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Relevant aspects on biomonitoring of heavy metal concentration in environmental air in Asunción city

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A B S T R A C T

Introduction. Bryophytes (mosses) have long been used to determine the concentration of heavy metals as an alternative to the collection of atmospheric aerosols. **Objective.** To evaluate the environmental concentration of lead, cadmium, mercury and arsenic in autochthonous species of moss and to analyze some methodological aspects of biomonitoring in Paraguay. **Methodology.** In an observational study moss samples were obtained from sub rural zone to be transplanted in 5 sites of high vehicular traffic in Asunción city. The samples were left outdoors for 58 days and then collected and subjected to study using the inductive coupling plasma source mass spectrometry technique. The bryophytes were characterized and all the climatological variables during the study period were consigned. **Results.** Lead concentrations detected in moss explants exposed to the urban environment were higher than mosses from natural forest, while arsenic levels in the latter were higher than those found in bryophytes transferred to the city. No conspicuous levels of cadmium and mercury were found. The bryophytes used belonged to two families: Hypnaceae and Pilotrichaceae. The range of temperature, relative humidity, wind and precipitation did not reach extreme levels during the studied period. **Conclusion.** The different lead levels measured here, could be surrogates of urban pollution while the notorious arsenic level in natural forest moss points to other sources like wildfires. Several aspects of the biomonitoring methodology are discussed.

Keywords: bryophyta; lead; arsenic; environmental monitoring; mass spectrometry

Aspectos relevantes del Biomonitoreo de la concentración de metales pesados en el aire ambiental de la ciudad de Asunción

R E S U M E N

Introducción. Las briofitas (musgos) se han utilizado durante mucho tiempo para determinar la concentración de metales pesados como alternativa a la recolección de aerosoles atmosféricos. **Objetivo.** Evaluar la concentración ambiental de plomo, cadmio, mercurio y arsénico en especies autóctonas de musgo y analizar algunos aspectos metodológicos de la biomonitorización en Paraguay. **Metodología.** En un estudio observacional se obtuvieron muestras de musgo de

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una zona sub-rural para ser trasplantadas en cinco sitios de alto tráfico vehicular en Asunción. Las muestras se dejaron a la intemperie durante 58 días y luego se recogieron para la medición de metales pesados por espectrometría de masas con fuente de plasma de acoplamiento inductivo. Se caracterizaron las briofitas y se consignaron todas las variables climatológicas durante el período de estudio. **Resultados.** Las concentraciones de plomo detectadas en los explantes de musgo expuestos al medio urbano fueron superiores a las de los musgos del bosque natural, mientras que los niveles de arsénico en estos últimos fueron superiores a los encontrados en los briófitos trasladados a la ciudad. No se encontraron niveles llamativos de cadmio y mercurio. Las briofitas utilizadas pertenecían a dos familias: Hypnaceae y Pilotrichaceae. Los rangos de temperatura, humedad relativa, viento y precipitación no alcanzaron niveles extremos durante el periodo estudiado. **Conclusión.** Los diferentes niveles de plomo medidos podrían ser subrogados de polución urbana mientras que el notorio nivel de arsénico en musgo de bosque natural apunta a otro tipo de fuentes como los incendios forestales. Se discuten varios aspectos de la metodología de biomonitorización.

Palabras clave: briófita; plomo; arsénico; monitoreo ambiental; espectrometría de masas

INTRODUCTION

A preliminary and very recent study found that the median daily environmental concentration (ug / m^3) of MP2.5 (2.5ug Particulate Matter) exceeded permissible limits (WHO) for 36% of the monitoring time. In this pilot test devices with filters located in several places of the capital were used and when they studied the composition of the particles, they reported very low concentration of heavy metal. The explanation given by the study on the probable source's points to the combustion of biomass, important taxpayer of our regional pollution. However, because it was a pilot study, limited results could have been given by the scarce monitoring interval[1].

The US Environmental Protection Agency (US EPA), because of its health effects, classifies six major pollutants: a- particulate matter (MP10 and MP2.5), b- sulfur dioxide (SO2), C- Nitrogen oxides (Nitrogen monoxide NO and nitrogen dioxide N2O), d- Carbon monoxide (CO), e- Ozone (O3), and f- Lead. The atmospheric emission of these compounds is produced in great quantity and has a great capacity of dispersion with the consequent exposure potential[2]. Since 1990, the European moss survey has been repeated at five-yearly intervals. Since that time the concentration in mosses has showed an interesting variation for arsenic, cadmium, iron, lead, vanadium, copper, zinc, mercury and chromium[3].

Some living beings are able to accumulate materials from environmental pollution. The monitoring of environmental pollution using biological indicators or biomonitoring is a potentially effective and economical alternative to carry out measurements in the environment[4]. In this sense, bryophytes (mosses) have been used for a long time to determine the concentration of heavy metals as an alternative to the collection of atmospheric aerosols on devices with filters that need costly equipment[5].

Mosses have little or no cuticle development which is why surface ions have direct access to cationic exchanges in cell membranes. They have a great capacity for tracer retention[6]. Different species have different catchment capacities and the deposition of material on its surface is also conditioned by the rainfall, temperature, and humidity conditions of the studied region[7,8].

Research on environmental pollution in Paraguay is in an embryonic state. To detect the concentration of environmental heavy metals through the use of native bryophytes and the possible conditioning variables in this peculiar methodology, we have elaborated the following study.

MATERIAL AND METHODS

Selection and collection of bryophytes

For the collection of biomonitors, mosses were taken from Cerro Patiño, belonging to the village with the same name, located in the Central Department, located at 34.4kms from the capital of the country. Six moss samples (Fig. 1) were collected in an integral manner, including the trunk or bark that housed them using appropriate gloves and placing each sample in individual plastic bags during their transfer to our capital. In a second phase, a sample (Moss control 2) from another natural forest (430 km from Asunción) was collected for a comparative analyzes.

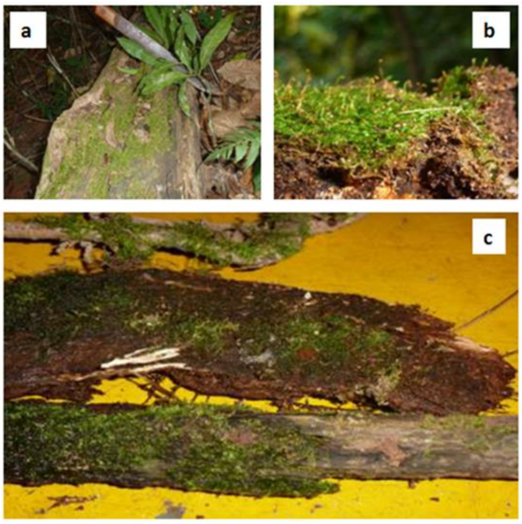

Figure. 1. a) Moss in its natural habitat; b) Moss habit; c) Epiphytic bryophytes prepared for transplantation at selected sites.

Location of biomonitors and bioaccumulators.

One sample was stored as control (Moss control 1). The remaining trees were planted (transplanted) in existing trees in the premises to be subjected to the study, including the bark or trunks that received them and later secured with fine wire (Fig. 2). Five Asunción sites were selected, which include Av. Mcal. López and Avda. Madame Elisa Alicia Lynch (Moss 1); Avda. Aviadores del Chaco and Avda. Madame Elisa Alicia Lynch (Moss 2); Avda. General José Gervasio Artigas and Avda. Primer Presidente (Moss 3); Avda. Mcal. López and Avda. Perú (Moss 4); and Avda. Perú and Avda. Rodríguez de Francia (Moss 5) considering the vehicular traffic that was present in the area. The biomonitorers remained in those places during a period of 58 days (2 September 2013 -29 October 2013).

Figure 2. Preparation fixed to selected site tree (transplant).

Treatment of samples

After the *in-situ* study time (58 days), the samples were collected from the five previously selected sites and immediately transferred to Eco Natura (Food and Environmental Control Laboratory) where they were subjected to specific analyzes.

Analysis of heavy metals

For the determination of heavy metals, acid digestion is carried out in a microwave oven, in which the sample is placed in Teflon cups, and the reagents are added thereto. They are then capped and placed in a rotor inside the equipment (ETHOS digester, MILESTONE®). The objective was to measure the concentration of lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) using microwave acid digestion method and inductive coupling plasma source mass spectrometry technique[9].

Concentration of heavy metals in mosses

Samples are homogenized in mortar and stored in polypropylene flasks, then subjected to digestion. The moisture content is expressed as the moisture determination has not been performed. For digestion: a reagent blank (6 ml HNO3 65% + 1 ml HCl) is prepared to check for interferences in reagents, white controls (0.5 g of moss + 6 ml HNO3 65% +1 ml HCl), Positive controls (appropriate volume of standard chemical elements + 0.5g of moss + 6 ml HNO3 65% + 1 ml HCl) and samples (0.5g of moss + 6 ml HNO3 65% + 1 ml HCl). The preparation is subjected to a temperature of 150 ° C for 30 minutes, where the organic matter is destroyed and the metals pass into a state of dissolution. Subsequently the preparations are removed from the microwave equipment and a dilution thereof is carried out in the appropriate proportion. To determine the concentration of heavy metals, the mass spectrometry technique with Inductive Coupling Plasma Source (ICP-MS) was used for which the Agilent Technologies-7500Series® analyzer was used. For ICP-MS analysis, a method is created to monitor the atomic mass of lead and its isomers 208, 206, 204 of cadmium 111 of arsenic 75 and mercury 200,202. A calibration curve, controls, wash and samples are injected. The results are expressed as counts per second (CPS), and relating this value to the concentration can graph a calibration curve and obtain the equations of the line approaching analytical curve concentrations ranged from 1.0 to 40, 1.2 to 24, and 1.0 to 20 μg L−1 for arsenic, cadmium, and lead, respectively. The procedure was carried out in the Eco Natura (Food Control and Environment Laboratory) and Diaz-Gill Laboratory.

Taxonomic characterization of mosses

It was carried out in the Department of Botany Faculty of Chemistry of the National University of Asunción (FCQ,UNA). Specimens were identified using the taxonomic rules of Bruck[10].

Meteorological conditions.

Environmental conditions such as precipitation, temperature, wind, and humidity can affect biomonitoring. It is extremely important to know if these variables were not outside the expected range during the observation period. The data was supplied by the Meteorological Service of Dirección de Meteorología e Hidrología, DMH-DINAC, Asunción, Paraguay, https://www.meteorologia.gov.py/.

RESULTS

Concentration of heavy metals

In the mosses that were transplanted at the intersection of Av. Mcal. López and Avda. Madame Elisa Alicia Lynch (Moss 1); Avda. Aviadores del Chaco and Avda. Madame Elisa Alicia Lynch (Moss 2); And Avda. Perú and Avda. Rodríguez de Francia (Moss 5), a higher lead concentration was found when compared with

Moss control 1. Similarly, in mosses 1 and 5, arsenic levels were higher than Moss control 1, as seen in Table 1. No significant cadmium or mercury traces were detected in these samples.

Table 1. Concentration of heavy metals in 500 mg of moss detected by ICP-MS, after 58 days of stay in sites of high vehicular traffic in Asunción (moss1-4), Paraguay.

Samples	Pb (mg/k)	Cd (mg/kg)	Hg (mg/kg)	As (mg/kg)
Moss 1	0.039	< 0.001	< 0.001	0.028
Moss 2	0.024	< 0.001	< 0.001	0.013
Moss 3	0.012	< 0.001	< 0.001	0.007
Moss 4	0.009	< 0.001	< 0.001	0.006
Moss 5	0.026	< 0.001	< 0.001	0.018
Naive moss 1	0.021	< 0.001	< 0.001	0.017
Naive moss 2	0.008	< 0.001	< 0.001	0.056

ICP-MS: Inductive Coupling Plasma Source Mass Spectrometry technique

Naïve moss 1: bryophytes from sub-urban zone; Naïve moss 2: from natural forest

Note that mosses 3 and 4 showed lower lead levels to Moss control 1, as observed for arsenic levels in mosses 2, 3 and 4.

Taxonomic characterization of mosses

The reported botanical typing is as follows: Moss 1 = *Versicularia versicularis*, Moss 2 = *Isopterygium tenerum*; Moss 3 = *Isopterygium tenerum*, Moss 4 = *Versicularia versicularis*, Moss 5 = *Callicostella Pallida*. Naive moss 1 = *Callicostella pallida* and naive moss 2 = *Chryso-hypnum diminutivum*. All belong to the family Hypnaceae, except the *Callicostella pallida* which forms part of the Pilotrichaceae. (Table 2)

Table 2. Description of families and species of bryophytes corresponding a moss samples used for monitoring of air concentration of heavy metals in Asuncion, Paraguay.

Samples		
Moss 1	Hypnaceae	*Versicularia versicularis*
Moss 2	Hypnaceae	*Isopterygium tenerum*
Moss 3	Hypnaceae	*Isopterygium tenerum*
Moss 4	Hypnaceae	*Versicularia versicularis*
Moss 5	Pilotrichaceae	*Callicostella pallida*
Naive moss 1	Pilotrichaceae	*Callicostella pallida*
Naive moss 2	Hypnaceae	*Chryso-hypnum diminutivum*

Meteorological conditions

The data of the climatological variables during the study period were supplied by the Meteorological Service of Dirección de Meteorología e Hidrología, DMH-DINAC, Asunción, Paraguay, expressed in median and range, were as follows: temperature 22 ° C (12.6-29.5 ° C), relative humidity: 65.5% (35 -92%, in 12 different days were recorded> 80%), wind: 13km / h (13-25.9km / h, in 7 different days were recorded> 20km / h). There were 5 matched days and an isolated rainy day (11 days) during which rainfall of 5.9mm (0.1-26.5mm) was observed.

Phase 2 (collection of naive moss 2)

In view of the dispersion of the data and especially when the transplanted moss samples were two different families and three different species, we have taken another sample from a site farther from the capital (430 km). This time a *Chryso.hypnum diminutivum* (Hypnaceae) was collected which shows very low levels of lead, as expected but striking concentration of arsenic.

DISCUSSION

Bryophytes are resistant to many substances that are highly toxic to other plant species and are capable of surviving in diverse and extreme environments; this aspect make them excellent indicators for a wide range of pollutants. Environmental contaminants are deposited on the mosses in the form of aqueous solutions, in the form of gases or particles[11]. These sedentary organisms possess a different pattern of physiological adaptations that makes them conducive to environmental studies in the medium and long term[12].

Since lead-enriched gasoline, the concentration of this and other metals in the ambient air became relevant, recognizing as a public health problem in several countries, for decades, biomonitoring with bryophytes has become routine, leading to longitudinal reports of their values. Thus, from 1990 to 2006, concentrations of lead, cadmium and mercury in mosses (mg / kg) decreased from 12.9 to 3.7, 0.31 to 0.16, and 0.044 to 0.035 respectively in Germany and from 11.3 to 2.15, 0.24 to 0.14 and 0.065 to 0.017 in Sweden[3]. Recent work done in Ecuador has also detected lead in the environment and, although no control sample was used, concentrations detected in mosses after 58 days of monitoring ranged from 0.73-3.86 mg/m^3[13].

We have found a difference between lead levels taken up by mosses exposed in the city (0,009-0,039 mg/kg) compared to basal concentrations in natural forest plant (0,008 mg/kg). The amount of arsenic found here in natural forest moss (0,056 mg/kg) is higher than the concentrations mentioned in the literature (0,007-0,028 mg/kg). The usual content, expressed as mg kg-1 in dry matter, of heavy metals in mushrooms from unpolluted areas (2000-2009) and accumulating species are below 5 mg kg^{-1} for Pb[14]. It is appropriate to note that comparisons of these variables with ranges cited in the literature should take into account the differential impact of the environment in different regions of the planet. In this respect, the fluctuation of concentrations over time should also be assessed[15] .

Lead level measured here, presents an interesting dispersion, phenomena that may have several edges of interpretation: a) Lead levels are really low in the city: this hypothesis is in line with the only recent air quality pilot test in Asuncion. In this work the mean daily concentration (MPa / m3) of MP2.5 at three different points was 24.4 ± 8.81, 23.4 ± 8.76 and 14 ± 5.26 and in the particle, the concentrations (ng / m3) of Pb were 1.4 ± 1.8, 4.2 ± 2.3 and 3.4 ± 1.7, respectively. As and Cd concentrations were less than 0.2. However, even low levels of lead in the environment can be toxic in certain situations. The current threshold for the toxicity of this metal is defined as a blood concentration <10 ug / dL, a cut-off point adopted by the USA in 1991 and by the WHO in 1995[16]; B) Lead levels are low because intrinsic issues to method: transplanted epiphyte moss may have an attenuation of deposition when placed under trees and although weather conditions (precipitation, temperature, wind and humidity) were maintained without very aggressive extremes in the biomonitoring period there may be variations in leaching uniform of the elements when the mosses are protected by the forest. A large proportion of the pollutants accumulate in the bryophytes through wet deposition; thus, the amount, duration and intensity of precipitation affect the accumulation and leaching (extraction of solute from a solid). Dry deposition increases during the transition from humid to arid climates; c). The concentration of lead is different in various parts of Asuncion: moss 3 has lower levels than the control sample and this could be because that intersection (Avda. Artigas and Primer Presidente) borders on an extensive green area (Jardin Botánico) which could have partially attenuated the deposition of the tracers, and moss 4 findings were also low and the crossing does not border on a leafy forest. We must consider that in several points of Asunción are distributed houses of spare parts or different sources of potential of pollution, collaborating thus to a probable heterogeneous distribution of pollutants.

The amount of arsenic found here in natural forest moss (0,056 mg/kg) is higher than those found in bryophytes transferred to the city (0,007-0,028 mg/kg) and concentrations mentioned in the literature (0.5 to 5 mg kg^{-1}). The usual content is for As[14], although one should always reflect on the reckless comparisons between different regions and the influence of time on the measurements of heavy metals in bryophytes. Arsenic can contaminate groundwater and can negatively impact human health through drinking and irrigation practices. The World Health Organization sets the interim guideline of 10 ppb (0.01 mg/L) as the standard for drinking water[17].

One of the most important sources of arsenic in forests are the ashes and residues from wildfires[18], and these unfortunate environmental events are becoming increasingly frequent in several forest reserves in Paraguay[19]. This is the first explanation of our findings.

The erratic distribution of heavy metal concentrations found in our study could also be due to the fact that different species and families of bryophytes were used. The species most frequently cited in surveillance studies are *Hypnum cupressiforme*, *Hylocomium splendens*, and *Pleurozium schreberi*: species particularly abundant in parts of Europe[20]. There are differences in chemical composition between species and even between separate parts of an individual moss. The mineral particles of the soil can also be translocated to the bryophyte. Other factors that may affect the concentration are: nutritional status of the moss, vegetation of the area, altitude, moss age (mosses older "age", have a higher concentration of metal[21]. In our study, two families were used Hypnaceae (*Versicularia versicularis, Isopterygium tenerum* and *Chryso-hypnum diminutivum*) and Pylothrycaceae (*Callicostella palida*). This is the first time that this type of studies has been carried out in our country, highlighting the use of native mosses.

The dispersion of the values could also be due to the heterogeneous uptake of the tracers by mosses. The efficiency of moss picking for the elements varies considerably and many articles postulate the following order: lead> cobalt and chromium> copper, cadmium, nickel and vanadium> zinc> arsenic. There are variations in the leaching of the elements as they are attached to the cell wall or accumulated on the surface of the moss[22,23]. It is necessary to clarify that these studies do not refer to mosses of the flora of Paraguay.

One of the most important challenges in biomonitoring studies is to establish monitored protocols for sampling, sample preparation and analysis systems in order to generally achieve comparable results, at least on a regional scale. Moss species should be collected in selected areas with predictable storage capacity under diverse environmental circumstances[24].

This study has several limitations: First, the small size of sample that was used to perform this research. A big number of bryophytes could help us to determine the real potential of mosses as biomonitoring in a Paraguayan city as a first step; and latter implement this method in a large scale including others cities.

Second: the differences between mosses species using in this research show very different results, not allowing us to conclude in a concrete way regarding the levels of contaminants captured by them. In a second study we should standardize the species in order to provide more reliable results and reflect the current situation of the area.

Third: As there are very few environmental studies in our country, we have arbitrarily chosen the areas to be investigated considering only vehicular traffic. This research represents the starting point to other environmental studies in our country.

In conclusion: different traces of lead and arsenic were found between mosses exposes to Asuncion environment and forest bryophytes, although the study poses important methodological issues that should be derived and validated for native plants. The implementation of a protocol to carry out these types of work is extremely important and should be considered as a priority to improve the quality of research in these fields.

Conflicts of interest: The authors declare no conflict of interest.

Contribution of the authors: Coronel Teixeira R, Cañiza B, Fretes J, Rodríguez M, Pasten M, Magis Escurra C, Pérez Bejarano D. had the same participation in: the idea and design of the research, data collection, statistical processing, analysis and discussion of the results, drafting of the work and approval of the final version.

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