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Syntrichia ruralis as a suitable bioindicator for urban areas – the case study of Tallinn city

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Abstract: Environmental pollution is one of the most important problems in urban environment. Mosses are good indicators of air pollution. In Estonia, heavy metals have been measured from *Pleurozium schreberi* and *Hylocomium splendens*, which do not grow in areas of Tallinn with a higher pollution load. In the present study, Cu, Fe and Cd were measured from five moss species growing in contaminated as well less polluted areas of Tallinn. Based on stationary and street pollution source inventory and air pollution dispersion modelling, the long-term average concentrations of fine particles (PM_{10}) and nitrogen oxides (NOx) in air were estimated. The work revealed that it is possible to find a moss species that is common in Tallinn and grows in both polluted and less polluted areas – *Syntrichia ruralis*, which is the most suitable species for bioindication based on this work. Moss species *Ceratodon purpureus* accumulated the most Cd, Cu, and Fe, then *Brachythecium rutabulum*/*Sciuro-hypnum curtum*, and *Rhytidiadelphus squarrosus* the least. Statistically significant higher Fe concentrations were in the *Syntrichia ruralis*, compared to the *Sciuro-hypnum curtum* and *Rhytidiadelphus squarrosus*. The *Syntrichia ruralis* also had significantly higher Cd content compared to the *Brachythecium rutabulum*/*Sciuro-hypnum curtum*. The results of the GLM analysis showed that the content of various heavy metals depends on the moss species and the degree of fine particles in the environment, and it didn't depend on whether the moss grows on the soil or a hard substrate such as concrete, stone or asphalt.

Kokkuvõte: Sammal *Syntrichia ruralis* kui sobiv indikaatorliik õhu kvaliteedi hindamiseks linnalistes asulates Tallinna näitel

Õhusaaste on tõsiseks probleemiks paljudes linnades, sh Tallinn, kuid otsemõõtmiste kaudu on küllalt vähe infot õhusaaste kohta linna erinevates piirkondades. Eestis on mõõdetud raskmetalle palusamblast (*Pleurozium schreberi*) ja laanikust (*Hylocomium splendens*), mida aga Tallinnas suurema saastekoormusega aladel ei kasva. Käesolevas uuringus mõõdeti Cu, Fe ja Cd sisaldusi viiest samblaliigist saastatud ja vähemsaastatud Tallinna piirkondadest. Lähtudes paiksete õhusaasteallikate ja linnatänavate heitkogustest ja kasutades õhusaaste hajumismudelit, hinnati peenosakeste (PM_{10}) ja lämmastikoksiidide (NOx) pikaajalisi keskmisi kontsentratsioone linnaõhus. Töös selgus, et on võimalik leida samblaliike, mis on Tallinnas levinud ja kasvavad nii saastatud kui ka vähem saastatud piirkondades, nt harilik karvkeerik (*Syntrichia ruralis*), mis on käesoleva töö põhjal bioindikatsiooniks kõige sobivam liik. Töö tulemuste põhjal sisaldas kõige enam Cd, Cu ja Fe harilik punaharjak (*Ceratodon purpureus*), seejärel harilik karvkeerik, harilik lühikupar/lame oravulmik (*Brachythecium rutabulum*/*Sciuro-hypnum curtum*) ja kõige vähem niidukäharik (*Rhytidiadelphus squarrosus*). Statistiliselt olulised kõrgemad Fe sisaldused olid karvkeerikus, võrreldes oravulmiku ja niidukäharikuga. Harilikus karvkeerikus oli ka oluliselt kõrgem Cd sisaldus võrreldes lühikupra/oravulmikuga. GLM analüüsi tulemused näitasid, et erinevate raskmetallide sisaldus sõltub samblaliigist ja peente osakeste määrast keskkonnas ja ei ole oluline, kas sammal kasvab mullal või kõval substraadil nagu betoon, kivi või asfalt.

Keywords: bioindication, mosses, bryophytes, heavy metals, Cu, Cd, Fe, particulate matter, urban environment, pollution

INTRODUCTION

Bryophytes have been used as a significant biological monitoring system for heavy metal pollution since 1968 (Tremper et al., 2004). Another group of organisms often used for bioindication is lichens but usually through species mapping and indicator species registration (Farkas, 1990; Marmor & Randlane, 2007; Das et al., 2020). In urban areas, the main lichenobiota grow on trees. As epiphytic lichens are the most sensitive among all the ecological groups of lichens (Das et al., 2020), then in big cities, most of its territory may be a lichen desert without any epiphytic lichens (Farkas, 1990).

Moss biomonitoring seems to be more popular because it causes fewer technical and analytical problems than lichens and tree bark (Szczepaniak & Biziuk, 2003).

Bryophytes have no roots and cuticula like vascular plants (Glime, 2021). Pollutants accumulate both within and among cells of bryophytes. The extracellular fraction reflects current pollution levels in the environment, whereas the intracellular fraction is more constant and testifies to the average pollution load that is present in the environment (Vanderpoorten & Goffinet, 2009). Metals from the atmosphere can reach the surface of terrestrial bryophytes in a solution (precipitation) or in the form of dry deposition which may later dissolve or be washed away. Even though terrestrial bryophytes take most of the substances from the atmosphere, soil contributes significantly to the heavy metal contents. This is particularly discernible during the rainy seasons or snowmelt periods when many substances from the soil can be transported in the form of solutes, wetting the plant. The bioavailability and mobility of heavy metals in soil are strongly correlated to its acidity, the amount of organic matter, and the chemical composition (Stanković, 2018).

Most bryophyte species are sensitive to environmental pollution and do not occur in contaminated areas – e.g., 87% of the British and Irish moss species have no tolerance for heavy metals (Hill et al., 2007). Many bryophytes have become extinct from urban and industrial environments because of their sensitivity to polluted air (Leblanc & Rao, 1974). At the same time, those species that tolerate pollution can concentrate heavy metals in large amounts greatly surpassing the absorbing capacity of vascular plants and expressing the past and present air quality (Govindapyari, 2010). Accumulated heavy metals can have an impact on bryophyte physiology (Kaur et al., 2010). Species diversity varies not only with the distance from a source of pollution but also with the type of habitat (Gilbert, 1968). According to Leblanc and Rao (1974), the order of increasing sensitivity is from terricolous to saxicolous and corticolous species – species growing on trees appear to be far more sensitive than those on other substrata, and the shady and alkaline niches are the most preferred by bryophytes in the city ecosystem.

The tolerance of mosses to polluted air depends to a greater extent on the ratio of metals taken up than on the absolute metal level. There is a marked difference in the tolerance levels of bryophytes for individual metals and a combination of cations. The reproductive potential of a species determines its degree of success in a polluted environment. The survival of *Bryum argenteum*, *Ceratodon purpureus*, *Funaria hygrometrica* and *Marchantia polymorpha* lies in their high reproductive capacity and fast subsequent growth. These species produce spores or gemmae on a very large scale (Govindapyari, 2010).

The ability of mosses to accumulate heavy metals depends upon the total leaf surface and the number of thin-walled parenchymatous cells (Govindapyari, 2010). The species have different growth rates and ages of green parts (Pakarinen & Rinne, 1979). There is a wide variation in metal accumulation from species to species (Folkeson 1979; Berg & Steinnes, 1997; Zechmeister, 1998; Govindapyari, 2010). Nevertheless, fluctuations in the annual increment of one species can be enormous between different habitats having different climatic and moisture conditions (Pakarinen & Rinne, 1979; Zechmeister, 1998). Stankovic et al. (2018) have pointed out that various species are often used together to get comparable information about HM pollution in different places, although their ability to collect heavy metals is different.

The preferred biomonitors are those that are able to accumulate pollutants, be available throughout the year, are easy to capture and identify, and are cosmopolitan in distribution (Govindapyari, 2010). If we want information on both contaminated and less polluted areas and we want to compare the results with natural areas, we need to choose a species that is sufficiently well represented in all the mentioned places. Previous studies and comparative data from other regions may also be useful. In Estonia, periodic moss heavy metal content studies are carried out within the framework of the international program ICP Vegetation, using *Pleurozium schreberi* and *Hylocomium splendens* (Harmens et al., 2015; Liiv & Kösta, 2017). Due to the relatively low pollution tolerance of these species, they can only be sampled from areas with cleaner air around the city, and therefore do not give an overview of the actual situation of pollution in the city.

The aims of this study are: a) to find the bryophyte species that are common in Tallinn and grow both in more and less polluted areas and to assess which bryophyte species is the most suitable for bioindication in Tallinn; b) to compare the concentrations of heavy metals in bryophyte species with different tolerances of heavy metals; and c) to find out, which environmental parameters affect the contents of heavy metals in bryophytes the most.

MATERIAL AND METHODS

Sampling sites and sample collection

The study site selection was based on the existing moss species data about Tallinn (eElurikkus, 2017). Accordingly, we selected five species that were studied in nine contaminated and nine less polluted sites in different regions of Tallinn, and at a control site in a rural area Jäneda (Fig. 1). As the main source of pollution in Tallinn is traffic (Pindus et al., 2016), the sampling sites were divided into two zones according to the traffic load: A – areas were located near (up to 5 m) heavy traffic streets (multiple lanes in one direction) and/or crossings; B – areas were located away from heavy traffic streets (at least 100 m). The selected five species were *Ceratodon purpureus, Syntrichia ruralis, Sciuro-hypnum curtum, Brachythecium rutabulum and Rhytidiadelphus squarrosus*. *Sciuro-hypnum curtum* and *Brachythecium rutabulum* are considered together in some analyses since they are systematically close species (formerly in one genus *Brachythecium*). Nomenclature of bryophytes is according to Vellak et al. (2015).

The fieldwork was carried out in April 2018 during six days. Bryophyte species within a radius of 5 m were recorded and samples of selected five species were collected if the amount was sufficient for analyses. At each site, samples of a species were collected from at least three different locations. Altogether 49 bryophyte samples from 15 sites and 22 soil samples from 13 sites were collected. Soil samples were collected at a depth of 2–4 cm and air-dried. Living parts of moss were washed with ultrapure water, air-dried and lyophilised. All samples were stored at room temperature. Iron (Fe), copper (Cu) and cadmium (Cd) contents were measured from bryophytes by flame and graphite atomic absorption spectroscopy (AAS).

Chemicals and reagents

All reagents used were of the highest purity available and all solutions were prepared in ultrapure water, with a resistivity of 18.2 MΩ obtained from a Milli-Q system Millipore (Bedford, USA). The 1000 mg/1 single metal stock solutions for iron (Fe), copper (Cu) and cadmium (Cd) (Atomic Spectroscopy Standard Solutions) were obtained from Honeywell Fluka™. Calibration solutions in the range of 2–10 ng mL^{-1} for cadmium and mercury and 0.1–1.0 µg mL^{-1} for copper and iron were prepared daily by sequential dilution of the stock ones. The reduction solution for cold vapour AAS analysis was prepared at 25% (m/v) $SnCl_2·2H_2O$ (Merck, Germany) in 20% HCl (v/v) (Merck, Germany). Nitric acid 68% (m/v) Suprapur from Merck and 35% (m/v) H_2O_2 from Fluka were employed for the digestion of samples. The applicability of the method to real samples was demonstrated by the analysis of CRM testing material LGC6187 "Trace elements in river sediment" (LGC standards).

Atomic absorption spectrometry (AAS) analysis

AAS metal analysis was carried out according to the overall procedure described in ISO standard EN 14084:2003 (Foodstuffs - Determination of trace elements - Determination of lead, cadmium, zinc, copper, and iron by atomic absorption spectrometry (AAS) after microwave digestion). Moss and soil samples were microwave digested using Microwave GO (SN: 81801381) equipment according to ISO standard procedures EN 13805:2002 (Foodsuffs - Determination of trace elements - Pressure digestion) and EN 13804:2002 (Foodsuffs - Determination of trace elements - Performance criteria and general considerations). For that, about 0.2 g of solid sample was weighed and 5 ml of nitric acid and 1 ml of hydrogen peroxide were added to the microwave bomb. The bomb was sealed and heated according to the program Organic A: ramp 20 min to 180 °C, hold 10 min, cooling 10 min. Digested samples were diluted to 50 ml and subjected for AAS analysis according to the procedure described in ISO standard. The determination of Fe and Cu contents was

Fig. 1. Study sites.

performed using a flame (AASF) atomic absorption spectrometer (SpectrAA 220F, Varian). Cd concentration was measured by using graphite-AAS (SpectrAA 220Z, Varian). Argon 4.0 was used in AASG and air–acetylene flame for the AASF method. The spectrometers were calibrated by means of calibrations solutions prepared by diluting stock solutions (1000 mg/l). The absorption lines 248.3 nm (iron), 324.8 nm (copper) and 228.8 nm (cadmium) were used. Liquid samples were subjected for analysis and the corresponding absorbance values were measured to obtain the respective concentrations of the metals. For each sample, two replicated measurements were carried out and the results given in this work are the averaged concentrations.

Modelled PM_{10} and NO_x

The long-term average concentrations of the air pollutants fine particulate matter (PM_{10}) and nitrogen oxides (NO_x) in Tallinn were modelled with AEROPOL 5.3 model (Kaasik et al., 2017). The meteorological data set was based on observations (2015–2018) from the Tallinn-Harku Meteorological Station of the Estonian Weather Service. The street emissions were based on the traffic flow modelling made by AS Stratum for urban planning purposes, the reference year 2017. The domestic heating emissions were included (based on expected energy consumption in locally heated urban areas), but these were found to be of minor importance, according to modelling results. Although wood and coal heating emissions are often a reason for high concentrations of particulate matter in certain urban areas, this appears not to be the case of this study, as most of the samples were taken near major traffic streets in areas of district heating and some of them, in contrary, originate from relatively clean sites.

In the urban domain of Tallinn, the concentrations of PM_{10} and NO_x in the air were modelled with 50 m grid resolution. The concentration in a certain sampling point was calculated as a distance-weighted average of the four nearest grid points. In the central part of the city (3 km by 3 km) the concentrations were modelled with 10 m grid resolution and the concentration in the nearest grid point was taken as the proxy for the sampling point. Before the application to certain sampling points, the concentrations in the entire grid were validated against three monitoring stations in the urban domain (one of these in the central part), and regression against measured values was applied to correct systematic discrepancies.

The concentrations of NO_x and PM_{10} outside of Tallinn were estimated as the annual average of rural background concentration measured in the nearest rural air pollution monitoring station Lahemaa (the rural background concentrations vary within a narrow range on the territory of Estonia: 1.8–2.1 $\mu g/m^3$ of NO_x and 3.9–4.5 $\mu g/m^3$ of PM_{10}) plus the dispersion of emissions from major roads nearby.

Data processing

To find out which factors influence the content of heavy metal in moss, the General Linear Model (GLM) analysis was performed using the CANOCO 5 program (ter Braak & Šmilauer, 2012). The effects of the following factors were studied: five moss species, substrata, modelled NO_x and PM_{10}. Differences between groups were evaluated by one-way ANOVA. For multiple comparisons, the Tukey-Kramer post-hoc test was performed. The homogeneity of variances was tested with Bartlett's test. Welch's ANOVA was used in the case of the heteroscedasticity of this data (McDonald, 2014).

RESULTS

Bryophyte species in Tallinn and species suitable for bioindication

A total of 28 species of bryophytes were found on the 19 sites. The most common of these were *Ceratodon purpureus*, *Syntrichia ruralis*, *Ptychostomum* spp. and *Sciuro-hypnum curtum*, which were found in 19, 17, 15 and 14 study areas, respectively. The most common species on concrete structures were *Ceratodon purpureus*, *Syntrichia ruralis* and *Bryum argenteum*. *Sciuro-hypnum curtum* and *Rhytidiadelphus squarrosus* grew mainly on the soil. On the asphalt grew *Ceratodon purpureus*, *Ptychostomum* spp and *Syntrichia ruralis*. Several bryophyte species, which were less common on the sites, grew on trees of some sites, such as the *Pylaisia polyantha*, *Orthotrichum speciosum*, *Sanionia uncinata* and *Amblystegium* spp. On the stones, for example, grew *Syntrichia ruralis*,

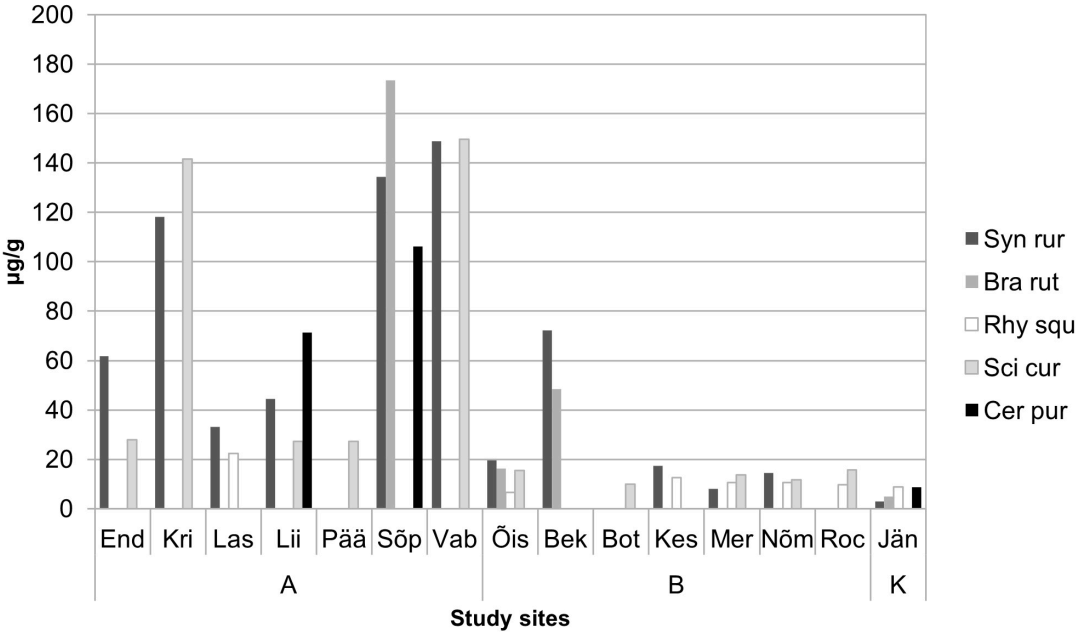

Fig. 2. Cu content in bryophytes by species and sites. A – near multi-lane heavy traffic streets and crossings (up to 5 m); B – far from heavy traffic streets (at least 100 m) and K – control area. *Syn rur – Syntrichia ruralis, Bra rut – Brachythecium rutabulum, Rhy squ – Rhytidiadelphus squarrosus, Sci cur – Sciuro-hypnum curtum,* and *Cer pur – Ceratodon purpureus*. Abbreviations for sites: End – Endla, Kri – Kristiine, Las – Lasnamäe, Lii – Liivalaia, Pää – Pääsküla, Sõp – Sõpruse, Vab – Vabaduse väljak, Õis – Õismäe, Bek – Bekkeri, Bot – Botaanikaaed, Kes – Keskhaigla, Mer – Merivälja, Nõm – Nõmme, Roc – Rocca al Mare, Jän – Jäneda.

Brachythecium spp and *Schistidium apocarpum*. In the areas with the highest pollution load (Nordic Hotel Forum, Jõe, Pronksi), only the *Ceratodon purpureus* and *Bryum* species occurred. On the trees nearby there did not grow mosses.

Our results show that it is possible to find moss species that are common in Tallinn and grow in both polluted and less polluted areas. According to our study, the species most suitable for bioindication is *Syntrichia ruralis*, which was found at almost all research sites, only in some of the most polluted research sites in the city centre did not grow.

Heavy metals in different bryophyte species

The highest Fe content was measured in *Ceratodon purpureus* (3522 µg/g) near a multi-lane heavy-traffic street Liivalaia and the lowest in *Brachythecium rutabulum* (128 µg/g) in the control area in Jäneda. Relatively low Fe concentrations were in *Rhytidiadelphus squarrosus* in the study areas of Õismäe, Botaanikaaed, Keskhaigla and Nõmme. As the result of the ANOVA analysis, the difference in the mean iron content of *Syntrichia ruralis* (M = 2485 µg/g, SD = 408) and *Sciuro-hypnum curtum* (M =1356 µg/g, SD = 265) in the A-areas is statistically significant (F (1,9) = 28.13, $p < 0.001$). Fe content in *Syntrichia ruralis*, *Sciuro-hypnum curtum* and *Rhytidiadelphus squarrosus* were compared in areas B, where the iron content of *Syntrichia ruralis* was significantly higher (M =1437 µg/g, SD = 636) than that of *Rhytidiadelphus squarrosus* (M = 505 µg/g, SD = 219) and *Sciuro-hypnum curtum* (M = 743 µg/g, SD = 299), being statistically significant (ANOVA, F (2,14) = 7.44, p = 0.006).

	Fe, F (7,30) = 12.17, p < 0.001				Cd, F (7,30) = 2.19, p = 0.063				Cu, F (7, 28) = 6.74, p < 0.001			
	b	SE	T	p(T)	b	SE	T	p(T)	b	SE	T	p(T)
Intercept	116	319	0.4	0.719	**0.131**	**0.051**	**2.6**	**0.015**	**-61.49**	**22.6**	**-2.7**	**0.011**
Spe.Bra	-316	301	-1.1	0.302	-0.026	0.048	-0.6	0.586	28.98	21.18	1.4	0.182
Spe.Rhy	**-793**	**288**	**-2.8**	**0.01**	-0.091	0.046	-2	0.054	-12.29	20.5	-0.6	0.554
Spe.Sci	**-772**	**267**	**-2.9**	**0.007**	-0.035	0.042	-0.8	0.414	-8.17	18.67	-0.4	0.665
Spe.Cer	186	329	0.6	0.577	0.104	0.052	2	0.055	9.28	23.05	0.4	0.69
Sub.soil	-144	249	-0.6	0.566	0.047	0.039	1.2	0.245	-10.31	17.47	-0.6	0.56
NO_x	13	22	0.6	0.567	0.002	0.003	0.6	0.548	0.04	1.51	0	0.981
PM_{10}	**119**	**43**	**2.8**	**0.01**	-0.003	0.007	-0.4	0.698	**9.06**	**3.01**	**3**	**0.005**

Table 1. GLM analysis – factors influence Fe, Cd and Cu content in moss species. Statistically significant results are marked in bold. Spe – species: *Bra – Brachythecium rutabulum, Rhy – Rhytidiadelphus squarrosus, Sci – Sciuro-hypnum curtum,* and *Cer – Ceratodon purpureus.* NO_x – the modelled indicator of nitrogen oxides (µg/m^3) and PM_{10} – the modelled indicator of fine particles below 10µg (particulate matter, µg/m^3). Sub – substrata: soil and hard substrata (concrete, stone, asphalt).

The highest Cd content was measured in Nõmme (*Sciuro-hypnum curtum*), in Liivalaia (*Ceratodon purpureus*) and in Sõpruse (*Ceratodon purpureus*), respectively 0.37, 0.37 and 0.34 µg/g. The lowest Cd content was measured in Jäneda (*Brachythecium rutabulum*, 0.04 µg/g) and Õismäe and Lasnamäe (*Rhytidiadelphus squarrosus*). According to ANOVA analysis, the Cd content of *Syntrichia ruralis* in A areas was significantly higher (M = 0.165 µg/g, SD = 0.058) than that of *Sciuro-hypnum curtum/ Brachythecium rutabulum* (M = 0.101 µg/g, SD = 0.034), being statistically significant (F (1,10) = 5.35, p = 0.043). Cd content differences between species in B areas were not statistically significant.

The highest Cu content was measured in Sõpruse (*Brachythecium rutabulum*, 173 µg/g) and in Vabaduse square (*Sciuro-hypnum curtum,* 150 µg/g and *Syntrichia ruralis,* 149 µg/g*)*. The lowest Cu content was measured in Jäneda (*Syntrichia ruralis*, 3 µg/g and *Brachythecium rutabulum,* 5 µg/g) and Õismäe (*Rhytidiadelphus squarrosus*, 7 µg/g) (Fig 2). The higher figures of Cu content were near the crossings of major multi-lane streets – Sõpruse, Vabaduse square and Kristiine. The mean Cu content in *Syntrichia ruralis* (M = 90 µg/g, SD = 49) in areas A was higher than in *Sciuro-hypnum curtum* (M = 75 µg/g, SD = 65), but this difference is not statistically significant. There are also no significant differences in areas B between *Syntrichia ruralis* (M = 15 µg/g, SD = 5), *Sciuro-hypnum curtum* (M = 13 µg/g, SD = 2) and *Rhytidiadelphus squarrosus* (M = 10 µg/g, SD = 2).

Environmental factors affecting the content of heavy metals

The results of the GLM analysis (Table 1) show that the content of Fe in mosses is significantly influenced by the moss species and environmental pollution – in species *Sciuro-hypnum curtum* and *Rhytidiadelphus squarrosus*, the Fe content was significantly lower than in other species, and in an environment contaminated by fine particles, the content of Fe in moss was significantly higher. In the case of Cd, the GLM analysis did not show any significant influence factors among the studied – the moss species *Ceratodon purpureus* and *Rhytidiadelphus squarrosus* were borderline insignificant, with higher levels of Cd in *Ceratodon purpureus* and rather lower Cd concentrations in *Rhytidiadelphus squarrosus*. GLM results for Cu showed a significant effect of PM_{10} on metal content in mosses being significantly higher in PM_{10}-contaminated environments.

DISCUSSION

Bryophyte species in Tallinn and species suitable for bioindication

The moss *Ceratodon purpureus* and some *Bryum* species were also related to higher levels of environmental pollution by other researchers (Gilbert, 1968; Köhler, 2006). Naszradi et al. (2007) found *Syntrichia ruralis* (*Tortula ruralis*) to be a suitable moss for indication of heavy metal pollution due to its resistant nature against stress factors, inc. heavy metals. This species is rather tall among mosses, it is relatively easy to recognize and grows on various substrates, soils, concrete walls, and asphalt. *Syntrichia ruralis* tolerates a wide range of soil and moisture conditions and likes lighted habitat. *Syntrichia ruralis* is one of the eight selected species by Mallen-Cooper et al. (2023) that are abundant in ecosystems in temperate to arctic regions and play major functional roles. The moss *Ceratodon purpureus* is a species that is frequent and often abundant on substrates with moderate or high concentrations of heavy metals, sometimes occurring as dominants over large areas, but is also frequent in other habitats (Hill et al., 2007). Although *Ceratodon purpureus* grew even at the most polluted research sites in our study, samples of this species are difficult to collect because its shoots are shallow, and it is difficult to obtain a sufficient amount for analysis. *Brachythecium* species and *Sciuro-hypnum curtum* are very similar, so there may be problems with the differentiation of species, and also *Brachythecium* species and *Sciuro-hypnum curtum* are more associated with soil surfaces that are not found everywhere in cities. *Rhytidiadelphus squarrosus* is not found in many polluted areas, and the distribution of this species is also limited by the lack of suitable habitats in the city.

Heavy metals in different bryophyte species

Comparing Fe content results of this work with a study carried out in 1994 (Mäkinen & Liiv, 1996), where *Pleurozium schreberi's* maximum 1140 µg/g Fe was measured on the outskirts of Tallinn, here it is comparable to the maximum result of this study *Rhytidiadelphus squarrosus* in Lasnamäe – both species are pleurocarps. However, the highest iron content in our work from *Ceratodon purpureus* is far behind the iron content of the 90s in northeastern Estonia from *Pleurozium schreberi* – 8840 µg/g (Mäkinen & Liiv, 1996). In the Liiv and Kösta (2017) study, where the areas were selected that were at least 300 m from the trunk road and at least 100 m from the smaller road, the average of *Pleurozium schreberi* and *Hylocomium splendens* Fe collected from the surroundings of Tallinn is 291 µg/g and the Estonian average is 208 µg/g. As in the present study, Fe content was higher in the city centre than elsewhere in Tallinn at the Mäkinen and Liiv (1996) study peat moss balls, which were placed in the city for 2.5 months.

The Estonian average Cd content in *Pleurozium schreberi* and *Hylocomium splendens* is 0.11 µg/g and the average for the vicinity of Tallinn is 0.13 µg/g (Liiv & Kösta, 2017). In the mid-1990s in Tallinn, for example in Pääsküla, Pelgulinna, Kloostrimetsa and Muuga, the Cd content was higher than the Estonian average of that time (0.2 µg/g), but lower compared to other European countries. Cd levels were particularly high (0.7 µg/g per month) in Helsinki in 1981 in moss balls near a garbage incineration plant (Mäkinen & Liiv, 1996).

In 1994, the maximum copper concentration in the samples of *Pleurozium schreberi* on the outskirts of Tallinn was 38.2 µg/g. The highest concentrations of Cu were determined in peat moss balls kept in the city for 2.5 months, which were exhibited in the passenger port area (Mäkinen & Liiv, 1996).

The heavy metal content of acrocarp species *Ceratodon purpureus* and *Syntrichia ruralis* tended to be higher than in pleurocarps (*Brachythecium rutabulum*, *Sciuro-hypnum curtum*, *Rhytidiadelphus squarrosus)* in the case of 3 investigated metals – Fe, Cd and Cu. Our results are opposite to Govindapyari's (2010) findings that among mosses the profusely branched and ramifying pleurocarps and the densely packed acrocarps are more efficient entrappers and absorbers of metal particles than the unbranched and erect acrocarps. Folkeson (1979) studied heavy metal concentrations (inc. Fe, Cu and Cd) of two acrocarps and three pleurocarps and the concentrations varied decidedly between species (especially for iron) – one acrocarp and one pleurocarp species had a much higher Fe concentration than the other studied species. The differences between species are better explained by the differences in the growth rate and morphology of the species and the variability in the age of the green part (Pakarinen & Rinne, 1979; Govindapyari, 2010).

Environmental factors affecting the content of heavy metals

The results of the GLM analysis give the conclusion that the content of various heavy metals depends on the degree of fine particles in the environment and moss species, and it does not matter whether the moss grows on the soil or a hard substrate such as concrete, stone or asphalt. Stanković et al. (2018) have pointed out that moss species are often used together in the works, although their ability to collect heavy metals is different. Folkeson (1979) developed calibration factors between five moss species he studied, and he also does not advise the use of different monitoring species in a deposition survey without interspecies calibration.

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