	513–774	513	121	3700
Co	0.04–0.05	0.31–0.72	0.04–0.11	0.02–0.05	0.02	0.26	0.41
Cr	0.15–1.49	9.02–18.6	1.16–2.53	0.33–0.50	0.33	1.57	3.89
Cs	<LOD	0.02–0.08	0.04–0.10	<LOD	<LOD	<LOD	0.81
Cu	3.70–5.18	14.9–69.6	9.60–13.0	7.32–8.96	n.d.	15.3	15.4
Eu	n.d.	0.02–0.06	0.03	n.d.	n.d.	n.d.	1.27
Fe	21.9–29.4	313–1176	72.9–78.4	38.3–75.7	38.3	14.0	936
Hg	0.02	0.07–0.12	0.02	<LOD	<LOD	0.06	<LOD
K **	0.64–0.93	0.87–0.93	3.88–4.72	0.46–0.68	0.39	0.83	4.88
La	0.04–0.10	0.07–1.83	0.07–0.12	0.01–0.03	0.01	<LOD	<LOD
Mn	2.8–7.4	49.1–554	9.60–16.6	27.9–60.5	35.3	n.d.	89.2
Mo	0.17–0.57	n.d.	0.08–1.52	0.78–0.82	n.d.	n.d.	n.d.
Ni*	11.6–12.6	0.10	1.20–5.16	2.54–3.79	n.d.	0.82	15.2
Pb*	0.44–3.65	6.95–12.9	1.82–7.04	3.95–4.70	n.d.	<LOD	16.6
Rb	0.61–14.1	<LOD	14.8–33.6	4.41–4.90	2.37	16.1	0.57
Sb	0.03–0.07	0.06–0.14	0.02–0.03	0.01–0.06	0.06	<LOD	0.27
Sc	0.002–0.004	0.027–0.248	0.001–0.010	0.002–0.004	0.001	0.004	0.17
Se	<LOD	0.09–0.20	0.02–0.08	0.05–0.11	<LOD	<LOD	0.06
Sr	<LOD	<LOD	5.00–8.41	3.19–6.10	<LOD	<LOD	80.5
V*	8.6–9.5	3.16	2.71	<LOD	<LOD	0.12	<LOD
Zn	2.6–6.9	14.2–375	24.1–28.0	24.5–43.3	24.5	15.0	34.2

TABLE IV. WEEKLY INTAKE OF ELEMENTS THROUGH WHEAT, APPLES, PEARS AND TOMATOES PRODUCED IN SOME ITALIAN FARMS, COMPARED WITH NAS [10] AND WHO [11] RECOMMENDATION, FOR A 70 kg MAN AND A MEDITERRANEAN DIET: a) EXPRESSED AS VITAMIN B_{12}; b) MAXIMUM ACCEPTABLE DAILY LOAD; c) EXPRESSED AS METHYL-Hg.

Element	Weekly intake (mg)	WHO recommended intake (mg)
Al	**127–340**	**490**
As	**0.18–1.05**	**1.05**
Co	**0.18–2.14**	**0.02**[a]
Cr	**1.76–7.23**	**0.35–1.40**
Cu	**28.6–68.4**	**14–21; 35**[b]
Hg	**0.025–0.037**	**0.343; 0.245**[c]
Se	**0.09–0.25**	**0.35–1.40**



```
               MCA #1 - Canberra S100 - TABII1H.MCA - Ferrandi II Mis.
Tag Number  : 200                   Plotted On       : Fri 13 Mar 1992 @ 16:20:32
Dead Time   : 1.24%                 Acquire Started  : Thu 12 Sep 1991 @ 08:42:23
                                                                   Full :LOG= 256K
Cursor=1173.1keV     From -6.5keV       To 2009.4keV        Pset(Lm)=        60.00
Counts=4794        Int=4148505      Area=0+-0.00%           Elap(Lm)=       120.00
```

FIG. 2. γ-Spectrum of neutron irradiated tobacco sample. It is reported some energy peaks: 145 keV, ^{141}Ce; 320 keV, ^{51}Cr; 514 keV, ^{85}Sr; 889 keV, ^{46}Sc; 1076 kev, ^{86}Rb; 1115 keV, ^{65}Zn; 1173 keV, ^{60}Co; 1291 keV, ^{59}Fe; 1332 keV, ^{60}Co; 1408 keV, ^{152}Eu; 1690 keV, ^{124}Sb

The algae involved *Spirulina platensis* and *maxima*, have a morphological structure that allows to entrap the pollutant: they are helicoidal and empty inside so the metals can be irreversibly trapped. For these reasons an analysis of Table III shows that *Spirulina* samples have the highest values for Br, Ca, Co, Cr, Cu, Fe, Hg, La, Mn, Pb, Sb, Se, and Zn among the crops examined. The durum wheat is a very sensitive biomaterial but some theories show that it is not sensitive to the atmospheric aerosols. The raw tomato is a good example of double pollution exposition from both soil and air. Although the tomato is helpful to describe the soil pollution because there is proportional and no-reversible metal absorption, in aerosol pollution it is not true. In our experience supported by other authors [12–14] during the washing step and/or the pretreatment to obtain the biomass contamination may occur and the no-waiting artifacts can influence the measurements. Apples and pears are not so good as biomaterials for their hygroscopy due to the fructose content: the effect on the metal content is a general loosing of some important metals in the spectrum composition (Table III). Finally, the tobacco is a biomaterial very close to algae for its peculiarity: in fact it has very large leaves exposed to the atmospheric aerosols and it can trap elements chemically too. However, the disadvantage is that the exposed area is really too large and this can cause some artifacts during the analysis.

4. CONCLUSIONS

From the analytical point of view INAA has been widely involved in the determinations and it is shown very helpful to solve the problem. The method consists in a neutron irradiation of the sample to transform a natural element in its radioactive isotope and the radioactive nuclides so obtained are measured by gamma spectrometry to evaluate the elemental concentrations. In this paper two typical applications of this method were reported: i) the determination of some elements in CII, IAEA reference materials, particularly at low concentration, not easily determined by the usual analytical methods; ii) the control of ecotoxic elements in fruits, tomatoes, cereals, algae, etc. sampled in different Italian regions, relevant to the industrial or traffic pollution. Furthermore, the role of INAA is underlined as a reference method for quality control [15] in the atmospheric pollution field. Among the different vegetal sample analyzed, the highest values were found for tobacco leaves but we consider the algae *Spirulina* as a reasonable candidate to be biomaterial for biomonitoring on line the atmospheric pollution. In this way we are trying to find out the correlation between the metal concentration and the adsorption by the vegetal [16].

REFERENCES

[1] AVINO, P., BROCCO, D., LEPORE, L., VENTRONE, I., Fundamental aspects of carbonaceous particulate measurements in the study of air pollution in urban area. In: Air Pollution 2000 (C. Brebbia, Ed.), WIT Press, Wessex, UK, 2000, 301–309.

[2] CIRILLO, M.C., BOCOLA, W., TRIOLO, L., Environment impact of air pollutants with emphasis on crops. The Piacenza case study. In: Advances in environmental modelling (A. Marani, Ed.), Elsevier, Amsterdam, 1988, 293–312.

[3] KOS, V. BUDIC, B., HUDNIK, V., LOBNIK, F., ZUPAN, M., Determination of heavy metal concentrations in plants exposed to different degrees of pollution using ICP-AES, Fresenius J. Anal. Chem. 354 (1996) 648–652.

[4] FREITAS, M.C., NOBRE, A.S., Bioaccumulation of heavy metals using *Parmelia caperata* for air pollution studies, J. Radioanal. Chem. 217 (1997) 17–20.

[5] QUEROL, X., ALAUSTEY, A., LÓPEZ-SOLER, A., BOIX, A., SANFELIU, T., MARTYNOV, V.V., PIVEN, P.I., KABINA, L.P., SOUSCHOV, P.A., Trace element contents in atmospheric suspended particles: inferences from instrumental neutron activation analysis, Fresenius J. Anal. Chem. 357 (1997) 934–940.

[6] CURRIE, L., Limits for qualitative detection and quantitative determination. Application ro radiochemistry., Anal. Chem. 40 (1968) 586–593.

[7] MARKERT, B., The pattern of distribution of lanthanide elements in soils and plants, Phytochemistry 26 (1987) 3167–3170.

[8] WILLIAMS, R.J.P., Gadolinium and friends, Nature 344 (1990) 595.

[9] GUZZI, G., COLOMBO, A., GIRARDI, F., PIETRA, R., ROSSI, G., TOUSSAINT, N., Comparison of various analytical techniques for homogeneity test of candidate standard reference materials, J. Radioanal. Chem. 39 (1977) 263–276.

[10] Recommended Dietary Allowances, Ninth Edition, National Academy of Sciences, Washington DC, 1980.

[11] WHO Technical Report Series, WHO, Geneva: Nos. 683 (1982), 776 (1989).

[12] SANSONI, B., Multi-elemental analysis for environmental characterization, Pure Appl. Chem. 59 (1987) 579–610.

[13] LIETH, B., and MARKERT, B., Methods of assessment and evaluation of elemental concentration cadasters in ecosystems, (B. Lieth and B. Markert Eds.), VCH, Weinheim, 1990, pp.448.

[14] MARKERT, B., Aspects of cleaning environmental materials for multi-element analysis, e.g. plant samples, Fresenius J. Anal. Chem. 342 (1992) 402–412.

[15] HEYDORN, K., Quality control in activation analysis, J. Radioanal. Chem. 151 (1991) 139–148.

[16] AVINO, P., CAMPANELLA, L., CRESCENTINI, G., CUBADDA, F., SAMMARTINO, M.P., Biomonitoraggio marino con sistemi algali, Proceeding of the 1st National Conference on Chemistry for the Environment, Ferrara, Italy, 26–28 February 1997, C22.

WATER SOLUBLE METALLOPROTEINS IN *HYPOGYMNIA PHYSODES*

I. FALNOGA[1], M. TUŠEK-ŽNIDARIČ[1], Z. JERAN[1], J. PAVIČIĆ[2],
D. IVANKOVIĆ[2], J. ŠČANČAR[1]

[1] Jožef Stefan Institute, Ljubljana, Slovenia
[2] Institute Ruđer Bošković, Zagreb, Croatia

Abstract

The survival of some lichens in environments with elevated levels of metals points to the existence of resistance and tolerance mechanisms in the thalli. In general the major molecules involved in *intracellular sequestration* and consequent detoxification of metal ions belong to the family of cysteine-rich proteins called metallothioneins. In our investigation the isolation and partial characterisation of such S-containing proteins or polypeptides from the lichen *HYPOGYMNIA PHYSODES* was attempted. The lichen was taken close to a lead smelter and contained high concentration of lead, zinc and cadmium (263.5, 96.3, 2.8 μg/g). After homogenisation and ultra filtration of the sample the presence of metal binding low molecular weight proteins was identified by SDS electrophoresis and size exclusion chromatography (Sephadex G-75) where UV absorption and the concentration of metals were determined in column eluents.

1. INTRODUCTION

Metal-binding proteins are important in the modulation of intracellular concentrations of both essential and nonessential metal ions. A variety of metal-binding cytosolic polypeptides with cystein thiolate groups have such roles and include the metallothioneins (MTs), characterised by low molecular mass, high metal content, high cysteine (cys) content, a lack of the aromatic amino acids and histidine, and by the spectroscopic properties of metal thiolates. They were discovered in mammalian systems and other animals but can also be found in plants, fungi and algae. In fungi they have been characterised as classical proteinaceous class I MTs or class II MTs, closely or distantly related to earlier discovered equine renal protein and as atypical, class III MTs, gamma-glutamyl-cysteinyl isopolypeptides, found mainly in plants and algae [1].

Regarding literature data such proteins have not yet been identified in lichens, although the survival of some lichens in environments with elevated levels of metals points to the existence of resistance and tolerance mechanisms in the thalli. Therefore, it is supposed that the major molecules involved in intracellular sequestration and consequent detoxification of metal ions belong to the family of cysteine-rich proteins or isopolypeptides called MTs [2].

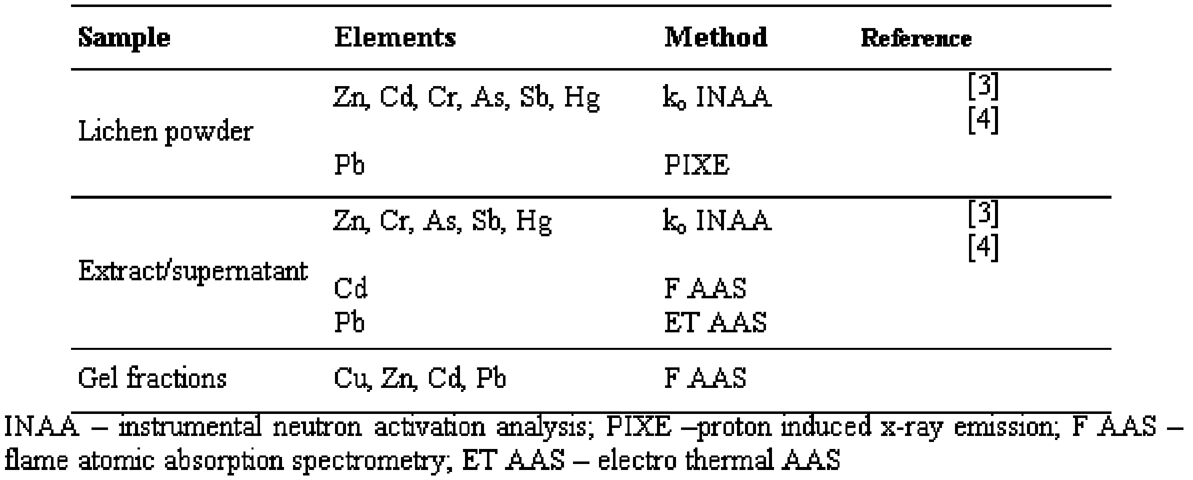
The aim of the study was to investigate the presence of and partially characterise low molecular weight MT — like proteins from an epiphytic lichen *Hypogymnia physodes (L.) Nyl*. The lichen was collected from the northern part of Slovenia close to a lead smeltery.

2. MATERIAL AND METHODS

Lichen sample

Hypogymnia physodes, (L.) Nyl. — naturally exposed epiphytic lichen was collected from an apple tree at 780m above sea level in northern Slovenia in the vicinity (about 10 km) of a Pb smelter. In the laboratory lichen thalli were freeze-dried with addition of liquid N_2 and powdered in an agate mortar and pestle.

TABLE I. SAMPLES, ELEMENTS AND METHODS



Sample	Elements	Method	Reference
Lichen powder	Zn, Cd, Cr, As, Sb, Hg	k_0 INAA	[3] [4]
	Pb	PIXE	
Extract/supernatant	Zn, Cr, As, Sb, Hg	k_0 INAA	[3] [4]
	Cd	F AAS	
	Pb	ET AAS	
Gel fractions	Cu, Zn, Cd, Pb	F AAS	

INAA – instrumental neutron activation analysis; PIXE –proton induced x-ray emission; F AAS – flame atomic absorption spectrometry; ET AAS – electro thermal AAS

Extraction and isolation of water soluble proteins

The powdered sample was homogenized (1:10, w/v) with a glass homogenizer and a motor driven Teflon pestle in N_2-saturated 10 mM Tris-HCl buffer (pH 7.6, 1–4°C) with 10mM DTT (dithiothreitol), 1mM PMSF (phenylmethylsulphonyl fluoride) and 0.25 M saccharose. The homogenate was ultracentrifuged at 100 000 g for 1h at 4°C (Kontron Instruments). The supernatant was strained through a 250-um nylon net.

Part of the supernatant (extract) was heat treated for 5 minutes in a water bath at 90°C, cooled and centrifuged at 40 000g for 20 minutes at 4°C.

Sephadex G-75 gel chromatography of the basic (crude) and heat treated supernatant was performed on calibrated columns, sized 2.4 × 65 cm and 1 × 70 cm, respectively. The elution buffer was 10 mM Tris-HCl buffer (pH 7.6, 4°C) containing 1mM DTT and N_2-saturated, the flow rate was 13.8 ml/min. UV absorption at 254 and 280 nm and the concentrations of metals (Zn, Cu, Cd, Pb) were determined in gel fractions (6–7 and 4–5ml, respectively).

The columns were standardized with marker proteins of known molecular weight (Pharmacia, Serva): blue dextran (2 000 000), human albumin (67 000), chymotripsinogen A (25 000) and cytochrome c (12 400).

SDS-PAGE electrophoresis

We used MINI-PROTEAN II, BIORAD (200V, 45 minutes) SDS-PAGE electrophoresis. The sample of supernatant or MT standard (Sigma) was mixed 1:1 with a Laemmli sample buffer, concentrated 2x, heated to 95°C for 4 minutes and cooled in ice. The volume of the sample was 20 uL. For dyeing and undyeing Coomasie Stain Solution (BIO-RAD); Coomasie R-250 (BIO-RAD) Destain Solution and 10–20% polyacrylamide gel (Ready gel) were used.

Metal determination

Various metals and metalloids were determined in the lichen and in its fractions obtained after the biochemical separation procedure as specified in Table I.

3. RESULTS AND DISCUSSION

Metal concentrations in lichen thalli and its water soluble extract

Table II shows metal (Pb, Zn, Cd, Cr, Hg) and metalloid (As, Sb) concentrations in *H.physodes* collected near the Pb smelter. The elements investigated were chosen with regard to possibly elevated levels in the smelter area and their capacity for metallothionein induction. Results are accompanied by literature data obtained for the same lichen species collected in an unpolluted area (background air pollution). Comparing the values it is evident that in the 'experimental' sample the levels of all elements are increased, especially for Pb, Cd, As, and Sb (one order of magnitude).

In the water soluble extract of the freeze-dried sample the percentage of metal extraction was quite low, less than 20%; the lowest for Pb (0.6%) and the highest for Cd (18%) (Table III). This means that major metal accumulation and/or retention in lichen is associated with metal sequestration in water insoluble forms such as metal-rich particulate entrapment on the lichen surface or in intercellular spaces, and extracellular complexation to cell walls.

Water soluble metalloproteins: metallothionein-like proteins

Figure 1 depicts metals and UV absorbance elution profiles of crude lichen supernatant on a calibrated Sephadex G-75 column where the molecular weight of cytochrome c (cyt, 12 000) is characteristic of a typical MT. The metals Cu, Zn, Cd and Pb are distributed among two different but connected sharp peaks around the range of cytochrome, and Zn is also found in the long shoulder-shaped peak at the end of the chromatogram. The second and the highest peaks where the binding of Cu, Zn and Cd coincide have a molecular weight of about 10 000.

Beside molecular weight and metal binding, heat resistance is another characteristic of MTs. In Figure 2 Sephadex G-75 gel chromatography of heat treated supernatant is shown. The molecular weight range of the Cu and Zn peaks (Cd was not measured) is higher than in the chromatogram of the crude supernatant. We suppose that dimerization of the protein occurred due to air oxidation during heat treatment. This fact was later checked by repeating the whole procedure with another *H. physodes* sample.

In crude supernatant the presence of MT-like proteins was also examined by SDS-PAGE electrophores using an MT standard (Sigma) for comparison.

TABLE II. METAL CONCENTRATION IN Pb EXPOSED H.PHYSODES (FREEZE-DRIED,µg/g) IN COMPARISON WITH LITERATURE DATA FOR *H. PHYSODES* FROM UNPOLLUTED REGIONS (BACKGROUND AIR POLLUTION).

H. physodes (freeze-dried)	**Pb**	**Zn**	**Cd**	**As**	**Cr**	**Hg**	**Sb**
Pb polluted area	**263**	**96.3**	**2.78**	**2.09**	3.97	0.09	**3.44**
Unpolluted regions							
[5]	28	44	0.22		1.75		
[6]	26	57.3	0.76	0.51	2.62	0.08	0.19

TABLE III. Pb EXPOSED *H PHYSODES* — PERCENTAGE OF METAL EXTRATION FROM TISSUE (FREEZE-DRIED)

H. physodes (Pb polluted area)	**Pb**	**Zn**	**Cd**	**As**	**Cr**	**Hg**	**Sb**
Extract conc. (µg/g)	**0.16**	**0.90**	**0.05**	**0.015**	**< 0.01**	**< 0.001**	**0.016**
Percentage Extraction (%)	0.6	9.3	18	7.2	< 2.6		4.7

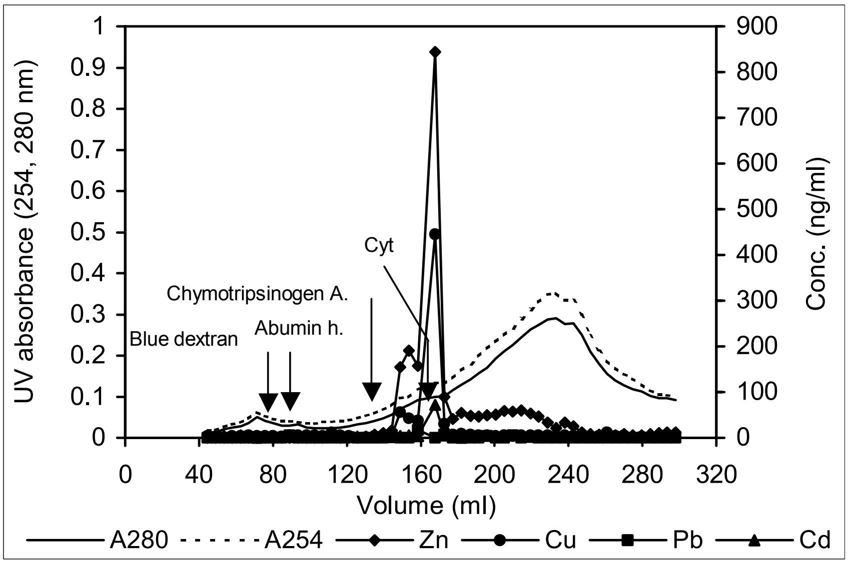


FIG. 1 Gel filtration on Sephadex G-75 (2.4 × 65 cm) of crude lichen supernatant (5ml). UV absorbances (220nm, 254nm) and metal (Cu, Zn, Pb, Cd) distribution profiles are given. Pb concentrations were mostly under the limit of detection (FAAS; 20 ng/g).

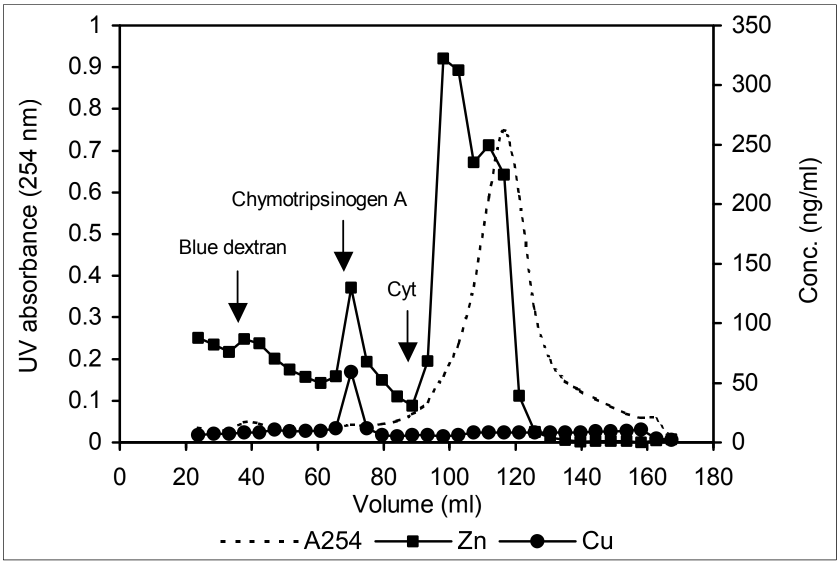


FIG. 2 Gel filtration on Sephadex G-75 (1 × 70 cm) of heat treated lichen supernatant (2 ml). UV absorbance (254 nm) and metal (Cu, Zn) distribution in elution fractions are given.

4. CONCLUSIONS

From the results it is possible to conclude that a kind of low molecular weight protein from the metallothionein family is certainly involved in the adoption of metals by lichen cells. Metallothionein-like proteins (Cu,Zn,Cd-thioneins) from Pb exposed *H. physodes* supernatant were isolated and partially characterised. To determine whether we are dealing with classical proteinaceous MT or atypical MT (isopolypeptides), the verification of its amino acid content or sequence should be performed.

REFERENCES

[1] GADD, GM., Interactions of fungi with toxic metals. New Phytol. **124** (1993) 25–60

[2] SARRET, G., MACEAU, A., CUNY, D., VAN HALUWYN, C., DERUELLE, S., HAZEMANN, J-L., SOLDO,Y., EYBERT-BERARD, L., MENTHONNEX, J–J., Mechanisms of lichen resistance to metallic pollution. Environ. Sci. Technol. **32** (1989) 3325–3330.

[3] JERAN, Z., JAĆIMOVIĆ, R., BATIČ, F., SMODIŠ, B., WOLTERBEEK, H.TH., Atmospheric heavy metal pollution in Slovenia derived from results of epiphytic lichens. Fresenius J. Anal. Chem. 354 (1996) 681–687.

[4] KEYZERO/SOLCOI for reactor-neutron activation analysis (NAA) using the Ko-standardization method. Version 4. User's manual. DMS Research, Geelen, Netherlands (1996).

[5] HERZIG, R., LIEBFNDORFER, L., URECH, M., AMMANN, K.,BUECHEVA, M., LANDOLT, W., Passive biomonitoring with lichens as a part of an integrated biological measuring system for monitoring air pollution in Switzerland. Intern. J. Environ. Anal. Chem. **35** (1989) 43–57.

[6] JERAN, Z., JAĆIMOVIĆ, R., BATIČ, F., MAVSAR, R., Lichens as integrating air pollution monitors. In preparation (2000).

AN EVALUATION OF OLIVE-TREE BARK FOR BIOMONITORING AIRBORNE CONTAMINANTS

A.M.G. PACHECO, L.I.C. BARROS
CVRM-IST, Lisbon, Portugal

M.C. FREITAS, M.A. REIS, C. HIPÓLITO, O. OLIVEIRA
DEA-ITN, Sacavém, Portugal

Abstract

There is a need for start looking into the real ability of tree bark for biomonitoring. Bark from olive trees (*Olea europaea* Linn.) has been collected over an area in north-western Portugal, featuring a blend of rural lands, industrial towns, and coastal environments. Samples were analysed through INAA and PIXE for their elemental contents. Results from both techniques were reconciled and then compared with an extensive database on 46 trace elements in *Parmelia* spp. thalli from the same sites *and* mostly from the very bark substrates. Distribution-free, nonparametric statistics show that, despite signal magnitude, variation patterns of bark and lichen concentrations follow one another in a most significant way. Selected elements in bark also correlate to a superior extent. As far as this investigation goes, there is no reason whatsoever for discarding bark as an alternative to lower epiphytes.

1. INTRODUCTION

Even though its origin can be traced back to the mid 19th century, to some early observations relating what is now called biodiversity to air pollution, the use of vegetable organisms (or parts of them) in atmospheric studies has only surged well into the 20th century. As early as 1866, the Finnish naturalist William Nylander wrapped up a few scattered references to the effects of polluted atmospheres on lichens, together with his own perception of their near-disappearance from the Luxembourg Gardens in Paris [1], to break new ground in the field of environmental assessment. Urban-industrial conurbations were the cradle of biological monitoring, as mosses were also found to be sensitive to air pollution in such an environment nearly one century ago [2]. Since then, the newborn practice has grown into a serious alternative — or, at the very least, an useful complement — to traditional (instrumental) methods of studying the deposition of airborne substances, either from anthropogenic or natural sources. There is now an impressive amount of biomonitoring work that spans a whole lot of pollution inputs to about every ecosystem on Earth, including barren or remote lands like volcanic cones or high latitudes [3–7], and that has been done with a myriad of plants at the region, community, population, individual, tissue and even cell levels [8]. The former entries are nothing but a few illustrations: a comprehensive review on the use of plants for air-monitoring purposes was given by Nimis in 1990 [9]. Since then, and according to Garty [10], the number of major new references dealing only with lichens has fallen just short of 150.

The expansive growth of plant biomonitoring has been gaining momentum mainly from lower organisms — lichens, bryophytes and, to a lesser extent, non-lichenised fungi — whereas the contribution from higher, vascular plants appears much more discreet. And yet, despite an arguably superior ability to accumulate and track down contaminants without an interference from soil elements– which is actually true only for epiphytic species — lichen communities are known for their slow regeneration, so an intensive sampling may put them in short supply or in the very threshold of extinction, and mosses seem hardly an option in dry areas. Furthermore, both are prone to uneven, patchy distributions, while their collection almost invariably requires specialists to differentiate between close-looking varieties. Of course, when it comes to vascular plants one has to cope with an active uptake through the root system, a much more elaborate physiology and metabolism, and, last but definitely not least, an identity between several air-pollution factors and plant-body constituents (*e.g.* some sulphur and nitrogen compounds). All things considered, though, biomonitoring with higher plants — especially, tree parts — seems rather underrated in view of its potential as to an availability of biological material year-round, an easier identification and sampling, and an ubiquity of some genera, such as *Quercus* (oak) or *Pinus* (pine), that makes it feasible to survey extensive areas over and repeatedly, to mention just a few assets.

Even in the realm of vascular plants, bark stands far behind leaves — or needles, for that matter — in what concerns atmospheric assessment. Long time regarded as an accumulator of airborne pollutants, hence a candidate for air monitoring, tree bark has yet to live up to such an expectation. Until recently, studies were scarce and mostly related to environmental acidification [11–16], though that situation has been going through changes in the last decade — see Schulz *et al.* [17,18] and pertinent references therein. Still, there is a need for an insight into the true capability of using bark to monitor air pollution and thereby, should it be the case, to bridge the widening gaps between the use of bark and epiphytes in atmospheric studies. As a contribution to that purpose, bark from olive trees (*Olea europaea* Linn.) has been sampled throughout mainland Portugal and searched for its elemental contents by means of two nuclear-analytical techniques: instrumental neutron activation analysis (INAA) and proton-induced X ray emission (PIXE). Results from both techniques were first reconciled and then compared with an extensive database on the concentration patterns of 46 trace elements in *Parmelia* spp. thalli from the same locations *and* mostly from the same bark substrates. Robust, nonparametric statistics have been used for measuring (quantifying) the degree of association between concentrations in barks and lichens, as well as between source-related elements in bark samples. Due to format requirements, however, only data from a subset of the whole grid along the Portuguese west coast and up to 80 km inland (228 sites), already defined in Freitas *et al.* [19], and relatively few elements can be dealt with herein.

2. EXPERIMENTAL

Better known as a distinctive feature of the archetypal Mediterranean landscape, the olive tree is actually also native to tropical and central Asia, as well as to various parts of Europe and Africa outside the Mediterranean basin. A member of the *Oleaceae* family, *O. europaea* L. is a graceful yet tenacious, long-lived evergreen tree (up to 500 years and beyond), with a pale-grey bark and a history of human-friendly utilisation that can be traced back to the very beginning of Western civilisation. Today, olive trees are grown commercially all around the world, from their autochthonous areas to North and South America (western United States; Chile and Peru), Australia, and South Africa. In mainland Portugal, that is thought to have been an original habitat for its early predecessor — the Oleaster *Olea sylvestris* — the olive tree has an ubiquitous distribution across the country, which makes it a serious candidate for comprehensive surveys of airborne contaminants. For that purpose, bark from the trunk seems more appropriate than its leaves, that have a tannin-rich skin with a waxy texture, particularly on the green (upper) side, and undergo replacement every 2–3 years.

The target area for this study — located in the north-west and depicted in Figure 1 — was chosen to approach a "worst-case situation" as to the reliability of bark indication. It is a densely-populated, highly-travelled area, and though small-scale agriculture and industry are prevalent, it also comprises the second-largest urban area in the country (Porto), together with a fair number of mid-size cities. Other than a strong marine influence near the coast and a significant dust mobilisation area-wide, due to farming and construction activities, major industrial sources include a pulp mill (Deocriste, 10 km east of Viana do Castelo), a power plant (Tapada do Outeiro, 20 km east of Porto) and a chemical complex around Estarreja (30 km south of Porto). All this comes down to a complex concoction of elemental inputs to the lower troposphere, likely to provide a broad range of disparate conditions for testing the performance of olive-tree bark relative to an epiphytic lichen — *Parmelia* spp., mostly *Parmelia sulcata* Taylor — which has proved to be quite sensitive to atmospheric pollution [20].

Details on bark collection, sample preparation and INAA procedures can be found in a former, preliminary study based only on raw data [21]. As for PIXE analysis, an average of less than 1 mg of bark was pelletised into boric acid, then irradiated with 2.0 MeV and 1.2 MeV protons for filtered and unfiltered conditions, respectively. X ray spectra were acquired from a 200 eV Si(Li) detector and put through computer analysis for elemental concentrations. Reference materials were the IAEA-336 "*Evernia prunastri* (L.) Ach." (epiphytic lichen) and the BCR-62 "olive leaves". As a whole, INAA and PIXE add up to 46 elements, 16 of which are determined by both. However, due to the aforementioned manuscript limitations, the present study is focused on a smaller yet representative selection of elements usually associated with crustal and marine sources, plus an element (Cu) of local importance for farm management. The selection is tentatively random as to the analytical methodology used (recommended) for each element — INAA, PIXE or both — unless otherwise stated.

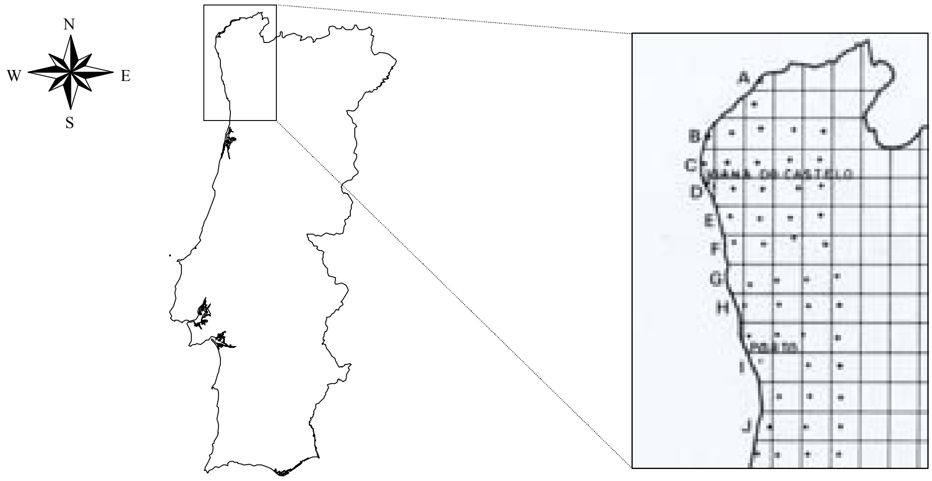


FIG. 1. Map of the study area in north-western Portugal, showing the 10 × 10 km grid for biomonitoring. Grid squares were labelled sequentially, inward then southward then inward and so on. Samples were collected at the centre of each square or at close vicinity, and named after the corresponding location.

A nonparametric approach to assessing the significance of bark results *vs* lichen's is taken here, which means that sample statistics are evaluated from data ranks rather than from data proper. Why such an approach…? First, tests based on order statistics imply fewer and/or less stringent assumptions about data (if any…) than parametric measures of correlation usually do. Second, even though all observations attain an interval scale of measurement, this is an exploratory evaluation where data structure seems much more important than numerical magnitude, so the assignment of ranks to data does not result in some waste of information

[22]. Third, ranks are less sensitive to experimental errors. Last, yet by no means least, the search for an explicit (parametric) relationship is not an issue here, as no calibration between bark and lichens is being attempted and only the consistency of data trends is at stake. Needless to say that any calibration of biomonitors needs reference data from physical-chemical devices and a long-term routine: dose-response functions are prone to suffer from lack of generality, as site-to-site changes affect not only the model coefficients but also the models themselves [23].

3. RESULTS AND DISCUSSION

3.1. Crustal elements and data normalisation

Elements commonly associated with soil sources are likely to play a major role in atmospheric circulation at regional level within the study area. This is an area of dense population, small property, and bustling construction in both public and private lands. It is also criss-crossed with major highways and country roads, plenty of which are dirt lanes. Relative enrichment of the lower troposphere with crustal elements from dust-mobilisation activities should show up through deposition and plant uptake, and indeed it does. According to Nriagu [24], major elements in airborne soil particles are aluminium, silicon, scandium, titanium, manganese, and iron. Of these six potential soil tracers, Mn is unfit for bark monitoring [21], even if unquestionably crustal [25], while the concentration of Si in bark samples fell below the detection limit of PIXE. Descriptive statistics for the remainder, that will be used for bark evaluation, are given in Table I.

TABLE I. BASIC STATISTICS FOR THE CONCENTRATION (μg g^{-1}) OF SELECTED CRUSTAL ELEMENTS IN *PARMELIA* SPP. THALLI (FROM REF. [20] AND THIS WORK)

	Al	**Sc**	**Ti**	**Fe**
Nation-wide data (297 samples)[a]				
Maximum	2.88E+04	2.83E+00	1.04E+03	7.57E+03
Minimum	2.17E+00	1.35E-01	5.44E+01	3.90E+02
Mean	5.40E+03	6.53E-01	3.20E+02	2.10E+03
Median	4.63E+03	2.48E-01	1.14E+02	1.85E+03
Area-wide data (41 samples)[b]				
Maximum	2.88E+04	2.83E+00	9.45E+02	7.57E+03
Minimum	2.59E+03	2.26E-01	1.06E+02	7.77E+02
Mean	6.82E+03	6.64E-01	3.17E+02	2.26E+03
Median	5.30E+03	4.87E-01	2.55E+02	1.61E+03
Std. Dev.	4.66E+03	5.21E-01	2.16E+02	1.54E+03
Skewness	0.98	1.02	0.86	1.28

[a] For comparative purposes only. Albeit small, the country is too diverse to be worth considering here measures of variability and asymmetry other than the range and the mean-median gap, respectively.

[b] The region is fairly homogeneous as to terrain and weather features. An enhanced measure of variability (standard deviation) and an asymmetry index (Pearson's coefficient of skewness) are now added to sample statistics.

As anticipated, soil elements are most relevant in this area. With an exception for Ti, all regional averages stand above the corresponding national averages, and absolute maxima for the whole country are found here. Therefore, sampling distributions all are consistently skewed in the positive direction. Even for Ti data, that features the smallest degree of skewness, means are close enough to preclude significance in their difference, and the regional median is strongly pulled on to a much higher value.

The relative performance of bark in indicating the former elements can be appraised through Table II, that lists the pairwise strength of association between bark and lichen variates — elemental contents of samples from the same spot — as measured by Spearman rank-order correlations (R_S). Given the characteristics of the data, there is no need for correcting for ties or using another statistic that might account for them (e.g., gamma G). Every coefficient and the corresponding probability of some spurious correlation (p-level) were computed to the 6th decimal and then rounded off to the nearest thousandth.

TABLE II. NONPARAMETRIC STATISTICS AND ASSOCIATE PROBABILITY LEVELS FOR THE CONCENTRATIONS OF SOIL ELEMENTS IN *OLEA EUROPAEA* BARK AND *PARMELIA* SPP. THALLI ACROSS THE STUDY AREA [a]

	Spearman R_S	p-level	Correlation
Elements in bark *and* lichen			
$[\mathrm{Al}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Al}]_{\mathrm{LICHEN}}$	0.205	.295	Faint to none
$[\mathrm{Sc}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Sc}]_{\mathrm{LICHEN}}$	0.385	.043	High
$[\mathrm{Ti}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Ti}]_{\mathrm{LICHEN}}$	0.223	.255	Faint to none
$[\mathrm{Fe}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Fe}]_{\mathrm{LICHEN}}$	0.425	.024	High
Elements in bark *only*			
$[\mathrm{Al}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Sc}]_{\mathrm{BARK}}$	0.860	.000(000)	Excellent
$[\mathrm{Al}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Ti}]_{\mathrm{BARK}}$	0.847	.000(000)	Excellent
$[\mathrm{Al}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Fe}]_{\mathrm{BARK}}$	0.897	.000(000)	Excellent
$[\mathrm{Sc}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Ti}]_{\mathrm{BARK}}$	0.843	.000(000)	Excellent
$[\mathrm{Sc}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Fe}]_{\mathrm{BARK}}$	0.956	.000(000)	Excellent
$[\mathrm{Ti}]_{\mathrm{BARK}}$ *vs* $[\mathrm{Fe}]_{\mathrm{BARK}}$	0.915	.000(000)	Excellent

[a] Pairwise deletion of missing data ≡ 28 valid cases.

From the Table results, one gets an impression of mixed performance: Sc and Fe are strongly correlated in bark and lichen samples, while the risk of random behaviour cannot be ruled out for Al and Ti. On the other hand, the association of all elements in bark is outstanding: randomness could still be rejected at an asymptotic (statistical) significance of zero per cent. Playing devil's advocate, one could say that coefficients of such magnitude just mean that bark is not indicating the deposition of soil elements from the atmosphere, but rather their uptake from the very soil, followed by translocation into the bark itself. But then again, this would not explain why the bark-lichen correlations hold good for Sc and Fe, should such an uptake be that serious and thus mask any soil-related input from the atmosphere.

Perhaps an explanation for the above discrepancy should be sought elsewhere. A closer look at the correlation matrix, whose full analysis is out of the present scope, reveals "suspiciously" strong, consistent correlations of both Al and Ti with elements usually found in emissions from power stations. The probability of error in asserting the correlations Al or Ti *vs* V and Al or Ti *vs* Ni is practically null, whereas the Spearman coefficients for Al or Ti *vs* S and Al or Ti *vs* As are all significant far beyond the 0.01 α-level. Statistically speaking, and given the number of elements involved, there is the slimmest chance (if any) that mere artifacts of data could account for a pattern of such an extreme consistency. Now, even though they have all been related to the burning of fossil fuels, there is a split between V, Ni, S, and As: the former two are usually derived from oil combustion and the latter from coal burning. They are specific tracers — for instance, the joint occurrence of V and Ni is viewed as an oil fingerprint, so to speak [26–28] — which are likely to show up simultaneously only in emissions from burning mixed fuels, *precisely* what Tapada do Outeiro has been doing ever since its inception. The plant was designed to run on local, low-grade coals (lignites), together with heavy fuel-oil to improve on the calorific yield. The plant has now reached the end of its productive life and is facing permanent shut-down as we write. During its operation, though, it has been known as a source of local pollution.

All things considered, there is enough evidence to suspect that Al and Ti may have come from an industrial component superimposed on the crustal one, picked up to a dissimilar extent by lichens and bark due to orientation and/or shelter effects. Needless to say that lichens are *not* an absolute reference, neither to remember that lichen and bark samples were collected from the same spots but *not* always from the same substrate (trees) within a spot. Interference in atmospheric indication from root uptake is an eventuality when dealing with vascular species, yet, in this case, Al and Ti may have a strong source other than soil. Besides, should soil elements in bark be erratic at large or their concentrations heavily biased through systemic features, then any attempt at using either of them to discriminate crustal against marine and pollution sources would turn out meaningless. And yet, this is not the case here: accounting ("correcting") for soil contributions into the elemental contents of bark and lichens eventually leads to an overall improvement in their correlations. In other words, the degree of association between bark and lichen concentrations is significantly higher — much higher, for some elements — when elemental ratios (enrichment factors) are compared instead of raw data. Scandium is used for that purpose hereinafter, as an unquestionable soil tracer, and a few examples of [X]/ [Sc] plots and nonparametrics are given next.

3.2. Marine and local elements

The relative enrichment of primary sea-salt tracers — Na and Cl — in bark and lichens across the study area is depicted in Fig. 2. Both elements were determined by INAA and their concentration is plotted as an elemental ratio to Sc at each sampling site. Despite signal magnitude, there is an overall impression of fair agreement between patterns of identical elements in bark and lichen samples from the same locations. Nonparametric statistics substantiates that impression: the correlation for [Na]/[Sc] is outstanding (R_S: 0.749; significant at any level) and for [Cl]/[Sc] the risk of randomness is just above 0.01 (R_S: 0.444). The correlation for Na enrichment is only marginally better than for Na concentrations in terms of raw data (R_S: 0.737; still significant at any level), but greatly improves for chlorine whose raw data from bark and lichens has actually proved unrelated, showing no correlation at all (R_S: 0.070). The results mean that, after accounting for crustal interference, the trends in bark indication with respect to marine salts are at least as reliable as lichens'. But they also mean something else: the normalisation device remains valid as far as it goes, that is unlikely to bias the association between enrichment factors of non-crustal elements in bark and lichens. This may be viewed as a *post hoc* basis for our former arguments. In other words, should soil-related data in bark be seriously flawed by systemic features, the statistical strength of such an association would not hold, much less generally improve from raw data.

The above discussion points to what may be called an extrinsic reliability of bark indication when comparing bark *and* lichen data. Additionally, an intrinsic reliability could be searched for in terms of bark data *only*. Figure 3 shows the site-to-site variation in the elemental ratios of Na and Cl in bark, this time referring to each other. Nonparametric statistics for ranked scores yields a Spearman coefficient of 0.597, that means an excellent correlation between the primary sea-salt tracers, significant at $\alpha = 0.001$. Again, this is the kind of result hardly to be expected from fortuitous artefacts of data, let alone flawed patterns. Moreover, the mean ratio of Cl to Na in bark turns into another index of intrinsic reliability, averaging 1.2 over the study area, which is quite remarkable for biomonitoring. The mean value for "standard" ocean water is about 1.8 [29], but then much more dramatic deviations have been found (from physical surveys) since the pioneering work of Rossby and Egnér [30]. In fact, with prevailing westerlies, ratios are more likely to linger within 1.2–1.5, rather than around the sea-water average [31].

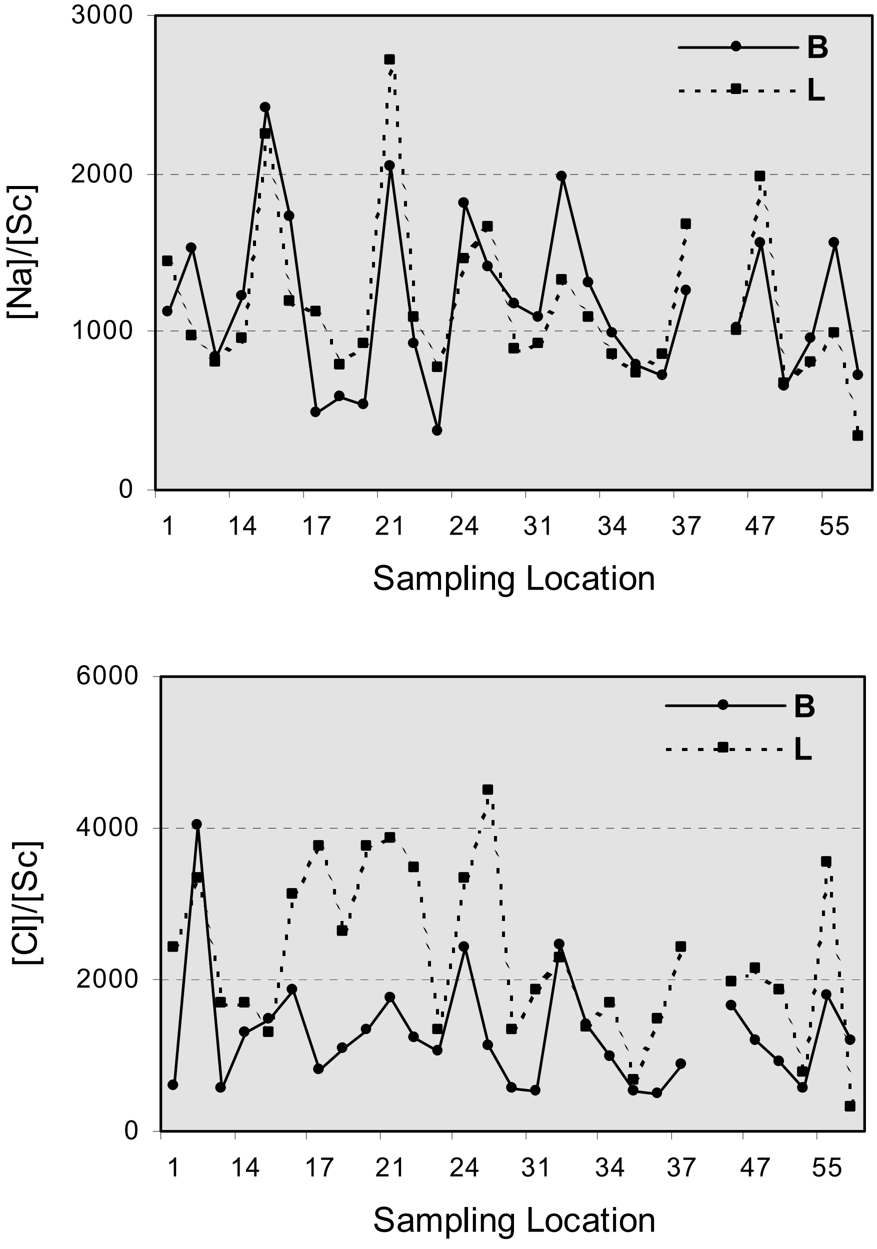


FIG. 2. Relative enrichment of sodium and chlorine in Olea europaea *bark (B) and* Parmelia *spp. Thalli (L) across the study area (28 sampling sites), with scandium as the crustal reference element.*

The former analysis could go on as correlations for other sea-salt elements in bark *and* lichens or in bark *only* are really strong and thoroughly consistent, leaving little room for questioning bark's ability to indicate marine sources in a reliable way. For the sake of argument, however, we would rather look into an element of local importance and find out whether bark is able to track down diffuse, less-common inputs of pollutants to the environment. The element is copper, for which there are neither industrial (mining, smelting, metallurgical) sources nor even a tradition of handicraft work in the area. Copper does play an important role in regional agriculture though, namely for pest control in vineyards, where $CuSO_4$-based fungicides are much used for combating mildew and other moulds attacking vines. North-western Portugal is famed for its speciality wine — *vinho verde*, an acidic, light, slightly gaseous wine — made from grapes that grow from very unusual vines. Unlike common cultivars that stem along the ground or reach a small height, those are lofty plants that climb along poles and trees to the extent that (step)ladders are required for picking up their grapes. Chemicals are thus sprayed up high overhead and not downwards, as usual, which is likely to put them into the local circulation at an enhanced rate.

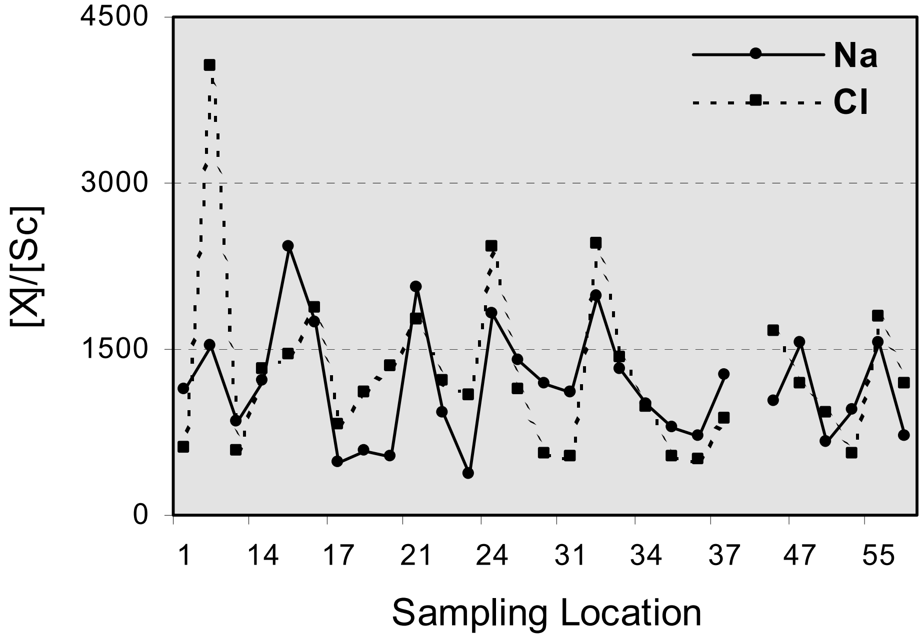


FIG. 3. Relative enrichment of sodium and chlorine in Olea europaea *bark across the study area(28 sampling sites), with scandium as the crustal reference element.*

Copper was determined by PIXE, as INAA is prone to analytical uncertainties regarding this element. The elemental ratio of Cu to Sc in bark and lichen samples is plotted in Fig. 4. The Spearman coefficient R_S reads 0.418, that makes it significantly different from zero at the 0.05 level. In fact, an association between Cu in bark and lichens at large can be asserted well beyond that level, as the risk of being misrepresented by the present sampling runs just short of 2.7%. Nonparametric statistics for Cu does not improve from raw data: figures match up to the third-decimal place. Copper is one of the two elements for which that actually happens — the other is vanadium, an industrial element not discussed here *per se*. They are both unlikely to have a crustal component within their depositional fluxes, so they appear indifferent to correcting for such a component. Judging from the present results, Cu enrichment in bark is visibly greater than in lichens, and bark data really peaks at a few points. Signal inflation due to direct spraying of fungicides onto tree trunks and/or to sample heterogeneity in PIXE analysis may indeed occur. However, even if agricultural and experimental features might be accounted for, an overall pattern of higher values are still evident, which comes somewhat at odds with the widespread belief that bark signals are invariably lower than epiphytes'. This seems largely an *a priori* conception or even an actual misconception, since there is not enough evidence from bark studies to support that notion. For the elemental ratios discussed here, one finds no definite hierarchy in bark and lichen signals, even when they refer to elements with a common source — see the salt tracers in Fig. 2, for instance. Such an assumption could as well be seen to underlie trends in raw scores [21], yet it cannot be endorsed on a general basis by the present, “corrected” data.

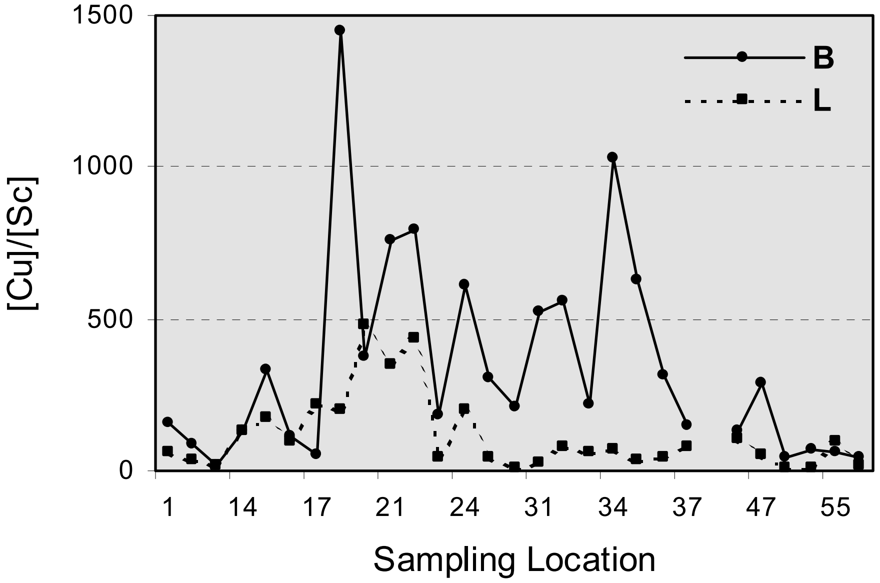


FIG. 4. Relative enrichment of copper in Olea europaea *bark (B) and* Parmelia *spp. thalli (L) across the study area (28 sampling sites), with scandium as the crustal reference element.*

4. CONCLUSIONS

Biological monitoring of airborne contaminants has made quite an impressive progress since the early observations of environmentally-induced stress on plants, and its applications have grown to an extent hardly envisaged just a few decades ago. Along that way, though, studies on higher plants have been comparatively few and far between, especially in what concerns the use of tree bark. Reasons for that are likely to stem from the fact that vascular plants feed on nutrients from soil and also that there may be an identity between some air-pollution factors and plant-body constituents. Surely, there is an active uptake through the root system and, of course, there are elements that make up plants’ structure and, for that motive, are kept under histological and/or physiological control. And yet, several genera of those underrated higher plants share an ubiquity, availability and easiness of sampling that is unmatched by lower epiphytes. A careful selection of species and elements to survey on them, an understanding of botanical and pedological features, and an enhanced analysis of biological data — actually, nothing that should not be done for any other biomonitoring system — may just be all that it takes to turn candidate species into atmospheric monitors. Needless to say that the outcome could be just as rewarding in both logistical and economical terms.

Albeit preliminary, the present results indicate that bark from olive trees could fit into that role, at least in a large expanse of Europe and other areas of the globe where *Olea europaea* is well known and widely distributed. Trends in the concentration of major elements in bark have been found very reliable when compared to a frame of reference of lichen data for the same sampling area. Olive-tree bark can be analysed in the same way as lichens and for many of the same elements. Statistically speaking, an extrinsic reliability of bark *vs* lichens can be asserted at an overall level of significance that leaves little room for questioning bark indication. Other than that, high degrees of association between elements with a common source provide an additional, intrinsic reliability that complements the former trends. All things considered, sample dimension notwithstanding, there seems to be more than just potential for olive-tree bark to become an alternative to lichens and bryophytes, not only in particular zones actually devoid of them due to pollution or dryness, but generally to prevent an intensive collection of species that can be in short supply or even endangered on biodiversity grounds.

REFERENCES

[1] NYLANDER, W., Les lichens du Jardin de Luxembourg, Bull. Soc. Bot. Fr. **13** (1866) 364–372.

[2] ARNOLD, F., Zur Lichenenflora von Munchen, Ber. Bayer. bot. Ges. **1–2**, **5–8** (1901) (suppl.).

[3] BARGHIGIANI, C., BARGAGLI, R., SIEGEL, B.Z., SIEGEL, S.M., A comparative study of mercury distribution on the Aeolian volcanoes, Vulcano and Stromboli, Water, Air, and Soil Pollut. **53** (1990) 179–188.

[4] DONGARA, G., VARRICA, D., The presence of heavy metals in air particulate at Vulcano island (Italy), Sci. Total Environ. **212** (1998) 1–9.

[5] LANDERS, D.H., FORD, J., GUBALA, C., MONETTI, M., LASORSA, B.K., MARTINSON, J., Mercury in vegetation and lake sediments from the U.S. arctic, Water, Air, and Soil Pollut. **80** (1995) 591–601.

[6] FRANCE, R., COQUERY, M., Lead concentrations in lichens from the Canadian high arctic in relation to latitudinal pollution gradient, Water, Air, and Soil Pollut. **90** (1996) 469–474.

[7] POBLET, A., ANDRADE, S., SCAGLIOLA, M., VODOPIVEZ, C., CURTOSI, A., PUCCI, A., MARCOVECCHIO, J., The use of epilithic Antarctic lichens (*Usnea aurantiacoatra* and *U. antartica*) to determine deposition patterns of heavy metals in the Shetland Islands, Antarctica, Sci. Total Environ. **207** (1997) 187–194.

[8] FIGUEIRA, R., SOUSA, A.J., BROWN, D.H., CATARINO, F., PACHECO, A.M.G., Natural levels of saline elements on lichens: determination of cellular fractions and their importance as saline tracers, Lichenologist **31** (1999) 183–196.

[9] NIMIS, P.L., "Air quality indicators and indices: the use of plants as bioindicators for monitoring air pollution", EUR 13060 EN Report (COLOMBO, A.G., PREMAZZI, G., Eds.), Commission of the European Communities, Luxembourg (1990) 93–126.

[10] GARTY, J., "Environment and elemental content of lichens", Trace Elements — Their Distribution and Effects in the Environment (MARKERT, B., FRIESE, K., Eds.), Elsevier, Amsterdam (2000) 245–276.

[11] STAXANG, B., Acidification of bark of some deciduous trees, Oikos **20** (1969) 224–230.

[12] O'HARE, G.P., Lichens and bark acidification as indicators of air pollution in west central Scotland, J. Biogeogr. **1** (1974) 135–146.

[13] GRODZINSKA, K., Acidity of tree bark as a bioindicator of forest pollution in southern Poland, Water, Air, and Soil Pollut. **7** (1978) 3–7.

[14] GRODZINSKA, K., "Monitoring of air pollutants by mosses and tree bark", Monitoring of Air Pollutants by Plants — Methods and Problems (STEUBING, L., JÄGER, H.-J., Eds.), Dr W. Junk Publishers, The Hague (1982) 33–42.

[15] HÄRTEL, O., "Pollutants accumulation by bark", Monitoring of Air Pollutants by Plants — Methods and Problems (STEUBING, L., JÄGER, H.-J., Eds.), Dr W. Junk Publishers, The Hague (1982) 137–147.

[16] TAKALA, K., OLKKONEN, H., JÄÄSKELÄINEN, J., SELKÄINAHO, K., Total chlorine content of epiphytic and terricolous lichens and birch bark in Finland, Ann. Bot. Fennici **27** (1990) 131–137.

[17] SCHULZ, H., POPP, P., HUHN, G., STÄRK, H.-J., SCHÜRMANN, G, "Biomonitoring of airborne inorganic and organic pollutants by means of pine tree barks. I. Temporal and spatial variations", Biomonitoring of Atmospheric Pollution (with emphasis on trace elements) — BioMAP, IAEA-TECDOC-1152, IAEA, Vienna (2000) 149–158.

[18] SCHULZ, H., SCHULZ, U., HUHN, G., SCHÜRMANN, G, "Biomonitoring of airborne inorganic and organic pollutants by means of pine tree barks. II. Deposition types and impact levels", Biomonitoring of Atmospheric Pollution (with emphasis on trace elements) — BioMAP, IAEA-TECDOC-1152, IAEA, Vienna (2000) 159–167.

[19] FREITAS, M.C., REIS, M.A., ALVES, L.C., WOLTERBEEK, H.Th., Distribution in Portugal of some pollutants in the lichen *Parmelia sulcata*, Environ. Pollut. **106** (1999) 229–235.

[20] FREITAS, M.C., REIS, M.A., ALVES, L.C., WOLTERBEEK, H.Th., "Nuclear analytical techniques in atmospheric trace element studies in Portugal", Trace Elements — Their Distribution and Effects in the Environment (MARKERT, B., FRIESE, K., Eds.), Elsevier, Amsterdam (2000) 187–213.

[21] PACHECO, A.M.G., FREITAS, M.C., BARROS, L.I.C., FIGUEIRA, R., Investigating tree bark as an air-pollution biomonitor by means of neutron activation analysis, J. Radioanal. Nucl. Chem. **in press**.

[22] TOWNSEND, J.T., ASHBY, F.G., Measurement scales and statistics: The misconception misconceived, Psychological Bull. **96** (1984) 394–401.

[23] FIGUEIRA, R., PACHECO, A.M.G., SOUSA, A.J., CATARINO, F., Development and calibration of epiphytic lichens as saltfall biomonitors, these Proceedings.

[24] NRIAGU, J.O., "Natural versus anthropogenic emissions of trace metals to the atmosphere", Control and Fate of Atmospheric Trace Metals (PACYNA, J.M., OFFAR, B., Eds.), Kluwer Academic Publishers, Dordrecht (1989) 3–13.

[25] NRIAGU, J.O., A global assessment of natural sources of atmospheric trace metals, Nature **338** (1989) 47–49.

[26] GANOR, E., ALTSHULLER, S., FONER, H.A., BRENNER, S., GABBAY, J., Vanadium and nickel in dustfall as indicators of power plant pollution, Water, Air, and Soil Pollut. **42** (1988) 241–252.

[27] FONER, H.A., GANOR, E., The chemical and mineralogical composition of some urban atmospheric aerosols in Israel, Atmos. Environ. **26B** (1992) 125–133.

[28] RAHN, K.A., HUANG, S., A graphical technique for distinguishing soil and atmospheric deposition in biomonitors from the plant material, Sci. Total Environ. **232** (1999) 79–104.

[29] WELLS, N., The Atmosphere and Ocean — A Physical Introduction, Wiley, Chichester (1997) 394 pp.

[30] ROSSBY, C.-G., EGNÉR, H., On the chemical climate and its variation with the atmospheric circulation pattern, Tellus **7** (1955) 118–133.

[31] ERIKSSON, E., The yearly circulation of chloride and sulfur in Nature; meteorological, geochemical and pedological implications. Part II, Tellus **12** (1960) 63–109.

ENVIRONMENTAL STUDIES BY MEANS OF THE USE OF BIOMONITORS (*TILLANDSIA* SP.) AND NUCLEAR TECHNIQUES

S. KOCHMANN[1], I. RIQUELME[1], M. ORTÍZ[2], F. YUBERO,
Y. GONZÁLEZ[3], G. BARÚA DE SOUBERLICH[4], G.

Asuncion National University:
[1] Atomic Energy National Commission, Teaching and Research Direction,
Nuclear Analytical Techniques Division, San Lorenzo, Asuncion, Paraguay
Chemistry Sciences Faculty:
[2] Investigation Direction — Botanic Department
[3] Academic Direction — Biologic Physic and Chemistry Laboratory
[4] Asuncion Municipality — Environmental Protection Direction —
Conservation and Environmental Education Center

Abstract

The lack of healthful atmosphere is one of the main causes of health deterioration. The present study uses biomonitors, with the objective of knowing the levels of pollutant accumulation in nucleus zones, by means of *Tillandsia´*s spp. sampling (a *Bromeliaceae*, epiphyte that captures the atmosphere's nutrients and concomitantly accumulates heavy metals) during the spring station. They correspond to intersections between avenues with high vehicular flow and particular characteristics as for mobile emission's sources, in Asuncion City; subdivided in such form that, each plant has the same probability to be selected, being 5 species of *Tillandsia*: *T. recurvata L.* (94%); *T. meridionalis Baker* (59%); *T loliaceae Mart.* (29%); *T. duratii Visiani* (12%), and *T. tricholepis Baker* (6%). These species were evaluated for element levels by means of X ray fluorescence energy dispersive, with conventional geometry.

1. INTRODUCTION

Paraguay, country located in the south center of South America; it possesses a surface of 406,752 km^2, a population of 5,152,588 inhabitants, and a density of 10.2 inhabitants/km^2. The capital is Asuncion, with a surface 117 km^2, and a population of 502,426 inhabitants, according to demographic data of 1992 census, and a density of 4,292 inhabitants/km^2 (Fig. 1).

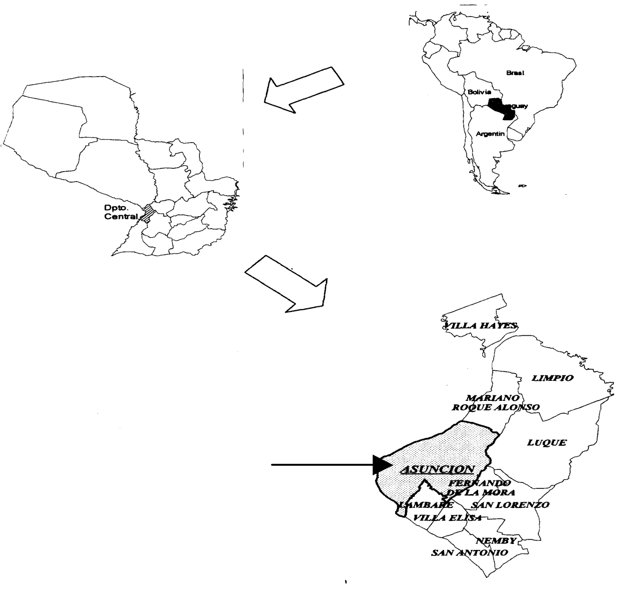


FIG. 1. Study area location

In 1996, Asuncion's Municipality had 5.84% green areas identified as parks, squares, and walks with trees; also in 1995, 95,530 self-driven vehicles were registered. Likewise, the metropolitan area (711 km), constituted by Asuncion and 10 cities, contributes with approximately 250,000 people and 30,000 vehicles that move daily in the business days to carry out Asuncion activities of all types.

According to the meteorological station (installed in the International Airport Silvio Pettirossi in the Asuncion's outskirts), the average of wind speed is 5.0 K.p.h. approximately, mainly in the northeast to south address during the whole year. The humidity oscillates between 60% in September and October, and 80% in May and June. The precipitation is influenced by both hot and humid wind coming from Matto Grosso, Brazil, and the dry wind of the south. It is considered the average of 1,400 mm annual precipitation, concentrated generally in the months of November and April.

The air pollution is part of the modern life; recent estimates point out that more than one hundred million people in Latin America urban areas are probably exposed to concentrations of atmospheric pollutants that surpass the securities guide recommended by the WHO (World Heath Organization).

In recent years, in answer to recommendations of the Schedule 21st and the commitments assumed in the Summit of the America (1994), the WHO together with the efforts of other multilateral and bilateral organisms has promoted, coordinated, and leaned several activities relating the improvement of air quality, for example, the elimination of lead in the gasoline, which has not yet been completely implemented in Paraguay.

The air pollution in the Asuncion city is mainly due to: continuous increase of the population; the use of combustible with Pb; the high number and the continuous increase of vehicles, mainly second hand ones, circulating in the Municipality; the inadequate traffic systems application; the lack of treatment of effluents by the industries; the absence of control of polluting emissions to the atmosphere by the responsible Institutions; garbage's burns; the information lack of the population concerning the effects in people health due to air pollution; the topographical, climatic, meteorological, and city-planning influences.

One of the main causes of the contamination in Asuncion is the high quantity of vehicles in the city. In 1997, the Municipality has settled the ordinance 19/97 for contamination control that takes charge particularly of CO control and other chemical elements in the air and it tries to standardize the regulations and sanctions against any violation. However it has not yet established the standard air pollution control, for what lacks quantitative information, although indicators are that the air in the Asuncion City is polluted. It is considered that the vehicle quantity in the hours of bigger demand of traffic in Asuncion centre, could generate more than 6,000 $\mu g/hr/m^3$ of air particles, quantity much superior to the securities considered as limits for the protection of the human health.

Some punctual emissions controls of CO and NOx, have been carried out on part of the Asuncion Municipality and the Public Health Ministry through the Environmental Reparation National Service (SENASA), in the intersections of streets and main Asuncion avenues. This was only executed in 1995 and 1996 at certain points and times, but in none of the studies, the sampling minimal frequency was completed. All the buses operate diesel motors and the emission is not controlled due to the lack of equipment for inspection and control on behalf of the public sector.

In a parallel and independent study in order to evaluate the current situation of air pollution of the study area, a team of the Japanese Agency of International Cooperation (JICA) has carried out a simple control to examine the values of NOx and NO_2, obtained by means of 20 captators, located in the main intersections of streets and in the Micro Center, and exposed 50 hours in each observation point. It was found that the automobiles are the biggest responsible of the CO emissions, NOx, hydrocarbons, and particles.

2. MATERIALS AND METHODS

The study is carried out initially in the Asuncion City (Phase I — Capital), foreseeing the extension to the Central Department (Phase II — 19 departments). It has been chosen 17 nucleus zones (Fig. 2), representing approximately 50% of the intersections of the city's bigger traffic avenues (according to the plane of the Asuncion City made in the Military Geographical Service — 1996). It is considered the Botanical Garden as Control Zone where the levels of potential toxic elements concentration are expected to be low, due to an important mass of trees; also the area is qualified as important in terms of biodiversity; the election has also relationship with the predominant direction of the winds in Asuncion.

From the viewpoint of the Contaminant Criterion (WHO), those for which standards of the air quality exist to protect the human health purpose, and, taking in consideration the analytical capacities of the Nuclear Analytical Techniques Laboratory (Nuclear Energy National Commission), it has been chosen the lead (Pb) fundamentally by their determination for X ray fluorescence. Since this is a multi elemental technique, it is also quantified elements whose concentrations are significant in the samples.

Biomonitors use makes possible the continuous passive monitoring and in some cases, retrospectively, at relatively low cost.

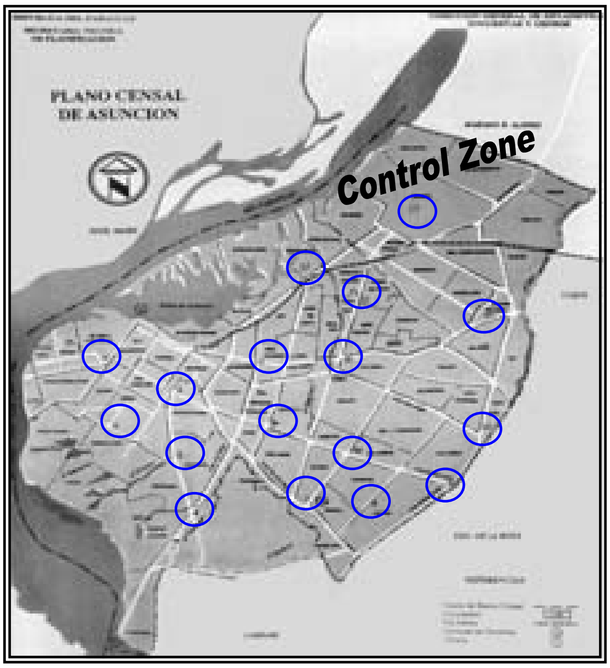


FIG. 2. Asuncion City plane indicating the sampling places

2.1. Sampling and sample preparation

Considering the general and specific objectives of the study, it was carried out biomonitors sampling (*Tillandsia, spp.*) during the spring station in the Nucleus Zones, for which the specific sampling area was subdivided in a grid series of such a form that each utilized plant as biomonitor, has the same probability of being selected [1,2].

Zone 1 has dimensions of 40 × 40 m, trees do not exist and it corresponds to each Nucleus Zone chosen; here data on climatic factors (as temperature, wind, precipitation) are considered in the Record Sampling. Zone 2 (damping) is often exposed to mechanical and anthropogenic alterations due to the proximity to Zone 1; there is no arboreal covering. Its maximum width is about 20 to 30 m.

Tillandsia spp. samples were taken from Zone 3, the largest arboreal covering and, therefore, high probability of finding samples, subdivided in smaller 100 grids, where there was *Tillandsia* spp. One worked with a pool represented by several *Tillandsia* species of each sampling place (Table I). Soil samples were also collected, with the purpose of establishing the correlation between the element levels in soil and in the biomonitor.

The samples were transported in paper bags, for their taxonomic characterization, being preserved herbal exemplars. For the preparation, only the leaves (in their entirety) were used; they were washed with deionised and distilled water during 3 minutes, and they were kept frozen until being dried by lyophilization in plastic or glass trays during 24 hours minimally.

TABLE I. RELATIONSHIP BETWEEN NUCLEUS ZONES AND RELATIVE Pb LEVELS IN *Tillandsia* spp. POOLS

Falling classification in function to Pb relative concentration (*Tillandsia* spp. Pool / Area)	**Nucleus Zone**	**Avenues Intersection**
T. loliaceae Mart.; T. recurvata L.	3	Dr. J. G. Rodríguez de Francia y J.
T. meridionalis Baker; T. recurvata L.	10	Rca. Argentina y Dr. E. Ayala (*)
T. meridionalis Baker; T. recurvata L.	6	Mcal. López y Gral. M. Santos (**)
T. recurvata L.	2	J. F. Bogado (#) y Costa Rica
T. recurvata L.; T. meridionalis Baker	7	Dr. E. Ayala y J. Kubistchek (*)
T. recurvata L.; T. loliaceae Mart.	12	Dr. E. Ayala y Madame Lynch (*)
T. meridionalis Baker; T. recurvata L.	9	De la Victoria e Yby´a
T. meridionalis Baker; T. loliaceae Mart.;T. recurvata L.	4	Pte. C. A. López y Colón
T. recurvata L.; T. loliaceae Mart.; T. meridionalis Baker	1	Perón y Ave. J. F. Bogado (#)
T. loliaceae Mart.; T. meridionalis Baker; T. recurvata L.	**11**	Choferes del Chaco y Mcal. López
T. recurvata L.; T. meridionalis Baker	16	Artigas y Brasilia (#)
T. meridionalis Baker; T. recurvata L.	**8**	Rca. Argentina y F. de la Mora
T. recurvata L.; T. duratii Visiani	13	Mcal. López y Madame Lynch (**)
T. recurvata L.; T. meridionalis Baker	14	Mcal. López y Aviadores del Chaco
T. recurvata L.	5	J. Falcón y Cementerio del Sur
T. meridionalis Baker; T. recurvata L.	15	Santísima Trinidad y Dr. M. Peña
T. duratii Visiani	17	Botanic Garden (Control Zone)

(#) Processes of larger speed; (* *) Ave. of larger vehicular flow
(*) Ave. in second place in relation to the total vehicular flow, where it is larger the flow of buses

2.2. Analytical methods — spectrometry X ray detection

a. *Tillandsia* spp. samples: once dried the leaves were reduced to powder, homogenized, and sifted through a mesh 50 (48 mesh; 0.300 mm) to obtain an uniform powder. The powder was pressed to form pills (about 300 mg), with a static pressure of 10 tons (about 2 tons/cm^2), using stainless steel disks for press, 2.5 cm diameter. They were exposed to the X ray generator during 3,000 seconds in triplicate.

b. Soil samples: the powder was dried between 8 and 24 hr. at 110ºC before preparing the pills under the same conditions as indicated in (a) except in what exposure time is concerned (here 2,000sec. instead of 3,000sec.). The pills were mounted in a sample container forming an angle of incidence of 45º, in relation to the incident beam, in order to obtain reproducible geometry. The system utilized conventional excitement, it consisted on Molybdenum X ray with sub target of the same material; the induced fluorescence was detected through a detector Si(Li) semi-conductor. After collecting the spectra (conditions 30 kV, 30 mA, and a resolution 170 eV Fe — Kα peak). The analysis qualitative and quantitative of the spectra was carried out, for all the detected elements, through the programs Spectrum Analysis by Least Squares Fitting Method and Emission Transmission Method (AXIAL) V. 3.5.

3. RESULTS AND DISCUSSION

The sampling area, a subtropical region, contributes to the proliferation of numerous *Tillandsia* spp. species (Fig. 3), a *Bromeliaceae* epiphyte that fulfils in principle the requirements for being considered biomonitor. For this species, according to previous references, its accumulative capacity had not been studied yet.

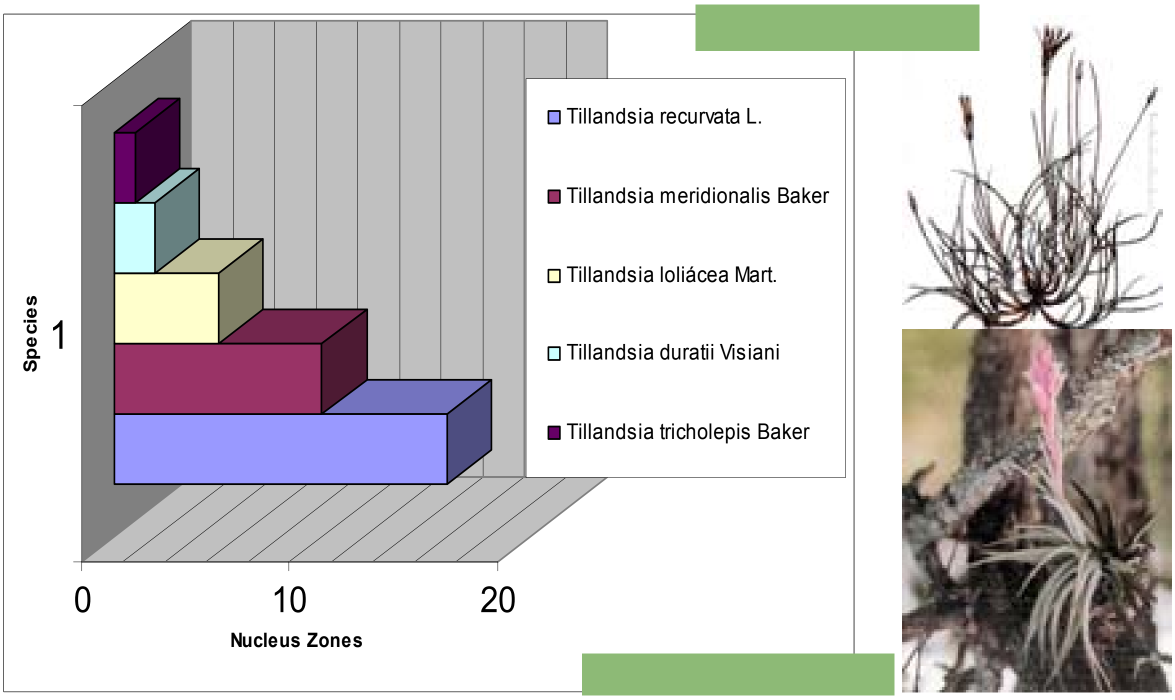


FIG. 3. Tillandsia spp. *found in the study area*

Results of the relative Pb concentration for the different Nucleus Zones of the biomonitors study are shown in Fig. 4. The comparison of either soil or plants element concentrations, was carried out by means of Microcal Origin V. 3,54. It is relevant to compare the avenues where the flow of traffic (Table II) has larger proportion of files, and with higher percentage of vehicles using leaded fuels (Table III).

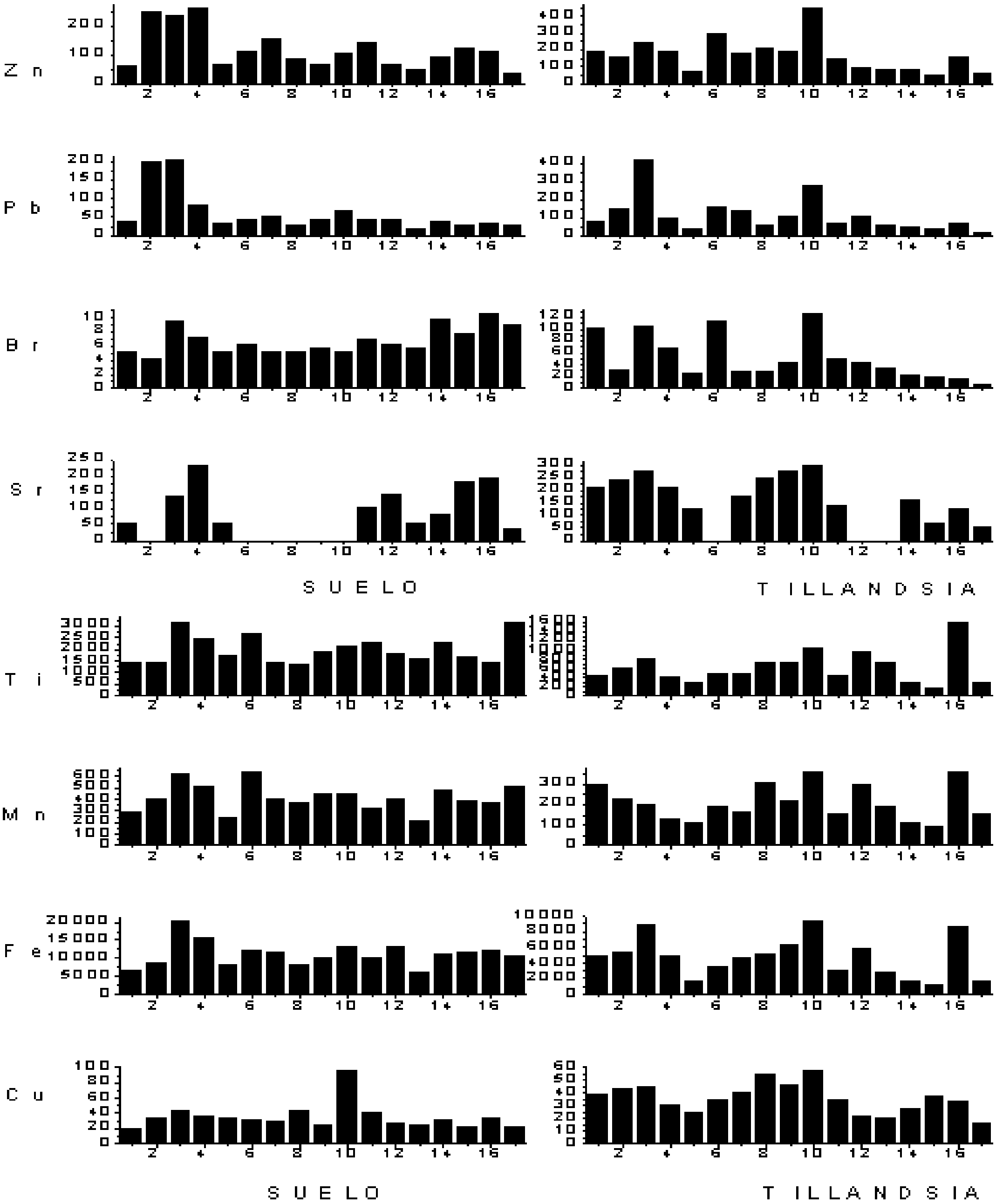


FIG. 4. Element concentrations (ppm) found in the diverse sampling places.

Asuncion shows the higher index vehicular flow in the process radial loops (according to JICA report data): 37,600 veh./14 hr. in the Ave. Mcal. López; 26,800 veh./14 hr in the Ave. E. Ayala. In circular or traverse processes, the highest flow is observed in the Ave. Choferes del Chaco between Mcal. López and E. Ayala. The flow of buses is elevated in the main process loop having the highest flow in the Ave. E. Ayala and the lowest among the main radial processes, in the Ave. España. The Ave. Artigas and J. F. Bogado are the avenues of larger speed with more than 30 km/h.

The vehicles registered quantity (Table IV) does not coincide with real quantity of circulating and existent vehicles inside the Municipality, because the solicitors should not necessarily live inside it Then it is usually bigger relative to the vehicles quantity registered, and also because the patent cost is superior in the Asuncion Municipality

TABLE II. TRAFFIC COMPOSITION

Avenues	**Car**	**Van**	**Truck**	**Bus**
Artigas	59.4	24.8	7.5	8.3
España	64.5	27.1	4.2	4.2
Mcal. López	62.1	27.8	2.5	7.6
E. Ayala	45.7	25.1	6.8	22.4
F. de la Mora	59.4	24.8	7.5	8.3
J. F. Bogado	57.4	25.1	7.3	10.3

TABLE III. VEHICLE AND COMBUSTIBLE TYPE COMPOSITION

	Car	**Bus**	**BUS LOOPS**	**Light Truck**	**Heavy Truck**
Common Naphtha	50				
Super Naphtha	20			20	
DIESEL	30	100	100	80	100

TABLE IV. REGISTERED VEHICLES IN 1996

Municipality	**Cars**	**Van**	**Trucks**	**Others**	**Sub Total**	**Population**	**Vehicle possession Veh/1000**
Asuncion	60.107	20.721	3.943	2.349	87.120	545.964	159,6

Basically three types of combustible are used in Paraguay: diesel, common, and super naphtha. The fuels come from PETROPAR (Paraguay's Petroleum's) and from the import of the MERCOSUR Countries. The 97 and 95 octane naphtha are unleaded and imported; to the PETROPAR produced tetraethyl Pb is added, and there is also super 95 naphtha with 0.2 g/l Pb.

4. CONCLUSIONS

The main objective of this preliminary study rotates around the element determination in the chosen epiphyte as biomonitor *(Tillandsia* spp.) in the study area, by means of a nuclear analytical technique (FRX). Of the 5 species of *Tillandsia* found in the sampling area, it is convenient to reduce the number at 2 of them, specifically to those distributed in more proportion, in order to be able to collect them in all sampling points.

The selection of the Nucleus Zones of the biomonitoring work has been carried out having into account 1) streets with high traffic, 2) the availability of the defined requirements for Zone 3 or of sampling concerning presence of trees (since epiphytes plants are used). This explains why no sampling was made in the micro centre, where NOx measurements were available.

Strictly speaking only 18% of the sampled points coincide exactly with the observations points of the studies faced by the Asuncion Municipality for the NOx and NO_2 control. As the observations have revealed, the NOx value is relatively high in the micro centre area and in it main processes of access and exit, and the emission corresponds in general to the vehicles using diesel engine (bus transports). The same occurs with Pb concentrations, for which the expectation is that they increase in the areas corresponding mainly to a high automobile flow using leaded naphtha. That is to say that the results of Pb relative concentrations in Nucleus Zones is quite consistent, having into account the composition and the flow of traffic and the proportion of combustible employees in the Asuncion City.

Considering that it is the first experience in this environment, it would be of vital importance to repeat the sampling in the same year station (starting from September month), and then:

- to operate just with two *Tillandsia* species, specifically the most abundantly distributed. In this way it would also be facilitated the task for species identification;
- to determine the percentage of each species present in each point (for relative abundance evaluation);
- to analyse the element concentration in each sampling point;
- to make measurements indicative of a potential physiologic damage (conductivity and pH).

This would allow to determine: 1) if the species in question are good accumulative of some element in particular and to establish the difference in the answer of different species; 2) if the levels of the different elements in the different species are indeed a measure of the atmospheric levels in that place; 3) if the species accumulation did not reach a saturation point; and 4) if, due to the high concentration of onsite elements, a physiologic damage has occurred that avoids further absorption, since the complexity can not be obviated of the biological systems.

By means of the statistical result analysis, it is possible to observe significant correlation between Pb measured in both *Tillandsia* and soil. For the other elements ANOVA shows significant differences among the sampling places, therefore the species (*T. recurvata* L., 94%) accumulates quite well.

It is also concluded that the zone chosen initially as control corresponds indeed to the place of smaller concentration of lead.

ACKNOWLEDGEMENTS

- To Mr. Aurelio Schinini of the IBONE (Institute of Northeast Botanical), Corrientes, Argentina, for the help in the *Tillandsia loliaceae* and *recurvata* species determination.
- To Dra. María Luisa Pignata of the Ability of Exact, Physical and Natural Sciences of the Cordoba National University, Argentina, for the orientation in the species selection, the evaluation of the experimental design, and the statistical analysis of the obtained data.
- To Dr. Esteban Ferro and his group, Vice Dean of the Chemical Sciences Faculty of the Asuncion National University for its authorization to make the samples dry by lyophillization in the Phitochemical Department dependence.

REFERENCES

[1] DIAZ, BARRIGA, F, COREY, ORELLANA, G., Evaluación del Riesgo por exposición al Plomo. CEPIS/OPS/OMS. Perú, 1999.

[2] EMISIONES DE TOXICOS EN EL AIRE., EPA, 1999.

[3] ESTUDIO DE OBSERVACIÓN ACERCA DE LA PLANIFICACIÓN DEL TRANSPORTE URBANO EN EL AREA METROPOLITANA DE ASUNCIÓN. INFORME FINAL, Yachito Engineering Co, Ltd, Central Consultant Inc. JICA/Municipalidad de Asunción/ República del Paraguay, 1999.

[4] GORDILLO, HERNANDEZ, D., Ecología y Contaminación Ambiental. interamericana McGraw-Hill, México, 1995.

[5] KORC, E., M.; SAENZ, R., Monitoreo de la calidad del aire en América Latina. Programa de Control de la Contaminación del aire, CEPIS/OPS-OMS, Lima, 1999.

[6] KUCERA, J., Biological Monitors of Air Pollution. Czech Ecological Institute, NAA Laboratory, Praga, 1999.

[7] MALM, O., FONSECA, M.D., MIGUEL, P.H., BASTOS, W.R., PINTO, F., N., Use of Epiphyte plants as biomonitors to map atmospheric mercury in a gold trade center city, Amazon, Brazil.. Science of the Total Environment **213 (1–3)** (1988) 57–64.

[8] MARKERT, B., Quality Assurance in Enviromental Monitoring — Sampling and Sample Pretreatment. VCH-Publisher, Nueva York, 1995.

[9] MARKERT, B., Instrumental Multielement Analysis of Enviromental Samples. Contaminated Soils, 3rd International Conference on the Biogeochemistry of Trace Elements, Institut National de la Recherche Agronomique (INRA), París, 1995.

[10] MARKERT, BERND., Distribution and Biogeochemistry of Inorganic Chemicals in the Environment. Ecotoxicology, John Wiley & Sons, Inc. and Spektrum Akademischer Verlag, 1998.

[11] MARKERT, BERND, OEHLMANN, JORG, ROTH, METCHTILD, General Aspectcs of Heavy Metal Monitoring by Plants and Animals. K.S. Subramanian and G.V. Iyengar (eds.): Enviromental Biomonitoring — Exposure Assessment and Specimen banking, ACS Symposium Series 654, American Chemical Society, Washington, 1997.

[12] PADILLA, ROMAN, Cuba, Manual de Espectrometría de Fluorescencia de rayos X, 1999.

[13] PLAN REGIONAL SOBRE CALIDAD DEL AIRE URBANO Y SALUD PARA EL PERIODO 2000–2009. Año 2000. OPS-CEPIS/99.21(AIRE), Washington, DC.

[14] PYATT, F.B., GRATTAN, J.P., LACY, D., PYATT, A.J., SEAWARD, M.R.D., Comparative Effectiveness of *Tillandsia* usneoides L. and *Parmotrema* praesorediosum(NYL.) as bio-indicators of atmospheric pollution in Louisiana., Water, Air and Soil Pollution **111 (1–4)** (1999) 317–326.

[15] SIMABUCO, S., OLIVEIRA DE JESÚS, E.F., DOS ANJOS, M.J., FERREIRA PINTO, J. & LOPES, R.T., 1999, Comparaçao dos teores de elementos traços em folhas de *Nerium oleander* no monitoramento da poluiçao ambiental nas cidades de Campinas e Río de Janeiro por Fluorescencia de Raios X. NURT´99, 2nd International Symposium on Nuclear and related techniques in Agriculture, Industry and Environment, Havana., Cuba, 1999.

[16] SMITH, L.B., Flora del Uruguay IV. Bromeliácea. Instituto Smithsoniano. Museo Nacional de Historia Natural, Montevideo, 1972.

[17] SCHWELA, D., Guías para la calidad del aire de la Organización Mundial de la Salud. OMS, Geneva.

[18] VAN, GRIEKEN, R.E., MARKCOWICZ, A., Handbook of X ray spectrometry. Methods and Techniques. De. Marcel Dekker, Inc., 1993.

[19] VUTCHKOV, M., LALOR, G.C., PRESTON, Biomonitoring of air pollution in Jamaica trough trace element analysis of epiphytic plants using nuclear and related analytical techniques. International Atomic Energy Agency, Section on nutrition, 1998.

LIST OF PARTICIPANTS

Albarrán, C.	Cátedra de Química General. Facultad de Ciencias Exactas, Físicas y Naturales. Universidad Nacional de Córdoba, Córdoba, Argentina.
Alberghini, F.	Department of Biology, Sect. of Botany, University of Ferrara, Ferrara, Italy
Almeida, S.M.	Instituto Tecnologico e Nuclear, ITN, EN10 Sacavém, Portugal
Alves, L.C.	Instituto Tecnologico e Nuclear, ITN, EN10 Sacavém, Portugal
Amanov, M.	Tashkent State Agrarian University, Kibray Region, Tashkent district, Uzbekistan
Amaral, R.S.	Departamento de Energia Nuclear, UFPE, Recife PE, Brazil
Avila-Perez, P.	National Institute of Nuclear Research, Ocoyoacac, Mexico
Avino, P.	DIPIA-ISPESL-Dipartimento Insediamenti Produttivi ed Interazione con l'Ambiente, Monte Porzio Catone Rome, Italy
Ayrault, S.	Laboratoire Pierre Süe, CEA-CNRS, CEA de Saclay, Gif-sur-Yvette, France
Bacchi, M.A.	Centro de Energia Nuclear na Agricultura — Universidade de São Paulo — CENA/USP Piracicaba SP Brazil
Barros, L.I.C.	CVRM-IST, Lisbon, Portugal
Barúa De Souberlich, G.	Atomic Energy National Commission, Teaching and Research Direction, Nuclear Analytical Techniques Division, San Lorenzo, Asuncion — Paraguay
Batic, F.	Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
Béguinel, P.	SRSE, CEA de Saclay, Gif-sur-Yvette, France
Beltramo, J.-L.	Université de Bourgogne, Csaab, Dijon, France
Benedetti, S.	A.R.P.A. Emilia-Romagna, Area Specialistica Fitofarmaci, Ferrara, Italy
Bergamaschi, L.	CNR Centro di Radiochimica e Analisi per Attivazione, Universita di Pavia, Pavia, Italy
Bleise, A.	International Atomic Energy Agency, Vienna

Blum, O.	National Botanical Garden, National Academy of Sciences of Ukraine, Kiev, Ukraine
Borrego, C.	Department of Environment and Planning, University of Aveiro, Aveiro, Portugal
Bovolenta, A.	A.R.P.A. Emilia-Romagna, Area Specialistica Fitofarmaci, Ferrara, Italy
Bragazza, L.	Department of Biology, Sect. of Botany, University of Ferrara, Ferrara, Italy
Cabrera, L.	National University of Mexico, Faculty of Chemistry, Mexico City, Mexico
Carconi, P.L.	National Agency of New Technologies, Energy and Environment (ENEA), Casaccia Center, Rome, Italy,
Carrot, F.	Laboratoire Pierre Süe, CEA-CNRS, CEA de Saclay, Gif-sur-Yvette, France
Carvalho, A.C.	Department of Environment and Planning, University of Aveiro, AVEIRO, Portugal
Catarino, F.	Museu, Laboratório e Jardim Botânico, University of Lisbon, Lisbon, Portugal
Chai, Z.F.	Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100080, China
Chen, J.B.	Systematical Mycology and Licheonology Lab, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.
Coddeville, P.	Département de Chimie et Environnement, Ecole des Mines de Douai, Douai, France
Constantin, J.	Department of Biological and Environmental Engineering, University of Trás-os-Montes e Alto Douro, Portugal
Coppi, S.	A.R.P.A. Emilia-Romagna, Area Specialistica Fitofarmaci, Ferrara, Italy
Costa, C.J.	Instituto Tecnologico e Nuclear, ITN, EN10 Sacavém, Portugal
Coutinho, M.	IDAD — Institute for Environment and Development, Aveiro, Portugal
Daillant, O.	Observatoire Mycologique, Néronde, Mazille, France

Ducruet, J.M.	Laboratoire de Bioénergétique, CEA de Saclay, Gif-sur-Yvette, France
Eguskiza, M.	European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium
Els, E.	Cátedra de Química General. Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina
Evans, G.J.	Facility for Aerosol LAMS, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Ontario, Canada
Falnoga, I.	Jožef Stefan Institute, Ljubljana, Slovenia
Farinha, M.M.	Instituto Tecnologico e Nuclear, ITN, EN10 Sacavém, Portugal
Férard, J.F.	EBSE, Campus Bridoux, Metz, France
Fernandes, E.A. De Nadai	Centro de Energia Nuclear na Agricultura — Universidade de São Paulo — CENA/USP, Piracicaba SP Brazil
Ferreira, J.	IDAD — Institute for Environment and Development, Aveiro, Portugal
Figueira, R.	Museu, Laboratório e Jardim Botânico (University of Lisbon), Lisbon, Portugal
França, E.J.	Centro de Energia Nuclear na Agricultura — Universidade de São Paulo — CENA/USP Piracicaba SP Brazil
Fränzle, St.	Umweltforschungszentrum Leipzig/Halle (UFZ), Leipzig, Germany
Freitas, M.C.	Instituto Tecnologico e Nuclear, ITN, EN10 Sacavém, Portugal
Gallorini, M.	CNR Centro di Radiochimica e Analisi per Attivazione, Pavia, Italy
Galsomiès, L.	ADEME, Département Air et transport, 27 rue Louis Vicat, F-75737 Paris Cedex 15, France
Gandolfi, S.	Escola Superior de Agricultura Luiz de Queiroz — Universidade de São Paulo — ESALQ/USP, Piracicaba SP Brazil
Garrec, J.P.	INRA — Centre de Recherche Forestière de Nancy, Laboratoire Pollution atmosphérique, Champenoux, France

Gerdol, R.	Department of Biology, Sect. of Botany, University of Ferrara, Ferrara, Italy
Glebov, A.	National Botanical Garden, National Academy of Science of Ukraine, Kiev, Ukraine
Goeij, J. De	IRI-TUDelft, Mekkelweg 15, NL-2629 JB Delft, Netherlands
González, Y.	Atomic Energy National Commission, Teaching and Research Direction, Nuclear Analytical Techniques Division, San Lorenzo, Paraguay
Gravenhorst, G.	Faculty of Forestry and Forest Ecology, University of Göttingen, Germany
Gross, G.A.	Laboratoire Pierre Süe, CEA-CNRS, CEA de Saclay, Gif-sur-Yvette, France
Gudiño, G.L.	Cátedra de Química General, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina.
Gueidan, C.	Observatoire Mycologique, Néronde, Mazille, France
Hazin, C.A.	Departamento de Energia Nuclear, Universidade Federal de Pernambuco, UFPE, Recife PE, Brazil
He, S.	Chinese Academy of Forestry, Research Institute of Forest Ecology and Environmental Science, Beijing, China
Hipólito, C.S.	Instituto Tecnologico e Nuclear, ITN, EN10 Sacavém, Portugal
Horimoto, L.K	Instituto de Botânica, Seção de Micologia e Liquenologia, São Paulo, SP, Brazil
Horvat, M.	Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
Ibrom, A.	Department of Biological and Environmental Engineering, University of Trás-os-Montes e Alto Douro, Portugal
Ivanković, D.	Institute Ruđer Bošković, Zagreb, Croatia
Jaćimović, R.	Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
Jacquiot, L.	Observatoire Mycologique, Néronde, Mazille, France

Jasan, R.C.	Técnicas Analíticas Nucleares, Comisión Nacional de Energía Atómica, Buenos Aires (1429), Argentina
Jeran, Z.	Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
Jervis, R.E.	Facility for Aerosol LAMS, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Ontario, Canada
Joyeux, E.	Observatoire Mycologique, Néronde, Mazille, France
Kadirova, M.	Institute of Nuclear Physics, Uzbekistan Academy of Sciences, Ulugbek, Tashkent Uzbekistan
Kennedy, G.	Nuclear Engineering Institute, École Polytechnique, Montreal, Quebec, Canada
Kirchner, G.	University of Bremen, FB 1, Bremen, Germany
Kochmann, S.	Atomic Energy National Commission, Teaching and Research Direction, Nuclear Analytical Techniques Division, San Lorenzo, Asuncion, Paraguay
Kopta, R.F.	Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba (5000), Argentina
Kremer, E.	Facility for Aerosol LAMS, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Ontario, Canada
Lartigue, J.	National University of Mexico, Faculty of Chemistry, Mexico City, Mexico
Lepore, L.	ISPESL — Dipartimento Insediamenti Produttivi ed Interazione con l'Ambiente, Monte Porzio Catone Rome, Italy
Lima, R.A.	Departamento de Energia Nuclear, UFPE, Recife PE Brazil
Loizeau, M.N.	Direction Départementale des Affaires Sanitaires et Sociales, Cité Administrative, Macon, France
Macacini, J.F.	Comissão Nacional de Energia Nuclear, Laboratório de Poços de Caldas — CNEN/COLAB, Poços de Caldas MG, Brazil
Maguregui, M.I.	European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium

Mao, X.Y.	Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100080, China
Marcelli, M.P.	Instituto de Botânica, Seção de Micologia e Liquenologia, São Paulo, SP, Brazil
Marchesini, R.	Department of Biology, Sect. of Botany, University of Ferrara, Ferrara, Italy
Markert, B.	Internationales Hochschulinstitut (IHI) Zittau, Germany
Marques, A.P.	Instituto Tecnologico e Nuclear, ITN, EN10 Sacavém, Portugal
Marques, M.C.	Department of Biological and Environmental Engineering, University of Trás-os-Montes e Alto Douro, Portugal
Martinez, T.	National University of Mexico, Faculty of Chemistry, Mexico City, Mexico
Maul, A.	STID, IUT Metz University, France
Mavsar, R.	Slovenian Forestry Institute, Ljubljana, Slovenia
Medeiros, C.	Departamento de Oceanografia, UFPE, Recife PE Brazil
Medici, A.	Department of Chemistry, University of Ferrara, Ferrara, Italy
Miranda, A.I.	Department of Environment and Planning, University of Aveiro, AVEIRO, Portugal
Mirsagatova, A.A.	Institute of Nuclear Physics, Uzbekistan Academy of Sciences, Ulugbek, Tashkent Uzbekistan
Moauro, A.	National Agency of New Technologies, Energy and Environment (ENEA), Casaccia Center, Rome, Italy
Mukhamedshina, N.M.	Institute of Nuclear Physics, Uzbekistan Academy of Sciences, Ulugbek, Tashkent Uzbekistan
Norboev, N.	Tashkent State Agrarian University, Uzbekistan, Kibray Region, Tashkent district, Uzbekistan
Oliveira, O. de	Instituto Tecnologico e Nuclear, ITN, EN10 Sacavém, Portugal
Orellana, L.	Instituto de Cálculo, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
Ortíz, M.	Atomic Energy National Commission, Teaching and Research Direction, Nuclear Analytical Techniques Division, San Lorenzo, Paraguay

Os, B.Van	National Geological Survey, Haarlen, Netherlands
Owega, S.	Facility for Aerosol LAMS, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Ontario, Canada
Pacheco, A.M.G.	CVRM-IST, Lisbon, Portugal
Paris, A.	Observatoire Mycologique, Néronde, Mazille, France
Pavičić, J.	Institute Ruđer Bošković, Zagreb, Croatia
Pedrini, P.	Department of Chemistry, University of Ferrara, Ferrara, Italy
Pignata, M.L	Cátedra de Química General, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina
Plá, R.R.	Comisión Nacional de Energía Atómica (CNEA), Unidad Radioquímica, Sector Técnicas Analíticas Nucleares, Buenos Aires, Argentina
Pognat	Observatoire Mycologique, Néronde, Mazille, France
Pommé, P.	SCK•CEN, Belgian Nuclear Research Centre, Mol, Belgium
Profumo, A.	CNR Centro di Radiochimica e Analisi per Attivazione, Pavia, Italy
Radnai, F.	INRA — Centre de Recherche Forestière de Nancy, Laboratoire Pollution Atmosphérique, Champenoux, France
Ramírez, A.	National University of Mexico, Faculty of Chemistry, Mexico City, Mexico
Reis, M.A.	Instituto Tecnologico e Nuclear, ITN, EN10 Sacavém, Portugal
Riquelme, I.	Atomic Energy National Commission, Teaching and Research Direction, Nuclear Analytical Techniques Division, San Lorenzo, Asuncion, Paraguay
Rizzio, E.	CNR Centro di Radiochimica e Analisi per Attivazione, Pavia, Italy
Robouch, P.	European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium
Rose, C.	INRA — Centre de Recherche Forestière de Nancy, Laboratoire Pollution atmosphérique, Champenoux, France

Rühling, Å.	University of Lund, Department of Ecology, Lund, Sweden
Saiki, M.	Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP, Supervisão de Radioquímica, São Paulo, SP, Brazil
Ščančar, J.	Jožef Stefan Institute, Ljubljana, Slovenia
Smodiš, B.	International Atomic Energy Agency, Vienna
Sousa, A.J.	CVRM-IST, Lisbon, Portugal
Spiric, Z.	INA- Naftaplin, Zagreb, Croatia
Taddei, M.H.T.	Comissão Nacional de Energia Nuclear, Laboratório de Poços de Caldas — CNEN/COLAB, Poços de Caldas MG, Brazil
Tan, P.V.	Facility for Aerosol, LAMSDepartment of Chemical Engineering and Applied Chemistry, University of Toronto, Ontario, Canada
Tejeda, S.	National Institute of Nuclear Research, Ocoyoacac, Mexico
Tillier, C.	Direction Départementale des Affaires Sanitaires et Sociales, Cité Administrative, Macon, France
Tsai, J.	Facility for Aerosol LAMS, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Ontario, Canada
Tušek-Žnidarič, M.	Jožef Stefan Institute, Ljubljana, Slovenia
Valcuvia, M.G.	Dip. di Ecologia del Territorio e degli Ambienti, Università di Pavia, Italy
Valentim, E.	Departamento de Energia Nuclear, UFPE, Recife PE, Brazil
Vasconcellos, M.B.A.	Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP, Supervisão de Radioquímica, São Paulo, SP, Brazil
Verza, G.	CNR Centro di Radiochimica e Analisi per Attivazione, Pavia, Italy
Wanna, Z.E.D.	Cátedra de Química General, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina.
Wolterbeek, B.	Delft University of Technology, Interfaculty Reactor Institute (IRI), Delft, Netherlands

Yubero, F.	Atomic Energy National Commission, Teaching and Research Direction, Nuclear Analytical Techniques Division, San Lorenzo, Asuncion, Paraguay
Zarazua, G.	National Institute of Nuclear Research, Ocoyoacac, Mexico
Zayed, J.	Department of Occupational and Environmental Health, University of Montreal, Montreal, Quebec, Canada
Zhang, Zh.	Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100080, China