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Mercury in *Pleurozium schreberi* and *Polytrichum commune* from areas with various levels of Hg pollution – an accumulation and desorption experiment with microscopic observations

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ABSTRACT

Because of its high mobility in ecosystems, mercury is one of the main toxic threats to the environment, and its concentration must be carefully controlled. To fulfill this need, we selected terrestrial mosses with different characteristic life forms: orthotropic and endohydric *Polytrichum commune* and plagiotropic and ectohydric *Pleurozium schreberi*. The concentrations of mercury were determined in both species growing together at sites situated approximately 0.75, 1.5, 3 and 6 km to the north, south, east and west, respectively of five known mercury polluters. The mercury concentrations reflected the emissions produced by the surrounding industry, reaching values of 0.44 mg kg^{-1} in *P. schreberi* and 0.79 mg kg^{-1} in *P. commune* in the vicinity of the chlor-alkali industry. To determine how long a load of Hg would remain in the mosses after mercury emitters restricted releases of Hg to the atmosphere, accumulation and desorption experiments were performed. We compared the two moss species collected from clean and moderately and heavily mercury-polluted sites. After eight days of exposure to mercury, *P. schreberi* accumulated up to 25 mg kg^{-1} of Hg, and *P. commune* accumulated up to 31 mg kg^{-1}. Both in the field and in the experiment, *P. commune* accumulated significantly higher concentrations of Hg than did *P. schreberi*, most likely because of its surface morphology, which is likely to enhance the capture of metal from the atmosphere. After sixteen days of exposure, mercury changed the structure of the plasma membrane and affected organelles such as the nuclei and chloroplasts, leading to cell disintegration and death. The negative effects of mercury on the functioning of living cells appeared first in the older leaves of *P. schreberi*. After 64 days growing in the absence of Hg, *P. schreberi* clearly retained only 10–14% of the initially accumulated Hg, while *P. commune* retained 10–21%.

1. Introduction

Mercury and many of its compounds are among the main toxic environmental pollutants, being dangerous to both humans and wildlife even at low levels (Hyman, 2004; Sonke et al., 2013). Once released in the air, mercury can be dispersed and transported globally and then removed from the atmosphere through both wet and dry processes (Kono and Tomiyasu, 2009; Lodenius, 2013). Because of its high mobility in ecosystems, there is a great need to monitor the deposition loads of Hg (Hintelmann et al., 2002; Lodenius, 2013). The most important anthropogenic releases of this metal occur through hard and brown coal combustion as well as through municipal and industrial incineration, chlor-alkali plants, cement production, non-ferrous metal manufacturing, porcelain factories and glass smelters (Merian, 1991). Monitoring the sources of the emitted quantities of Hg in the environment can be achieved with mosses (Lodenius, 2013). Because these plants are able to accumulate toxic elements (accumulative bioindicators), they are particularly suitable for monitoring the spatial patterns and temporal trends of metal bioaccumulation over large areas (Markert et al., 1996; Samecka-Cymerman et al., 2006). They provide a measure of element deposition from the atmosphere to terrestrial ecosystems (Remon et al., 2013). When terrestrial mosses are used to monitor atmospheric contamination, the samples are usually analyzed without first being washed, so that

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the contaminants deposited on the moss can be quantified (Aboal et al. 2011). According to Pérez-Llamazares et al. (2011a), the extracellular fraction can provide information about the potential risks to the organism associated with the possible solubilization of these particles and their posterior absorption. The same author proposed that the extracellular concentration tends to reflect the immediate environmental conditions (Pérez-Llamazares et al., 2011a, 2011b). These plants have a high surface-to-volume ratio, enabling particles to be trapped, as well as a high cation exchange capacity, which leads to the accumulation of large amounts of elements, including mercury (Sun et al., 2007; Aboal et al., 2011; Lodenius, 2013). However, much less is known about the transfer of mercury from these plants back to the atmosphere (Lodenius et al., 2003). This exchange between vegetation and the atmosphere is partly bidirectional and is affected by a number of different physical, chemical and biological factors (Lodenius, 2013). Among mosses, different characteristic life-forms can be distinguished: those growing vertically from the substrate, known as orthotropic; endohydric species, such as *Polytrichum commune*, possessing a differentiated internal conducting system; and species such as *Pleurozium schreberi*, which grow horizontally and are plagiotropic and ectohydric, lacking an internal conduction system and absorbing water over the entire surface of the plant (Markert and Weckert, 1993; Victoria et al., 2009). Additionally, the presence of lamellae in *Polytrichum* leaves greatly increases their surface area which serves as a possible pathway for the absorption of various polluters (Glime 2007; Goffinet and Shaw 2009). Due to its complex leaf structure *P. commune* should be able to accumulate a significantly higher concentration of Hg than *P. schreberi* (which lacks lamellae on its leaves). For this investigation, we selected both species growing together in the vicinity of emitters of mercury to compare their abilities as bioaccumulators of Hg pollution. *P. schreberi* has been used successfully in recent decades and has proved to be a suitable bioindicator of inorganic substances and, therefore, is widely applied to map and control metal pollution in European countries (Niemelä et al., 2007; Kosior et al., 2010). *P. commune* has been tested as an acceptable bioindicator for different chemical elements (Markert and Weckert, 1993; Gough et al., 2006). The aim of this paper was to (1) measure the mercury concentration in both species used as bioaccumulators in the surroundings of five emitters of various loads of Hg; (2) compare the mercury accumulation by both species under experimental conditions as well as the possible occurrence of decontamination processes when this element is no longer present in the environment; and for better understanding of the toxic influence of Hg on mosses (3) to determine the effect of mercury exposure on the cellular organization in *P. schreberi* leaves. Microscopic observations visualize the impact of Hg on leaf cell vitality in *P. schreberi* and are interdisciplinary approach of the examined research problem.

We tested the hypotheses that (1) the orthotropic and endohydric *P. commune* is a better bioindicator of Hg pollution than the plagiotropic and ectohydric *P. schreberi* in mercury contaminated areas because of the leave structures and especially of the lamellae providing a larger area for Hg uptake; (2) once accumulated, atmospheric deposited Hg is released from both species when Hg is no longer present in the environment; and (3) mercury exposure negatively influences the functioning of living cells in *P. schreberi* leaves, resulting in cell disintegration and death.

This investigation contributes to the use of *P. schreberi* and *P. commune* as bioindicators in areas no longer polluted by mercury or with intermittent mercury accidents. In cases where the moment of total desorption has not yet been reached, bioindication enables the determination of the period of time between the cessation of the pollution episode and the actual stage of the environment.

2. Materials and methods

2.1. Sampling design

In the Lower Silesia, Opole and Wielkopolska districts of Poland, five mercury polluters were selected: 1. the chlor-alkali industry in Brzeg Dolny (51° 16′ 29″ N, 16° 44′ 21″ E), producing per year, among others, 100,000 t of polyols, 20,000 t of polyurethane systems, 63,000 t of caustic soda, and 120,000 t of chloride; 2. the glass smelter in Poniec (51° 45′ 37″ N,16° 48′ 57″ E); 3. the power plants in Brzezie (50° 45′ 07″ N, 17° 53′ 16″ E), using twenty million tons of coal and producing a power of 1532 MW (10 TWh per year), and 4. Kędzierzyn-Koźle (50° 21′ 29″ N, 18° 17′ 16″ E), using four million tons of coal and producing a power of 244 MW; 5. the ceramics and porcelain factory in Bolesławiec (51° 16′ 11″ N,15° 34′ 03″ E). An unpolluted control site was selected in a forest clear-cut North from the Uliczno village (Lower Silesia N: 50° 48′ 59″; E: 16° 42′ 42″). In all areas, sites were selected where *Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Polytrichum commune* Hewd. were growing together. Moss samples and soil samples from under each species were taken along differently oriented transects from the center of the industrial sites. The sampling sites were at distances starting as close as possible, at ~0.75 km, and then moving away to 1.5, 3 and 6 km from the sources of mercury pollution in each direction (N, E, S and W) (Fernández et al., 2000a; Carballeira and Fernández, 2002; González-Miqueo et al., 2010). The distances were selected according to the moss and lichen investigation of Sensen and Richardson (2002), who estimated the sphere of influence of mercury deposition to extend 2.4–3.4 km from the emitter. At each site, we established squares 25 m × 25 m (Varela et al. 2010). Each of these squares was divided into sub-squares of 1 m × 1 m, of which five were selected randomly. From each of these five sub-squares, three subsamples of the green parts of the gametophytes of *P. schreberi* and *P. commune* were selected randomly. As required by the rules set by the Environmental Monitoring and Data Group (Markert et al., 1996) and within the European Heavy Metal Survey (ICP Vegetation, 2005), the mosses were not exposed directly to canopy through fall. Dead material, soil particles and litter were manually removed from the moss samples. The moss samples were not washed (Markert et al., 1996; ICP Vegetation, 2005; Aboal et al., 2011). Samples of the topsoil were also taken from each square: 0–5 cm after removal of the O horizons. Each sample consisted of a mixture of three subsamples. The plant remains and stones were removed from the soil. The total number of soil and plant samples was $N = 5$ polluters $\times 4$ directions $\times 4$ distances $\times 5$ replicates $+ 5$ control replicates $= 405$.

2.2. Plant and soil analysis

The soil and moss samples were dried and pestled after the coarse material had been removed using a 2 mm sieve. The plant samples were homogenized to a fine powder in an IKA Labortechnik M20 laboratory mill. The plant and soil samples were dried at 50 °C to a constant weight. According to Lodenius et al. (2003), this temperature is low enough to prevent the loss of mercury. Mercury was analyzed using an AMA 254 Advanced Mercury Analyzer. All measurements were carried out in three replicates with RSD < 3%. The accuracies of the methods were determined using the standard moss reference materials M2 and M3 (Finnish Forest Research Institute and Steinnes et al. (1997) and RTH 907 Dutch Anthropogenic Soil (Wageningen Evaluating Programs for Analytical Laboratories). The recovery efficiencies for the above procedures were as follows: Moss M2: certified $\pm$ SD 0.058 ± 0.005 mg kg^{-1}, found 0.059 ± 0.002 mg kg^{-1}, recovery 101.72%, Coefficient of Variance (CV) 3.4%; Moss M3: certified 0.035 ± 0.004 mg kg^{-1}, found 0.037 ± 0.001 mg kg^{-1}, recovery 105.71%, CV 8.3%; Dutch Anthropogenic Soil RTH907: certified 1.14 ± 0.13 mg kg^{-1}, found 1.17 ± 0.03 mg kg^{-1}, recovery 102.63%, CV 2.56%.

2.3. Accumulation and desorption experiments

Samples of *P. schreberi* and *P. commune* were collected from an unpolluted control site, from the most polluted site near the chlor-alkali industry in Brzeg Dolny, and from a moderately polluted site near the power plant in Kędzierzyn-Koźle. The moss material was cleaned but not washed according to Lodenius et al. (2003). Patches of a layer of clean mosses (about 100 g of fresh weight of *P. schreberi* and about 80 g of *P. commune*) without soil were placed on a nylon net (at 15 cm distance from the bottom) in closed transparent plastic chambers (volume 3 L) filled with 50 mL of redistilled water. A mercury droplet (10 mg) was applied to a petri dish in the chamber (Lodenius et al., 2003). The mosses in each pot were moisturized daily by redistilled water. Each species patch was placed in a separate pot. Control pots for each species from each collection site were also prepared in the same way but excluding mercury. Each experimental combination was prepared in five replicates. The total number of pots was 3 sites $\times 5 = 15$ plus 3×5 controls $= 30$ per species. The concentrations of mercury in the mosses were measured at time 0 and after two, four and eight days of exposure. At the end of the experiment, all patches of mosses were transplanted back to the control site and were left free from further Hg exposure to observe the possible desorption of Hg. A period of eight days of exposure was chosen because we wanted to observe the