

Lichens as biomonitors at indoor environments of primary schools

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Received: 2 June 2011 / Published online: 28 June 2011
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Abstract A biomonitoring study, using transplanted lichens *Flavoparmelia caperata*, was conducted to assess the indoor air quality in primary schools in urban (Lisbon) and rural (Ponte de Sor) Portuguese sites. The lichens exposure period occurred between April and June 2010 and two types of environments of the primary schools were studied: classrooms and outdoor/courtyard. Afterwards, the lichen samples were processed and analyzed by instrumental neutron activation analysis (INAA) to assess a total of 20 chemical elements. Accumulated elements in the exposed lichens were assessed and enrichment factors (EF) were determined. Indoor and outdoor biomonitoring results were compared to evaluate how biomonitors (as lichens) react at indoor environments and to assess the type of pollutants that are prevalent in those environments.

Keywords Lichens · Biomonitoring · Indoor environments · Air pollution · Rural and urban areas

Introduction

Indoor air quality is a subject that has gathered consensus concern due to its health effects on people since they spend most of their time in indoor environments. Children, in

particular, are a very susceptible group because they spend most of their time at home or at school/preschool and have airways that are not fully developed. The greater impact in health and, by consequence, in the educational performance of children as well reveals the importance of indoor air studies to assess the exposure of children to air pollutants [1, 2].

However, the use of standard sampling methods to monitoring the indoor air quality required by the countries legislation (such as air pumping and use of air filters) [1] raises some problems towards their application and adequacy in indoor environments such as schools. Mainly, the noise and the sampling apparatus interfere with the classroom activities, decreasing the students performance and attention to the lessons/teacher.

Due to these reasons, a passive sampling method has been developed to collect total particulate matter (TPM), based on the passive deposition of particles (dust), for a period of time within 1–3 months. Results of this study were already published elsewhere [2, 3] and showed it to be possible to assess the exposure of children to inorganic pollutants [2] and an association between TPM mass and rhinitis was found [3].

This passive method also has the advantage of its low price, minimum requirement of equipment and allows to do simultaneous sampling in several schools (14 in cited study), which allows to do a qualitative comparison between them afterwards.

To evaluate complementary techniques to this sampling method, biomonitoring with lichens appears as a possible solution. Its application is widespread as practical biomonitors of inorganic atmospheric contamination due to the lichens ability to accumulate levels of elements in excess of physiological requirements in close correlation with atmospheric elemental levels [4, 5]. However, lichens

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applications as biomonitors are usually conducted in outdoor environments because in indoors, physical conditions as temperature and humidity can cause physiological stress (electric conductivity [6], e.g.). However, indoor biomonitoring with higher plants has already been studied with success, namely with the plant *Scindapsus aureus* [7].

The aim of this study is to evaluate if, besides the physiological stress that the lichens go under in indoor environments, the ability of the lichen to accumulate elements present in the air is not affected in this type of environment. Instrumental neutron activation analysis (INAA) was the chosen analytical technique due its multi-elemental potential analysis.

Experimental

Transplanted lichens

The lichen *Flavoparmelia caperata* and tree bark (*Olea europaea*, commonly known as olive tree) were collected from a clean area of Portugal which is defined as a rural area (39°03'20 N, 8°10'42 W, about 93 km away from Lisbon city), in 13 April 2010.

In the laboratory, the lichen samples were set into tree bark pieces of around 6 cm × 6 cm each and they were hanged at the courtyards trees of the studied schools, using

nylon strings, and on trays inside the classrooms. The exposure period was from 20 April 2010 to 18 June 2010, in a total of 59 days.

Description of the studied sites

The location of the studied schools in the urban and rural areas of Portugal is shown on Fig. 1. The urban site was represented by Lisbon area where three primary schools were selected (U1: 38°42'15 N, 9°11'06 W; U2: 38°45'22 N, 9°10'02 W; U3: 38°44'32 N, 9°12'38 W) and the rural site was represented by Ponte de Sor municipality where 2 primary schools were selected (R1: 39°10'39 N, 8°14'25 W; R2: 39°15'02 N, 7°55'35 W). In the primary schools of the urban area, the transplanted lichens were exposed inside two classrooms (on a tray at about 1.80 m height) and in the courtyard (at tree branches at about 1.80 m height) per school. In the rural area, the transplanted lichens were exposed only in one classroom and in the courtyard of each school (in the same conditions).

Sample preparation and chemical analysis

After the exposure periods, lichen samples were sorted and cleared of extraneous material at the laboratory. For the conductivity measurements, the established procedure [6] was followed: 100 mg of each sample (washed and 24 h

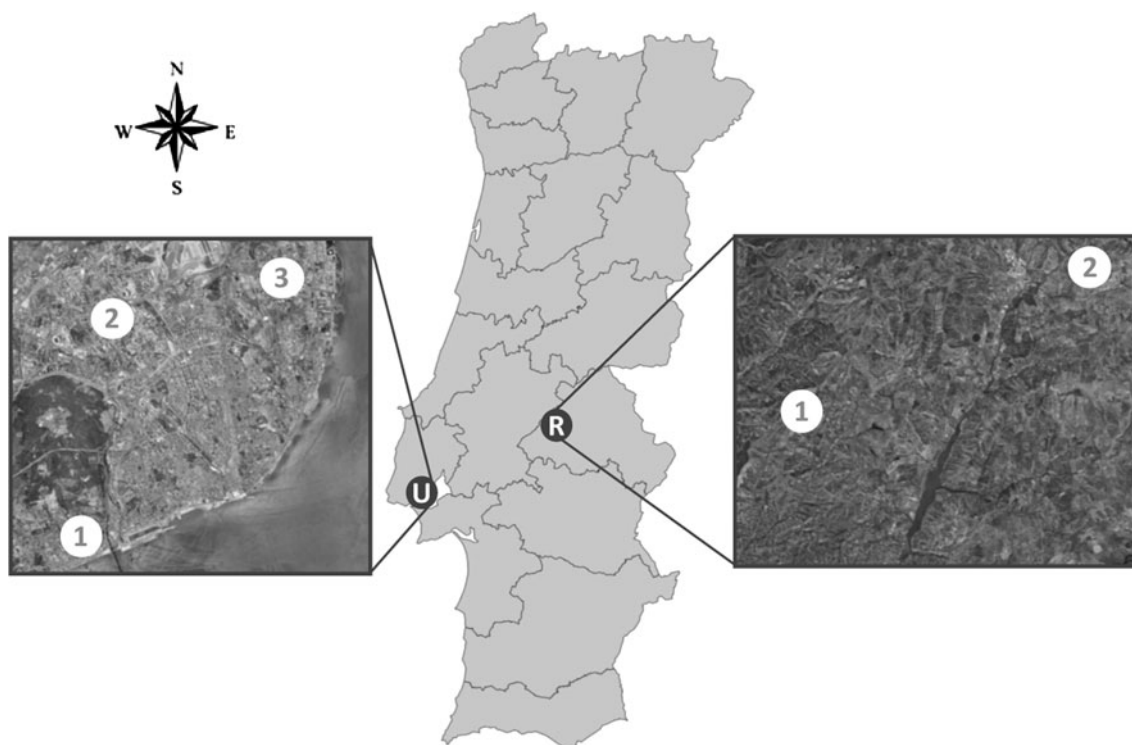


Fig. 1 Outline of mainland Portugal with focus on the urban (U) and rural (R) areas, where the studied schools were located

air-dried material) was immersed in 10 mL double distilled water for 60 min. The electric conductivity of each solution was measured afterwards by an electric conductivity meter (712 Metrohm Conductometer). The rest of the samples were freeze-dried and grounded in Teflon™ (balls and capsule) mills. After thorough homogenization, pellets of 250–300 mg were prepared and pelletized for neutron irradiation.

All samples were irradiated for 5 h at a thermal neutron flux density of $2.6\text{--}2.9 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$, at the Portuguese Research Reactor (RPI-ITN; nominal power 1 MW). Gamma spectra of the irradiated samples were acquired with high-resolution, hyperpure Ge detectors, for a period of 2 and 3.5 h after 4 days and 4 weeks, respectively. The k_0 -INAA method [8] was used and 0.1% Au–Al alloy discs were used as comparators and irradiated concurrently with the samples. Quality control was asserted by analyzing lichen reference material (RM) IAEA-336, which was prepared as the samples and co-irradiated with them. A total of 5 RM replicates were done.

Results and discussion

Quality control of the results

The ratio between the results obtained in this work and the certified values for the RM-IAEA-336, with uncertainties at 95% confidence level, is shown in Fig. 2. Overall, the ratio data is acceptable although it was verified that Hg, Sb and Se presented average values below the reference value (with a deviation of 43, 27 and 23%, respectively). This fact is probable due to the volatilization of the elements during the irradiation process. A deviation of 16% was found for Ce and Th but, for all the other elements, the overall deviation was within $\pm 15\%$. The u score [9] test was applied for all the elements and their values were always below 1.64, which means that the obtained results do not differ significantly from the recommended ones.

Lichens vitality

The leachate conductivity is considered the more sensitive indicator of general lichen vitality [5, 10], which is

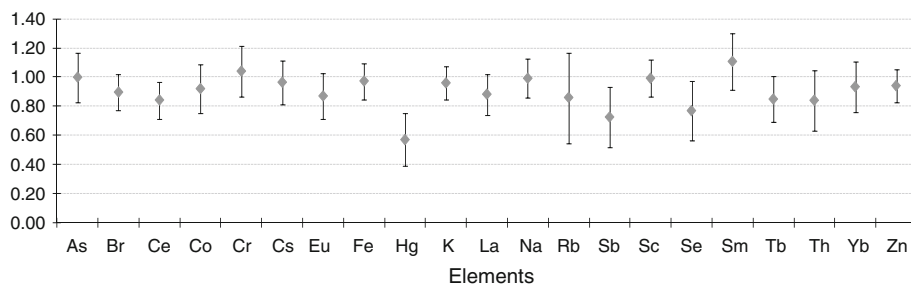
influenced by the stress that the lichen is under due to the pollution and to the atmospheric conditions of the site where the lichen was transplanted to. In our study, the outdoor values of the lichens conductivity of two of the urban schools (U1 and U2) are slightly higher than the rural schools and the urban school U3 (which are similar to the electric conductivity value of the unexposed lichens: $8.4 \text{ mS m}^{-1} \text{ g}^{-1}$). In fact, schools U1 and U2 are near to central Lisbon roads which may cause a higher stress into the lichens. Usually, transplanted lichens are used only in outdoor environments and, due to the physical specifications of indoor environments (such as less light, ventilation, accumulation of gaseous pollutants as CO_2 and CO due to human occupancy), it was supposed to obtain higher conductivity values, as it was verified by this work. In fact, indoor conductivities were always higher than $19.3 \text{ mS m}^{-1} \text{ g}^{-1}$, while the outdoor average was only $10.4 \text{ mS m}^{-1} \text{ g}^{-1}$. Differences between classrooms of the same school can be observed, which shows that the ventilation habits and human occupancy (which vary between classrooms) play a major role in the lichens vitality.

Element accumulation

Figure 3 shows the ratio of concentrations of each element after exposure and in control samples prior to exposure. This ratio is also known as the EC ratio [11] (exposed-to-control ratio) and it reveals the accumulation of some elements. The use of ratios allows us to interpret changes in element content without assuming a linear or non-linear model describing element accumulation and/or release during the time of exposure [12]. The classification [11] of the EC ratio values refers to the accumulation/loss and it is divided in 5 classes: (a) 0–0.25 severe loss; (b) 0.25–0.75 loss; (c) 0.75–1.25 normal; (d) 1.25–1.75 accumulation and (e) >1.75 severe accumulation.

For the school U1, the classroom A presented accumulation towards Na, Rb, Sb and Ta, while classroom B presented accumulation for Ca and Rb. The outdoor of U1 only showed accumulation of Sb. Classroom A of school U2 did not presented any accumulation, while classroom B showed accumulation for Ce, Cs, Hf, La, Sm, Ta and Yb and severe accumulation for Rb. The outdoor of U2 only

Fig. 2 Ratios between the concentrations obtained in this work for the RM-IAEA-336 and their recommended values, with uncertainties at the 95% confidence level



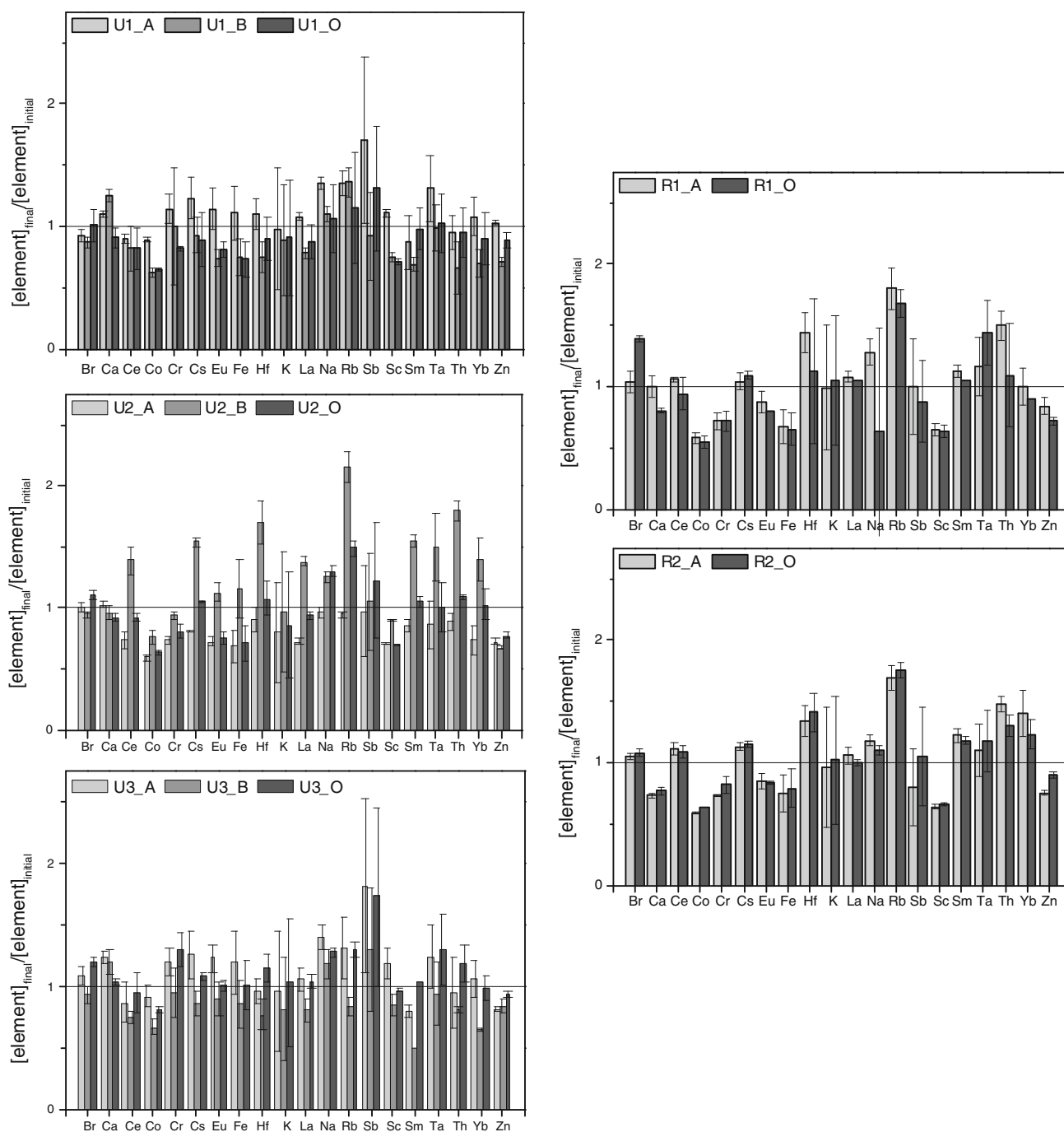


Fig. 3 Ratios of element concentrations in transplanted and native *F. caperata* originating from a relatively unpolluted site in a rural area. *R* rural schools, *U* urban schools, *O* outdoor/courtyard, *A* classroom A and *B* classroom B

showed accumulation of Na and Rb. For School U3, the classroom A showed accumulation for Cs, Na and Rb, while classroom B only showed accumulation for Sb. The outdoor of U3 showed accumulation of Cr, Na, Rb, Sb and Ta.

In the rural area, the classroom A of school R1 showed accumulation for Hf, Na and Th and severe accumulation for Rb, while its outdoor showed accumulation for Br, Rb

and Ta. The classroom A of school R2 showed accumulation of Hf, Rb, Th and Yb, while the schools outdoor showed accumulation of Hf, Rb, Th.

Antimony was only accumulated by the lichens in the urban area (p value <0.05 when comparing with rural results) revealing its source as probably traffic, since one of the major differences between rural and urban areas is the intensity of the traffic.

Enrichment factors

Enrichment factors [13] (EF) were calculated, using Sc as a crustal reference element (EF_{Sc}) and the Mason and Moore [14] soil composition values. EFs were calculated based on the following equation:

$$EF_X = ([X]/[SC])_{lichen} / ([X]/[SC])_{crust}$$

The crustal EF analysis is used to try to evaluate the strength of the crustal and non-crustal origin of the elements. Figure 4 shows the EF for the exposed and unexposed lichen samples.

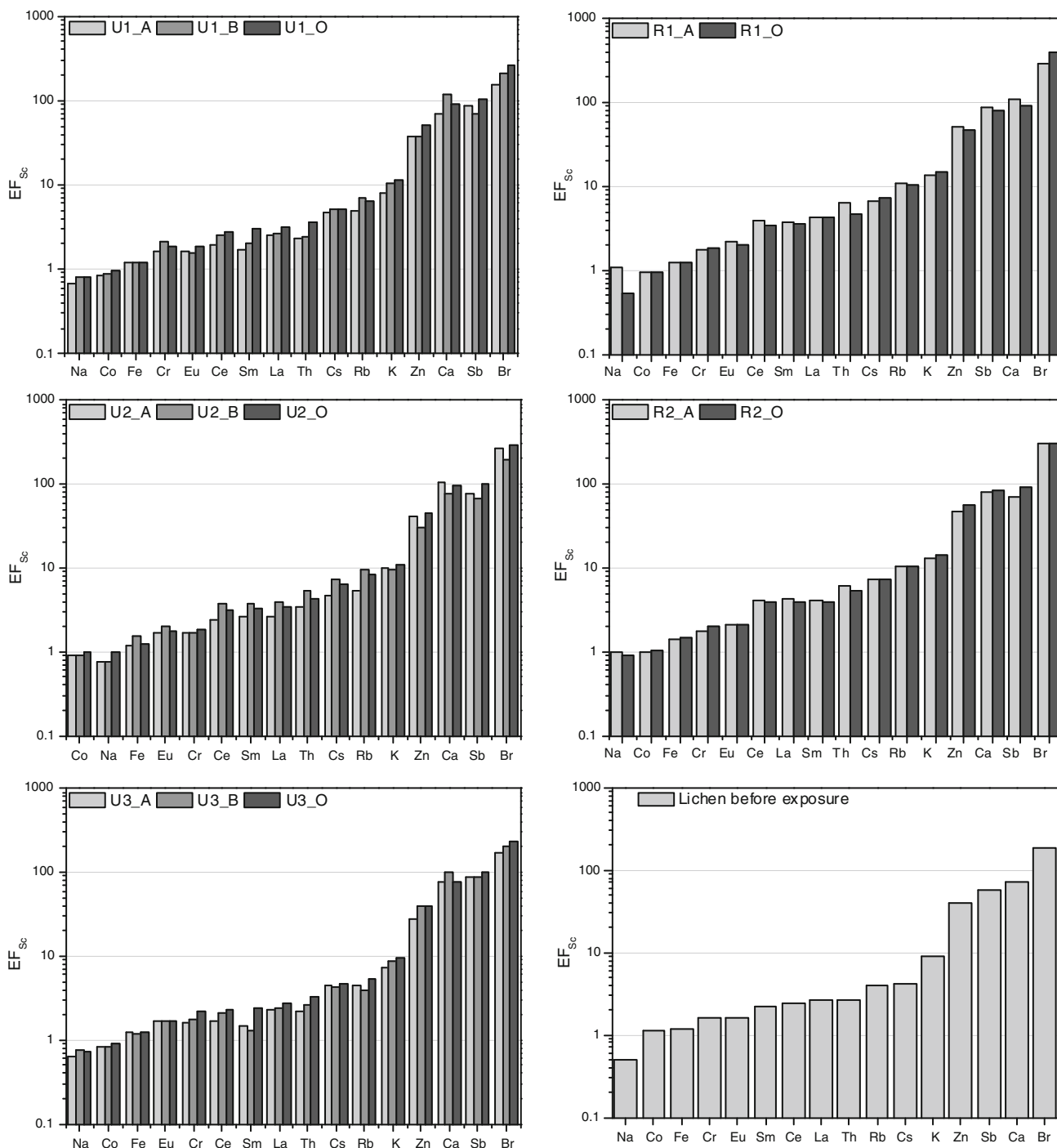


Fig. 4 Crustal EF for the exposed and unexposed lichen samples. *R* rural schools, *U* urban schools, *O* outdoor/courtyard, *A* classroom A and *B* classroom B

EFs higher than 10 can be observed for Zn, Sb, Ca and Br in the exposed and unexposed lichens. This fact suggests that there is a non-crustal origin for these elements. Usually, Zn and Sb (EF values of 41 and 57, respectively) are associated with sources such as combustion, incineration and traffic (mainly tires and brake wear) [15]. This fact reveals that the unexposed lichens were under the influence of some traffic influence, although they were collected from a supposed clean rural area.

For Sb, all the exposed samples showed an increase of the EF value (with a minimum EF value of 66, for the classroom B of school U2). In the urban area, all the outdoor EF_{Sb} were higher than the ones obtained inside the classrooms. Calcium and Br enrichment is probably due to the physiological characteristics of the lichens.

In the rural area, EFs higher than 10 in the exposed lichens were also observed for Rb and K. In the urban area, only K showed some enrichment, namely for schools U1 and U2.

Conclusions

A biomonitoring study was conducted in rural/urban and indoor/outdoor environments using the lichen *F. caperata*. A total of 20 chemical elements from the exposed and unexposed lichen samples were obtained by the INAA technique. Elemental accumulation was found in the exposed lichens in both outdoor and indoor environments. This fact shows that the use of lichens is possible in indoor environments, despite the higher physiological stress that the lichens are under in these types of environments (showed by the higher values of electric conductivity that were obtained).

In the urban exposed samples, a Sb accumulation was found which reveals a traffic source that affects the air quality in both indoor and outdoor environments. A variability of the elements accumulation was found between classrooms of the same school, which shows that the classrooms specific characteristics affect the local pollutants in the air. EF confirmed the Sb accumulation in the urban samples and, as well, a rural traffic source in the unexposed lichens was found.

Further work with this biomonitoring method will be developed in order to study a wider range of schools to assess the variability between schools and their indoor/outdoor environments.

Acknowledgments Financial support by Fundação para a Ciência e a Tecnologia (FCT; Portugal) through research contract PTDC/

SAU-ESA/65597/2006 is gratefully acknowledged. N. Canha thanks FCT for his PhD grant.

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