

# Mosses as indicators of atmospheric metal deposition in an industrial area of southern Brazil

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## RESUMO

(Musgos como indicadores da deposição atmosférica de metais em uma área industrial do sul do Brasil) Caxias do Sul sedia o segundo maior pólo metal mecânico do Brasil, apresentando elevado risco de contaminação atmosférica. Com o objetivo de identificar espécies úteis como indicadoras da deposição atmosférica de metais, foram analisadas 15 espécies de musgos procedentes da zona urbana e da área rural e espécimens depositados no Herbário da Universidade de Caxias do Sul. Os metais (Zn, Ni, Cd, Cr, Cu e Pb) foram determinados por meio de Espectrometria de Absorção Atômica (AAS). O Teste de Mann-Whitney indicou diferenças significativas nas concentrações de Zn, Cu, Pb e Cr entre as áreas urbana e rural. Um aumento significativo na presença de Zn, Cd, Cu e Pb foi evidenciado nas amostras recentes. As espécies *Thuidium tomentosum* Besch., *Sematophyllum subpinnatum* (Brid.) E. Britton, *Helicodontium capillare* (Hedw.) A. Jaeger, *Schlotheimia jamesonii* (W.-Arnott) Brid. e *Meteorium deppei* (Hornsch. ex Müll. Hal.) Mitt. são indicadas como potenciais biomonitoras da deposição atmosférica de metais.

**Palavras-chave:** bioindicadores, Bryophyta, metais pesados, poluição atmosférica, Rio Grande do Sul

## ABSTRACT

(Mosses as indicators of atmospheric metal deposition in an industrial area of southern Brazil). Caxias do Sul hosts the second largest metal-mechanic pole in Brazil, which increases the risk of atmospheric contamination. With the aim of identifying species that might be useful as indicators of atmospheric deposition of metals, 15 species of mosses from an urban and a rural setting were analyzed and compared with specimens deposited at the Herbarium of the Universidade de Caxias do Sul. Metal concentrations (Zn, Ni, Cd, Cr, Cu and Pb) were determined using Atomic Absorption Spectrometry (AAS). The Mann-Whitney test indicated concentrations of Zn, Cu, Pb and Cr that were significantly different between the urban and rural areas. Additionally, Concentrations of Zn, Cd, Cu and Pb were significantly higher in recently collected samples. The species *Thuidium tomentosum* Besch., *Sematophyllum subpinnatum* (Brid.) E. Britton, *Helicodontium capillare* (Hedw.) A. Jaeger, *Schlotheimia jamesonii* (W.-Arnott) Brid. and *Meteorium deppei* (Hornsch. ex Müll. Hal.) Mitt. are potential biomonitoras of atmospheric metal deposition.

**Key words:** atmospheric pollution, bioindicators, Bryophyta, heavy metals, Rio Grande do Sul

## Introduction

Organisms *in vivo* are often used as active or passive bioindicators of environmental stress (Markert *et al.* 2003). Certain plant species in particular have been useful in determining the degree of atmospheric pollution, and have been used as biomonitoras of specific contaminants such as ozone (Vergé 2002), fluoride (Oliva & Figueiredo 2005), fossil fuel byproducts (Klumpp *et al.* 2001), and trace metals (Husk *et al.* 2004).

Bryophytes in the broad sense, are cryptogamous, non-vascular, and generally small plants found in almost every environment, particularly where humidity is high. They play an important role in soil formation and plant succession. With 17,800 described species, bryophytes are the second largest group of terrestrial plants (Gradstein *et al.* 2001). They are currently divided into three distinct groups: Bryophyta (mosses), Marchantiophyta (hepatics or liverworts) and Anthocerotophyta (hornworts) (Buck & Goffinet 2000). Of these, mosses are the largest, with ca.

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12,000 species, encompassing the broadest morphological variation (Vanderpoorten & Goffinet 2009).

Despite certain limitations stemming from abiotic (e.g., precipitation, elevation above the sea level) and biotic factors (moss species, age of moss, growth rate of a species), the use of mosses is still one of the best methods available to estimate the trace element burden in the environment (Grodzinska & Szarek-Lukaszewska 2001). Mosses are particularly useful in evaluating environmental conditions that are being constantly modified by humans for several reasons. For instance, mosses lack a cuticle and a vascular system, which causes them to accumulate metals passively, acting as ion exchangers (Grodzinska 1999; Onianwa 2000). Also, they are broadly distributed, and occur in both clean and industrial areas (Grodzinska & Szarek-Lukaszewska 2001; Korzekwa *et al.* 2007; Pedroso 2007).

The use of mosses as indicators of atmospheric pollution by heavy metals was developed in Sweden (Rühling & Tyler 1973) and is now well established in many European countries (Galsomies *et al.* 1999; Siewers *et al.* 2000; Grodzinska & Szarek-Lukaszewska 2001; Reimann *et al.* 2001; Pesch & Schroeder 2006). Mosses are used to determine the present extent of environmental pollution in vast as well as in small areas (Zechmeister 1997; Galsomies *et al.* 1999; Grodzinska *et al.* 1999; Steinnes *et al.* 2008), and to help estimate the environmental concentrations and effects of contaminants (Siewers *et al.* 2000; Steinnes *et al.* 2005, Pesch & Schroeder 2006). Every five years, since 1990, at least 21 European states have conducted projects aimed to detect spatial and temporal trends of metal accumulation in terrestrial ecosystems (Pesch & Schroeder 2006).

A total of 885 moss taxa are known to occur in Brazil (Costa *et al.* 2010), of which 536 have been recorded from Rio Grande do Sul (Bordin & Yano 2010). Despite their diversity in the country, mosses have rarely been used in air quality biomonitoring (Carneiro 2004). One exception is *Sphagnum*, which has been used to monitor environmental pollution levels (Gutberlet 1996; Carneiro 2004; Henriques 2007).

Caxias do Sul, the second largest city in the state of Rio Grande do Sul, is one of the largest metal mechanic industry centers in Brazil. Because it harbors several metallurgical and galvanic industries, the atmosphere of Caxias do Sul is contaminated with trace metals (SIMECS 2002) that interfere with the ecosystem and impact human health.

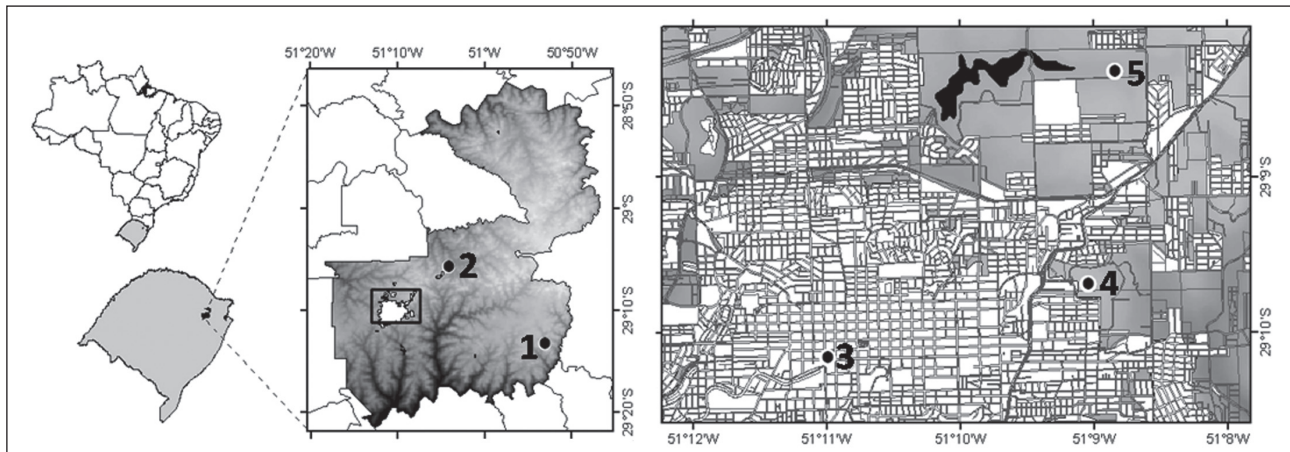
Our study is an attempt to identify potential moss species that can be used as biomonitors of atmospheric deposition of metals in Brazil. It is important for two reasons: first, results can be used to demonstrate the increased metal air pollution in this urban-industrial center; second, species that are identified as potentially useful in air quality control can be prioritized in further studies, because taxonomic difficulties, lack of surveys and little knowledge about the biology, physiology and geographical distribution of most moss species have hampered their use as indicators of air quality in the country.

## Materials and methods

Caxias do Sul is located in southern Brazil (29°10'4"S, 51°10'44"W), at altitudes ranging from 740m to 820m. It has an estimated population of 410,166 inhabitants (IBGE, 2009). The climate is Cfb (Kottek *et al.* 2006), with average annual temperature of 15.9°C and a well-distributed annual precipitation of 1,821 mm/year.

We selected four sampling sites in an urban environment, where automobile traffic and factories are a strong influence, and four sites in a rural area, located 45 km from the urban center, where agriculture is the main economic activity (Fig. 1).

Out of the 91 species of mosses cited by Bordin & Yano (2009) for Caxias do Sul, we selected 15 for analysis: *Meteorium depeei* (Hornsch. ex Müll. Hal.) Mitt., *Macromitrium guatemaliense* Müll. Hal., *Orthostichella rigida* (Müll. Hal.) B.H. Allen & Magill, *Vesicularia vesicularis* (Schwägr.) Bro-



**Figure 1.** Sampling points in the city of Caxias do Sul. (1). Rural area: District de Vila Oliva; (2-5). urban area: (2). Neighborhood of Ana Rech, (3). City center, (4). University of Caxias do Sul, (5). Botanical Garden of Caxias do Sul.

th., *Sematophyllum subpinatum* (Brid.) E. Britton, *Helicodonium capillare* (Hedw.) A. Jaeger, *Schloteimia jamesonii* (W.-Arnott) Brid., *Thuidium tomentosum* Besch., *Squamidium nigricans* (Hook.) Broth., *Pilotrichella flexilis* (Hedw.) Ångstr., *Phyllogonium viride* Brid., *Prionodon densus* (Hedw.) Müll. Hal., *Zelometerium ambiguum* (Hornsch. in Mart.) Manuel, *Leucobryum crispum* Müll. Hal. and *Pyrrhobryum spiniforme* (Hedw.) Mitt. Among these, only *O. rigida* and *T. tomentosum* occur both in urban and rural settings.

We collected samples in 2003 and 2004 (during fall and winter). Mosses were collected from the stems of trees, identified and stored according to a technique commonly used for the group (Yano 1984). Vouchers were deposited in the Herbarium of the University of Caxias do Sul (HUCS). Some duplicate vouchers were deposited in the Herbarium of the State "Maria P. Eneyda Kauffmann Fidalgo (SP) "in São Paulo.

We examined nine samples from the urban area and nine samples from the rural area, totaling eight and nine species, respectively. In order to ascertain the atmospheric deposition of metals in specimens from the HUCS, we analyzed nine samples collected between 1947 and 1992. Of this total, six samples including four species came from the urban area and three species were collected in the rural area.

Dry and macerated samples were sent to the Center for Environmental Research, University of Saarland (Germany) for analysis. The material was ground, cold-dried, pressurized and liquefied by digestion with HNO<sub>3</sub>. A subsample (150 g) was weighed and heated under pressure at 17°C for 10 hours. Lead (Pb), Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni) and zinc (Zn) were detected and quantified by flame atomic absorption spectroscopy (FAAS), using an AAS Perkin & Elmer PE 3000. The concentration of metals was expressed in µg.g<sup>-1</sup> dry weight.

The comparison between the samples was carried out considering the metal concentration in all species. Percentile calculations were used to ascertain metal accumulation in

mosses. The values above 90% in the percentile calculation were considered high.

The differences in metal deposition were evaluated by the Mann-Whitney test ( $p = 0.05$ ). Normality was ascertained using the Kolmogorov-Smirnov test ( $p = 0.05$ ). Statistical analyses did not include herbarium species from the rural area, due to the small number of samples.

## Results and discussion

### Trace metal concentrations in naturally-occurring moss

The results pertaining to metal concentrations in species are summarized in Tables 1 and 2.

Mosses from the urban area had about two times more Ni, Cd, Pb and Cr, and almost six times more Zn (Tables 1 and 2) than samples from the rural area. Studies conducted by the authors in Caxias do Sul analyzing metal deposition in *Lolium multiflorum* (unpublished data) showed that the concentration of Zn are classified as critical in the urban area. According to Onianwa (2001), the highest concentration of metals in naturally-occurring moss is usually found in the vicinities of pollution sources, gradually decreasing as the distance from the source increases.

The metallurgical and galvanic industries in Caxias do Sul employ materials such as cadmium, copper, nickel, tin, gold, silver, chromium and zinc. Galvanization with iron is the main source of Zn emission (Duarte & Pasqual 2000)

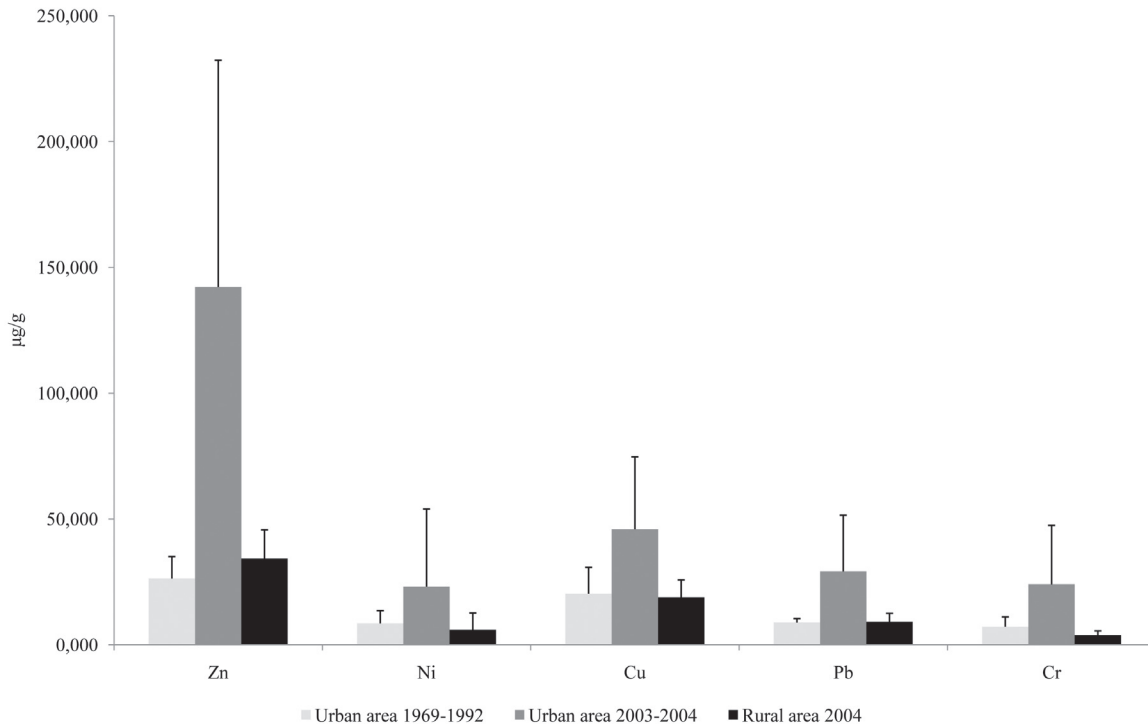
When urban and rural areas were compared with regard to metal accumulation in moss, a significant difference was verified in the concentration of Zn, Cu, Pb and Cr (Fig. 2). The sources of these metals in the urban area are vehicular traffic and industries (Grodzinska *et al.* 2001; Pesch & Schroeder 2006).

**Table 1.** Metal concentration in mosses (µg.mg<sup>-1</sup>) in the urban area of Caxias do Sul (2003-2004)

	Urban area					
	Zn	Cd	Ni	Cu	Pb	Cr
Mean	142,2011	0,337778	23,18889	45,92778	29,17111	24,03889
Median	180	0,297	7	36,8	20,73	9,5
Maximum	250	0,563	93	86	77	66
Minimum	24,73	0,1	0,69	18	7,54	5,14

**Table 2.** Metal concentration in mosses (µg.mg<sup>-1</sup>) in the rural area of Caxias do Sul (2003-2004)

	Rural area					
	Zn	Cd	Ni	Cu	Pb	Cr
Mean	34,2577	0,2706	5,8922	18,8522	9,1777	3,7966
Median	30,67	0,2275	2,91	16,83	9,65	3,77
Maximum	50,57	0,542	21,07	35,15	15,79	7,78
Minimum	19,74	0,05	0,2	12,95	4,86	1,48



**Figura 2.** Comparison between the concentrations of six trace metals determined in naturally occurring mosses collected in urban and rural areas and in herbarium mosses.

The concentrations of Cd and Ni were high in both areas. The presence of these metals in rural areas may result from the use of phosphate fertilizers (Gonçalves Jr. *et al* 2000).

Of the species surveyed, *S. subpinnatum*, *H. capillaris*, *T. tomentosum*, *S. jamesonii* and *M. deppei*, had the highest concentrations of Zn. The first three species were heavily contaminated with virtually every metal surveyed.

The five species mentioned above belong to the class Bryopsida, also known as ectohydric mosses. They are considered good indicators of environmental quality because they obtain almost all their water and nutrients directly from the air, through their thin leaves. This is because their water conductive tissues are either poorly developed or completely lacking. Species that have well-developed internal conductive vessels are not good atmospheric indicators (Goffinett & Shaw 2008).

Many bryophytes have developed adaptive strategies to accumulate water and survive in inhospitable places. For instance, *Hylocomium splendens* (Hedw.) B.S.G. and *Thuidium tamariscinum* (Hedw.) Bryol. Ieur. have paraphyllia. The latter has been used in France (Galsomiès *et al.* 1999) to biomonitor atmospheric deposition. Paraphyllia (leaflike appendages between the leaves) create capillary spaces much like a tomentum that decreases water loss and possibly the loss of other substances (Glime 2006). Water retention is also accomplished by papillae, which form interstitial spaces between them that function as capillary system drivers (Proctor 1984). *Thuidium tomentosum* and *M. deppei* have paraphyllia and papillae, which may facilitate the accumu-

lation of metals, thus explaining the high concentrations of metals (especially Zn) found in samples of these species from the urban area near metal-mechanical facilities (Fig. 2).

*Thuidium tomentosum*, present in all regions of Brazil (Bordin & Yano 2009; 2010), was the only species found in both urban and in rural areas. The wide geographical distribution of this species, associated with its peculiar biological characteristics, makes it a good candidate for biomonitoring. Another species of the same genus, *T. gratum* (P. Beauv.) Jacq. showed levels of Pb up to 250 ppm near busy roadsides in Nigeria (Onianwa 2000). *Sematophyllum subpinnatum*, *H. capillaris*, *S. jamesonii* and *M. deppei*, found in almost all Brazilian regions (Bordin & Yano 2010), are also potential biomonitors of atmospheric metal deposition.

#### Trace metal concentrations in herbarium moss

Metallurgical and chemical industries emerged in Caxias do Sul by 1913, and grew significantly from 1920 on (Lazzaroto 1981). Most of the industrial expansion, however, took place after 1960. The rate of urban population growth in the region between 1960 and 1996 was approximately 300% (Breitbach, 2002). According to data from IBGE (2009), the population of Caxias do Sul increased 29% over the past 18 years.

Herbarium moss samples have been demonstrated as a useful resource to ascertain past levels of environmental contamination (Herpin *et al.* 1997). The herbarium samples of *Orthostichopsis tenuis* (A. Jaeger) Broth., *Pterobryon*

*densum* (Schwägr.) Hornsch, *P. flexilis* and *P. densus*, used in this contribution, were collected between 1969 and 1992 from an urban area, and those of *Syrrhopodon gaudichaudii* Mont., *O. tenuis* and *P. viride* were collected 1947, in a rural setting.

When compared with herbarium samples obtained from the urban area, the recently collected moss samples showed a significant increase ( $p < 0.05$ ) in concentrations of Zn, Cd, Cu and Pb (Fig. 2). Among the non-essential and toxic elements, Pb is strongly associated with urban growth (Galsomies *et al* 1999). However, the most significant increase was observed in the concentration of Zn, six times higher than in herbarium samples.

Contrary to what has been observed in this study, the air quality in most European cities, ascertained through moss monitoring in 1990, 1995 and 2000 (Siewers *et al.*, 2000, Grodzinski & Szarek-Lukaszewska, 2001, Pesch & Schroeder, 2006), has significantly improved as a result of emissions control.

The four herbarium species (*O. tenuis*, *P. flexilis*, *P. densus* and *P. densum*) we surveyed are not among the species sampled recently, and were not reported by Bordin & Yano (2009) for urban centers. *Pilotrichella flexilis* and *P. densus* were collected in 2003 and 2004 in rural areas. These species usually grow in forests (Buck 1988) and are likely to be sensitive to urban pollution. This is however a hypothesis and needs to be tested.

None of the trace metals were found in high concentrations in herbarium mosses collected in the urban setting. Furthermore, Cd was not detected in herbarium mosses from the latter. One urban sample from the herbarium had a high concentration of Ni, probably a result of pesticide use.

#### *Biomonitoring of atmospheric deposition of heavy metals*

Urbanization and growth of the metallurgical industry are directly correlated with increased air pollution by metals in the urban center. This hypothesis is corroborated by the statistically significant similarity between the values found in mosses from urban and rural settings in 2003 and 2004 (Fig. 2).

Emission control measurements require long-term sampling in a large number of sampling sites (Wolterbeek 2002). They also require the selection of species that occur in both settings and whose biology and ecology are well-known.

The main species used to survey atmospheric deposition of metals in Europe, *Pleurozium schreberi* (Willdenow ex Bridel) Mitten and *Hylocomium splendens*, are well studied (Ceburnis 1997; Berg & Steinnes 1997; Siewers & Herpin 1998; Grodzinska 1999; Taminnen 2004), having well-defined growth rates and reproductive cycles (Longton & Greene 2005).

The potential for the use of mosses in the assessment of air quality in Brazil is promising, given their richness in species. The fact that *P. flexilis* and *P. densus* are absent in current urban samples, but present in rural areas, indicates that the environmental integrity of the latter has been preserved. We suggest that *S. subpinnatum*, *H. capillaris*,

*T. tomentosum*, *S. jamesonii* and *M. deppii* are good candidates for biomonitoring atmospheric deposition of metals due to their ability to accumulate different metals, and the fact that they are widely distributed.

## Conclusions

The following species have a potential as bioindicators: *S. subpinnatum*, *H. capillaris*, *T. tomentosum*, *S. jamesonii*, *M. deppii*, *P. flexilis* and *P. densus*. However, there is a need to broaden our knowledge of the geographic distribution, biology, ecology and ecophysiology of these species, in order to better understand the processes of accumulation and transport of substances important to air pollution control.

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