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Research into Heavy Metals Pollution of Atmosphere Applying Moss as Bioindicator: a Literature Review

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Atmospheric pollution causes serious damage for human health and to all natural ecosystems. Nowadays, the biggest provocative of atmospheric pollution is anthropogenic human activities and transport sector, main pollutants being heavy metals (HM).

Biomonitoring of HM pollution of atmosphere by mosses is one of the most popular, perspective and cost-effective method to control, detect and evaluate changes in the air quality. The most important environmental features of mosses as a suitable tool of biomonitoring are: rootless, large surface, wide-spread population, a habit to grow in groups, long life-cycle, survival in a high-polluted environment, an ability to obtain nutrients from wet and dry deposition.

This literature review presents environmental properties of mosses, what makes them to be suitable for biomonitoring, HM deposition trends in some European countries during 1990-2005/6, methodology of sampling and chemical analysis, a summary of strengths and weaknesses of the most popular HM analysis techniques in Europe.

Key words: Heavy metal, mosses, atmospheric pollution, bioindicator, ion exchange, uptake efficiency, sampling, ICP - AES, ICP - MS, FAAS, GFAAS.

1. Introduction

Air pollution has been one of the major threats to human health and the environment since the last century. The degree and extent of environmental changes over the last decades has given a new urgency and relevance to the detection and understanding of environmental change, due to human activities, which have altered global biogeochemical cycling of HM and other pollutants. Approximately 5 million chemicals are presently known and 80,000 in use; 500 - 1,000 are added per year resulting in a progressive increase in the flux of bioavailable chemical forms to the atmosphere (Hock and Seifert, 2003; Obbard et al., 2005; Batzias and Siontorou, 2006; Dmuchowski and Bytnerowicz, 2009).

Monitoring toxic air pollutants is needed for understanding their spatial and temporal distribution and ultimately to minimize their harmful effects. In addition to direct physical and chemical methods of air pollution monitoring, bioindication has also been used to evaluate air pollution risk (Dmuchowski and Bytnerowicz, 2009).

Biological monitoring of airborne contaminants has made a great 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 (Kuang, et al., 2007).

But it is important to note that a unique species that can be a suitable bioindicator for biomonitoring of toxic metal pollution all over the world has not been found yet. For this reason, different species of mosses are useful as bioindicators in different parts of the world (Coskun, 2006).

Biomonitoring consists of the use of responses of individual plants or plant associations at several biological organization levels in order to detect or predict changes in the environment and to follow their evolution as a function of time (Kuang, et al., 2007).

Some plant species are sensitive to single pollutants or to mixtures of pollutants. Those species or cultivars are likely to be used in order to monitor the effects of air pollutants as bioindicator plants. They have a great advantage to show clearly the effects of phytotoxic compounds present in the ambient air. As
such, they are ideal for demonstration purposes. However, they can also be used to monitor temporal and spatial distributions of pollution effects (Temmerman, et al., 2005).

Two Swedish scientists Åke Rühling and Ger mun d Tyler (1960) have discovered that mosses are good bioindicator of HM pollution in the atmosphere, after this successful discovery many European countries have used mosses in national and multinational surveys of atmospheric-metal deposition. Such moss surveys can uncover regional differences and temporal trends of airborne pollution, enabling in certain cases the possibilities to establish comparison between contamination levels in geographically different areas (Alvarez, et al., 2006; Charakrabortty and Parker 2006; Lee et al., 2003).

2. Mosses as airborne pollution bioindicator

The term bioindicator is used to refer to an organism, or a part of it that depicts the occurrence of pollutants on the basis of specific symptoms, reactions, morphological changes or concentrations. Bioindicator generally refers to all organisms that provide information on the environment or the quality of environmental changes (Poikolainen, 2004).

Biomonitoring with mosses is based on the fact that terrestrial carpet-forming species obtain most of their nutrients directly from wet and dry deposition, they clearly reflect the atmospheric deposition, especially well suited to HM pollution on a larger time scale (Čeburnis et al., 2002; Čeburnis et al., 2000).

The broad and, in some cases, cosmopolitan distribution of many moss species suggests that these gametophyte are dominant plants among Earth’s most adaptable taxa. Mosses can be found on every continent and in every terrestrial ecosystem, from tropical rain forests to arid deserts and in polar tundra as well. Mosses play a crucial role in preventing soil erosion and conserving large amounts of water thereby regulating the water budget of local ecosystems (Poikolainen, 2004; Fernandez et al., 2006; Wang, et al., 2008; Cui, et al., 2009).

Mosses as bioindicators reflect elevated sulphur dioxide (SO₂) concentration, accumulation of HM and other contaminants emitted to the atmosphere from natural and anthropogenic sources. It has been reported in a large number of studies including local investigations as well as regional surveys in different parts of the world (Giordano et al., 2004; Čeburnis et al., 1999a).

In the scientific articles and international mosses surveys reports the most commonly used mosses as bioindicators are: Hypnum cupressiforme, Hylocomium splendens, and Pleurozium schreberi. These species in particular are largely abundant in some parts of Europe (Onianwa, 2000).

Mosses possess many properties that make them suitable for monitoring air pollutants. These species obtain nutrients needed for vital processes from wet and dry deposition and they do not have real roots. Nutrient uptake from the atmosphere is promoted by their weakly developed cuticle, most bryophytes are small and the leaves of many mosses and folious liverworts consist of only one cell layer. Substantial properties of mosses as good indicator are: large surface to weight ratio, their slow growth rate and a habit of growing in groups. Other suitable properties of mosses include minimal morphological changes during the mosses lifetime, ease sampling, an ability to survive in highly polluted environment and the possibility to determine concentrations in the annual growth segments (Čeburnis et al., 2002; Cenci et al., 2003; Poikolainen, 2004; Wang et al., 2008 Dragović and Mihailović, 2009).

Bryophytes are resistant to many substances which are highly toxic for other plants species – they are able to survive in such diverse and often extreme environment, these sedentary organisms possess an equally diverse set of physiological adaptations. Mosses have been shown to be capable of surviving complete desiccation and temperatures as extreme as 110°C (Cenci et al., 2003; Fernandez, et al., 2006; Dragović and Mihailović, 2009).

Mosses as bioindicator are popular not only due to their environmental features, but economic advantages are important as well. Biological indicators are applied as the cheapest and simplest indicators for monitoring the HM concentrations in the atmosphere. The technique of moss analysis provides a surrogate, time-integrated measure of metal deposition from the atmosphere to terrestrial systems. It is easier and cheaper than a conventional precipitation analysis as it avoids the need for deploying large numbers of precipitation collectors with an associated long-term program of routine sample collection and analysis. Therefore, a much higher sampling density can be achieved than with conventional precipitation analysis (Harmens, et al., 2008).

3. Ion accumulation and cation exchange processes

Air pollutants are deposited on mosses in aqueous solution, in gaseous form or attached to particles. The attachment of particles in mosses is affected e.g., by the size of the particles and the surface structure of the mosses. Ion exchange is a fast physiological-chemical process that is affected e.g., by the number and type of free cation exchange sites, the age of the cells and their reaction to desiccation, growing conditions, temperature, precipitation pH, composition of the pollutants and leaching. In the ion exchange process, cations and anions become attached to functional organic groups in the cell walls among other things through chelation. Mosses cannot prevent ions penetrating into their tissues because they have high counter-gradient mechanisms by which they accumulate significant concentrations of metals in their bodies (Shakya, et al., 2008; Chakrabortty et al., 2006).
The accumulation of pollutants in mosses occurs through a number of different mechanisms: as layers of particles or entrapment on the surface of the cells, incorporation into the outer walls of the cells through ion exchange processes, and metabolically controlled passage into the cells (Poikolainen, 2004).

The cell wall has a high polyuronic acid content which makes moss a very good natural ion exchanger. The cell walls of bryophytes possess many negatively charged anionic sites to which cations are bound in exchangeable form. Studies of electron microscope charged anionic sites to which cations are bound in the extracellular region outside of the cytoplasm, bound to the cell wall, and due to the highly reduced presence or absence of cuticle in the moss, ions have a direct access to the cell wall, mosses surfaces and rhizoids do not perform any active heavy metal ion discrimination (Lee, C. K., 1994; Shakya et al., 2008; Reimann et al., 2006; Onianwa, 2000).

The stability of metal organic complexes and chelates and the great cation exchange capacity of the tissues are primarily conditions for the sorption of HM by mosses. The degree of metal uptake efficiency retention proved to decrease in the order Cu > Pb > Ni > Co > Cd > Zn, Mn. Lead is very strongly fixed in the moss, and for which the correlation between concentration in moss and bulk deposition is particularly high (Čeburnis et al., 1999; Rosman, et al., 1998).

A high proportion of the pollutant load accumulates in mosses through wet deposition. The amount, duration and intensity of precipitation affect accumulation and leaching. The contribution of dry deposition increases on moving from humid to arid climates (Poikolainen, 2004).

Evidence exists that metals, including both mineral nutrients and heavy metals, move between the annual increments of feather moss and are lost due to leaching, depending on the meteorological conditions and seasonal growth (Brümelis and Brown...1997).

Theoretically, if these concentrations are due only to exchangeable ions on the cell wall, and assuming 100% absorption and retention, but no vertical movement of metals, then the concentrations in a particular aged segment should be equal to age multiplied by the concentration in the first segment. In the available literature this is never the case and this may be explained by redistribution of elements between segments and losses by leaching. It is not known if the cellular concentrations of metals in feather moss reject short-term readjustment to chemical equilibrium conditions with the environment, or an integrated estimate of past deposition. The relationship between metal transfer and the available pool sizes indicates that recycling can potentially redistribute metals between segments, and also the underlying organic horizon, depending on the conditions of water transfer (Brümelis and Brown...1997).

Regional and sub-regional patterns of deposition of aerial metal burden in many parts of the world have been mapped from the levels accumulated in mosses. The regions so studied have spanned from small parts of a country to entire subcontinents. From many of such studies have been produced contour maps, isoleths and colours - coded maps depicting variations in regional levels of atmospheric HM pollution (Onianwa, 2000).

4. HM deposition in Europe using mosses as bioindicator

The first European moss survey was conducted in 1990/1 and has since then been repeated at five-yearly intervals. The most recent survey was conducted in 2005/6, with mosses collected from over 6,000 sites in 28 countries. Samples were collected according to a standardized protocol and concentrations for 10 – 12 HM were determined in the last three years’ growth segments. European maps were produced based on the EMEP 50x50 km2 grid, displaying the mean heavy metal concentration for each cell (Harmens et al., 2009)

According to Harmens (2008), since 1990, the HM concentration in mosses has declined the most (45-72%) for arsenic, cadmium, iron, lead and vanadium, followed by copper, nickel and zinc (20-30%), with no significant reduction being observed for chromium (2%) and mercury (12% since 1995). As in previous European surveys, the lowest concentrations of heavy metals in mosses were generally found in (north) Scandinavia, the Baltic States and northern parts of the United Kingdom in 2005/6. Therefore, even in times of generally decreasing metal deposition across Europe, temporal trends are different for different geographical scales (Harmens, et al., 2008).

In Table 1 lead and cadmium levels (mg/kg–1) in some European countries in 1990 - 2005/6 accumulated by mosses are presented, mercury data is presented only from 1995 - 2005/6 surveys.

From the data in Table 1 it is evident that the biggest amounts of HM in mosses were found 1990. Later, when environment protection became a more important issue, concentrations of HM has declined in all countries, for instance, in Slovenia in 1990 were detected 40.9 mg/kg–1 and 12.3 mg/kg–1 of lead during 2005/6 mosses survey.

According to Harmens et al. (2008), in 2005, “road transportation” had become the second source of lead emissions with a contribution of 17% and “manufacturing industries and construction” (41%) was the main source.

The biggest deposition of cadmium in 1990 (in selected countries in Table 1) were detected in Slovakia – 1.36 (mg/kg–1) has declined till 0.50 during 2005/6 mosses survey.

The biggest deposition of mercury 0.070 (mg/kg–1) was detected also in Slovakia, but in 2005/6 it has declined to 0.088 (mg/kg–1).

Only in data from the Ukraine it is evident that depositions of Pb and Cd increase during time scale.

Most methods in HM monitoring employ mosses as bioaccumulators and involve sample collection followed by laboratory analysis techniques (Stihi et al., 2006).
Table 1. HM levels (mg kg⁻¹) in some European countries in 1990 - 2005/6 accumulated by mosses (Harmens et al., 2008; Harmens et al., 2008 (a))

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<td>Lead (Pb)</td>
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<td>Mercury (Hg)</td>
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<td>5.8</td>
<td>3.7</td>
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<td>0.22</td>
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<td>16.6</td>
<td>11.0</td>
<td>5.7</td>
<td>4.94</td>
<td>0.32</td>
<td>0.31</td>
<td>0.23</td>
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<td>2.60</td>
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<td>0.18</td>
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<td>Finland</td>
<td>9.9</td>
<td>5.7</td>
<td>3.0</td>
<td>2.70</td>
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<td>3.69</td>
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<td>2.9</td>
<td>3.79</td>
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<td>Lithuania</td>
<td>7.6</td>
<td>11.4</td>
<td>8.3</td>
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<td>Sweden</td>
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<td>Russian Fed.</td>
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<td>Slovakia</td>
<td>40.9</td>
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1 - data was taken from St. Petersburg;
2 - data was taken from Sergiev Posad, Tula, Tver, Udmurt Republic

5. Field sampling

According to Harmens recommendations of mosses sampling for European manual survey (2010), the performance of sampling in the field should be performed according to the following principles:

- Each sampling point should be situated at least 3 m away from the nearest projected tree canopy in forests or plantations primarily in small gaps, without pronounced influence from canopy drip from trees, preferably on the ground or on the level surface of decaying stumps.
- In habitats such as open heathland, grassland or peatland, sampling below a canopy of shrubs or large-leafed herbs should be avoided, as well as the areas with running water on slopes.
- The sampling points should be located at sites representative of non-urban areas of the respective countries. In remote areas the sampling points should be at least 300 m from main roads (highways), villages and industries and at least 100 m away from smaller roads and houses.
- In mountainous areas such as the Alps the sampling points should be below the timberline in order to eliminate confounding influences of altitude on the HM concentration in mosses.
- In order to enable comparison of the data from this survey with previous surveys, it is suggested to collect moss samples from the same (or nearby, i.e. no more than 2 km away but with the same biotope conditions) sampling points as used in previous surveys (at least the same sampling points as used in the 2000 and 2005 survey). In addition, sampling of mosses near (long-term) monitoring stations of atmospheric HM, nitrogen or POPs deposition is recommended in order to directly compare their concentration in mosses with the accumulated atmospheric deposition.
- It is recommended to make one composite sample from each sampling point, consisting of five to ten (ten for POPs) subsamples, if possible, collected within an area of about 50 x 50 m. In the composite sample only one moss species should be represented. The sub-samples should be placed side by side or on top of each other in large paper or plastic bags (POPs: polythene bags or glass jars), tightly closed to prevent contamination during transportation. The amount of moss needed is about one liter (or two liters when POPs analysis will be conducted as well). As some POPs are susceptible to volatilization and photochemical breakdown, samples for POPs analysis should be kept cool and in the dark at all times.
- Smoking is forbidden during sampling and further handling of samples, and disposable plastic, non-talcum gloves should be used when picking up the mosses. Do not use vinyl examination gloves if they are powdered with talcum as this will contaminate the samples.
- Samples should preferably be collected during the period April - October. In arid regions of Europe it is advised to collect the samples during the wet season. Although the HM concentration in 
  *Hylocomium splendens* and *Pleurozium schreberi* appear not to vary with season, this might not be true for other moss species and all climates in Europe. Therefore, it is suggested to sample the mosses in the shortest time window possible.
- Each locality must be given co-ordinates, preferably longitude and latitude (Greenwich coordinates, 360° system), suitable for common data processing.
In order to determine the overall variability associated with the entire procedure (sampling + analysis), multiple moss samples (at least 3 samples per site) must be collected from at least two sites with different levels of overall contamination. These multiple moss samples must be collected, processed, and analyzed individually in order to characterize the overall variability of the data.

6. Samples preparation

The cleaning procedure is an important step in the samples preparation technique because it can affect the final results. From samples of mosses is important to remove all forest debris such as soil, leaves, needles, and other litters. The material should therefore be handled by clean laboratory equipment on clean laboratory paper, glass shields or clean polythene in order to avoid contamination from smoke and laboratory tables. Non-talcum, disposable plastic gloves should be worn and no metal tools should be used (Harmens, 2010).

In the analytical programme for the 2005/2006 survey based on the recommendations of Rühling, the following is stated in reference to the cleaning of moss samples: if the samples cannot be cleaned immediately after sampling, they should be placed in paper bags and dried and stored at room temperature (20–25°C) until further treatment. Alternatively, samples can be deep-frozen. Although in some surveys the moss samples were cleaned directly in the field, but most researchers do not usually pick over the moss samples directly in the field, due to the long time that it takes. They usually collect a large volume of moss, place this in a sampling bag and store the bag for an undetermined number of days or weeks, under often undetermined conditions, until the samples are finally cleaned in the laboratory prior to analysis (Aboal et al., 2008).

7. Digestion

Wet ashing of a homogeneous sub-sample is recommended for the decomposition of organic material. Dry ashing is not acceptable. The preferred method of digestion is microwave digestion. Wet ashing, using nitric acid, has been used in most countries in the past and has proven to give reproducible results. If excess acid is evaporated, samples should not be allowed to become completely dry. It is important to note that wet ashing should not be applied when INAA (Instrumental Neutron Activation Analysis) is used as analytical technique; a homogenous, dried sub-sample should be analyzed without further pretreatment (Harmens, 2010).

8. Chemical analysis

Mosses which had been made to sorb various HM should be equilibrated with varying concentrations of extractants such as EDTA, acetic acid, dilute mineral acid, calcium, magnesium, sodium and potassium ions (Onianwa, 2000).

Contemporary instrumental techniques, such as inductively coupled plasma optical emission spectrometry (ICP-OES) or atomic absorption spectrometry (AAS) allow for simultaneous or sequential determination of large number of elements, if only they exceed a threshold concentration, defined by corresponding limit of detection and by adequate spectral resolution of the instrument. The requirement for the analyzed element concentration to exceed its limit of detection is rarely met in trace analysis (Feist et al., 2008).

According to Harmens et al. (2008 (a)) manuscript, mostly European countries use ICP-ES, ICP-MS and GFAAS techniques for HM determination in mosses.

The basic difference between the two techniques (AAS and ICP) is that one relies upon an atomic absorption process while the other is an atomic/ionic emission spectroscopic technique. The next essential difference is the means by which the atomic or ionic species are generated. A combustion flame or graphite furnace is typically used for AA while ICP-ES uses plasma (Tyler, 1991).

The following Table (2) summarizes the main relative strengths and weaknesses of AAS and ICP techniques.

The detection limits in AAS technologies may be very good or excellent for some elements, whereas ICP techniques may be performed for most elements very successfully. According to an elementary overview of elemental analysis, ICP-MS produces the best detection limits (typically 1-10 ppt), followed by GFAAS, (usually in the sub-ppb range) then ICP-AES (of the order of 1-10 ppb) and finally FAAS (in the sub-ppm range) (An elementary...2010).

The duration of sample throughput (Table 2) at GFAAS may take 3 - 4 minutes per element, whereas FAAS takes only 10 – 15 seconds per element. ICP systems may save more time due to their technical abilities, for instance, sample throughput by using ICP-AES may take 1 – 60 samples per minute, moreover, ICP-MS characteristics allow to take all elements in less than 1 minute.

ICP-MS typically operates at much lower concentration levels so that linear ranges up to 10⁻³ can be achieved for some analytes. In standard practice, however, ICP-MS is a technique for ultra-trace to trace levels to ppb levels (An elementary...2010).

Selecting the most appropriate tool for the job can sometimes appear to be a daunting task, especially since there is considerable overlap of capabilities. In fact, all of the techniques may be able to perform in particular analysis at acceptable levels of accuracy and precision (An elementary...2010).

According to an elementary overview of elemental analysis, the short-term precision of FAAS is in the range of 0.1-1.0%. The long-term precision depends on the spectrometer optics; double beam types are capable of long-term precision of 1-2%, where single-beam optics are typically in the 5% range. Pri-
marily because of difficulties in injecting very small volumes, GFAAS short-term precision is generally in the range of 0.5-5%. Long-term precision is highly dependent on the tube type and condition. However, several repeats per sample may be necessary with GFAAS to obtain satisfactory precision. ICP-AES short-term precision is reasonably good, around 0.1-2%, and even over periods of several hours, should be no worse than 1-5%. Short-term for ICP-MS is in the range 0.5-2%, with long-term precision may be around the 4% level.
According to an Elementary overview of elemental analysis, AAS and ICP techniques suffer from interference caused effects. The severity of these effects can cause a big difference in results for real samples. According to Table 2 at least affected by interference from all the systems is ICP-MS, where some or few spectral, chemical and physical interference problems can be.

One of the strengths of ICP-AES is presented at high total dissolved solids field (Table 2) that dissolved solids in solution can be 0 - 20 %, whereas ICP-MS meets here a limitation, dissolved solids in solution can be only 0.1 - 0.4 % (An elementary...2010).

Only FAAS meets here a limit, due to large volume of samples requirements. Very small - medium volume of sample is required for GFAAS, ICP-MS and ICP-AES techniques.

The easiest technique for users (Table 2) is FAAS, on the other hand, other remaining techniques are likewise easy or moderately easy to use.

From Table 2 comparisons are obvious that ICP-AES and ICP-MS have more strengths than AAS systems. On the other hand, not all companies can afford to run ICP equipment due to their high running costs.

According to G. Tyler (1994) it is important to note that there is no technique, which will satisfy all researchers’ needs and requirements. All techniques are complementary. There will always be samples where one technique is better suited for the analysis than the other.

9. Conclusions

In biomonitoring of atmospheric deposition of HM it is popular to use terrestrial mosses. The most popular mosses dealt with in research articles are: Hypnum cupressiforme, Hylocomium splendens, and Pleurozium schreberi.

The most important environmental features of mosses as a good tool of air pollution deposition reflection are: mosses do not have any roots, their surface is large, they grow in wide-spread population in groups, they have long life cycle, they survive in the high-polluted environment, they are able to obtain nutrients from wet and dry deposition and clearly reflect the atmospheric deposition. All these environmental characteristics prove that mosses are a good tool in airborne pollution monitoring, especially in HM monitoring.

European researchers use mosses surveys in order to reveal airborne pollution trends. This literature review has presented data only from a few countries. According to the data, HM deposition in mosses in 1990-2005/6 has significantly declined. The biggest HM pollution were detected in 1990, later HM depositions declined due to a greater concern over environment protection, therefore lead from transport sector became a second source of pollution.

Sampling and chemical analysis are fast and inexpensive operations, it is easy to collect samples, and there is no need for any special modeling programs, extra training or specific instructions for employees. The most important part of the study is both to interpret correctly the data due to critical issues such as species identification of mosses and to convert and evaluate the data from different species of mosses in one survey. It is important to note that a critical step is sample preparation, for example, samples’ preparation with entrapped forest debris or not very clean laboratory equipment can deform the final results.

For chemical analysis it is important to choose the best equipment according to your personal needs.
and particularity of research work. In EU countries ICP-ES, ICP-MS and GFAAS are most used.

Sometimes all mentioned in this article techniques (FAAS, GFAAS, ICP - AES and ICP - MS) suit to the analysis. Researcher has to be careful in evaluating technical possibilities of equipment and project costs.

References


Oro taršos sunkiaisiais metalais tyrimai naudojant samanas kaip bioindikatorių

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Kasmet į atmosferą įžievinti įvairių pramonės šakų, transporto sektoriaus patenka daug teršalų, tarp jų ir sunkieji metalai (SM), kurie yra vieni iš pavojingiausių ne tik žmogui, bet ir visai ekosistemai.


Pagrindinis savybės, dėl kurių samanos yra puikus bioindikatorius: neturi šaknų, SM akumuluoja tiesiogiai iš oro, didelis paviršinio plotas, sugeba išgyventi stipriai užterštose teritorijose, neсудėtingas ir nebrangus mėginių rinkimas bei tyrimas.

Straipsnyje yra pristatyti samanų rinkimo ir analizės metodai, pateikti SM koncentracijų pokyčių Europoje; tyrimų įrangos techninės charakteristikos.