

Active and Passive Moss Biomonitoring for Water and Air Pollution Control.

Urinova Sadoqatoy Kobuljanovna
Andijan Institute of Mechanical Engineering
Faculty of Transport and Logistics
Department of "Safety of life"
«Occupational safety production and technological
process security (by industry) master
sadoqaturinova1983@gmail.com

Abstract : Present paper dedicated to moss biomonitoring technique issues as a multi-media tool of pollution control. In recent years, this method has become a popular way of controlling the level of environmental pollution. In many studies, method of biomonitoring with mosses has proven to be relatively accurate, practical and simple method. Nevertheless, a number of shortcomings and questions have been identified through series of studies. One of the most important questions is what kind of moss biomonitoring method we should use – passive or active biomonitoring? Despite the large number of publications and the continued use of the transplant method, there are also a number of serious unresolved issues. In particular, there is no consensus on the optimal exposure duration, moss type, moss bag form, quantity of samples and pre-experimental preparations. All the problems that were listed below a considerable barrier on the way of standardization of this technique. This review paper collected and analyzed moss biomonitoring studies in order to present different experimental methods and experimental preparations of biomonitoring control of air and water pollution used in different study, and to present advantages and disadvantages of this technique and show the future perspective and possible solutions.

Keywords: water and air quality control, moss biomonitoring

Introduction

The industrial boom of the 20th century, namely the ubiquity of vehicles with internal combustion engines, the growth of industrial centers and cities, and the progress of heavy and chemical industries has led not only to improved quality of life, but also to environmental degradation worldwide, but strongest in the developed world. In the pursuit of improving product quality, increasing production capacity and minimizing costs, the harmful environmental impact of emissions and waste on the environment was most often ignored. It was only towards the end of the 20th century and the beginning of the degradation of some ecosystems that the effects of anthropogenic pressure on the environment, including on the human body, began to be studied. Among the objects of the environment, the greatest interest is caused by atmospheric air pollution and water pollution. This is due to the fact that pollutants present in the air and water are absorbed not only by the soil, animals and plants, but also by humans. Humans passes a large volume of air through their lungs and also water (mostly through drinking) and eat different kinds of seafood, as a result, various harmful substances, such as heavy metals, enter their body. The danger of heavy metals is that they can accumulate in human organs and thus cause serious harm to health. The concentrations of heavy metals in the air and water are difficult to determine due to their small size, so traditional instrumental methods are not applicable in this case. In addition, modern scientists are interested in the degree of air pollution and water pollution in large areas (megacities and agglomerations) with very different levels of pollution. Environmental monitoring of heavy metal content by traditional methods in such territories is an almost impossible task, so the method of biomonitoring is widely used.

Biomonitoring is the quality control of an ecosystem using living organisms (biomonitors). This method is not a direct measurement of the ecological state of the system, but is a measurement of the response of living organisms to the quality of the environment and changes in this quality. There are a large number of

biomonitor organisms in nature, and their choice depends on the ecosystem and environment that we want to explore.

Mosses have been widely used in many studies to assess the quality of atmospheric air and quality of water due to their high accumulation capacity and wide distribution in nature. There is passive and active biomonitoring using mosses. Passive biomonitoring uses mosses growing in the study area to analyze medium pollution level. This method is suitable for tracking changes in the ecosystem over a long period of time and over large territories. But the research is complicated by the fact that it is not always possible to find the necessary amount of moss growing at different points in the study area. In the case when there is no or not enough local moss, resort to the use of moss transplanted from an ecologically clean area – the so-called method of active biomonitoring. The advantages of this method are flexibility in choosing the placement site, the ability to control the exposure time, and the ability to determine the initial concentrations of chemical elements. When transplanting mosses from relatively clean territories to urbanized ones, there are not always enough sites for transplanting. Therefore, most often mosses are placed in mesh bags made of chemically neutral materials, the so-called “moss bag” technique. But the moss dries up during exposure and often crumbles. To overcome these disadvantages, mosses are placed on a special support, watering and shading are installed. But for large enough territories, this method is too time-consuming and expensive.

Despite the large number of publications and the continued use of the transplant method, there are still a number of unresolved issues. In particular, there is no consensus on the optimal exposure duration, moss type, moss bag form, quantity of samples and pre-experimental preparations. The choice of the exposure period is one of the main problems of evaluating using the "moss-bag" technique, since mosses are prone to drying out and the effectiveness of retention of elements by mosses may vary as a result. This study was conducted in order to present different experimental methods and experimental preparations of biomonitoring control of air or water pollution.

Experimental methods and preparations in literature

C. Carballeira, A. Carballeira (2019) ^[1] chose active biomonitoring with moss *Fontinalis antipyretica* in order to control water pollution in river. The aquatic moss *Fontinalis antipyretica* Hedw. was collected in a rural site, far from any sources of pollution, in the river Moss plants were rinsed in situ with fresh water (to remove sediment, epifauna and some adhered particles) before being placed in polyethylene bags and transported (within a few minutes) to the laboratory. The plants were placed in glass vessels containing oxygenated, cooled (5 °C) bidistilled water and held under standard light/dark conditions (12 h/12 h). The green parts of the moss plants were separated, washed twice with bidistilled water and again placed in glass containers to keep them alive until all samples were processed. For preparation of the moss bags, the moss samples were either kept alive or were devitalized. Thus, some moss samples were devitalized by drying in an oven in stages (at temperatures of 50, 80 and 100 °C, each held for 8 h), as described by Deben et al. (2017). Flat moss bags (10 x 10 cm) were made from fibreglass mesh (mesh size 2 x 2 mm), which was previously steeped in 10% HNO₃ for 24 h, rinsed several times with bidistilled water and dried at 40 °C. An aliquot of living moss (3 g f.w.) or devitalized moss (0.5 g d.w.) was placed in each bag (n = 192 for each type). The bags were then sewn shut with nylon cord. Moss bags containing living moss were stored in glass containers, as described above, until use. The moss bags were exposed for two different lengths of time: 10 (t1) and 30 (t2) days, with a 10 day overlapping period. Twelve moss bags (6 for each treatment) were each attached to a concrete brick with plastic ties and placed in each exposure site. Authors had made following conclusions: devitalized moss proved useful for biomonitoring trout farms during an exposure period of 10 days.

Fabrizio Monaci, Stefania Ancora (2021) ^[2] used native and transplanted specimens of the aquatic moss *Platyhypnidium riparioides* in order to evaluate metal(loid) contamination in a river receiving multiple acidic and metalliferous drainages from sulphide mineralized areas and derelict mines. According to native mosses in the study area, eight sampling sites colonized by the moss *P. riparioides* were identified. An additional sampling site was placed in the reference site, with the purpose to derive local baseline metal(loid) data from that site. Representativity of the collected material was achieved by getting at least 100 g fresh weight of moss from 3 to 5 points of each sampling site for a stretch of river of about 30 m.

According to transplanted mosses, Moss bags were prepared at the reference site immediately after the collection by putting about 100 g of moss material inside a rectangular 15 × 20 cm bag, packaged with 3 mm mesh plastic garden net, previously washed in acidified distilled water. On the same day, the moss bags were deployed in triplicate at the reference site (blank) and each native moss sampling site of Merse by grounding the bag to rocks or other stable substrates with a plastic-covered wire. The exposure of all the deployed moss bags lasted 3 weeks. At the laboratory, native and transplanted moss was dried at 40 °C for 48 h, sorted to remove dead or senescent tissue and extraneous material and then stored at a dry place. Since there is evidence of zoning of accumulated metal in moss shoots according to age the upper 2-cm, green shoots of moss were detached by a single operator using latex gloves, and clean stainless-steel tweezers and scissors, and put in a paper packet. As a rule, this terminal part is the youngest and the most actively growing of the shoot and is likely to reflect recent ambient chemical conditions, hence a selection of samples of the same age is a prerequisite for reliable comparisons of results between native and transplanted moss. The selected material was then homogenized in an ultracentrifuge mill (Retsch ZM100, heavy metal-free) and stored in plastic containers until chemical analysis. For determination of elemental content author used Inductively Coupled Plasma Atomic Emission Spectrometry and High-Resolution Continuum Source Atomic Absorption Spectrometry.

Their results demonstrate the benefits of a combined approach in assessing river quality in abandoned mining areas impacted by multiple metalliferous sources, i.e. diffuse runoff from areas with pyrite and polymetallic ore deposits, effluents from derelict mines and drainages from mine wastes. the capacity of *P. riparioides* to uptake and retain a convenient spectrum of elements and its high response sensitivity made this species an effective sampling medium to identify those that are most likely to affect biological receptors, indicating potential ecological impact. combined use of native and transplanted moss, for detecting possible bias due to intense contamination or other abnormal stream-quality conditions.

Santiago Díaz, Rubén Villares (2012)^[3] used aquatic moss *Fontinalis antipyretica*. For this purpose, moss specimens from a clean site were exposed to concentrations of As, Hg, Sb, and Se ranging from 0.1 to 10,000 µg l⁻¹, for incubation times of between 1 and 22 days, and the tissue concentrations of the metals in the moss specimens were then measured. The moss used in the experiments was collected from a clean site. Samples were rinsed in situ and transported in river water in cool boxes. Only apical tips (2 cm) were used in the experiments to minimize any errors due to the different accumulation capacities of the different parts of the plant. The concentrations of the elements were determined by atomic fluorescence spectroscopy. Authors concluded, that high concentrations were generally reached in the mosses with in a few days of exposure, especially with Hg, which implies that *F. antipyretica* has a high capacity to magnify low levels of these elements in water, thus facilitating their detection. the minimum time recommended for use in active biomonitoring studies by means of transplants will be within a few days. Santiago Díaz, Rubén Villares (2012)^[3] also not recommend the use of *F. antipyretica* for biomonitoring low levels of Sb in water.

In the work of Mattia Cesa, Andrea Baldisseri (2013)^[4] an innovative network based on transplanted bryophytes providing a continuous monitoring of the priority substances Cd, Hg, Ni, and Pb and other trace elements (Co, Cr, Cu, Fe, Mn, Zn) was designed for the watercourses flowing across an industrial district of NE Italy where both permitted and illegal wastes cause sporadic, intermittent or chronic events of environmental alteration. An active biomonitoring strategy based on transplanted bryophytes (moss bags) was chosen. The bryophyte adopted for the present study was the aquatic moss *Platyhypnidium riparioides* (Hedw.). Mosses were collected from two uncontaminated reference sites. Mattia Cesa, Andrea Baldisseri (2013)^[4] conducted preparation of cages made of a plastic net (20 x 20 cm) with 4 x 4 mm holes, containing about 10 g of a mixture of moss branches collected from different river stretches of the reference site; Transportation to the survey area within 24 h and exposure to the water flow of biomonitoring sites (one bag containing the mixture for each station as shown by the electronic Supplementary figure); One bag was preserved to measure pre-exposure concentration at each monitoring action; Recovery of the moss bag (immediately replaced by a new bag) 28 ± 3 days after transplantation, preliminary washing in the river water, and transportation to the laboratory; Second washing with bi-distilled water to remove mud, sand, epifauna and metals not adsorbed to the cell wall; Selection of apical parts (2-4 cm), which are the richest in leaves and have the widest sorption surface; Desiccation to constant weight at 40 °C (2 days, residual water content 4-

6%). Metal concentrations were measured using inductively coupled plasma mass spectrometry. The biomonitoring network authors presented is able to provide a) a reliable classification of water bodies for some priority substances (Cd, Hg, Ni, Pb) and other trace elements, b) information about long-term trends of substance concentrations that tend to accumulate in biota in the context of monitoring programmes under the Water Framework Directive 2000/60/EC, and c) an integration of the official water-sampling based surveys, which are not calibrated or not able to assess local pollution in small tributaries and intermittent events.

S. Debén, J.A. Fernández (2018) ^[5] carried out study to determine the optimal number of moss bags of *Fontinalis antipyretica* required for biomonitoring stream water pollutants. Samples of the aquatic moss *Fontinalis antipyretica* Hedw. were collected from an unpolluted stream. The plants were rinsed first at the site with stream water and washed once again in the laboratory (5 L water per 150 g f.w. of moss, for 1 min, with shaking). The remaining material was devitalized by oven-drying with temperature ramp (50 °C for 5 h, 80 °C for 5 h and 100 °C for 10 h). Flat bags (10×20 cm) were made with fibreglass mesh (aperture 4mm²) free of trace contaminants due to a previous wash in HNO₃. The ratio between the moss weight and the surface area of the bag ranged between 3 and 6 mg cm⁻². In total, 215 moss bags were prepared and 50 of these were placed in each of the 4 sampling sites (SS). For each SS, 3 control moss bags were treated in the same way as the transplants but were not exposed in the streams. Finally, another 3 moss bags were vacuum-packed and stored for subsequent determination of the initial concentrations of elements. S. Debén, J.A. Fernández (2018) ^[5] recommend that the moss bags should be cleaned as thoroughly as possible in situ. They also recommend, that at least 6 moss bags should be used in biomonitoring studies. This guarantees an error of less than 20% in the estimation of the mean tissue concentrations of contaminants in the moss exposed at one SS. Greater number of moss bags should probably be used in highly contaminated sampling sites (EF > 23) or when the user wishes to differentiate sites with very similar levels of contaminants.

The active biomonitoring technique has been demonstrated to be an excellent tool for monitoring water quality in work of S. Debén, J.A. Fernández (2020) ^[6]. Their study was carried out to determine the best options for various methodological aspects of monitoring some metals and metalloids (i.e. Al, As, Cd, Co, Cu, Fe, Hg, Ni, Zn and Pb): 1) the type of transplant, 2) pre-exposure washing (with or without cellular extractants), 3) the ratio between moss weight and bag surface area, and 4) the depth at which the bags are exposed. Samples of the aquatic moss *Fontinalis antipyretica* Hedw. were collected from two streams do not affected by any type of known pollution sources. The samples were rinsed on site with stream water and transferred to the laboratory in a portable refrigerator. Green parts of the moss were selected and manually cleaned to remove most epiphytes and particles attached to the surface. Pre-exposure treatment of the samples included washing with distilled water (1 L water per 12 g dry mass of moss, for 20 min, with shaking), and a devitalizing treatment that consisted of oven-drying with a temperature ramp (50 °C for 8 h, 80 °C for 8 h and 100 °C for 8 h). The mosses were then placed in flat bags (6× 4 cm) prepared with fibreglass mesh (aperture 4 mm², previously washed with HNO₃ to remove any trace contaminants). In all cases, the transplants were attached with plastic ties to concrete blocks and placed in situ, in areas of flowing water, at a depth of 20 cm from the bottom of the streams. Exposition time was 7 days.

The moss bags were exposed simultaneously as follows: 1) uncovered; 2) covered with perforated PVC tubing (15 cm length and 5 cm diameter), closed at both ends; and 3) covered with unperforated PVC tubing (15 cm length and 5 cm diameter) open at the end opposite the water current. Five replicates of each type of transplant were placed in each of the sampling sites. Two types of moss bags were exposed for four different periods of time (1, 2, 4 and 7 days). Twenty-four moss bags (3 replicates for each exposure time and pre-exposure treatment) were exposed at each of the sampling site.

Moss bags were prepared with different amounts of moss (600, 1200 and 2400mg dry mass of moss) to determine the optimal ratio between the weight of the moss and the surface area of the bag (hereafter “W/S ratio”). The surface area of the flat bag was 48 cm², and the resulting W/ S ratios were 12.5, 25 and 50 mg cm⁻². The bags were placed at depths of 0.15, 2.5, 5 and 7.5m (from the surface to the bottom) in the reservoirs and at depths of 0.15, 0.5, 1 and 1.5m (from the surface to the bottom) in the rivers. The concentrations of metals were then determined by inductively coupled mass spectrometry (ICP-MS).

Authors concluded there were no significant differences in the loss of material caused by the different types of transplant, and tubular transplants thus failed to reduce the loss of material during exposure. On the

other hand, the accumulation of metals in the uncovered moss bags was significantly higher than inside the tubes when the concentrations were above the LOQT (i.e. for Al, Cu, Fe and Zn) showing that uncovered flat moss bags are the best type of support for preparing the transplants. Authors also support the recommendations regarding the pre-exposure washing with cellular extractants and the type of transplant. On the basis of the findings of the present study, authors recommend using a ratio of 12.5 mg cm^{-2} , because it is the lowest of the ratios studied that does not decrease the uptake.

Mattia Cesa, Alessandro Bizzotto (2009) ^[7] used the aquatic moss *Rhynchostegium riparioides* (Hedw.) C.E.O. Jensen transplanted under laboratory conditions to conduct experiments of Hg accumulation. Mosses were collected from clean springs of the river. The moss branches were rinsed in the water of the spring, to remove organic particles, mud and sand, and then homogenised. Moss bags containing 20–30 g of moist material and made of a plastic net (4 mm holes) were transported to the laboratory for experimentation within a few hours. Mercury bioaccumulation in moss bags of *Rhynchostegium riparioides* under laboratory conditions occurred with a great uptake ratio, but a wide variability range. The best performance provided by 2 – 4 d of exposure.

Another study of Grzegorz Kosior, Eiliv Steinnes (2017) ^[8] was conducted in Sudety (SW Poland) where the past uranium/polymetallic mining activities left abandoned mines, pits and dumps of waste rocks with trace elements and radionuclides which may erode or leach out and create a potential risk for the aquatic ecosystem, among others. In the present work four rivers affected by effluents from such mines were selected to evaluate the application of aquatic mosses for the bioindication of 56 elements. Naturally growing *F. antipyretica* and *P. riparioides* were compared with transplanted samples of the same species. Moss samples for transplantation (green gametophyte parts) were placed in 1 L nylon mesh bags (mesh size 2 mm), and transplanted in 5 replicates to each of the 20 monitoring sites described above. *F. antipyretica* and *P. riparioides* were collected for analysis after 30 days of experiment. This time was long enough, as mosses are able to accumulate trace elements over a short time. Concentrations of element were measured by inductively-coupled plasma mass spectrometry. Author observed that in the most polluted rivers, native *F. antipyretica* and *P. riparioides* contained significantly higher concentrations of As, Ba, Cu, Fe, La, Nd, Ni, Pb, U, and Zn than transplanted ones, whereas in less polluted sites the situation was reverse. The transplanted moss moved from a clean into an extremely polluted river probably protected itself against the accumulation of toxic elements, thus reducing their uptake. The results of this study show that moss-based bioindication in extremely polluted rivers in which concentrations of trace elements are estimated in transplanted mosses may be misleading.

S. Deben, J.A. Fernandez (2016) ^[9] present the results of an experiment carried out for the first time in situ to select a treatment to devitalize mosses for use in active biomonitoring of water pollution. Three devitalizing treatments for the aquatic moss *Fontinalis antipyretica* were tested and the effects of these on loss of material during exposure of the transplants and on the accumulation of different heavy metals and metalloids were determined. Flat bags (10 x 20 cm) were prepared with fibreglass mesh (aperture 2 mm), previously washed in HNO₃ to eliminate possible trace contaminants. The treated moss was placed inside the bags and the exact weight of moss used in each bag was measured with a precision balance. The mean ratio between moss weight and bag surface area was $8.2 \pm 1.9 \text{ mg cm}^{-2}$. In total, 147 moss bags were prepared (126 with *F. antipyretica* and 21 with *S. denticulatum*). The experiments carried out by S. Deben, J.A. Fernandez (2016) ^[9] have shown, for the first time in situ, that devitalization of aquatic moss samples does not inhibit the capacity of the moss to accumulate contaminants. However, the different devitalization treatments altered the structure of the moss in different ways, which affected the loss of material during the exposure period and also the accumulation of contaminants. In addition, the results were affected to a greater extent by the duration of exposure than by the conditions in the streams. For use of the moss *F. antipyretica* to biomonitor contamination of aquatic environments, devitalization of the samples by oven-drying with a temperature ramp is recommended.

Mari'a Dolores Va'zquez, Rube'n Villares (2013) ^[10] characterized the aquatic contamination by a series of physical and chemical parameters, which were then related to the physiological responses determined in transplants of the aquatic moss *Fontinalis antipyretica*. Moss samples were collected from the clean river and transported to the laboratory. In the laboratory, the samples were washed in distilled water; apical segments (5–6 cm) were removed and placed in plastic mesh bags (1 cm² mesh size). There was a clear

relationship between the degree of urban contamination characterized by the physical and chemical characteristics of the water and the degree of stress evaluated in terms of the physiological responses in the moss transplants. Of the physiological parameters measured in the transplants of *F. antipyretica*, the following appeared to be the most suitable as indicators of urban contamination: the increase in the concentration of phaeopigments and inhibition of the net photosynthetic rate. Author strongly stated, that active biomonitoring with transplants of aquatic moss, in this case *F. antipyretica*, is a very useful method because of its simplicity and relatively low cost and its capacity to characterize the quality of water affected by urban waste, in terms of the ecological risk induced.

Mattia Cesa, Alberto Bertossi (2015) ^[11] made a contribution for validating a standard method for trace element monitoring based on transplants and analysis of aquatic bryophytes, in the framework of the EC Directive 2000/60. It presents the results of an experiment carried out to assess significant differences in the amount and variability of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in three moss species (*Cinclidotus aquaticus*, *Fontinalis antipyretica*, *Platyhypnidium riparioides*) and two different parts of the moss (whole plant vs apical tips). Mosses were caged in bags made of a plastic net (hole size 4×4 mm) and transplanted for 2 weeks to an irrigation canal impacted by a wastewater treatment plant. Trace element concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Mattia Cesa, Alberto Bertossi (2015) ^[11] concluded that *P. riparioides* provides the best performances in terms of uptake of the elements considered. *C. aquaticus* is the weakest accumulator, while *F. antipyretica* shows an intermediate efficiency.

F. Capozzi, S. Giordano (2016) ^[12] tried to develop an internationally standardized protocol for the moss bag technique application and of the moss bags exposure in terms of bag characteristics (shape of the bags, mesh size, weight/surface ratio), duration and height of exposure by comparing traditional moss bags to a new concept bag, “Mossphere”. Two couplets of moss-bags of different shape were compared at parity of mesh size (2 mm), quantity of devitalized moss filled in, and external surface of the device: rounded bag vs. Mossphere – both made with a dry mass/surface ratio of 30 mg cm⁻² and flat bag vs. Mossphere – both made with a dry mass/surface ratio of 15 mg cm⁻². The Mossphere is a device designed by F. Capozzi, S. Giordano (2016) ^[12] consisting of two coaxial empty spheres, each formed by two hemispheres, made of pierced high-density polyethylene (the internal sphere), and of a 2 mm mesh nylon net (the external sphere). The rounded bags – a square of plastic net of 22 x 22 cm was filled with the moss material, and secured with a nylon thread. Rectangular flat bags (approximately 700 cm²) were made with plastic net (2 mm mesh size). The moss was distributed homogeneously inside the bag. Mosspheres with different mesh size (1 mm, 2 mm, and 4 mm) were filled with 11.40 g of dry moss material (dry mass/surface ratio: 30 mg cm⁻²), total number of samples = 189 (3 mesh size x 3 countries x 7 sites x 3 replicates). In order to investigate the effect of weight, Mosspheres with a nylon mesh of 2 mm were filled with 5.70, 11.40 or 17.10 g d.w. of moss material, in order to have weight/surface ratios of 15 mg cm⁻², 30 mg cm⁻² and 45 mg cm⁻², respectively. For duration exposure, Mosspheres with a dry mass/surface ratio of 30 mg cm⁻² and 2 mm mesh were exposed in triplicate at 4 m above the ground. Three different durations of exposure (3, 6 and 12 weeks) were tested in parallel in all the sites between March and June 2013, so there were in total 4 subsequent exposure periods of 3 weeks, 2 subsequent exposure periods of 6 weeks and 1 exposure period of 12 weeks. For this assay, Mosspheres with a dry mass/surface ratio of 30 mg cm⁻² and 2 mm mesh were exposed in triplicate at 4, 7 and 10 m above the ground. Concentrations of elements were determined by inductively coupled plasma mass spectrometry. According to the reported outcomes, authors suggest the use of a Mossphere, because it is reusable, not “home-made” and with a regular and fixed shape; it should be prepared with 1 or 2 mm mesh net (to avoid the loss of material) and a moss content allowing a weight/surface ratio not higher than 15 mg cm⁻² and it should be exposed at 4 m above the ground for a period not shorter than 6 weeks.

Cesa M., Nimis P.L. (2014) ^[13] used aquatic moss *Rhynchostegium riparioides* estimate Hg concentrations of <4 µg/L in groundwaters of a polluted area in NE Italy. The day before transplantation, bryophytes were collected from reference sites by using clean steel scissors, removing 3–5 cm of the apical parts of the plants. At each site, the mixture of shoots was homogenized by hand and subdivided into 29 bunches, which were placed in a big bottle filled with springwater; 28 bunches were transported to the survey area, placed inside ‘moss bags’ made of a plastic net (4×4-mm holes) and exposed to experimental water. The

remaining bunch was preserved for pre-exposure concentration analyses (blank). The present case study showed that aquatic bryophyte transplants are sensitive, ready-to-use (1–4 days) and cheap (slight additional costs compared with traditional water sampling) monitors of trace element pollution, both in surface waters and groundwaters. They could also be used as time-integrated sensors in the short term to (1) estimate metal concentration in the water when it is lower than the instrumental detection limit and (2) indicate presence/absence of that pollutant at the borders of the contamination plume.

Anna Di Palma, Aridane G. González (2019) ^[14] used moss *Sphagnum palustre* to estimate a unified protocol of biomonitoring for air pollution control. Oven-devitalization is proposed as unique treatment for moss to use as biomonitor.

Sofia Augusto, Cristina Máguas (2013) ^[15] conducted a review to allow designing a set of guidelines for biomonitoring of environmental POP pollution. They recommend to use one kind of moss to estimate pollution in area, but if it is not possible, calibration needed.

Rogova N.S. (2011) ^[16] studied the accumulation capacity of ten bryophytes. Four forest mosses, four swamp mosses and two epiphytic bryophytes were studied. She chose a 12 week exposure period. The results showed that the epiphytic bryophyte *Pylaisia polyantha* had the highest accumulation capacity. Rogova N.S. uses moss bag method (active moss monitoring method).

Anli (2006) ^[17] compared the heavy metal accumulation capacity of different bryophytes (using passive bryophyte monitoring method). Seven species of bryophytes were used in this study. According to the comparison of heavy metal contents in seven kinds of bryophytes, she suggested that different species of bryophytes have different enrichment ability for the same heavy metal element. The enrichment ability of the same species of bryophytes to different heavy metals was also different. Anli also suggested that *Haplocladium* could be used as a good biological monitor.

Ren Zhaojie et al. (2011) ^[18] investigated 62 species of bryophytes (passive bryophyte monitoring method). It is pointed out that the common bryophytes in the industrial area are *Gymnostomum calcareum*, *Weissia planifolia* Dix., and *Physomitrium eurystomum*. Therefore, they recommend these bryophytes as environmental pollution indicators.

Conclusion. Problems and future perspectives

Method of biomonitoring with mosses is modern and outstanding method of water and air pollution control. It has such advantages as wide variety of application spheres, wide variety of species to choose, simple application and cheap cost. Through a short period of time method of biomonitoring with mosses was accepted and adopted throughout the world. Terrestrial, aquatic and epiphyte mosses proved to be excellent for environmental quality control. Despite all these facts there is exist a problem of standardizing moss biomonitoring studies. Although, there were multiple attempts to conduct studies focused on standardization development ^[19, 20], there is still no clearly agreement on this topic. Through brief analyze of study methods from different papers listed above, we can conclude that most common species for monitoring aquatic environment pollution is *Fontinalis Antipyretica*, due to its ability to accumulate mercury. However, it is still a point of argument which kind of mosses we should use for atmospheric air quality control. There is also no agreement about moss bag type. In most of studies, it is also recommended to use more than six samples for one experimental point. It is obviously, after solving of standardization issue of the moss biomonitoring method, it will be used in future widely. Thus, conducting of accurate and wide standardization study is priority target for next few years in this field.

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