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Biomonitoring of urban air pollution (particulate matter, trace elements and polycyclic aromatic hydrocarbons) using mosses *Sphagnum girgensohnii* Russow and *Hypnum cupressiforme* Hedw.

Doctoral Dissertation

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УНИВЕРЗИТЕТ У БЕОГРАДУ

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Гордана П. Вуковић

Биомониторинг загађености ваздуха градске средине суспендованим честицама, елементима у траговима и полицикличним ароматичним угљоводоницима коришћењем маховина *Sphagnum girgensohnii* Russow и *Hypnum cupressiforme* Hedw.

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Gordana Vuković

Biomonitoring of urban air pollution (particulate matter, trace elements and polycyclic aromatic hydrocarbons) using mosses *Sphagnum girgensohnii* Russow and *Hypnum cupressiforme* Hedw.

Abstract

With concerns of public health escalating, monitoring programmes are increasingly being promoted globally as means for assessing urban air pollution. Although regulatory monitoring has reached the stage of development that is challenging and promising, data collection of air pollutant levels is often unevenly distributed across examined areas. In this context, active moss biomonitoring appears as a complementary method to standardized measurements of air pollutants by filling gaps in the regulatory monitoring network. In urban areas, where mosses are often scarce or even absent, the "moss bag technique" has been developed for assessment of air pollution. The aspects concerning methodological steps in the technique – selection, preparation of moss, preparation of bags and post-exposure treatments, were tested. However, further investigation of various aspects of the technique application in the field, i.e., urban environment, is still required.

This doctoral dissertation addresses several key aspects in the practical application of the moss bag technique, especially in the urban environment, demonstrating its usefulness. In the series of experiments, the moss bags were exposed over the city of Belgrade, for ten-week period, to assess content of particulate matter (PM), major, trace, and rare earth elements (REEs), and polycyclic aromatic hydrocarbons (PAHs). After exposure, the moss samples were analysed by different analytical methods: magnetic measurements, inductively coupled plasma-optical emission spectrometry (ICP-OES), inductively coupled plasma-mass spectrometry (ICP-MS) and gas chromatography–mass spectrometry (GC-MS).

In the experimental set-up in five street canyons, one city tunnel, and four parking garages, vertical and horizontal spatial distribution of the elements were tested during the summer/autumn 2011 and winter 2011/2012, respectively. Within these urban environments, an emphasis was put on investigation of the exposure height of moss bag. In the street canyons, the moss bags were exposed at the heights of 4 m, 8 m, and 16 m. Based on the results, height of 4 m was recommended as optimal for the moss bag exposure. The results also indicated that the moss bags are sensitive to small-scale spatial distribution of the elements. An ability of the moss bags for biomagnetic monitoring was also outlined through the measurements of saturation isothermal remanent magnetization (SIRM). Very high correlation coefficients ($r \geq 0.95$) were found between moss SIRM values and moss concentrations of Cr, Cu, Fe, and Ni.

For extensive screening of urban air pollution over the city, the moss bags were exposed at 153 sampling sites during the summer 2013. Two species, *Sphagnum girgensohnii* (a species of the most recommended biomonitoring moss genus) and *Hypnum cupressiforme* (a common moss in the study area), were used. The content of magnetisable PM, trace elements, and PAHs were determined. Based on the results, the studied moss species could not be interchangeably used for biomonitoring purposes in urban areas, except for Cu. Similar city zones related to high, intermediate and low levels of airborne element pollution were distinguished according to the relative accumulation factors calculated for both moss species. Furthermore, new pollution hotspots, omitted by the regulatory monitoring, were identified. Moss magnetic analysis was represented to be an effective first step tool for screening of particulate air pollution prior to more complex and expensive chemical analyses.

To study an ability of the moss bags to reflect seasonal variability of air pollutants, the moss bags were exposed at 22 sampling sites during the winter 2013/2014. The experiment was an extension to the previous experiment performed during the summer. The results clearly demonstrate the seasonal variations of the moss enrichment with the air pollutants, especially PAHs, Sb, Cu, V, Ni, and Zn. The patterns of the moss concentrations of the REEs normalised against North American Shale Composite (NASC) and Post-Archean Australian Shales (PAAS) were identical across the study area, but enhanced by anthropogenic activities.

Finally, the moss bags were exposed across the city at 48 crossroads, two-, and one-lane streets during the summer 2014. The concentrations of 44 elements were determined in the moss samples. For the majority of the elements, a common pattern of concentration decrease was identified from crossroads to one-lane streets. A strong correlation between the moss concentrations of Cr, Cu, Fe, and Sb, and the site-counted traffic flow makes these elements as reliable traffic tracers.

The results of the dissertation demonstrate that moss bag technique could be applied as a pragmatic approach for optimizing the representativeness of regulatory monitoring networks. In addition, moss magnetic analysis is represented as an effective first step for screening of particulate air pollution before more expensive chemical analyses.

Keywords: urban air pollution, active moss biomonitoring, moss bag technique, *S. girgensohnii*, *H. cupressiforme,* SIRM, major and trace elements, REEs, PAHs

Major scientific field: Mathematics and Natural Sciences (Chemistry)
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- the project “*Investigation of climate change and its influences on environment – monitoring the influences, adaptations, and offsets*”, No III43007, Ministry of Education and Science of the Republic of Serbia;
- the project: “*Active biomonitoring of airborne major and trace elements in the Belgrade urban area using moss bags technique*”, supported by the Secretariat for Environmental Protection of Belgrade;
- the project: “*Biomonitoring of heavy metals in the air along the main roads of the city of Belgrade*”*,* supported by the Oil Company NIS Novi Sad and the City of Belgrade;
- the bilateral cooperation with Joint Institute for Nuclear Research, Dubna, Russia; and
- the COST Action FP1204, GreenInUrbs.

Based on the results and conclusions presented in this doctoral dissertation the following scientific papers were published in the international journals:

1. **Vuković G.**, Aničić Urošević M., Razumenić I., Goryainova Z., Frontasyeva M., Tomašević M., Popović A., 2013: Active moss biomonitoring of small-scale spatial distribution of airborne major and trace elements in the Belgrade urban area, Environmental Science and Pollution Research 20:5461–5470.
2. **Vuković G.**, Aničić Urošević M., Razumenić I., Kuzmanoski M., Pergal M., Škrivanj S., Popović A., 2014: Air quality in urban parking garages (PM10, major and trace elements, PAHs): Instrumental measurements vs. Active moss biomonitoring, Atmospheric Environment 85:31–40.
3. **Vuković G.**, Aničić Urošević M., Tomašević M., Samson R., Popović A., 2015: Biomagnetic monitoring of urban air pollution using moss bags (*Sphagnum girgensohnii*), Ecological Indicators 52:40–47.
4. **Vuković G.**, Aničić Urošević M., Goryainova Z., Pergal M., Škrivanj S., Samson R., Popović A., 2015: Active moss biomonitoring for extensive screening of urban air pollution: Magnetic and chemical analyses, Science of the Total Environment 521–522:200–210.
5. **Vuković G.**, Aničić Urošević M., Pergal M., Janković M., Goryainova Z., Tomašević M., Popović A., 2015: Residential heating contribution to persistent air pollutants (PAHs, major, trace and rare earth elements): A moss bag case study, Environmental Science and Pollution Research, doi 10.1007/s11356-015-5096-0

Биомониторинг загађености ваздуха градске средине суспендованим честицама, елементима у траговима и полицикличним ароматичним угљоводоницима коришћењем маховина *Sphagnum girgensohnii* Russow и *Hypnum cupressiforme* Hedw.

Резиме

Мјерење и праћење параметара квалитета ваздуха у градској средини представља обавезу дефинисану међународним и домаћим законодавством. Постављање мреже за мониторинг са задовољавајућом просторном резолуцијом мјерних мјеста ограничено је високом цијеном потребних инструмената, као и њиховог одржавања. Стога, активни биомониторинг коришћењем маховина све више добија на значају као метода комплементарна стандардизованим мјерењима загађујућих супстанци у ваздуху. У градским срединама, тзв. условима „антропогене пустиње“, гдје маховине одсуствују, развијена је примјена технике „врећице с маховином“. До сада су, у примјени ове технике, утврђени методолошки кораци, као што су избор одговарајуће врсте маховине, начин припреме врећица и одабир третмана након излагања врећица с маховином. Међутим, и даље је неопходна валидација ове технике у реалним условима, односно у градској средини.

У овој докторској дисертацији је испитивано неколико кључних корака у практичној примјени врећица с маховином за процјену садржаја суспендованих честица, елемената у траговима, елемената ријетких земаља и полицикличних ароматичних угљоводоника (ПАУ) у ваздуху градске средине. У низу експеримената, маховине у врећици су излагане атмосферској депозицији на подручју Београда, у периоду од 10 недеља. Након излагања, узорци маховина су анализирани различитим аналитичким методама: мјерења магнетних параметара, индуктивно спрегнута плазма-оптичка емисиона спектроскопија (ICP-OES); индуктивно спрегнута плазма-масена спектроскопија (ICP-MS) и гасна хроматографија-масена спектроскопија (GC-MS).

Вертикална и хоризонтална дистрибуција елемената у траговима је испитивана у експерименту спроведеном у пет улица кањонског типа, градском тунелу и четири паркинг гараже (љето/јесен 2011; зима 2011/2012). С циљем утврђивања оптималне висине за излагање у градској средини, врећице с маховином су излагане атмосферској депозицији на 4 m, 8 m и 16 m у улицама кањонског типа. На основу добијених резултата, висина од 4 m је препоручена као оптимална за излагање врећица с маховином. Такође, испитивањем и мјерењем магнетних параметара излаганих маховина, потврђена је могућност употребе врећица с маховином у биомагнетном мониторингу.

Да би се испитао квалитет ваздуха на ширем подручју Београда, врећице с маховином су излагане на 153 мјерна мјеста (љето 2013). Коришћене су двије врсте маховина, *Sphagnum* *girgensohnii*, као најпрепоручљивија биомониторска врста, и *Hypnum cupressiforme,* најзаступљенија врста у Србији. Значајна зависност између концентрација одређиваних елемената у обје врсте нађена је само за Cu. Према нивоу обогађења маховина честицама, елементима у траговима и ПАУ, издвојене су зоне са високим, умјереним и ниским нивоом загађености ваздуха. Такође, мјеста са повишеним нивоом загађујућих супстанци су препозната у зонама у којима се не спроводи инструментални мониторинг.

С циљем утврђивања да ли врећице с маховином одражавају сезонске промјене у концентрацијама загађујућих супстанци, врећице су излагане атмосферској депозицији на 22 мјерна мјеста током зиме 2013/2014. Добијени резултати указују на значајне сезонске промјене свих испитиваних загађујућих супстанци, а посебно ПАУ, Sb, Cu, V, Ni и Zn. Расподјела концентрација елемената ријетких земаља нормализованих у односу на доступне стандарде (енг. North American Shale Composite и Post-Archean Australian Shales) је била униформна на испитиваном подручју, али ипак зависна од повећања садржаја уличне прашине чија је ресуспензија изазвана повећаним интезитетом саобраћаја.

Током љета 2014., врећице с маховином су излагане на високофреквентним раскрсницама, двосмјерним и једносмјерним улицама. Примјећено је да концентрације већине испитиваних елемената значајно опадају од раскрсница преко двосмјерних ка једносмјерним улицама. Значајна корелација између концентрација Cr, Cu, Fe и Sb у маховини и протока возила на мјерним мјестима, чини ове елементе поузданим индикаторима емисија поријеклом из саобраћаја.

На основу резултата ове дисертације, техника „врећице с маховином“ се може препоручити као подесна за оптимизацију постојеће мреже станица за мониторинг загађујућих супстанци у ваздуху. Такође, мјерење магнетних параметара је представљнео као ефикасан начин за прелиминарно утврђивање загађености ваздуха честицама прије примјене скупљих хемијских анализа.

Кључне ријечи: ваздух градске средине, активни биомониторинг, маховине у врећици, *S. girgensohnii*, *H. cupressiforme*, биомагнетна мјерења, елементи у траговима, елементи ријетких земаља, ПАУ

Научна област: Природно-математичке науке (Хемија)
Ужа научна област: Наука о животној средини (Атмосферско загађење ваздуха)
УДК: 504.3.054

Докторска дисертација: „Биомониторинг загађености ваздуха градске средине суспендованим честицама, елементима у траговима и полицикличним ароматичним угљоводоницима коришћењем маховина *Sphagnum girgensohnii* Russow и *Hypnum cupressiforme* Hedw.“ рађена је у оквиру:

- пројекта „*Истраживање климатских промена и њиховог утицаја на животну средину – праћење утицаја, адаптација и ублажавање*“, бр. ИИИ43007, област интегралних интердисциплинарних научних истраживања финансираних од стране Министарства просвете, науке и технолошког развоја Републике Србије;
- пројекта: „*Активни биомониторинг тешких метала и других елемената у ваздуху Београда коришћењем врећица с маховином (moss bag technique)*“, који је подржао Секретаријат за заштиту животне средине Града Београда;
- пројекта: „*Биомониторинг тешких метала у ваздуху/едукација о утицају саобраћаја на квалитет ваздуха*“*,* који је подржала компанија НИС а.д. Нови Сад и Град Београд;
- билатералном сарадњом са Франковом лабораторијом неутронске физике Обједињеног института за нуклеарна истраживања у Дубни (Русија);
- COST програма „FP1204, GreenInUrbs“.

На бази резултата и закључака презентованих у овој докторској дисертацији објављени су следећи радови у врхунским међународним часописима (М21):

1. **Vuković G.**, Aničić Urošević M., Razumenić I., Goryainova Z., Frontasyeva M., Tomašević M., Popović A., 2013: Active moss biomonitoring of small-scale spatial distribution of airborne major and trace elements in the Belgrade urban area, Environmental Science and Pollution Research 20:5461–5470.
2. **Vuković G.**, Aničić Urošević M., Razumenić I., Kuzmanoski M., Pergal M., Škrivanj S., Popović A., 2014: Air quality in urban parking garages (PM10, major and trace elements, PAHs): Instrumental measurements vs. Active moss biomonitoring, Atmospheric Environment 85:31–40.
3. **Vuković G.**, Aničić Urošević M., Tomašević M., Samson R., Popović A., 2015: Biomagnetic monitoring of urban air pollution using moss bags (*Sphagnum girgensohnii*), Ecological Indicators 52:40–47.
4. **Vuković G.**, Aničić Urošević M., Goryainova Z., Pergal M., Škrivanj S., Samson R., Popović A., 2015: Active moss biomonitoring for extensive screening of urban air pollution: Magnetic and chemical analyses, Science of the Total Environment 521–522:200–210.
5. **Vuković G.**, Aničić Urošević M., Pergal M., Janković M., Goryainova Z., Tomašević M., Popović A., 2015: Residential heating contribution to persistent air pollutants (PAHs, major, trace and rare earth elements): A moss bag case study, Environmental Science and Pollution Research, doi 10.1007/s11356-015-5096-0

Content

1. Introduction

Air quality, especially in urban areas, has been continuously deteriorated with elevated emissions of many air pollutants from various sources. Atmospheric burdens with particulate matter (PM), trace elements and persistent organic compounds, impose threat to human health. Exposure to the elevated concentrations of air pollutants has been linked to many types of respiratory, cardiovascular, immunological, hematological, neurological, and reproductive problems. Because air pollution "affects every resident, is seen by every resident, and is caused by nearly every resident" (Mage et al., 1996), it is of a great importance to monitor and regulate air pollutant emissions. In the EU, current air quality monitoring strategy is mainly driven by a need to achieve and to comply with limit values of few air pollutants – PM, Cd, Cr, Ni, and Pb along with benzo[a]pyren (Directive 2004/107/EC; Directive 2008/50/EC). Regulatory policy measures have permanently been revised (e.g., Clean Air Programme for Europe, revised National Emissions Ceiling Directive) to improve air quality due to emergence of new pollutants and need to tighten existing targets.

From the aspect of good monitoring practice, current data collection about the air pollutant levels is uneven and incomplete, and available data are more detailed in developed countries while developing countries face information shortfalls. Although existing monitoring techniques provide high quality of data, with low detection limits, monitoring is limited with several practical constraints. Namely, instrumental monitoring devices are primarily expensive, and require power supply and permanent maintenance. The instruments usually operate for one-day period because repetitive deployment for a few or more weeks is not financially feasible. Thus, instrumental measurements could not characterize long-term exposure of humans to air pollutants. The above-mentioned implies the need for development of other monitoring techniques such as low-cost multi-component analysers with low power consumption, which would be easy outdoor-installable (Kulbusch et al., 2014).

Over the past several decades, biomonitoring has been developed as a valuable tool for assessing environmental pollution. Biomonitoring represents usage of living organisms (plants, animals) as a measure of environmental quality. Among the terrestrial organisms employed as plant biomonitors, mosses have been proven as reliable bimonitors of air quality due to their morpho-physiological characteristic. Between the two types of moss biomonitoring, passive (using naturally growing mosses in a certain area) and active (using moss transplants), the active approach has been applied for studies in areas, such as industrial and urban, where naturally growing mosses are scarce or even absent. Since the early work of Goodman and Roberts (1971), "moss bag technique" was introduced as a method of active moss biomonitoring. The technique involves exposure of anthropogenically unpolluted moss material within mesh bags for monitoring of presence of pollutants in air. The moss bag technique gives a reliable picture of pollution patterns in a much more cost-effective way than regulatory monitoring devices, which thus could not provide dense network of monitoring sites, and require power supply and maintenance. In addition, the technique also integrates the air pollutant levels over longer time than most of instrumental measurements do. Although, several methodological steps regarding the technique are established (Ares et al., 2012), investigation of different aspects of the technique application, particularly in urban environment is still required.

This doctoral dissertation represents an upgrade of the state of the art in this field addressing the practical application of the "moss bag technique" as a method of active moss biomonitoring of the air pollutants under complex conditions of urban topography. The dissertation is an extension to the candidate's master thesis entitled "Активни биомониторинг елемената у траговима у ваздуху градских улица кањонског типа и тунелу коришћењем маховина *Sphagnum girgensohnii* Russow" (in Serbian). The main objective was to investigate several methodological features of the application of the "moss bag technique" in urban environment demonstrating its usefulness.

The specific focuses were:

1. To test an ability of the moss bags to reflect small-scale changes in the horizontal and vertical distribution of airborne major and trace elements in the urban microenvironments such as street canyons and city tunnel (Experiment 1);
2. To evaluate a reliability of the moss bags for monitoring of trace element air pollution within enclosed spaces such as parking garages (Experiment 2);
3. To evaluate an ability of the use of moss bags for biomagnetic monitoring through measurement of saturation isothermal remanent magnetization (SIRM), as a proxy of particulate air pollution, across the urban microenvironments (Experiment 3);
4. To test whether the moss bag data could be used to produce a credible city zonation of air pollution (Experiment 4);

5. To establish if the moss bags could reflect seasonal variation in the pollutant levels during the winter and summer, i.e., heating and non-heating season (Experiment 5);
6. To test whether moss bags could reflect distance-decline of the toxic element concentrations on a micro-scale from crossroads to two- and one-lane streets depending on variable traffic burden in urban area (Experiment 6).

Basic characteristics of the air pollutants – PM, major, trace and rare earth elements, and polycyclic aromatic hydrocarbons (PAHs), along with their origin in urban air, are described in the Theoretical part of this dissertation. The importance of research and motivation for monitoring of the above-listed pollutants are explained through their impact on human health. The current air quality standards are also presented. The Theoretical part of the dissertation provides an overview of literature data on biomonitoring with special emphasis on the active biomonitoring using moss bags. Basic principles and the value of biomagnetic monitoring of suspended particles are also given.

The set-up of six experiments along with chemical and magnetic analyses of the moss bag samples are described in the section Material and Methods. In the Results and Discussion section, the findings of each performed experiment were discussed. Detailed description of the vertical and horizontal distribution pattern of the element concentrations are given regarding specific topography and microclimate conditions in the street canyons and city tunnel (Experiment 1). The ability of the moss bags for air quality monitoring within enclosed hotspots such as parking garages was discussed (Experiment 2). In addition, data of the moss bag and instrumental monitoring simultaneously performed in the Experiment 2 were presented. Within the results of the Experiment 3, potential of the use of moss bags in biomagnetic monitoring of PM pollution in urban environments was elaborated. Following the small-scale studies, the moss bag data of the extensive screening of air pollution by particles, trace elements, and PAHs, were given (Experiment 4). The moss interspecies comparison of the uptake of certain air pollutants by the moss bags was also presented. Concerning available literature, an upgrade in the moss bag application for the PAH biomonitoring was discussed within the results of Experiment 5. Finally, better characterization of micro-scale siting for monitoring of distribution of the airborne toxic elements was assessed by the moss bags (Experiment 6). The whole discussion of the results of the dissertations is summarized in the Conclusion section.

2. Theoretical part

2.1. Urban air pollution: regulatory monitoring

Urban air pollution is a major environmental and health problem all over the world (Lim et al., 2012). More than 70% of the European population lives in urban areas (EEA, 2013). This results in increase of anthropogenic activities and consequently, elevated air pollution. According to World Health Organization (WHO, 2011), the mortality in cities with a high pollution level is higher by 10 – 20% than that observed in relatively cleaner cities. Thus, more than 2 million of premature deaths annually can be attributed to the effects of urban outdoor air pollution.

Concerning health issues, PM appears as one of the most important air pollutants. Numerous studies have linked exposure to the elevated concentrations of PM to the high rate of deaths from cardio-vascular and respiratory diseases, and cancers (e.g., Curtis et al., 2006; Kelly and Fussell, 2012). Organic and inorganic chemicals adsorbed on the surfaces of PM determine its toxicological characteristics. Because of the suspected carcinogenity of PAHs and some metals, i.e., Cd, Ni, Cr, and Pb, regulatory monitoring of these pollutants is recommended by EU legislation (Directive 2004/107/EC). Transition metals, such as V, Fe, Ni, Cu, and Zn, remain a group of components for which reduction measures will most likely lead to improvement of the population health (WHO, 2013). Exposure to another group of persistent pollutants – rare earth elements (REEs), has also arisen questions about their detrimental health effects (Pagano et al., 2015) since their utilization is growing worldwide (US EPA, 2012).

The European Union Member States are bound under Decision 97/101/EC to engage in a reciprocal exchange of information on ambient air quality. For this purpose, AirBase is formed as database system maintained by the European Environmental Agency (EEA). The air quality database consists of a multi-annual time series of air quality data and statistics for a number of air pollutants. The regularly revisions are also recommended for the evidence on the health effects of air pollutants and the implications for air quality policy (WHO, 2013). Despite the upswing in air quality monitoring, many cities in low- and middle-income countries, which are subjected to deteriorated environmental and urban air conditions, still lack a capacity to monitor air quality (WHO, 2014).

Data collection of the air pollutant levels is not only limited by the expensiveness of equipment, also by a lack in accessibility of sampling sites, electrical supply, and technical capacities (Engel-Cox et al., 2013). Regulatory network of air quality monitoring stations have been typically designed to assess regional and temporal air pollution variability. However, on a micro-scale specific urban topography may contribute to creation of poor air dispersion conditions, giving rise to contamination hotspots. High pollution levels have been observed in urban microenvironments such as city tunnels, crossroads, bus stations, street canyons, etc. In addition, recent research has indicated that monitoring stations may not accurately characterize the spatial complexities of the air pollutant dispersion across an urban area (Wilson et al., 2005; Brauer, 2010).

Besides the spatial changes, the levels of air pollutants greatly vary with time depending on atmospheric conditions and dominant sources of emissions. Namely, in many countries with moderate to continental climates, fossil fuel and wood combustion processes are particularly intensified during the winter, i.e., heating season, which lead to elevated concentrations of air pollutants. In addition, stagnant atmospheric conditions, low temperature and strong temperature inversion in winter (Engler et al., 2012) aid increasing of the concentrations of pollutants (Perišić et al., 2014; Wang et al., 2014).

Regarding to the all above-mentioned, assessment of air quality requires more detailed approaches. Biomonitoring, as a cost-effective and easy-performed method, could be integrated well with regulatory measurements, aiming to wider spatio-temporal coverage, and to larger and more detailed database of air pollutants (see section 2.2. Biomonitoring).

2.1.1. Particulate matter

Particulate matter is a term generally used for a multi-phase system comprising liquid and solid particles, suspended in air, with a wide range in size and chemical composition (EEA, 2012). The properties of atmospheric PM are presented in the following (Seinfeld and Pandis, 1998). Particulate air pollution originated from both, natural and anthropogenic sources. The natural sources of PM include wind-blown dust and soil particles, volcanic ash, sea salt spray, and products of forest fires, along with existing particles, which are chemically altered in the air. The anthropogenic sources of PM encompass:

- fossil fuel combustion by power plants;

- industrial processes such as production of metals, chemicals, lime, and cement, along with mining activities and construction work;
- diesel and gasoline vehicles exhaust emissions; and
- traffic-originated non-exhaust emissions such as brake and tyre wear, and road dust re-suspension.

The main local sources of PM in urban areas are vehicle exhaust, road dust re-suspension and burning of wood, fuel or coal for domestic heating. Important distinction of the PM air pollution is based on how the particles are released into the atmosphere:

- Primary particles originated directly from their sources, mainly by combustion processes. The main sources of these PM are traffic, stationary combustion (domestic coal burning) and industrial processes. Soil and dust re-suspended particles along with marine aerosol are also classified as primary PM;
- Secondary particles are formed by transformation of primary pollutants due to: 1) chemical reactions, involving so-called PM precursor gases – SO_2, NO_X, NH_3 and volatile organic compounds, for creation of either secondary inorganic or organic PM; 2) the processes of nucleation and condensation of gases with a low vapour pressure; and 3) the processes of coagulation.

A number of features such as size, density, shape, and chemical composition characterizes particulate matter. The behaviour of PM in the atmosphere, and the effects on the environment and human health, are strongly dependent on the PM diameter size, varying from 0.001 to 100 μm. Total suspended particles comprise those smaller than 40 μm. Particles which are the most important from the aspect of atmospheric chemistry and health effects can be classified as:

- coarse particles (PM10), refers to fraction with a diameter smaller than 10μm;
- fine particles (PM2.5), refers to fraction with a diameter smaller than 2.5μm; and
- ultra-fine particles (PM0.1), refers to fraction with a diameter smaller than 0.1 μm.

Particle size distribution varies significantly with the physical features and chemical composition. The coarse PM contributes to the major proportion of PM mass in urban areas (**Figure 2.1**). This class includes the most visible forms of PM, such as black smoke, pollen, soil and dust particles, along with mechanically generated particles. Note that PM10 comprises ambient PM including fine and ultra-fine PM. The PM2.5 fraction contains primary combustion and secondary PM, which have been caused by coagulation and condensation. The ultra-fine particles dominate surface area of PM but do not contribute large quantities to its mass. This size fraction originate from primary combustion emissions and secondary particles produced by gas-to-particle conversion processes. They are inherently unstable and grow into larger particles through coagulation and condensation.

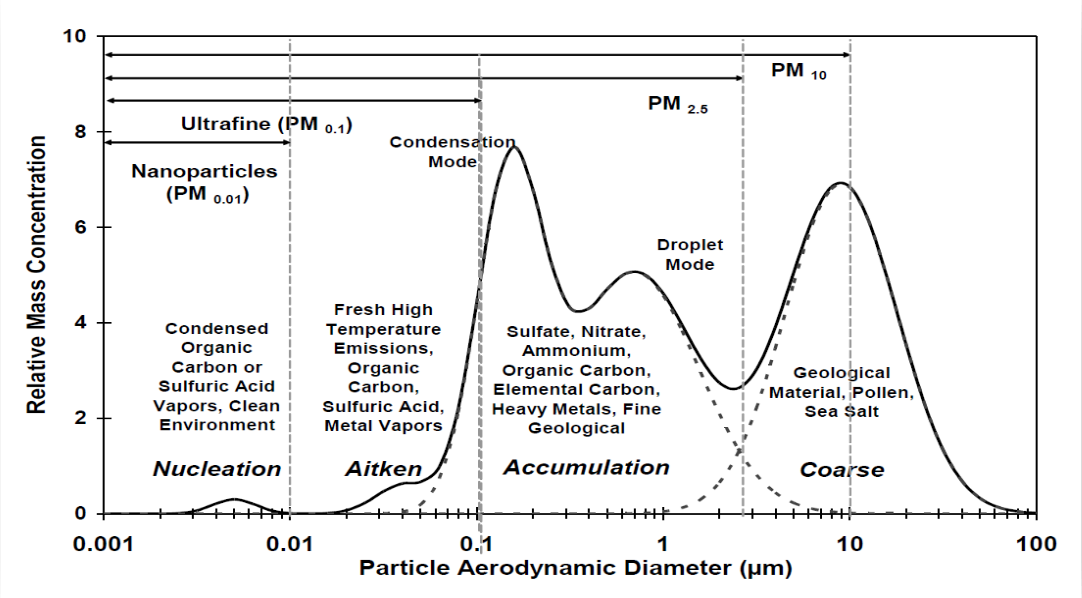

Figure 2.1. Relative ratio of the particle mass in air of urban areas and distinctive composition of the PM of individual sizes (Watson et al., 2002).

According to their size, PM is divided into groups based on inhalation and penetration processes into the lungs (Kelly and Fussell, 2012). The smaller the particles, the deeper they penetrate into the lungs. Coarse PM10 are sometimes termed "thoracic" as they can escape the initial defences of the nose and throat and penetrate beyond the larynx to deposit along the thorax. Fine PM2.5 is also referred to as "respirable" particles due their ability to penetrate into the alveolar gas exchange region of the lungs. Finally, PM0.1 presents a particular health threat because their small size allows greatest lung penetration and onward passage across the air blood barrier.

Besides size, the environmental and health effects of PM are also strongly dependent on their chemical composition. The main components of which PM consist are (Phalen, 2002):

- inorganic compounds – inorganic ions and trace elements;
- organic compounds – volatile organic compounds and PAHs;

- elemental carbon; and
- organic carbon.

In Europe, approximately 30% of the PM10 concentrations and 50% of the PM2.5 concentrations in the regional background consist of inorganic chemical substances. Organic substances contribute 20% in average to the PM10 concentrations and 30% to the PM2.5 concentrations (EEA, 2012).

Epidemiological studies attribute the most severe health effects from air pollution to PM. Particulate matters pose a health risk even at concentrations below current maximum allowable values. Particle-induced mortality and morbidity are clearly associated with the PM2.5 fraction, reflecting their detrimental effects on the respiratory and cardiovascular system (e.g., Mortimer et al., 2008). The PM2.5 fraction represents 40–80% of the PM10 mass concentration in ambient air in Europe (EEA, 2012). The PM 10 fraction has also been reported as damaging to human health. Up to 88% of the EU urban population is estimated to be exposed to PM concentrations exceeding the WHO reference values (EEA, 2013). The need for regularly monitoring of PM (Directive 2008/50/EC) is emphasized, and the PM10 and PM2.5 limit and target values to achieve health protection are set. Note that toxicological characteristics of PM greatly depend on various inorganic and organic chemicals adsorbed on PM surfaces (e.g., trace elements and PAHs). Thus, it is of great importance to characterize chemical composition of PM.

2.1.2. Trace elements

Several definitions are used to describe the term “trace elements” although precise definition has never been established by any authoritative institution such as the International Union of Pure and Applied Chemistry (IUPAC). In general, the term “trace” is related to the abundances of elements in any medium and includes elements that are present in very low concentrations, ppm or less, in some specified source (Bargagli, 1998). Therefore, some elements that are trace in biological materials are not trace in the environmental mediums such as water, soil, or air.

In the available literature, “trace elements” have been widely misnamed with the term “heavy metals”. The term “heavy metals” have often been associated with toxic effects that elements such as Cd, Ni, Pb, and Cr have on the living organisms. The term also refers to the elements that are essential for plants in a small amount, whereas in excess may be toxic (Cu, Mn, Fe, Co, Zn, and Mo). Based on the chemical characteristics, i.e., specific density, "heavy metals" include those metals or metalloids that have a density greater than 4.5 g cm^{-3}. In addition, some "lighter" metals such as Al (2.7 g cm^{-3}) and Be (1.85 g cm^{-3}), along with metalloids As (5.72 g cm^{-3}) and Sb (6.68 g cm^{-3}) and non-metal Se (4.8 g cm^{-3}), are classified in this group due to their high toxicity (Markert, 1993). However, the definition of "heavy metals" could be also based on relative atom mass, atomic number, or other chemical properties (Duffus, 2002).

Related to the element physiological functions, the other terms – "micronutrients", "essential elements", and "toxic elements", could be found. In the literature, the term "toxic elements" is rather imprecise because the degree of toxicity of elements varies greatly from element to element and from organism to organism. Toxicity should be defined refering to a dose–response curve for the species under consideration (Duffus, 2002). In this dissertation, the term "trace" would be used to indicate all elements which concentrations in the analyzed moss are lower than 1000 μg g^{-1} (Bargagli, 1998), irrespective of how essential or toxic these elements might be. The term "major" is related to Al, Ca, Fe, K, Na, Mg, and Zn.

Major and trace elements in the atmosphere are mainly associated with PM of diameter between 0.01 and 100 μm, because of the coagulation or adsorption processes. Some of the elements, such as Hg, can occur in gaseous state. Major and trace elements originate from both, anthropogenic and natural sources (**Table 7.1.1., Appendix 1**). The elements emitted from the natural sources are mainly involved in the slow biogeochemical cycles of non-reactive constituents of the atmosphere, which in a long period have uniform concentrations. Anthropogenic sources (**Table 7.1.1., Appendix 1**) of trace elements are numerous and encompass industrial activities such as mining and metallurgical processes, combustion of fossil fuels, agricultural production, and treatment of waste materials. Smaller sources of trace elements include cement factories, the production of batteries, paints and varnishes. In urban areas, road transport is among the dominant sources of airborne trace elements. There are four main traffic-related sources: 1) the diesel or gasoline fuel combustion; 2) the lubricant oils; 3) the engine wear or abrasion of after-treatment system; and 4) non-tail pipe emissions from tyre wear, brake wear and possibly from road abrasion (Pulles et al., 2012).

The atmospheric deposition of major and trace elements, both wet and dry, depends on the particles diameter. The particles of larger diameter have a tendency to precipitate quickly close to the emission sources whereas the particles less than 10 μm in size may exist in the air from days to months, and may be transported to distant regions. Accumulation or entrapment of trace elements by living organisms after emissions depends on the element's state during transfer: gaseous, liquid or solid; neutral or ionic; dissolved in an aerosol or adsorbed on solid matrices. During deposition and accumulation, the element's state may change deeply, e.g., through oxidation or reduction in the soil, precipitation of insoluble forms, solubilisation in water, adsorption on different types of clays or integration in organic matters issued from living beings (Clemens et al., 2002).

Exposure to airborne trace elements has been often cited to have adverse health effects. The growing interest is invariably paid on transition metals such as Cu, Cr, Ni, Fe, and V, based on their potential to produce reactive oxygen species in biological tissues. These metals contain unpaired electrons in d-orbital, and thus, are capable to generate free radical species. In addition to transition metals, non-redox active metals, such as Al, Pb, and Zn, can affect the toxic effects of transition metals, by either enhancement or reduction of the production of free radicals. Toxicology studies linked daily mortality, pulmonary injuries, and inflammation with increased concentration of Cr, Cu, Fe, Mn, Ni, V, and Zn (e.g., Kelly and Fussell, 2012). In addition, the International Agency for Research on Cancer (IARC, 2013) classified the Cd, Ni, As, and Cr (VI) compounds as carcinogenic to humans (Group 1), while Sb (III) oxide is classified as a possibly carcinogenic to humans (Group 2B).

The Protocol for Heavy Metals under the UNECE Convention for Long Range Transboundary Air Pollution (LRTAP) emphasizes the importance of monitoring of trace elements in terms of air pollution (UNECE, 1998), aiming at reduction of their levels in the air. Based on health impact criteria, important regulations related to the concentrations of As, Cd, Hg and Ni in ambient air are set by the Directive 2004/107/EC.

2.1.2.1. Rare earth elements

The IUPAC have identified 15 transition elements, from lanthanum ($_{57}$La) to lutetium ($_{71}$Lu), as lanthanides, which include $_{57}$La, $_{58}$Ce, $_{59}$Pr, $_{60}$Nd, $_{61}$Pm, $_{62}$Sm, $_{63}$Eu, $_{64}$Gd, $_{65}$Tb, $_{66}$Dy, $_{67}$Ho, $_{68}$Er, $_{69}$Tm, $_{70}$Yb, and $_{71}$Lu. Due to similar physiochemical properties, the lanthanides often occur together as elemental constituents of their host minerals. Two other metals commonly found in association with lanthanides in the same mineral assemblages are scandium ($_{21}$Sc) and yttrium ($_{39}$Y). The lanthanides along with Y and Sc are commonly referred to as rare earth elements (REEs). The term "rare" is a carryover from difficulties related to metallurgical processes needed for isolation of individual element (Gupta and Krishnamurthy, 2004). Specifically, the REEs do not occur as native elements in nature, only as a part of mineral ores. Since the REEs are very chemically similar to one another, separation of individual REE from the host mineral has been very complex.

The abundance of the REEs in the Earth's crust (**Table 7.1.2., Appendix 1)** varies from 0.3 mg kg^{-1} for Tm to 60 mg kg^{-1} for Ce (US EPA, 2012). The REEs with even atomic number, e.g., Ce, Nd, and Sm, are more common in nature than REEs with odd atomic number, e.g., Eu, La, and Pr. This property is known as Oddo-Harkins rule (Vernadsky, 1980). According to the crustal abundances of certain REEs, they are divided into light (Ce, Eu, Nd, Pm, Pr, Sc, Sm, and La) and heavy (Dy, Gd, Ho, Er, Tb, Tm, Yb, Lu and Y) REEs.

Given the global affinity for green and sustainable products in energy, military and manufacturing industries, the REE utilization is growing worldwide in various modern commercial and industrial processing, products and technologies (**Figure 7.1.1., Appendix 1**). The REEs are mostly used in metallurgical processes (29%) follows by its application in electronics (18%), chemical catalysts (14%), and audio and computer systems (12%). The REEs are also used in the industry of automotive catalytic converters (9%), (US EPA, 2012).

Despite the wide use of the REEs, hence increasing environmental and human exposures, these elements have been neglected as xenobiotics up to recent years. The limited epidemiological data are available about the specific health effects of the REEs (US EPA, 2012). The toxicological investigations on the REE health effects have been mostly focused on Ce and La, with less information available for Gd and Nd (Pagano et al., 2015). The data indicate that pulmonary toxicity of the REEs in humans may be of concern. These negative health effects could be associated with redox action mechanisms involving formation of reactive oxygen species, lipid peroxidation and modulation of antioxidant activities. A detailed review about health effects and toxicity mechanisms of the REEs is given by Pagano et al. (2015). Based on the above-mentioned, further studies are required, aiming to identify environmental compartments (air, water, and soil) and the corresponding loads (air emissions, water discharges etc.) of the REEs (US EPA, 2012).

2.1.3. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons are group of complex organic compounds constituted by carbon and hydrogen atoms, which form at least two condensed aromatic rings (**Figure 7.1.2., Appendix 1**). They are often classified as low- (2-ring), medium- (3- and 4-ring), and high- (5- and 6-ring) molecular weight PAHs. These compounds usually occur as a mixture rather than single compounds. At ambient outdoor temperature, 70 – 90% of PAHs are adsorbed on PM, mostly on small inhalable particles (Ravindra et al., 2008).

The PAHs are mainly formed during the incomplete combustion and pyrolisis of fossil fuels and biomass. A small quantity of PAHs in the atmosphere originates from natural sources such as volcanic emissions, forest and grassland fires. The main anthropogenic sources of PAHs can be divided into two groups: pyrogenic and petrogenic. Pyrogenic PAHs form during the combustion of fossil fuel and biomass from automobiles, power plants, industrial coal and petroleum burning, whereas petrogenic PAHs originate from combustion of crude oil and its products, including kerosene, gasoline, diesel fuel, and lubricating oil. Classifying by domestic, mobile, and industrial anthropogenic sources, as main contributors to PAH emissions has also been suggested (Ravindra et al., 2008). The examples of the PAH emission rates from various sources are given in **Table 7.1.3.** (**Appendix 1**).

The transport, deposition, and chemical transformation of PAHs in air depend on their gas/particle phase partitioning. The gas/particle partitioning of PAHs depends on: 1) the PAH vapor pressure; 2) ambient temperature; 3) the PAH concentrations; and 4) type of particles present in the atmosphere (Baek et al., 1991). The high-molecular-weight PAHs are adsorbed on airborne coarse PM and deposited close to the emission sources. The low-molecular-weight PAHs are presented in gas phase. These compounds can be removed from the atmosphere by wet or dry deposition, or by photochemical transformations including photo-degradation and photo-oxidation. In addition, increased concentrations of PAHs in winter are most likely due to: 1) reduced vertical air mixing caused by inversion; 2) less intensive atmospheric reactions; 3) enhanced sorption to particles at lower temperature (as a result of reduced vapor pressure and/or shifting in the gas/particle distribution induced by ambient temperature variation); and 4) increased emissions from residential heating system (Ravindra et al., 2006).

Increased attention has been paid to the PAH emissions in air due to their adverse health effects. Due to their persistence, mobility, tendency for bioaccumulation and toxic health effects, these compounds have been included in the Convention on Long Range Transboundary Air Pollution Protocol on Persistent Organic Pollutants (Council Decision 2004/259/EC). The US EPA has listed 16 priority PAHs (**Figure 7.1.2., Appendix 1**) for monitoring because: 1) they are more abundant; 2) exposure to these PAHs are more likely to occur; and 3) they are suspected to be more harmful. Eight of them have been classified (US EPA, 1997) as potentially carcinogenic: B[a]A, Chr, B[a]P, B[b]F, B[k]F, DB[ah]A, I[cd]P, and B[ghi]P. In addition, the IARC (2013) has classified B[a]P as carcinogenic to humans (Group 1), B[a]A as probable carcinogenic to humans (Group 2A), and Chr, B[b]F, B[k]F, DB[ah]A, I[cd]P as possibly carcinogenic to humans (Group 2B). Although many PAHs are selected to be priority pollutants, emission of only B[a]P is regulated by the Directive 2004/107/EC, as this compound is considered to be an indicator of the remaining PAHs. The maximum atmospheric concentration of B[a]P are set in the ambient air by this Directive.

2.2. Biomonitoring

Biomonitoring is defined as the use of living organisms, plants, or animals, to obtain information on the environmental quality over space and time, i.e., the response of living organisms to changes in their environment. In the following, the main terms that have been defined in the field of biomonitoring are given (Bargagli, 1998; Walterbeek et al., 2002; Markert et al., 2003). Organisms, populations, biocenoses and the whole ecosystems are influenced by numerous biotic and abiotic stress factors, which affect biological systems at different levels of organisation, from individual enzyme systems through cells, organs, single organisms, and population to entire ecosystems. The relevant information in biomonitoring is commonly deduced either from changes in the behaviour of the living organisms (e.g., presence or absence of species, the morphology changes etc.) or from the concentrations of specific substances in the organism tissue. Over the past several decades, biomonitoring has been developed and accepted as a valuable tool for assessing environmental pollution. In this dissertation, the use of lower plants – mosses, will be presented for the monitoring of airborne pollutants (suspended particles, trace elements, and PAHs).

Living organisms including plant species are diversified according to their responses to changes in environment and could be defined as:

- **bioindicator** – an organism (or part of an organism or a community of organisms) that contains information on the quality of the environment (or a part of the environment); and
- **biomonitor** – an organism (or part of an organism or a community of organisms) that contains information on the quantitative aspects of the quality of the environment.

Based on the responses, which exhibited in the presence of certain substances, bioindicator/biomonitors could be diversified on accumulative, and effect or impact organisms. Impact indicators/monitors demonstrate specific or nonspecific effects in the response to exposure to a certain compound or a number of substances. Accumulation indicators/monitors have capability to take up one or more compounds from their biotic and abiotic environment. Thus, two mechanism of accumulation are recognized:

- **biomagnification** refers to the absorption of the substances from nutrients through epithelia of the intestines. Therefore, this mechanisms is characteristic for many land animals, except in the case of volatile compounds which are taken up through respiratory system.; and
- **bioconcentration** is direct uptake of the substances from the surrounding media through tissues or organs. Plant biomonitors, i.e., moss, can only uptake substances in this way.

Referring to the selection of species for monitoring of air quality, an ideal plant biomonitor should satisfy several key aspects. The selected plant species should be: 1) tolerant to pollutants at relevant levels; 2) very sensitive to a specific air pollutant, i.e., the species should have a low threshold when exposed to the ambient concentrations of pollutants; and 3) available to give reproducible response at any time or season. Additional requirements, specific for the monitoring of air pollutants, are (Sloof, 1993): 1) the selected species should be common and widely distributed in a given area; 2) the pollutant uptake should be independent of local conditions prevailing in the surrounding environment; 3) the pollutant uptake should not be influenced by regulating biological mechanisms or antagonistic or synergistic effects; 4) the average concentrations of pollutants should be available over a suitable period as a result of integrated exposure over a time period; 5) the organism should not take up appreciable amounts of elements from sources other than atmospheric (e.g., when naturally growing plants are used, the uptake of pollutants from the surface should be minimized); 6) the initial concentrations of pollutants should be low; 7) the sampling and sample preparation should be easy-performed; 8) the accumulation should reach to the concentration levels which are accessible by referent analytical techniques.

In general, two approaches of biomonitoring have been applied:

- **passive biomonitoring** refers to *in situ* use of the naturally growing plant in a given study area; and
- **active biomonitoring** refers to the use of the selected or cultivated plant species exposed in a standardized form in the field for a defined period of time.

Among the terrestrial organisms applied as plant biomonitors, mosses, lichen and tree leaves have often been used for monitoring of air quality. Biomonitoring of air quality using mosses and lichens was promoted as a reliable tool for indirect monitoring of emissions (JRC Reference Report on Monitoring of emissions from IED-installations, 2013). This method could complement direct emission measurements and/or dispersion modelling, by demonstrating possible biological effect, in particular, if there are diffuse emissions, which do not allow direct emission measurements. In addition, the technical working groups (WG) of European Committee for Standardization (CEN) have been dealing with methods of biomonitoring of air pollution using mosses and lichens (CEN/TC 264/WG 31). The WG 31 works to provide a method for assessing epiphytic lichen diversity. It aims for "assessing the impact of anthropogenic intervention, particularly for estimating the effects of atmospheric pollution". Guidelines for biomonitoring of ambient air quality using mosses have recently been established by the standard numbered EN 16414:2014. It describes the sampling protocol and the sample preparation of *in situ* mosses to monitor the bioaccumulation of atmospheric pollutants. All necessary steps from the sampling of mosses until the preparation for the final analysis are covered. The standard addresses "all operators wishing to conduct air quality biomonitoring studies". The *in situ* mosses are used as collectors for different substances and described to be capable to identify and localize emission sources, as well as background pollution levels. Finally, an integrated approach using data obtained by both biomonitoring to mosses and lichens along with regulatory monitoring should be applied for air quality assessment.

2.2.1. Mosses as biomonitors

Bryophytes encompass the following divisions: the hornworts (Anthocerotopsida), the liverworts (Marchantiopsida) and mosses (Bryopsida). Bryophytes are autotrophic cryptogames comprising approximately 25,000 species, which can be found all over the world, from humid to arid regions. Bryophytes occur at almost every kind of terrestrial substrate such as: peat bogs, water springs, alpine grassland, bare stones, bark, and skeletons, and grow in fresh water but are absent from saline habitats (seas, oceans).

Mosses do not have clearly differentiated main organs such as root, stem, and leaf. The analogue parts of these organs are rhizoids, cauloide, and phyllodes, respectively. Although some mosses have specialized tissue for the transport of water, it is not considered true vascular tissue. Mosses are poikilohydric organisms, i.e., they have capacity to tolerate dehydration, and to recover from it without physiological damage. The most of mosses are ectohydric – lacking a root system and cuticle on leaves, they absorb water, nutrients, and toxic substances mainly via the entire plant tissue from dry and wet deposition (Brown and Bates 1990).

Compared to the other plants, mosses have the greatest comparative advantages as bioindicators of air pollution by organic and inorganic substances due to its morpho-physiological characteristics, as well as cosmopolitan abundance (Bargagli, 1998, Markert et al., 2003). The main advantages are the following:

- the lack of a fully developed root system minimize the accumulation of elements from the substrate;
- the airborne elements are adopted via the entire moss surface due to absent or poorly developed cuticle on the surface of rhizoids and phyllodes;
- high ion (cation)-exchange capacity of the cell membrane;
- high mass to surface ratio which enables the high capacity for adsorption and retention of both organic and inorganic pollutants; and
- mosses exist as the homogeneous population in nature, i.e., communities built by individuals of the same species. Thus, only variation less than 10% in the pollutant initial content in the same moss turf could occur.

The sensitivity of moss was compared to the other biomonitors and materials used as pollution indicators with respect to their accumulation capacities and ability to reflect different pollution levels. Based on the cytological stress signals and vitality tests, lichens proved to have a better resistance to environmental stress, but researchers highlighted higher capacity of the mosses to intercept most of the airborne elements compared to the lichens (e.g., Spagnuolo et al., 2011). The mosses also showed better entrapment capacity in comparison with artificial materials, such as cellulose and cation exchange filters (Adamo et al., 2007; Giordano et al., 2013). Differences in the surface structure have been proposed as one of the main factors explaining these diversities between the used biomonitors and materials (Adamo et al., 2007).

Mineral requirements of the mosses are similar to those in vascular plants. Mineral uptake by the moss cell is controlled by a semipermeable membrane. The basic source of minerals for mosses is atmospheric deposition – wet and dry (Bates and Bakken, 1988). Positive correlation was found between the quantity of rainfall and nutrient concentrations in moss (Brown and Bates, 1990). Several mechanisms could be associated with the uptake of minerals/elements by the moss surface entrapment along with intercellular, extracellular, and intracellular uptake (Brown and Bates, 1990; Zechmeister et al., 2003).

The **surface entrapment** and **intercellular uptake** comprise the capture of elements associated with atmospheric particles (Brown and Bates, 1990). Exchangeable cations are bound to negatively charged sites at the cell wall, and are fixed by physical processes. Considering the fact that they are not chemically bonded, these elements can be easily removed by washing or mechanic treatment.

Extracellular uptake is a rapid process, which occurs during the first few minutes after the element deposition (Gjengedahl and Steinnes, 1990), based on the strict physico-chemical rules. The uptake depends on the presence of different functional groups on moss cell wall and the element covalent binding indices (Varela et al., 2015). The most of the membrane binding sites could be assigned to carboxyl, phosphodiester, phosphoryl and amine groups, and polyphenols (Gonzales and Pokrovsky, 2014). The carboxyl, phosphodiester, and phosphoryl groups contain oxygen and preferably bind elements with low covalence indices such as Ba^{2+}, Sr^{2+}, Be^{2+}, and Al^{3+} (Nieboer and Richardson, 1980). Elements with high covalence indices (e.g., As^{3+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Fe^{2+}, Pb^{2+}, and Ni^{2+}) bind to groups without oxygen such as amine and sulphydryl groups. The number of available exchange sites and morphological structures of moss differ from species to species. In comparison to *Hypnum* sp., *Pseudoscleropodium purum*, and *Brachytecium rutabulum, Sphagnum* spp. exhibited the highest proton and the element binding capacity of Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}, (Gonzales and Pokrovsky, 2014). Considering the application of passive moss biomonitoring, data about the moss element uptake are variable. The younger parts of moss are reported to show higher amounts of monovalent cations and nutrient anions than older parts whereas the reverse distribution was observed for divalent cations (Zechemister et al., 2003). Dead tissues retain polyvalent cation more effectively still.

However, recent research demonstrated that the concentrations of Cd, Cu, Hg, Pb and Zn in the moss tissues appear to be more closely related to the moss physicochemical characteristics and metabolism than to any preferential accumulation in young or old tissues (Boquete et al., 2014).

In contrast to the extracellular uptake mechanism, the **intracellular uptake** of elements is influenced by various aspects of plant metabolism. Entry to the cell plasma depends on: the affinity of elements for an appropriate carrier, the presence of competitive elements, the gradients in element concentration, and the energy status of the cell (Zechmeister et al., 2003). Non-physiological elements could affect the processes of cell metabolism. For example, trace elements induce the production of thiol-containing peptides such as glutathione, which therefore can be used as biomarker for the trace element pollution (e.g. Bruns et al., 2001). Nevertheless, the rates of the intercellular uptake are much lower than the elements binding at the extracellular sites (Brown and Bates, 1990).

In addition to the above-mentioned, water budget plays a dominant role in element uptake and redistribution within moss tissue because it is a powerful ion-exchanger (Bargagli, 1998). This was concluded based on sequential elution technique. Water can enhance or reduce uptake of soluble cations bound in an exchangeable form on the moss cell wall and outer surface of the plasma membrane. Insoluble elements associated with particles may be stable, but rain can cause its displacement mechanically.

Finally, limited data are available about the uptake and metabolism of organic substances in the moss tissue. The observation suggested that persistent compounds such as phenanthrene have a high affinity for the sites on the moss cell wall, preferentially filling this compartment before migrating into the cellular cytoplasm (Keyte et al., 2009).

2.3. Active moss biomonitoring of urban air pollution

In urban areas and industrial zones, i.e., “anthropogenic dessert”, naturally growing biomonitors – mosses/lichens are often absent not only due to the increased pollution levels, but also because of the mechanical removal along with paved and landscaped surfaces. Thus, between the two types of moss biomonitoring approach, active biomonitoring using moss transplants has been more often applied in these areas.

The active moss biomonitoring overcomes various problems associated with the passive biomonitoring using native mosses. It reduces a high degree of variability in the uptake of contaminants by naturally growing mosses. In the passive biomonitoring study of Boquete et al. (2014), the highly variable concentrations of the airborne elements were found between different parts of the moss shoots analyzed during several well-defined periods. The authors reported the error between 18% and 41%, associated with measurement of the concentrations of the elements in individual segments of the moss shoots. By the application of active moss biomonitoring, the possible edaphic influence on the concentrations of certain elements is minimized. In addition, active moss biomonitoring can be used more appropriately for interpretation of the temporal variability in the results because the initial concentration of pollutants is known. In addition, the exposure period to air pollution could be well defined to get reliable and reproducible biomonitoring data. Finally, active rather than passive moss biomonitoring is recommended because the mosses could undergo adaptive response (probably genotypic) to airborne pollutants. This was confirmed for the moss *P. purum* that have been continuously burdened with high atmospheric levels of trace elements in industrial areas (Boquete et al., 2013).

Active moss biomonitoring could be performed in several ways by:

- transplanting moss (*moss transplants technique*) with the substrate (a thin layer of soil, bark, etc.) from anthropogenic unpolluted area at sites of interest (e.g., Amblard-Gross et al., 2002; Foan et al., 2015);
- the use of specific chamber as the moss carrier (*test chamber technique*) at the experimental site (e.g., Couto et al., 2004); and
- handling the moss material within mesh net bags (*moss bags technique*); the moss is collected in unpolluted areas, and then exposed in any location of interest (Goodman and Roberts, 1971).

Among the listed techniques of the active moss biomonitoring, the moss bag technique is the most commonly applied for monitoring of air quality.

2.3.1. Moss bag biomonitoring

The “moss bag technique” involves exposure of mosses, collected from natural, unpolluted areas, within mesh bags for monitoring of the presence of pollutants in air. Basic principle is that the transplanted moss thallus preserves a capacity of absorbing air pollutants. The technique was firstly applied by Goodman and Roberts (1971), and later has been developed by many researchers. Thus far, the moss bags have been mainly used for monitoring of major and trace elements across industrial and urban areas (e.g., Vasconcelos and Tavares, 1998; Adamo et al., 2003; Aničić et al., 2009a; Fernández et al., 2009; Giordano et al., 2009; Saitanis et al., 2013; Ares et al., 2014). However, the research on REEs (Aničić et al., 2009b; Saitanis et al., 2013; Calabrese et al., 2015), and non-metals and metalloids (e.g., Cao et al., 2009; Kosior et al., 2015) has not been so widely explored by the moss bag technique. Regarding the monitoring of organic contaminants, a far fewer studies have been focused on monitoring of persistent organic pollutants such as dioxins and PAHs (Wu et al., 2014). In several studies, focused on single season, PAHs were assessed characteristic microenvironments – a street canyon (De Nicola et al., 2013), a city tunnel (Zechmeister et al., 2006a), roads and crossroads (Viskari et al., 1997; Orlinski, 2002), and an electrode plant (Wegener et al., 1992), and across the city affected, by both, industrial and traffic emissions (Ares et al., 2011).

There is no standardized protocol related to the application of the technique in a real environment, only a critical literature review of the moss bag methodology was carried out by Ares et al. (2012). The key aspects of the technique were discussed to harmonize the following steps: 1) the selection and preparation of the collected moss material; 2) preparation of the moss bags; 3) the exposure parameters such as positioning, number of bags, duration of exposure, etc.; and 4) the post-exposure treatments.

The moss species selected for biomonitoring should be widely distributed, and it should possess physico-chemical characteristics that provide efficient uptake of atmospheric pollutants (see explanation in the section 2.4.1.1. Interspecies comparison). The collected moss material should be packed in nylon net bags with 2-mm mesh size in one layer, with surface area of 30 mg cm^{-2} (Ares et al., 2012). Nylon is inert material and does not interfere with the uptake of the contaminants, and the mesh size should ensure minimal loss of the moss material during the exposure.

Oven-drying devitalizing pre-treatment (24h at 120°C) of mosses is recommended before exposure, but the fragility of *Sphagnum* oven-dried material remains disadvantageous (Ares et al., 2012). In addition, recent study (Giordano et al., 2013) reported that differences in the element concentrations among the moss bags exposed after water-washing and different devitalizing treatments (oven-drying, acid-washing, and water-boiling) were not significantly different. This step require further justification and could be applied depending on the objective of the biomonitoring survey, e.g., devitalised moss bags could be used when the aim of survey is to compare results from different climate regions.

Once exposed in the field, vitality of the moss in bags is dependent on environmental conditions such as air humidity, precipitation, solar radiation, wind speed, etc. Spagnuolo et al. (2011) demonstrated that the moss exposed in bags undergoes severe structural damages such as the membrane interruptions and dehydration. The moss in bag mainly exhibited passive mechanism of element uptake, prevalently retained in the extracellular form and as entrapped particles. The concentration of the intracellular elements can be considered a reliable indicator of bioavailability and potential ecological risk from pollutants irrespectively of biomonitor vitality. In addition, higher particle entrapment capacity was reported for the irrigated (wet) moss bags, but both wet and dry moss bags showed very similar trend in the moss enrichment with the particular elements (Aničić et al., 2009c). The efficiency of the element uptake remains constant, as capture is predominantly due to passive entrapment of the particles (Aničić et al., 2009b; 2009c).

The concentrations of the contaminants in the moss bags increased with the exposure time from one to five months (Aničić et al., 2009c). However, during the first ten weeks, the steepest increase of the majority of determined elements was observed, even for the REEs. Thus, the exposure period of ten weeks is proposed to enable satisfactory enrichment of the elements and adequate replicabillity of the results. Based on the above-mentioned, a standardized protocol was proposed (Ares et al., 2012) and serves as the basis for further studies aimed at improving the moss bag technique. However, investigation of many different aspects of the technique application under the complex conditions of urban environment is still required. Thus, an upgrade of the state of the art in this field was done in this dissertation.

2.3.1.1. Interspecies comparison

One of the criteria to select moss species for biomonitoring survey is its presence and abundance in the study region. In addition, the selected moss should have biomonitoring characteristics that will allow efficient entrapment and retention of the atmospheric particulates associated with associated air pollutants. The widely used moss species are those from *Sphagnum* genus (e.g., *S. palustre L.*, *S. girgensohnii* Russow, *S. squarrosum* Crome) followed by other species such as *Hypnum cupressiforme* Hedw., *Pseudoscleropodium purum* (Hedw.) Fleisch, *Pleurozium schreberi* (Brid.) Mitt., and *Hylocomium splendens* (Hedw.) Schimp. Moss bags have been recommended to be prepared primarily with species of the *Sphagnum* genus. The leaves of the *Sphagnum* moss constitute about two thirds of the dry biomass with a ratio of branches to leaves of about 6:1 (Bargagli, 1998). As the leaves are only one cell layer thick, a very large surface area for capture of airborne particles is provided. In most species, leaves are spirally arranged around the stem, enhancing the moss particles entrapment. In addition, the *Sphagnum* moss is characterized by numerous surface proton-binding sites, i.e., cation-exchange sites, which increase adsorbing efficiency (Clymo, 1963; González & Pokrovsky, 2014), and the hyalocysts have large pores that can act as a trapping system for airborne particulates (Giordano et al., 2005). In the southern latitudes of the north hemisphere, this species can be rarely found, only in damp and shadowed habitats at high altitudes (mountains). In southern and central European countries, other moss genera, such as *Hypnum* are widespread (ICP Vegetation, 2005).

Due to such practical constraints, different moss species are used in biomonitoring studies. As the pollutant entrapment differs from species to species, the low level of comparable results from different regions are available. To overcome this, interspecies calibration has been implied as possible solution. The interspecies comparison of mosses to compare the uptake of certain elements has been performed for several species (e.g., among *H. splendens, P. schreberi, H. cupressiforme* and *P. purum*) predominantly used in passive moss biomonitoring surveys (e.g., Rinne & Mäkinen, 1988; Wolterbeek et al., 1995; Berg and Steinnes, 1997; Halleraker et al., 1998; Reimann et al., 2001; Fernández et al., 2002; Galsomiès et al., 2003; Carballeira et al., 2008; Migaszewski et al., 2009). The results were highly variable depending on the used moss and determined pollutants.

Very few studies of bag-based active moss biomonitoring have elaborated upon species comparisons (Čeburnis and Valiulis, 1997; Yurokova and Ganeva, 1997; Culicov and Yurukova, 2006; Castello, 2007; Ares et al., 2014). However, the conclusions so far are ambiguous as outcome of limited datasets. In this dissertation, the moss bags of *S. girgensohnii* (a species of the most recommended biomonitoring moss genus) and *H. cupressiforme* (a common moss in Serbia), were exposed across entire Belgrade urban area, enabling extensive database for valuable statistical comparison of the element uptake by two moss species.

2.3.1.2. Moss bag biomonitoring vs. regulatory monitoring

Moss bag biomonitoring has applicable differences in comparison to the means of regulatory air quality monitoring. Contrary to the instruments used for regulatory monitoring, the moss bag does not require power supply and maintenance. Thus, the moss bag biomonitoring could be applied at any site of interest to provide satisfactory (dense) spatial resolution of sampling sites, and consequently an extensive database.

Sampling time is another important difference between the moss bag technique and instrumental measurements. Instrumental measurements are usually restricted to short-time periods, giving instantaneous concentration of pollutants, whereas moss act as long-term integrator of air pollutants over a period providing average pollutant concentration. Thus, short-term variations of air pollutants in a given time are cancelled out by the use of moss bags. Long-time sampling is prerequisite for assessment of cumulative exposure to a certain pollutant, which may have hazardous impact on human health. Finally, the relative ease of sampling, the absence of any need for complicated and expensive technical equipment, and time-integrative behaviour of the moss, render the moss bag technique mostly suited for extensive studies of pollutants monitoring on both, spatial and temporal scales.

However, moss bag technique has not been adopted by relevant authorities, i.e. policy makers, yet. Several studies were performed to compare the moss bag technique with standardized measurements of air pollution (EN15841:2009), i.e., the bulk deposition collectors (Aničić et al., 2009a, Ares et al., 2014, Ares et al., 2015). The aims were to test could moss bags be complement to the regulatory monitoring of air pollution. High correlations were found between the concentrations of V, Cu, As, and Ni in the moss bags of *S. girgensohnii* and bulk deposits (Aničić et al., 2009a). In addition, the enrichment factors of the elements for both types of monitors followed the same pattern at the corresponding sites. Significant correlations were also found between Cd, Cu, and Zn concentrations in the *S. denticulum* moss bags and bulk deposition load. For *P. purum* on the contrary, a lack of correlation between the element concentrations in devitalized moss bags and bulk deposition was reported recently (Ares et al., 2015). Inspired by all the above-mentioned, monitoring of air pollution using simultaneously moss bags and referent instrumental air samplers was performed in one experiment of this dissertation.

2.4. Environmental magnetism

2.4.1. Magnetic proxy parameters

Magnetism appears because of the orbital and spin motions of electrons, and the interactions of electrons with each other. In the following, the basic principles of magnetism are given (Dekkers, 1999). For magnetism, the spin moment is most important as each electron can be regarded as a microscopic magnet. All matter is magnetic, but various materials differently correspond to applied magnetic field. The main distinction is that in some materials there is no collective interaction of magnetic moments, whereas in other materials there is a very strong interaction between magnetic moments. In most compounds, electrons act independently and consequently a very low-magnetic moment is measured in applied magnetic fields and no permanent or remanent magnetic moment can exist. Diamagnetic compounds have paired electrons, and a very low negative magnetic moment is measured in applied fields. Paramagnetic compounds posses unpaired electron spins which yield a very low positive magnetic moment. On the other hand, based on exchange interaction, the outermost electrons of transition elements may act collectively in some compounds, if their orbitals sufficiently overlap. Several classes of this phenomenon are distinguished:

- **ferromagnetism** – in ferromagnetic compounds, parallel alignment of magnetic moments results in large net magnetization even in the absence of a magnetic field;
- **ferrimagnetism** – in ferrimagnetic compounds, opposing alignment of unequal magnetic moments causes very large net magnetization in applied magnetic fields, just as for the ferromagnetic materials; and
- **antiferromagnetism** – in antiferromagnetic compounds, equal magnetic moments are oppositely oriented which results in a net magnetic moment of zero, but in reality usually a weak moment persists.

Collective magnetic interactions cause the existence of permanent or remanent magnetization, i.e., magnetic moments that remain after removal of an external magnetic field. The magnetic properties of ferromagnetic compounds and their variations are dependent on mineral type, temperature, grain size, and strain state of the grain (Maher, 1999).

In nature, the most common magnetic minerals are strongly magnetic (magnetite-like, Fe_3O_4) and weakly magnetic (haematite-like, α-Fe_2O_3) iron oxides. Goethite (α-FeOOH), pyrrhotite (Fe_7S_8), and greigite (Fe_3S_4) are often referred to as minor magnetic minerals. One intrinsic parameter, which can be used to distinguish magnetic minerals, is the Curie point temperature above which collective behaviour disappears and a paramagnetic substance remains. This temperature could reach values of several hundreds of degree Celsius, e.g., approximately 580°C for magnetite. When cooling below the Curie temperature, collective spin coupling is set again.

Magnetic materials consist of small regions called magnetic domains. Domains are 1 μm – 100 μm in size. The existence of domains is hinted that some magnetic properties, such as coercivity and remanence, vary greatly with the grain size of magnetic materials. Based on the grain size, the four structure types of domain could be distinguished:

- **superparamagnetic** (SPM) – grains which cannot support a stable domain configuration, i.e., the spin configuration rapidly changes with variations in applied field. For example, at room temperature, magnetite grains smaller than 20 nm – 25 nm in size are superparamagnetic;
- **single-domain** (SD) – grains in size of 25 nm – 80 nm, which contain one magnetically stable domain. These grains are uniformly magnetized;
- **pseudo-single domain** (PSD) – grains in size of 80 nm to 10 μm – 15 μm which contains up to 10 domains. For magnetite, these domains occur in the size range between 0.1 μm –20 μm; and
- **multidomain** (MD) – grains in size larger than 10 μm –15 μm which contains more than 10 domains.

The number of domains depends on the grain's size and shape, and of the saturation magnetization of the magnetic material. The grains of strongly magnetic minerals such as magnetite contain more domains than a same size grain of weakly magnetic minerals such as hematite.

Due to the existence of magnetic domains in ferromagnetic materials, an alternating magnetic field applied to the material will cause magnetization, which will trace out a loop called a hysteresis loop (**Figure 2.2**). Several magnetic parameters presented at the loop are of the importance for environmental studies. The initial susceptibility (χ_{lf} and χ_{hf}) represents the magnetization of a sample in a small applied field (up to a few times the intensity of the geomagnetic field, 30–60 mT) divided by that field. The maximum value of magnetization, which can be reached by the application of external magnetic field, is the saturation magnetization (M_S). For example, ferro- and ferrimagnetic materials exhibited very high values of saturation magnetization (M_{SF}). Once the material has been driven to saturation, the applied magnetic field can be reduced to zero in reverse direction. In that case, ferromagnetic materials termed saturation isothermal remanent magnetization (SIRM or commonly labelled M_{rs}). The values of magnetic field where magnetization is zero are called coercive force or coercivity ($(B_0)_C$ or H_c) while the field value where the remanence corresponds to zero is labelled remanent coercive force ($(B_0)_{CR}$ or H_{cr}).

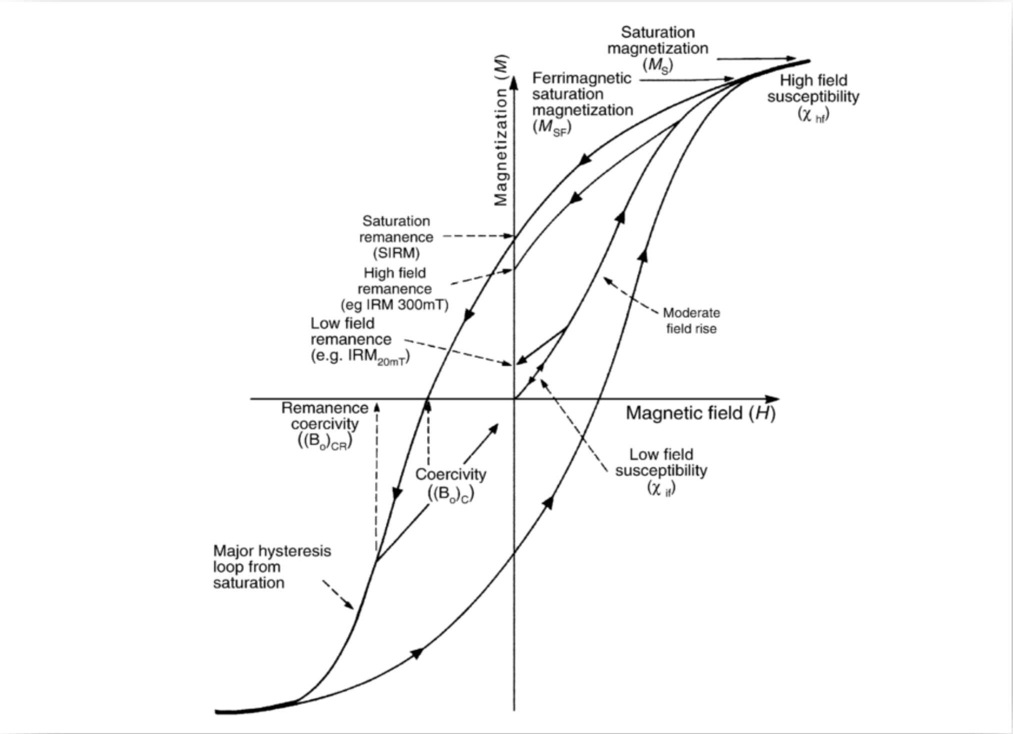

Figure 2.2. Magnetic response (M) to the applied magnetic field (H): an example of typical magnetic hysteresis loop (M–H) exhibited by natural material containing a mixture of ferromagnetic and paramagnetic minerals, and the proxy magnetic parameters often used in environmental studies.

Based on the magnetic parameters, so-called Day plot, i.e., a graph of the ratio of M_{rs} to M_s, against the ratio of H_{cr} to H_c, was proposed by Day et al. (1997) and further developed by Dunlop (2002a) as a method of discriminating domain state (SD, PSD, and MD), and by implication the grain's size. Thus, as the magnetic particles originated from different sources are different in grain size, the Day plots of their magnetic properties would differ. Theoretical Day plot for magnetite is given in **Figure 2.3** (Dunlop, 2002a). The present work suggests $M_{rs}/M_s = 0.02$, $H_{cr}/H_c = 5$ as boundary values between PSD and MD regions. In addition, region with $M_{rs}/M_s > 0.1$ and H_{cr}/H_c values as high as 100 is seen to be associated with mixtures of SP and SD grains. Lately, this plot was compared to a data for a wide range of materials such as marine, lake and rock sediments (Dunlop, 2002b), PM10 air filters (Sagnotti et al., 2006), plant samples and dust samples from the exhaust pipe of gasoline engines and disk brakes (Sagnotti and Winkler, 2012).

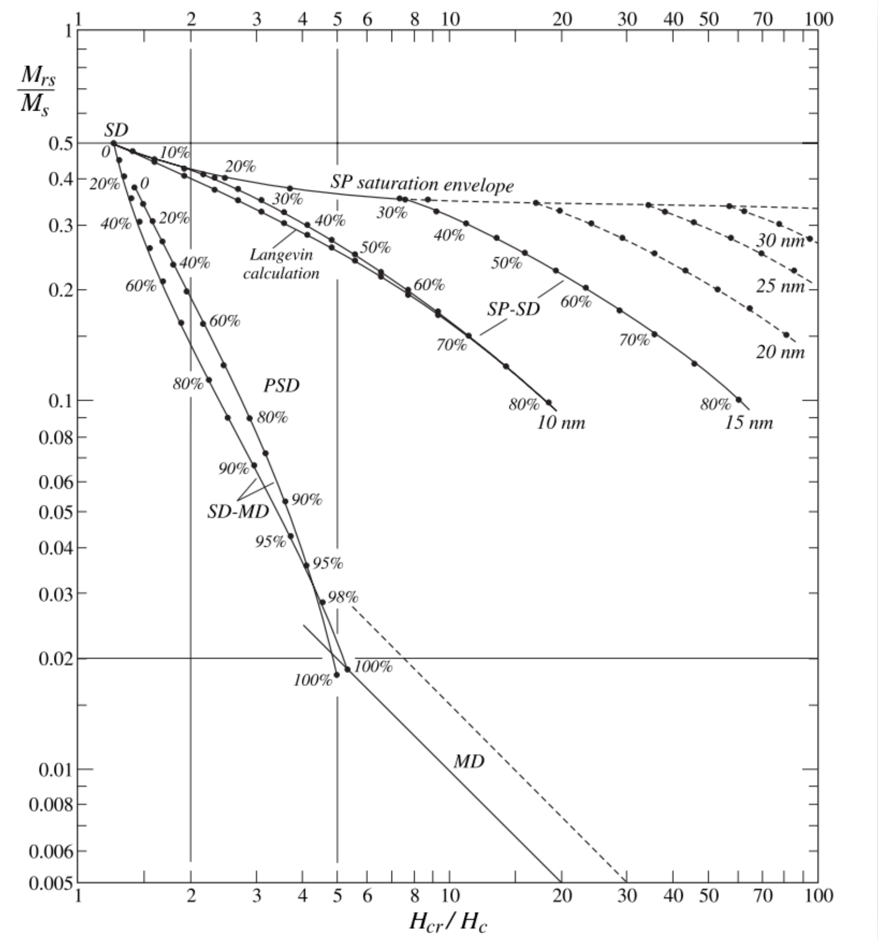

Figure 2.3. Theoretical Day plot curves calculated for magnetite. Numbers along curves are volume fractions of the soft component (SP or MD) in mixtures with SD grains (Dunlop, 2002a).

Changes in magnetic properties reflect changes in the composition of magnetic material, grain size, oxidation degree, etc., because of changing areas, climatic conditions, diagenetic regimes, and anthropogenic pollution (Maher, 1999). Thus, as particles originated from diversified sources contain different magnetic minerals, measurements of magnetic parameters could provide valuable information on the source pollution apportionment. The value of obtained data could be greatly enhanced utilizing magnetic parameters together with elemental analysis.

2.4.2. Biomagnetic monitoring

Biomonitoring can be based on either chemical analysis or evaluation of physical parameters of the biomonitor samples. The concept of using of magnetic parameters in environmental studies was developed by Oldfield and co-workers in the late 1970s

(Thompson et al., 1980). Thus far, the measurements of magnetic properties, such as susceptibility and remanence, have mainly been applied in biomonitoring using tree leaves (e.g., Matzha and Maher, 1995; Moreno et al., 2003; Hansard et al., 2011; Kardel et al., 2012) and rarely lichens (e.g., Chaparro et al., 2013). The leaf magnetic properties have been reported as a valuable proxy for ambient PM pollution (Hofman et al., 2013, 2014). Atmospheric particles that derived from both natural (e.g., volcanic eruptions and fires) and anthropogenic sources (e.g., the combustion of fossil fuels, abrasion of vehicular components and industrial emissions) contain variable amount of magnetisable particles. In addition, trace elements could be incorporated into the lattice structure of the magnetic particles during the combustion processes or could be adsorbed onto the surface of the pre-present magnetic particles in the environment (Lu et al., 2008). Biomagnetic techniques are most sensitive to the ferro(i)magnetic PM fraction which can be quantified by Saturated Isothermal Remanent Magnetization (SIRM). In addition, significant correlations have been reported between magnetic parameters and element concentrations in traffic burdened areas (Gautam et al., 2005; Maher et al., 2008). Thus, in urban areas, biomagnetic monitoring could be useful in characterization of the PM fraction associated with trace elements that derives from different sources of air pollution.

However, although mosses are widely used in biomonitoring of air pollution, magnetic analysis on these bioindicators were reported in few cases for terrestrial mosses (Jordanova et al., 2010; Fabian et al., 2011). In addition, the measurements of moss magnetic parameters have been rarely studied using moss bags, only in industrial areas (Salo et al., 2012; Salo, 2014; Salo and Mäkinen, 2014).

In this dissertation, the use of the moss bags was tested in biomagnetic monitoring of PM pollution in urban environments.

3. Materials and Methods

3.1. Study area

All experiments were performed in the city of Belgrade (44°50’ – 44°44'N, and 20°22' – 20°32'E; elevation: 70 – 250 m altitude). The city is the capital of Serbia, with approximately 1.7 million inhabitants, and is situated at the confluence of the rivers Sava and Danube.

Belgrade lies in the moderate continental zone with the average temperature of 0ºC in January and 22ºC in July. The average annual rainfall amounts 650 mm with minimums in February and in September – 45 and 55 mm, respectively. The dominant wind directions are SE (25%), W (15%), and NW (13%). During the winter, the prevailing wind is so-called “Košava”, with main SE direction and an average daily speed of 6 m s^{-1}. Meteorological conditions during each of the performed experiments are given in **Table 7.2.1** (**Appendix 2**).

During summer, traffic is considered the major source of air pollution. The total number of vehicles in the city consists of approximately 40,000 of passenger vehicles, 1,000 diesel-fuelled city buses, 400 heavy-duty lorries, 108 trams, and 76 trolleys per day. During winter, the heating plant system operates and supplies around 200,000 households in the city. The most used energy sources are natural gas (82%), oil (17%), and coal/pellets (1%). The total production capacity of the heating plants is about 2,800 MW. In addition, 140,000 households are using individual heating sources passed on burning coal or oil. There is no precise data on total fossil fuel consumption of individual heating sources in major urban and suburban areas, and consequently, on pollutant emissions. The details of the studied area were taken from the annual review of Environmental quality in the city of Belgrade (2013).

3.2. Moss sampling and moss bag preparation

For the purpose of all experiments (Experiments 1-6), the moss *Sphagnum girgensohnii* Russow was collected from a pristine wetland area located near Dubna, Russia (**Figure 3.2.1**). This area was pronounced an appropriate background site in the previous research (Aničić et al., 2009b; 2009c). For the Experiments 4 and 6, the moss *Hypnum cupressiforme* Hedw. was collected in the protected area “Vršačke planine“, Serbia (Frontasyeva et al., 2004) (**Figure 3.2.1**). In the laboratory, the green apical parts of the mosses were separated from the rest of brown tissue and carefully manually cleaned of soil particles, plant remains, and epiphytes. Subsequently, the moss material was rinsed three times with double-distilled water (approximately 10 L water per 100 g of moss dry weight and 10 min of shaking). Such prepared mosses were air-dried and gently hand-mixed to obtain homogeneous material. Then, approximately 1.5 g of the moss was packed loosely in 7×7 cm polyethylene net bags with a 2-mm mesh size which were previously washed in 0.1 M HNO_3 to eliminate any contamination.

Figure 3.2.1. Collection of the mosses *S. girgensohnii* and *H. cupressiforme* from the background areas in: a) Domkino, Russia and b) Vršačke planine, Serbia, and c) the moss bags prepared in the laboratory conditions.

To minimize the influence of possible moss growth on element uptake during experiment time, an oven-drying devitalizing pre-treatment of moss before to its exposure in the field were recommended by Giordano et al. (2009) and Ares et al. (2012). However, in previous survey (Aničić et al., 2009b), a poor vitality state of the moss in bags was evident after exposure time because of dry continental climate conditions in the study area. To test the recommended devitalizing moss pre-treatment, one-half of the prepared moss bags were oven-dried (Experiment 6) at 120°C for 24 h whereas no devitalizing treatment was applied to another half of the bags.

To avoid contamination of the moss material, the entire process of the moss sampling and bag preparation was conducted wearing polyethylene powder-free gloves. Several moss bags were stored in the laboratory conditions, at room temperature, as control samples for determination of the initial moss element concentrations.

3.3. Experimental set-up

3.3.1. Experiment 1: Ability of moss bags to reflect horizontal and vertical distribution of major and trace airborne elements on small-scale in urban microenvironments

The experiment was designed to evaluate the reliability of the use of the *S. girgensohnii* moss bags for monitoring of: 1) vertical distribution patterns of major and trace element concentration in the street canyons; 2) horizontal distribution patterns of major and trace elements in the city tunnel; and 3) time-dependence of the moss element enrichment in the city tunnel. Finally, the factors influencing typical spatial distribution were considered.

Different types of urban microenvironments were selected: five street canyons and one city tunnel (**Figure 3.3.1a** and **3.3.1b**). The chosen street canyons can be classified as regular, medium or short and rather symmetric (**Table 3.3.1**) according to height/width (H/W), as well as length/height ratio (L/H) described by Vardoulakis (2003). Four of them (SC_1, SC_2, SC_3, and SC_4) were characterised by traffic flows of 1,254; 502; 332 and 212 vehicles per hour, respectively, while the fifth street (SC_{ped}) is a pedestrian zone. The holders with eight moss bags were mounted on the buildings at a distance of ≈ 1−2 m from the building wall, away from any porches, balconies, etc. The moss bags were hung at heights (z) of about 4 m (I), 8 m (II), and 16 m (III), and referred as z/H ratio in all street canyons (0.14-0.16, 0.29-0.32, and 0.57-0.64, respectively). The first height (I) was selected in order to gain an insight into the pollution level in the pedestrian zone and to prevent loss by vandalism as well. The other two heights (II and III) were chosen arbitrary with the aim to study the vertical pollutant distribution.

The studied tunnel (Terazijski tunnel) is 300 m long and had an average traffic intensity of 6,991 vehicles per hour. Data on traffic flows were averaged on the morning rush hour (according to data obtained from the City Secretariat for Transport, *personal communication*). The holders with 12 moss bags were hung inside (IT), at 4 m from the ground and ≈ 100 m from the entrance; in front of the entrance (ET); and outside of the tunnel (OT), at the corner of the tunnel road and the neighbouring side street.

All moss bags were exposed for ten weeks during the summer and autumn of 2011. Searching for time-dependence of the element enrichment by the moss bags each half of the bags was removed from the exposure sites after five and ten weeks, respectively.

Figure 3.3.1. The moss bags exposed a, b) in the studied street canyons, and c) at the entrance and d) inside of the studied city tunnel.

Table 3.3.1. Dimensions and types of the studied street canyons in the centre of Belgrade

Street name	Street dimensions [m]			H/W	Type	L/H	Type	Street
	Height (H)	Width (W)	Length (L)					symmetry
St. Kraljice Natalije (SC$_1$)	27	20	160	1.35	regular	5.93	medium-long	symmetric
Masarikova St. (SC$_2$)	25	16	140	1.56	regular-deep	5.60	medium-long	asymmetric
St. Dragoslava Jovanovića (SC$_3$)	25	9	50	2.78	deep	2.00	short	symmetric
Obilićev venac St (SC$_4$)	28	22	80	1.27	regular	2.86	short	symmetric
Knez Mihailova St. (SCped)	25	15	500	1.67	regular-deep	20.00	long	symmetric

3.3.2. Experiment 2: Ability of moss bags for monitoring of trace element air pollution in enclosed spaces such as parking garages

The objectives of the experiment were to test: 1) an ability of the *S. girgensohnii* moss bags for monitoring of trace elements in the enclosed spaces such as parking garages, and 2) to compare results obtained by the moss bag technique with instrumental measurements.

The studied parking garages, PG, (Pionirski park – PG_U, Masarikova – PG_{SE1}, Zeleni venac – PG_{SE2}, and Oblićev venac – PG_O) have capacities of 460, 304, 472, and 619 parking spaces, respectively; traffic flows per hour during the experiment were 12, 18, 10, and 31, respectively (according to data obtained from public utility company "Parking services", City of Belgrade). They differ in design: PG_U is an underground garage; PG_{SE1} and PG_{SE2} are semi-enclosed, while PG_O is a garage with openings. The parking garages were situated at the street canyons studied in the Experiment 1 (**Figure 3.3.2.**). The set-up was uniform in all garages; at ≈ 2.5 m above ground, polyethylene string with 8 moss bags were suspended at two positions: near the entrance/tollbooth (TPG) and in the garage interior (IPG) (**Figure 3.3.2**). The moss exposure positions were chosen based on expected similar high traffic intensities between the different parking garages, but under different airflow/ventilation conditions. Poor ventilation conditions, and thus air quality, were expected inside the garage, i.e. for PG_U, PG_{SE1}, and PGSE2.

Along with the moss bag monitoring, instrumental measurements of PM10 were performed in two garages with inadequate functionality of ventilation systems (according to data obtained from PUC "Parking services", City of Belgrade), PG_U and PG_{SE1}. MiniVol portable air samplers (Springfield, OR, USA), provided with PM10 cut-off inlets with a flow rate of 5 L min^{-1}, were positioned near the tollgates (**Figure 3.3.2**). The sampler inlet was about 2 m above ground. PM10 (< 10 μm) were collected on preconditioned (48 h) and preweighed Teflon-coated Quartz filters (Whatman, 47-mm diameter, 2-μm pore size). The sampling time was 24 hours, from 14.00 one day to 14.00 the next day. Due to practical constraints, PM filters were changed every other day for a period of ten weeks. During the sampling, the total number of filter samples per one garage was 30. The PM10 mass concentrations were measured by gravimetric method. Both experiments, referred to instrumental and moss bag monitoring, were performed for the same period during the autumn/winter 2011.

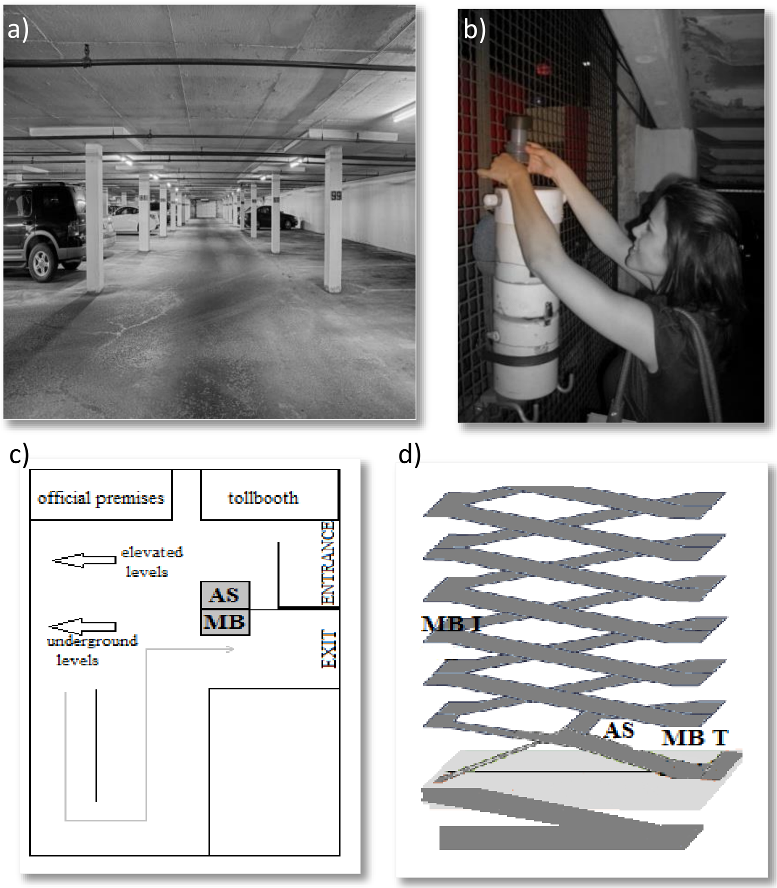

Figure 3.3.2. a) the studied underground parking garage, b) Mini Vol air sampler, c) scheme of the experimental set-up, and d) MB T – the exposure site of the moss bags near the tollgatee, MB I – the exposure site of the moss bags in the garage interior, AS – the exposure site of the air sampler.

3.3.3. Experiment 3: Ability of moss bags for biomagnetic monitoring through measurement of saturation isothermal remanent magnetization (SIRM)

The overall goal of the experiment was to verify the biomagnetic remanence characteristics of the moss *S. girgensohnii* for monitoring of urban air pollution in relation to the element concentrations determined in the moss bag samples in the Experiments 1 and 2 (Vuković et al., 2013; 2014). The more specific objectives were to evaluate: 1) the moss SIRM as an indicator of ambient PM pollution across various classes of urban microenvironments, i.e., street canyons, a city tunnel and parking garages; 2) the spatial distribution of PM, i.e. the vertical distribution in the street canyons and the horizontal distribution in semi-enclosed spaces such as a city tunnel and parking garages, based on SIRM values; and 3) the time-dependent particle enrichment of the moss *S. girgensohnii* in the city tunnel. The experimental set-up was the same as described in the Experiments 1 and 2.

3.3.4. **Experiment 4:** Ability of moss bags for extensive screening of air pollution across urban area

In this experiment, two mosses, *Sphagnum girgensohnii* Russow (a species of the most recommended biomonitoring moss genus) and *Hypnum cupressiforme* Hedw. (a common moss species in the study area), were used. The aim was to test whether *S. girgensohnii* and *H. cupressiforme* could be interchangeably used for the biomonitoring of air pollutants. For this purpose, the moss bags were used to assess the following: 1) particulate contamination through magnetic measurements, 2) trace metal concentrations and 3) PAH concentrations. Consequently, this experiment aimed to determine whether the active moss biomonitoring data could be used to produce a credible city zonation of air pollution.

Based on the regulatory monitoring data, the city zoning reflected differences in air quality over the city of Belgrade was proposed. Based on different traffic loads, the public transportation system is divided into the following zones: public transport I, public transport II, and public transport III, characterized by the high, moderate, and low vehicle flows, respectively (Public Transportation Company, *personal communication*). The following land use classes can be distinguished:

- urban central – zones of the city centre as well as public transport I (T I) and II (T II);
- suburban – residential zones (R) and public transport III (T III);
- industrial – zones mainly influenced by local industrial emissions (I);
- green zones – city parks and forests (G).

To identify small-scale spatial variations in air pollution throughout the study area, 153 biomonitoring sampling sites were defined in total (**Figure 3.3.3**). The entire territory was divided into a regular grid of 800 × 800 m with a sampling site in the centre of each square, i.e., 109 sampling sites were chosen to uniformly cover the study area. In the field, certain sampling sites were abandoned or relocated to avoid unusable locations, e.g., private properties, sport fields, forests, and rivers. Where it was possible, the moss biomonitoring grid was chosen to overlap with the existing regulatory monitoring network but certainly exceeded it. Representative regulatory sites may not accurately characterize the spatial complexities of the particulate dispersion across an urban area (Puustinen et al., 2007; Tretiach et al., 2011). For this reason, additional sampling sites (44) were chosen to cover specific urban microenvironments, such as crossroads, junctions, roadsides, street canyons, gas stations, residential areas and city parks, which were not well represented by the regular grid. Finally, the sites were uniformly selected over the study area to represent all considered land use classes: urban central, suburban, industrial, and urban green zones.

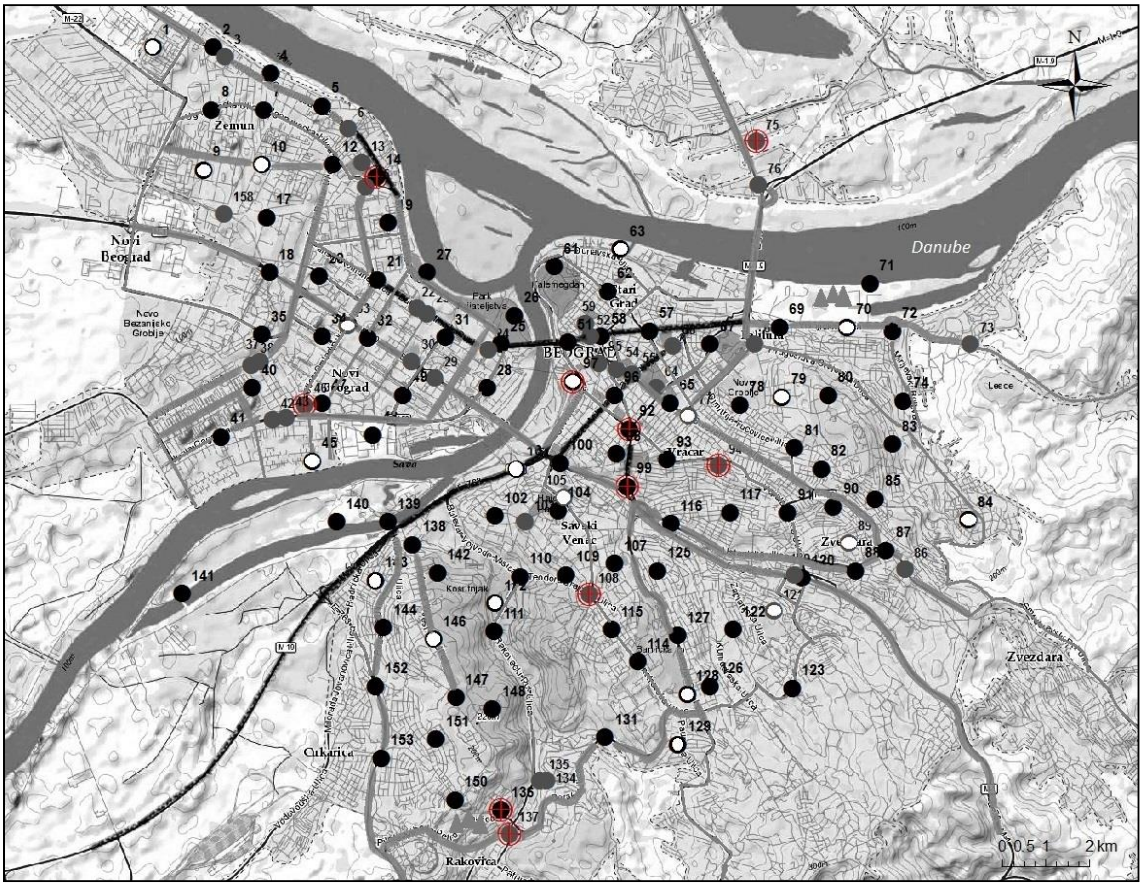

Figure 3.3.3. Map of the Belgrade study area with 153 biomonitoring sampling sites. Public transportation system according to the traffic load and land use classes is represented. Legend: black lines – public transport I, thick grey lines – public transport II (urban central), and thin grey lines – public transport III (suburban); grey ellipses – stationary emission sources (industrial); black and grey circles – regular and additional biomonitoring sampling sites, respectively; black-white circles – sampling sites of PAH pilot study; and red circles – regulatory monitoring stations.

Three moss bags of both species were hung on plasticized aluminium holders specifically designed for that purpose (**Figure 3.3.4**). The holders were mounted perpendicular to the lampposts, which are commonly distributed uniformly across entire urban areas, which enables researchers to cover any microenvironment of interest, e.g., roads, residential/housing areas, parks, etc. The holders with moss bags were placed at the representative height of 3−4 m in open spaces far from tree canopies, roofs, and electric cables. The height of exposure was proposed based on the results from Experiment 1 (Vuković et al., 2013).

For the PAH biomonitoring pilot study, additional moss bags were simultaneously exposed at 20 sampling sites (**Figure 3.3.3**). The number of sampling sites was reduced due to practical constraints, but low concentration of PAHs in urban air is likely during the summer season due to intense PAH degradation under high insolation/temperature conditions. The sites were uniformly positioned across the study area to represent all land use classes. A biomonitoring survey was conducted for ten weeks during the summer 2013 (between 15 June and 15 August).

Figure 3.3.4. The moss bags hung on a, c) plastized aluminium holders exposed over the city of Belgrade: b) bridge, d) junction, e) roundabout, f) crossroad, g) pedestrian zone, and h) city park.

3.3.5. **Experiment 5:** Ability of moss bags to reflect seasonal variations of air pollutants

The experiment was an extension to Experiment 4 performed during the summer of 2013 (Vuković et al., 2015a). It was of interest to use the *S. girgensohnii* moss bags for assessment of airborne pollutant levels – PAHs, major and trace elements, and REEs during the winter, i.e., heating season. The aim was to address the following questions: 1) whether the moss bags reflect pollutant emissions related to residential heating during the winter; 2) is there seasonal variation of the air pollutant levels between the winter and the summer (Experiment 4); and 3) could any pollutant be selected as a tracer for certain pollution source?

Three moss bags per sampling site were hung on plasticized aluminium holders (**Figure 3.3.4**). The holders were placed perpendicularly to lampposts at the representative height of 3 – 4 m (Experiment 1, Vuković et al., 2013), at open space, far from tree canopies, roofs and electric cables. The moss bags were exposed for ten weeks (between 25 December 2013 and 25 February 2014) which was selected to be appropriate period for significant moss REEs enrichment (Aničić et al., 2009b).

The sampling sites, 22 in total (**Figure 3.3.5**), overlapped with the sites selected for the PAH biomonitoring pilot study in Experiment 4. The sites were positioned over the study area to represent different land use classes: urban central, suburban, industrial, and green zones. Whenever it was possible, the sites were chosen to analyse if possible influence of heating sources, heating plants and individual heating sources, on the overall air quality exists during the winter. Thus, considering the height (approximately 150 m) of the heating plant chimney, the sampling sites were positioned downwind of the pollution source at a distance of approximately 500 m, in the nearest residential area. This is in agreement with the recommendations for positioning of monitoring sites in Directive 2004/107/EC related to regulatory monitoring of certain elements and PAHs in ambient air.

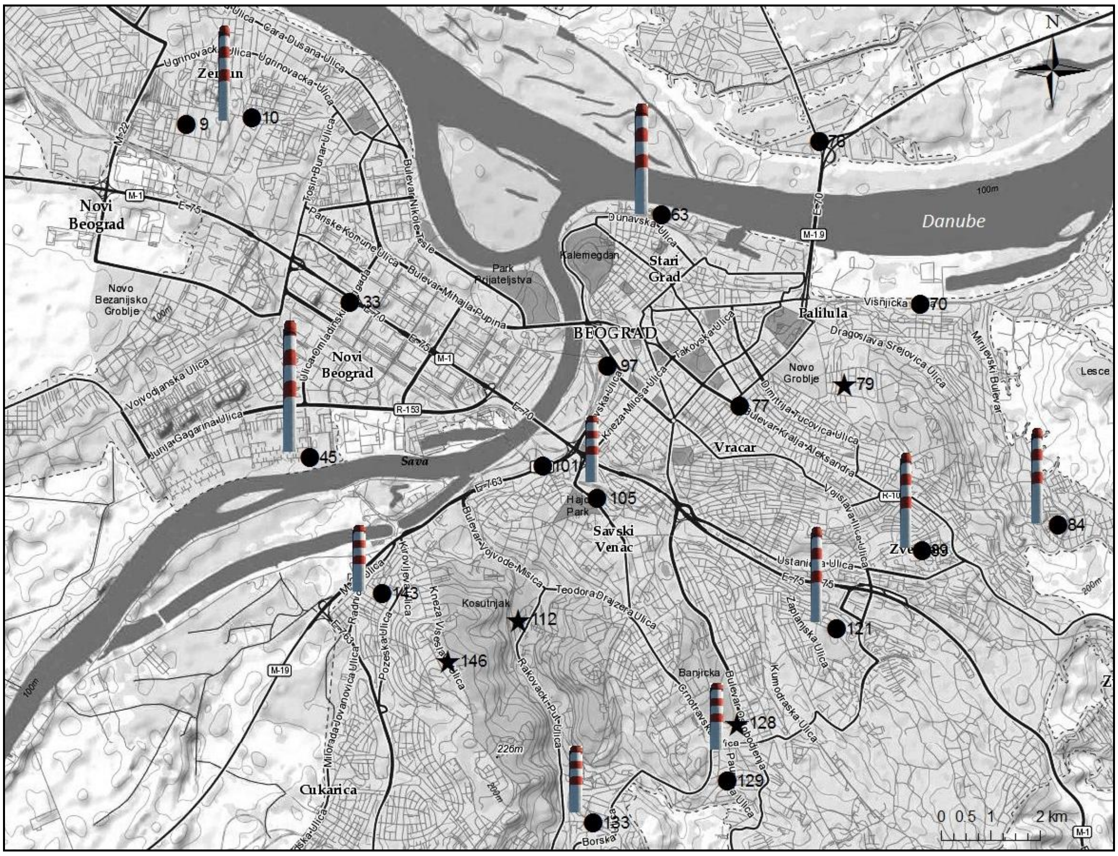

Figure 3.3.5. Map of the Belgrade study area with 22 biomonitoring sampling sites. The sampling sites overlapped with those in the Experiment 4 (Vuković et al., 2015a). Legend: black circles – urban sites, black stars – green zones, and chimney – heating plants.

3.3.6. Experiment 6: Ability of moss bags to reflect distance-decrease of trace element concentrations depending on different traffic burden across crossroads, two- and one-lane street

The purpose of this experiment was to assess level of airborne trace elements close to highly traffic-burdened crossroads, two- and one-lane streets using the moss bag technique. We hypothesized that distance-decline of the trace element concentrations exists from crossroads to two- and one-lane streets depending on the traffic burden. The aim was also to assess if the moss bags could reflect this dependence. The specific objectives were related to the methodological aspects of the application of moss bag technique. The main reason for selecting a particular moss species for moss bag technique is its presence and abundance in the study region. Thus, we tested whether (1) two moss species, *S. girgensohnii* and *H. cupressiforme* could be interchangeably used for biomonitoring purpose, and (2) recommended oven-drying pre-treatment of moss bags (Giordano et al., 2000; Ares et al., 2012) is necessary step before moss exposure in active moss biomonitoring studies.

Four bags of both moss species (oven-dried and live) were exposed for ten weeks (between 22 June and 22 August 2014) using plasticized aluminium holders, specifically designed for this purpose. The holders were mounted perpendicular to the lampposts, at open space, at the representative height of 3 – 4 m (Experiment 1, Vuković et al., 2013). The holders with moss bags were positioned across the sampling sites burdened with different traffic flows – crossroad, two- and one-lane street, were selected (**Figure 3.3.6**). Three pedestrian zones were chosen to represent urban background sites. The sites were selected in different parts of the city: urban-central and peri-urban zone, and within an urban forest.

3.3.6.1. Traffic flow estimation

During the experimental period, traffic is considered the major source of air pollution, particularly trace elements, because of the absence of the heating systems in operation. To estimate contribution of traffic intensity to the trace element concentrations in the air, traffic flows were counted. For each study site, traffic flows were recorded by video cameras for later off-site counting. The vehicle fleet were classified into categories of passenger cars, buses, trams, trolleys, motorcycles, light and heavy-duty vehicles. Traffic flows of each vehicle category were counted for 15 minutes during the rush hours (7.00 –

9.00 and 16.00 – 18.00) on Wednesdays and Sundays in June and July. The average traffic flows during the experimental period were estimated from these counts (**Table 3.3.2**), using a recommended procedure by the Secretariat for Transport of Belgrade (*personal communication*).

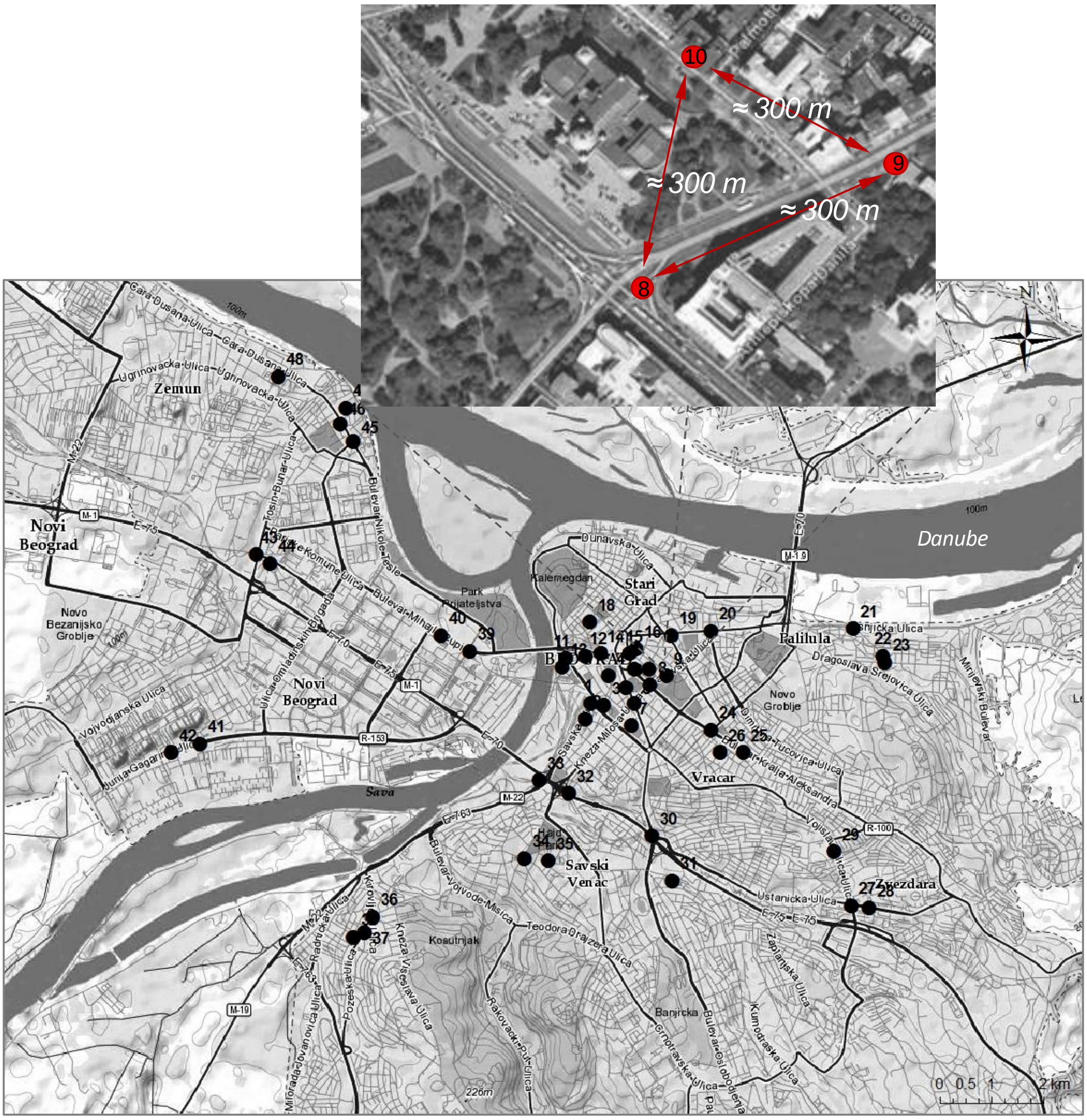

Figure 3.3.6. Map of the Belgrade study area with 48 biomonitoring sampling sites. The zoom view represents an example of the experimental set-up of three sampling sites – crossroad (8), two- (9) and one-lane (10) street.

Table 3.3.2. Characteristics of the biomonitoring sampling sites and traffic flows for vehicle categories (vehicles h^{-1}); LDV – light duty vehicles, HDV – heavy duty vehicles, * the moss bags were missing after the exposure period

	Type of sampling site	Geographic coordinates	Car	Bus	Tram	Trolley	LDV	HDV	Motorcycle	Total traffic flow [vehicles h^{-1}]
1	crossroad	44°48'30" N 20°27'22" E	1716	260	44		124	76	24	2244
2*	two-lane street	44°48'23" N 20°27'18" E	/	/	/	/	/	/	/	/
3	two-lane street	44°48'29" N 20°27'30" E	536	32	/	/	16	/	8	592
4	two-lane street	44°48'42" N 20°27'33" E	708	20	/	/	16	/	12	756
5	crossroad	44°48'30" N 20°27'49" E	3604	108	/	64	124	4	60	3964
6	two-lane street	44°48'37" N 20°27'44" E	1128	28	/	32	24	/	36	1248
7	two-lane street	44°48'20" N 20°27'47" E	504	8	20	/	28	/	4	564
8	crossroad	44°48'38" N 20°27'59" E	4456	128	/	24	148	/	64	4820
9	two-lane street	44°48'42" N 20°28'09" E	2264	28	/	28	112	/	8	2440
10	one-lane street	44°48'45" N 20°27'58" E	300	0	/	/	8	/	/	308
11	two-lane street	44°48'50" N 20°27'06" E	588	12	24	/	68	24	8	724
12	two-lane street	44°48'51" N 20°27'18" E	32	68	/	/	/	/	/	100
13	one-lane street	44°48'46" N 20°27'04" E	32	0	/	/	/	/	/	32
14	crossroad	44°48'52" N 20°27'28" E	3596	120	/	/	68	/	64	3848
15	crossroad	44°48'52" N 20°27'46" E	3012	88	/	/	88	4	68	3260
16	one-lane street	44°48'54" N 20°27'50" E	596	20	/	/	28	/	32	676
17	two-lane street	44°48'45" N 20°27'49" E	1592	32	/	/	44	/	48	1716
18	pedestrian zone	44°49'06" N 20°27'21" E	/	/	/	/	/	/	/	/
19	two-lane street	44°49'00" N 20°28'12" E	1032	120	/	/	60	12	16	1240
20	crossroad	44°49'02" N 20°28'37" E	2668	152	/	/	152	4	48	3024
21	crossroad	44°49'03" N 20°30'06" E	1760	68	/	/	80	20	32	1960
22	two-lane street	44°48'51" N 20°30'25" E	124	0	/	/	4	/	4	132
23	one-lane street	44°48'48" N 20°30'26" E	296	60	/	/	20	/	12	388
24	crossroad	44°48'18" N 20°28'37" E	2568	28	32	/	52	4	60	2744

Table 3.3.2. (continued).

	Type of sampling site	Geographic coordinates	Car	Bus	Tram	Trolley	LDV	HDV	Motorcycle	Total traffic flow [vehicles h^{-1}]
25	two-lane street	44°48'06" N 20°20'06" E	2020	0	28	/	32	/	44	2124
26	one-lane street	44°48'08" N 20°28'43" E	416	16	/	/	16	/	12	460
27	crossroad	44°46'60" N 20°30'05" E	3140	112	/	28	116	28	20	3444
28	two-lane street	44°46'59" N 20°30'16" E	1124	92	/	4	40	4	8	1272
29	two-lane street	44°47'24" N 20°29'54" E	1672	0	/	28	72	8	20	1800
30	crossroad	44°47'31" N 20°28'00" E	5788	184	/	/	176	/	52	6200
31	one-lane street	44°47'11" N 20°28'13" E	360	16	/	/	20	/	/	396
32	crossroad	44°47'50" N 20°27'08" E	1024	60	/	/	20	24	4	1132
33	crossroad	44°47'58" N 20°27'53" E	1104	84	/	/	64	12	12	1276
34	crossroad	44°47'21" N 20°26'40" E	348	4	/	/	4	/	20	376
35	urban background	44°47'20" N 20°26'55" E	/	/	/	/	/	/	/	/
36	crossroad	44°46'55" N 20°25'05" E	1232	144	16	/	60	8	16	1476
37	two-lane street	44°46'48" N 20°24'60" E	1488	144	12	/	52	/	8	1704
38	one-lane street	44°46'46" N 20°24'53" E	140	/	/	/	/	/	/	140
39	crossroad	44°48'53" N 20°26'06" E	4476	144	20	/	176	/	48	4864
40	two-lane street	44°49'00" N 20°25'48" E	2496	120		/	88	4	28	2736
41	crossroad	44°48'12" N 20°23'17" E	4472	80	40	/	224	12	20	4848
42	two-lane street	44°48'08" N 20°22'59" E	3616	72	40	/	144	/	16	3888
43	crossroad	44°49'36" N 20°23'52" E	2596	84	/	/	112	/	16	2808
44	two-lane street	44°49'32" N 20°24'01" E	872	68	/	/	40	/	/	980
45	crossroad	44°50'26" N 20°24'52" E	1392	160	/	/	32	/	32	1616
46	two-lane street	44°50'34" N 20°24'45" E	1036	112	/	/	16	4	16	1184
47	pedestrian zone	44°50'41" N 20°24'48" E	/	/	/	/	/	/	/	/
48	one-lane street	44°50'55" N 20°24'06" E	88	16	/	/	/	/	/	104

3.4. Chemical analyses

Unexpectedly, after the exposure period, due to vandal removal, the moss bags were missing from fifteen, six, and one sampling site in the Experiments 4, 5 and 6, respectively. The remaining moss samples were air-dried, homogenized, and treated for further analysis as in all experiments. For element determination (Experiments 1 – 6), the moss samples were kept at room temperature. Prior to the PAH determination performed in the Experiments 4 and 5, the moss samples were stored in a refrigerator at 4ºC.

3.4.1. Determination of element concentrations in moss samples

For the measurement of element concentration, in the Experiments 1 and 2, eight subsamples per position were analysed while in the Experiments 3 – 6, three subsamples per sampling sites were analysed. Approximately 0.3 g of each moss subsample was digested for 45 min in a microwave digester (ETHOS 1 Advanced Microwave Digestion System, Milestone, Italy) at 200°C with 7 mL of 65% HNO_3 (Sigma Aldrich, puriss. p.a., distilled by the apparatus for acid distillation – BERGHOF, Products+ Instruments GmbH, Germany) and 1 mL of 30% H_2O_2 (Sigma Aldrich, puriss. p.a.). Digested samples were diluted with double-distilled water to a total volume of 50 mL. The same procedure was applied for the analysis of a half of each air filter (Experiment 2).

In the experiments 1 – 6, the concentrations of Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, V, and Zn were determined using inductively coupled plasma-optical emission spectrometry, ICP-OES (Thermo Scientific iCAP 6500 Duo, Thermo Scientific, UK). For the calibration of ICP-OES a Multi-Element Plasma Standard Solution 4, Specpure (Alfa Aesar GmbH & Co KG, Germany) was used to prepare intermediate solutions: 10 μg L^{-1}, 50 μg L^{-1}, 100 μg L^{-1}, 500 μg L^{-1}, 1 mg L^{-1}, 5 mg L^{-1}, 10 mg L^{-1}, 25 mg L^{-1} and 50 mg L^{-1}.

In the experiments 5 and 6, the concentrations of trace (Be, Ga, Mo, Rb, Pd, Pt, Sb, Ti, Th, Tl, and Sb) and REEs (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sc, Sm, Tb, Tm, Y, and Yb) was determined using inductively coupled plasma-mass spectrometry, ICP-MS (Thermo Scientific iCAP Q, Thermo Scientific, UK). For the calibration of ICP-MS a Low Level Elements Calibration Stock, EPA Method Standard (VHG Labs, Manchester) was used to prepare intermediate solutions: 0.1 μg L^{-1}, 1 μg L^{-1}, 2 μg L^{-1}, 5 μg L^{-1}, 10 μg L^{-1}, 25 μg L^{-1}, and 50 μg L^{-1}.

In the experiments 1 and 2, the quality control was performed using the standard reference material lichen-336 (IAEA) while in the experiments 4 – 6 it was performed using certified reference materials of moss *Pleurozium schreberi*, M2 and M3 (Steinnes et al., 1997). The recovery of determined elements is given in **Table 7.2.2** (**Appendix 2**). If the element concentrations differed by more than 20% of the reference value, the results were considered only as indicative.

For the elemental determination, control samples, certified reference materials and analytical blanks, were analysed once every ten samples. The corresponding method detection limits and limits of quantification for the element determination are presented in **Tables 7.2.3 – 7.2.6** (**Appendix 2**).

3.4.2. Determination of PAH concentrations in moss samples

For the purpose of PAH determination (Experiments 4 and 5), three subsamples per site were analysed. Approximately 1.0 g of each moss subsample stored in a refrigerator was subjected to three subsequent extractions by ultrasonication (sonicator 4GT, Sonic, Niš, Serbia) in 50 mL of dichloromethane (Carlo Erba, HPLC pure) for 5 min. The extracts were filtered and merged into one that was vacuum rotary evaporated to 1 mL. Analysis was performed by GC-MSD 7890A/5975C, Agilent, USA (with HP-5MS capillary column 30 m x 0.25 mm x 0.25 μm film thickness). Helium (5.0, Linde Gas) was used as a carrier gas at a constant flow rate of 1.5 mL min^{-1}. In all cases, 1 μL of sample was injected in splitless mode.

The 16 US EPA priority PAHs were determined: naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Acp), fluorene (Fl), phenanthrene (Phen), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), indeno[1,2,3-c,d]pyrene (I[cd]P), dibenzo-[a,h]anthracene (DB[ah]A) and benzo[ghi]perylene (B[ghi]P). The PAH Mix standard containing the 16 analyzed PAHs (AccuStandard, USA, Z-014G-R) was used for calibration. Intermediate standard solutions were also prepared: 0.01 mg L^{-1}, 0.1 mg L^{-1}, 0.5 mg L^{-1}, 1 mg L^{-1}, and 3 mg L^{-1}.

For the PAH determination, control samples, certified reference materials and analytical blanks, were analysed once every ten samples. For all determined PAHs, the method detection limit and method quantification limit were 0.01 ng g^{-1} and 1.40 ng g^{-1}, respectively.

In each subsample, an internal standard containing 1,4-dichlorobenzene-d4, naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and benzo[ghi]perylene-d12 (AccuStandard, USA, Z-014J) was added. The recovery of each sample was calculated and used to correct the results because no reference material similar to moss is available for the determination of PAHs. Finally, the PAH concentration values were also automatically corrected using Agilent Technologies ChemStation with internal standard values. Recovery values varied between 90 − 100% for all of the PAHs contained in the internal standard, except for Nap (65%).

3.5. Magnetic measurements in moss samples

To measure the saturation isothermal remanent magnetization (SIRM) of the moss samples (Experiments 3 and 4), three subsamples per sampling site (approximately 0.5 g) were taken from the homogenized moss samples. The subsamples were tightly wrapped with cling film to avoid movement of any sample and pressed into a 10-cm^3 plastic container. The subsamples were individually magnetized in a direct current (DC) field of 1 T with a Molspin pulse magnetizer (Molspin Ltd, UK). Subsequently, the isothermal remanent magnetization (IRM) of the samples (A m^2) was measured twice using a Molspin Minispin magnetometer with high sensitivity ($\sim 0.1 \times 10^{-8}$ A m^2, Molspin Ltd, UK). Significant decrease of the IRM of the samples was observed only after 20 min of magnetization (**Figure 7.2.1, Appendix 2**). The instrument was calibrated after every ten measurements by means of a magnetically stable rock specimen.

Based on Hofman et al. (2014), the IRM at 1 T was assumed to be the saturation isothermal remanent magnetization (SIRM) of each measured sample. However, comparison of the IRM measured on the moss samples magnetized at 300 mT and 1 T revealed no significant differences (**Figure 7.2.2, Appendix 2**). Thus, in the further experiments, the IRM measured with an applied field of 300 mT can be considered as an SIRM. Finally, the SIRM was normalized to the moss dry mass (10^{-5} A m^2 kg^{-1}). Each SIRM value was the average of two measurements on the same sample.

3.6. Data processing

To assess the element enrichment in the moss samples (Experiments 1 – 6), relative accumulation factor (RAF) was calculated based on the moss content of each element after exposure ($C_{exposed}$) reduced by and then divided by the element content before exposure ($C_{initial}$):

$$\mathrm{RAF} = \frac{C_{exposed} - C_{initial}}{C_{initial}}$$

The element in PM10 samples were calculated following the equation:

$$\mathrm{C\ (ng\ m^{3})} = \frac{C_{determined}\ (ng\ mL^{-1}) \times 50\ mL}{7.2\ m^{3}},$$

where 7.2 m^3 represents volume of air sampling for 24 h and with a flow rate of 5 L min^{-1}.

To represent graphically abundances of the REEs (Experiments 5 and 6), concentrations of the REEs observed in the moss samples were normalised to those in different shales, North American Shale Composite (NASC) and Post-Archean Australian Shales (PAAS) (Dołęgowska and Migaszewski, 2013). The REEs concentrations in the shales are given in **Table 7.2.7** (**Appendix 2**).

To determine PAH pollution sources, PAH diagnostic ratios (Ravindra et al., 2008; Oliveira et al., 2011) were calculated for the dominant PAHs in the moss samples (Experiment 4 and 5).

The data were processed using STATISTICA 8.0 (StatSoft, Inc., Tulsa, OK, USA) and the free software R (R Development Core Team, 2014). The normality ($p < 0.05$) of the data was tested by the Kolmogorov-Smirnov test (Experiments 1 – 4) and Shapiro-Wilk test (Experiments 5 and 6). Spearman's correlation was applied ($p < 0.05$) to determine all discussed correlations.

The Mann-Whitney U test ($p < 0.05$) was used to determine significant differences in:

- the moss element concentrations between different heights in the street canyons (Experiment 1);
- the moss SIRM values between the different sampling positions within the particular urban microenvironment (Experiment 3); and
- the element and PAH concentrations in the moss bags exposed during the summer and winter (Experiment 5).

The Wilcoxon sign-rank test ($p < 0.05$) was used to determine significant differences in:

- the SIRM values between the two moss species, *S. girgensohnii* and *H. cupressiforme* (Experiment 4);
- the PAH concentrations between the two moss species , *S. girgensohnii* and *H. cupressiforme* (Experiment 4); and
- the elemental concentrations between oven-dried and live moss samples of both *S. girgensohnii* and *H. cupressiforme* (Experiment 6).

One-way analysis of variance (ANOVA) was applied to establish significant differences ($p < 0.05$) in:

- the moss element concentrations measured in four parking garages (Experiment 2); and
- the moss SIRM values, and the corresponding element concentrations in the three considered urban microenvironments, street canyons, city tunnel, and parking garages (Experiment 3).

For comparison of the metal concentrations in the tissue of the two moss species (Experiments 4 and 6), *S. girgensohnii* and *H. cupressiforme*, ANOVA is not applicable because the content of the metals is not independent but related to the location of the sampling sites and the amount of metal deposition (Fernández et al., 2002; Carballeira et al., 2008). For this reason, a type II regression model was applied by calculating the regression as a standardized major axis regression (Legendre, 2014).

A hierarchical cluster analysis (CA) using Ward's method with Euclidian distances was applied:

- to the moss SIRM values observed in the street canyons, city tunnel, and parking garages (Experiment 3), and
- to the moss SIRM, Cr and Cu concentrations, for which the highest correlation coefficients were observed (Experiment 4).

All maps were made using software ArcGIS 9.3.

4. Results and Discussion

4.1. Experiment 1: Ability of moss bags to reflect horizontal and vertical distribution of major and trace airborne elements on small-scale in urban microenvironments

4.1.1. Vertical moss element distribution in street canyons

The concentrations of the determined elements in the *S. girgensohnii* moss bags are given in **Table 7.3.1, Appendix 3**. The concentrations of the most elements were significantly ($p < 0.05$) higher in the exposed moss bags than in the unexposed ones, indicating that the studied street canyons were polluted by the elements in question (**Figure 4.1.1**). According to the RAF values, the most enriched element in the moss bags was Pb, then in descending order Zn > Cr > Cu > V > Ni > Ba > Al > Sr > Cd (**Table 4.1.1**). The observed Pb content was lower than in the previous study (Aničić et al., 2009a) with the moss bags of the same moss species, exposed over the city of Belgrade. This result coincides with the fact that leaded gasoline was banned in Serbia at the beginning of 2011. Because of the introduction of unleaded fuel, Pb can no longer be taken as an indicator for current traffic-induced air pollution (Zechmeister et al., 2005). RAF values of Mg and Mn were close to zero indicated that there was no enrichment of these elements in moss tissue. Potassium and Na showed a substantial decrease after the exposure of moss bags (**Table 4.1.1**). The result is in agreement with the previous research (Aničić et al., 2009b; Aničić et al., 2009c).

In two street canyons (SC_2 and SC_3), the vertical distribution patterns of Al, Ba, Co, Cr, Cu, Pb, V, and Zn, showed significant decrease from the first, 4 m, to the third height, 16m, of the moss bags exposure. However, in the other street canyons (SC_1, SC_4, and SC_{ped}), the highest concentrations of Al, Ba, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sr, and V were determined in the moss bags exposed at the second height. The vertical distribution could be explained by considering several important parameters that influence the pollutant dispersion process in street canyons, which include ambient conditions (wind speed and directions), building geometry and street dimensions, thermal stratification (solar insulation and orientation, building and street thermal capacitance), vehicular movement (size, number and frequency), etc. (Xie et al., 2005). In four street canyons (SC_1, SC_3, SC_4, and SC_{ped}), the moss bags were placed on the windward sides, while in SC_1 they were exposed on the leeward side (**Figure 4.1.2a**). During the exposure period, an average wind speed was hourly measured and found to be below 1.5 m s^{-1}, except in SC_3 (SEPA, 2011).

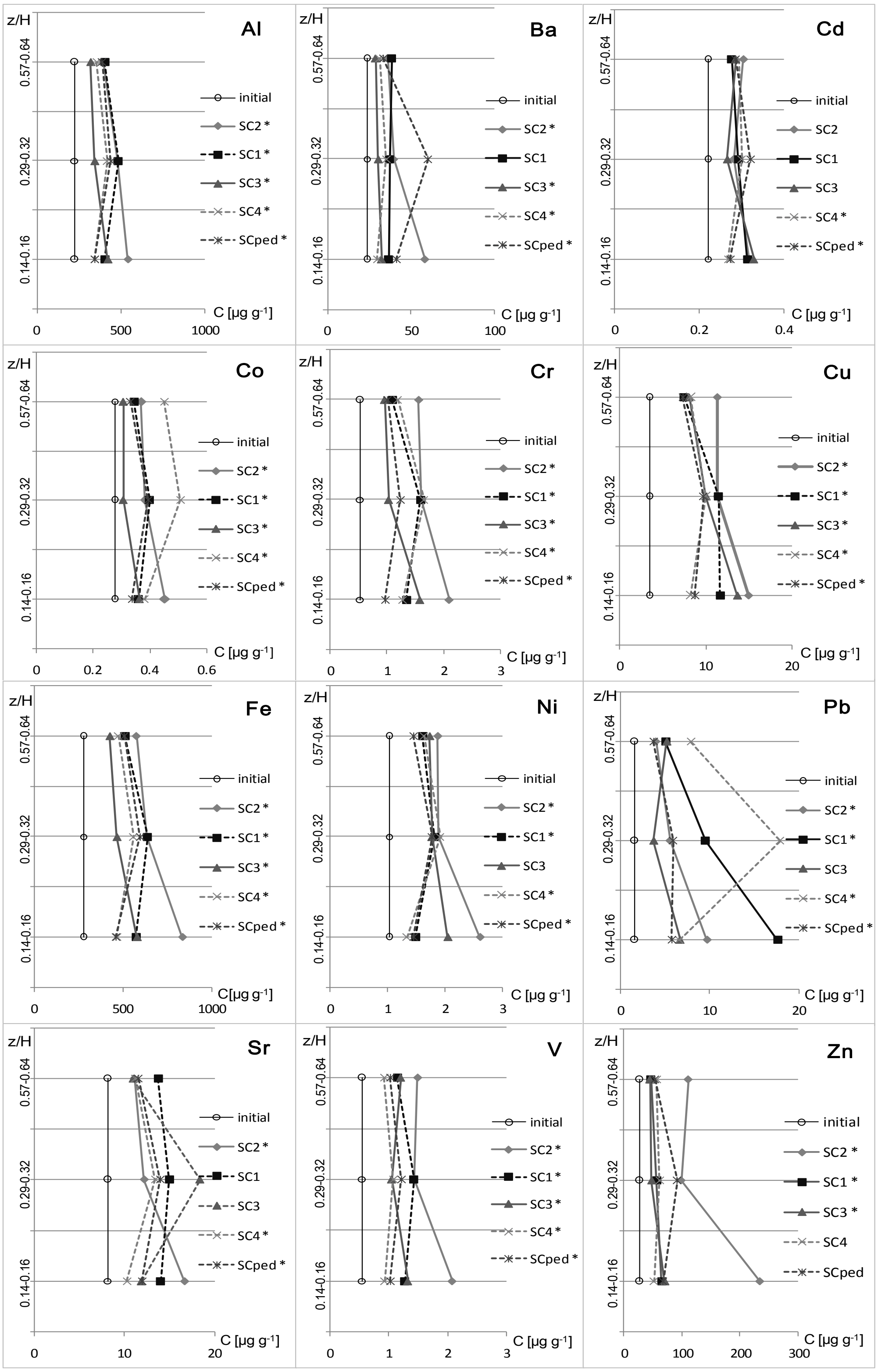

Figure 4.1.1. Vertical profiles of the concentrations of Al, Ba, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sr, V, and Zn (μg g^{-1}) in the *S. girgensohnii* moss bags exposed for ten weeks during the summer of 2011. Legend: SC_1, SC_2, SC_3, SC_4, and SC_{ped} – the studied street canyons; initial – the element concentrations in the unexposed moss; z/H – ratio reffering to the heights of about 4 m, 8 m, and 16 m; * significant difference ($p < 0.05$) in the moss element concentrations between the heights.

Table 4.1.1. Relative accumulation factors (RAF) of the elements in the *S. girgensohnii* moss after ten-week exposure during the summer of 2011 in five street canyons in the centre of Belgrade

RAF	Element
≤0	K, Mn, Mg
0-1	Ca, Cd, Co, Na, Sr
1-5	Al, Ba, Cu, Fe, Ni, V
5-10	Cr, Pb, Zn

When the speed of synoptic wind is below 1.5 m s^{-1}, the main wind vortex within the street canyon tends to disappear and the air stagnates in the street (De Paul and Sheih 1986). In that case, a thermal vortex in the street of aspect ratio of H/W ≈ 1 might play a dominant role whose impact depends on whether leeward or windward side is heating. When the windward side is heated, an upward buoyancy flux opposes the downward advection flux along the wall, and divides the flow structure into two counter rotating cells with a clockwise top vortex and a reverse lower vortex within the canyon (Xie et al. 2005; Wang et al. 2011) (**Figure 4.1.2b**). In SC_4 and SC_{ped}, the direction of a reverse lower vortex possibly brings pollution from the ground and favours deposition of trace elements near the second exposure height (at the z/H ≈ 0.29).

On the other side, in SC_1, the highest concentration of some elements (Al, Co, Cr, Cu, Cr, Fe, Ni, Pb, V, and Zn) at the second exposure height (z/H ≈ 0.29) could be related not only to thermal vortex but also to their association with fine aerosols. Moss-bag entraps a prevalence of potentially inhalable or breathable particles; the smallest particles classes (<2.5 and 2.5-10 μm) were largely predominant (78.8% and 19.4%, respectively) with only 0.2% of particles greater than 25 μm in diameter (Tretiach et al., 2011). The fine (PM2.5) and ultra-fine (PM<0.1) particles were reported to contain more of anthropogenic, traffic-related metals (Ni, Cr, V, Pb, and Ba) than particles of other sizes, although crustal metals accounted for over 90% of all particulate mass concentrations (Singh et al. 2002, Ntziachristos et al., 2007). In addition, 72 – 78% of the Pb+Cd+Ni+Cr were retained in the fine particles and the percentage remained significant in ultra-fine ones (24-50%) (Lin et al., 2005). Weber et al. (2006) demonstrated that the concentration of PM2.5 also changed significantly with height, decreasing from a maximum at z/H = 0.23 towards both the lowest (z/H = 0.15) and highest (z/H = 0.59) sampling heights.

In SC_3 which belongs to deep canyons (H/W ≈ 3), weak counter rotating secondary and tertiary vortices (**Figure 4.1.2c**) may be observed at street level (Pavageau et al., 1996;

Jeong and Andrews, 2002). These vortices seem to prevail over thermal vortex and they may be considered as the major contributors to higher deposition of trace elements at the z/H ≈ 0.15.

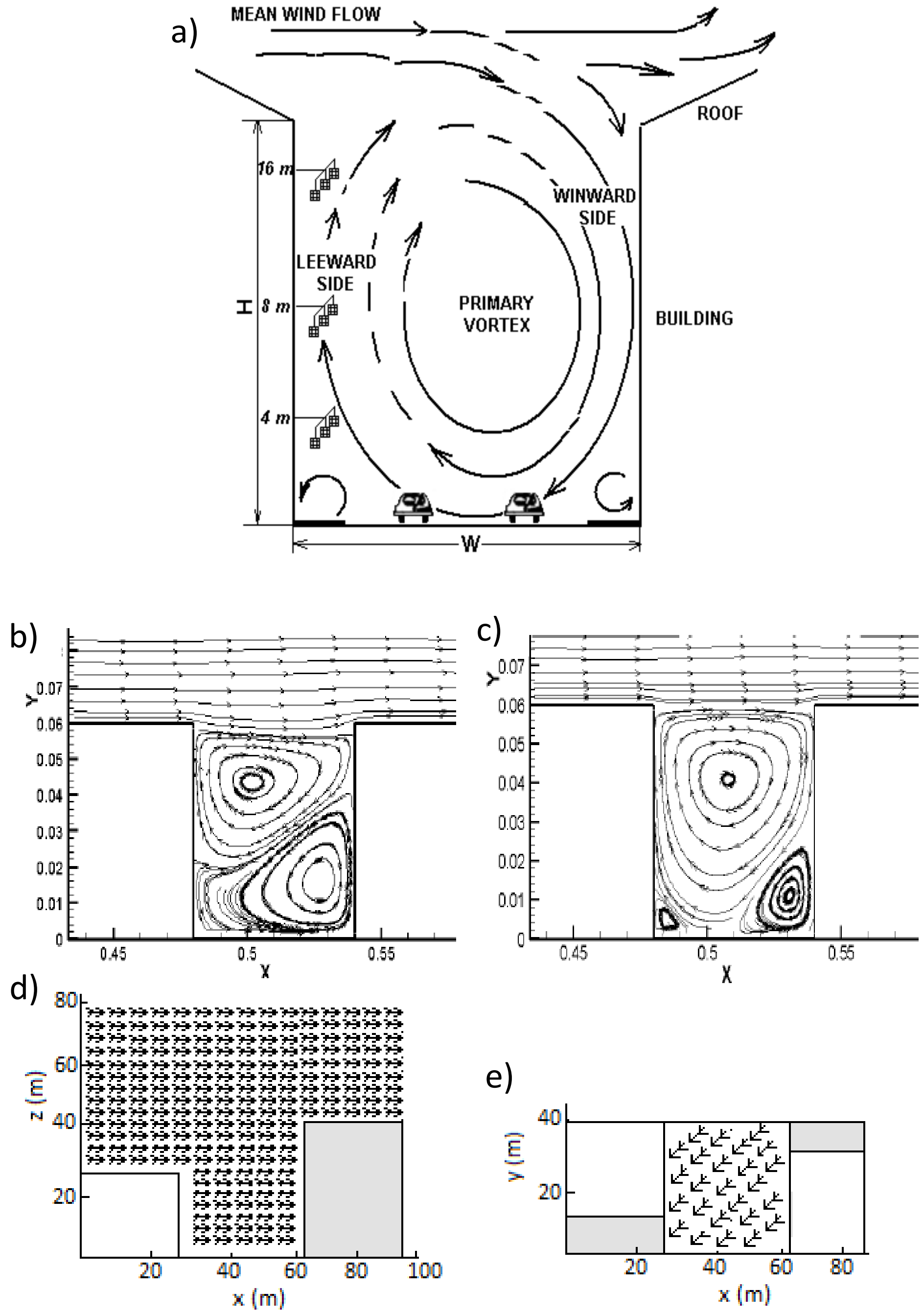

Figure 4.1.2. The specific airflow present in street canyons. Legend: a) a scheme of the primary vortex, and the position of exposed moss bags – 4 m, 8 m, and 16 m; b-c) the examples of thermal vortices in SC_4 and SC_{ped}, SC_3, respectively (adapted from Wang et al. 2011); d-e) the example of dynamic vortex in asymmetric street canyon SC_2 (adapted from Gu et al. 2011).

Unlike the other investigated street canyons, in SC_2 the exposure positions were placed on the leeward side whereas the windward was heating. Consequently, clockwise top vortex causes pollutant concentration to increase at the $z/H \approx 0.15$ on the leeward side. Similar to other streets, SC_2 has $H/W \approx 1$ ratio, but there is strong asymmetry due to very high buildings on one side compared to those on the opposite side of the street. The air flow is separated at the top area of the high building wall in the step-up section, forming a strong down washing (Gu et al., 2011) (**Figure 4.1.2d-e**). The low wind speed (about 1.5 m s^{-1}) may still contribute to the highest pollution level of the street canyon.

Contrary to the often observation, the results indicate that spatial distribution of air pollutants is not always uniform in microenvironments such as street canyons. Specifically, no differences were obtained between element concentrations in *H. cupressiforme* bags exposed in street canyons at heights of 3 m, 6 m, and 9 m (De Nicola et al., 2013). Any differences were not also obtained between element concentrations in *Hylocomium splendens* bags exposed at street canyons at heights of between 3 m and 21 m (Rivera et al., 2011). However, in this experiment, obtained vertical deployment of trace elements between 4 m and 16 m were significant. Although the results are highly variable, a specific height must be established to standardize this aspect. Based on the results from this experiment and due to practical consideration (e.g., to avoid vandalism) an exposure height of 4 m is recommended for the moss bag exposure in urban environment.

4.1.2. Horizontal moss element distribution in city tunnel

In the city tunnel, trace element enrichment in the *S. girgensonhii* moss bags was observed after both periods of exposure – five and ten weeks (**Table 7.3.2, Appendix 3**). As well as in the street canyons, the concentrations of Al, Ba, Co, Cr, Cu, Fe, Ni, and Zn increased significantly in the moss bags in comparison with the initial concentrations (**Table 4.1.2**). The concentrations of Mg and Mn were slightly increased while the concentrations of K and Na substantially decreased. Significant differences in the elements enrichment were observed between five- and ten-week of exposures (**Figure 4.1.3**).

Specific horizontal distribution of elements was observed in the moss bags depending on poor air ventilation in the city tunnel. Thus, after ten-week exposure, decreasing trend of concentrations in the moss bags from inside to the entrance and outside of the tunnel was obtained for Al, Ba, Cd, Co, Cr, Cu, Fe, and Zn (**Figure 4.1.4**).

The results indicate the capability of the moss bags to reveal potentially hotspots of pollution in urban areas, such as city tunnels. Due to poor air conditions in the tunnel, designing of ventilation systems and implementation of regular control measurements are needed.

Table 4.1.2. Relative accumulation factors (RAF) of the elements in the *S. girgensohnii* moss after ten-week exposure during the autumn of 2011 in the city tunnel in the centre of Belgrade

RAF	Element
≤0	K, Mn, Mg
0-1	Ca, Cd, Sr
1-5	Al, Ba, Co, Ni, Zn
5-10	Fe
10-20	Cu, Cr

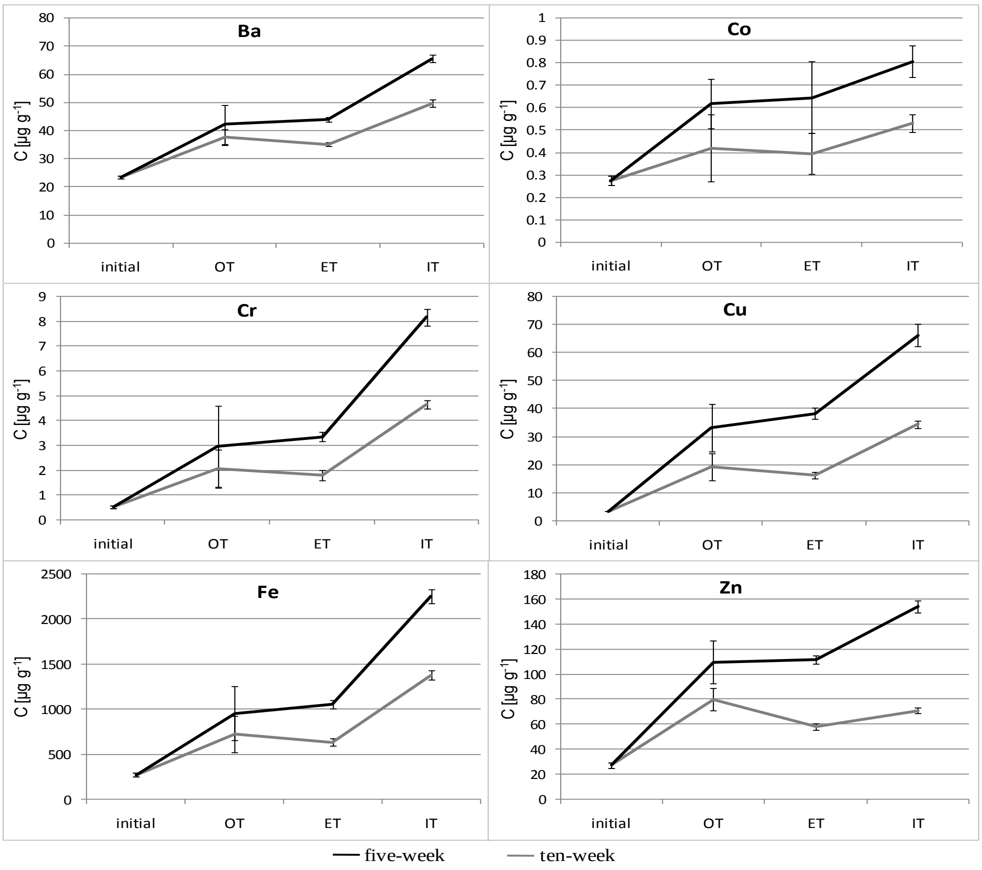

Figure 4.1.3. Average concentrations with standard deviation bars of Al, Ba, Co, Cr, Cu, Fe, and Zn in the *S. girgensohnii* moss after the five- and ten-week exposure during the autumn of 2011 in the city tunnel; IT – inside the tunnel; ET – entrance of the tunnel; OT – outside of the tunnel; and initial – the element concentrations in the unexposed moss.

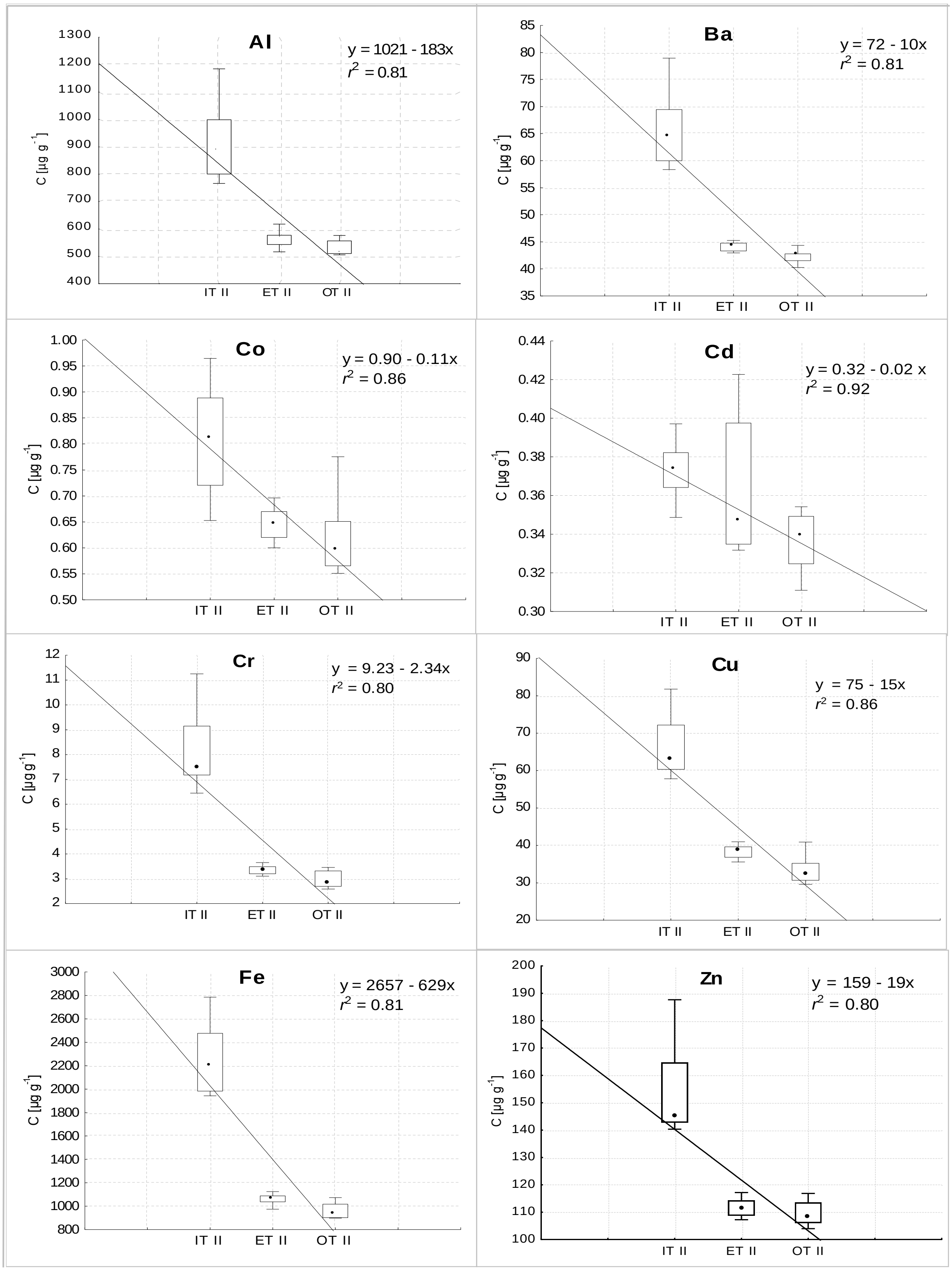

Figure 4.1.4. Concentrations (µg g^{-1}) of Al, Ba, Cd, Co, Cr, Cu, Fe, Ni, and Zn in the *S. girgensohnii* moss bags exposed for ten weeks during the autumn of 2011 in the city tunnel; IT – inside of the tunnel, ET – at the entrance of the tunnel, and OT – outside of the tunnel.

4.1.3. Traffic flow influence on moss elemnt enrichment

Significant high correlation coefficients were observed between pairs of elements: Al/Ba (0.76), Al/Fe (0.89), Al/V (0.80), Ba/Fe (0.88), Fe/V (0.89), Fe/Zn (0.80), Cu/Zn (0.80), and V/Zn (0.83) (**Table 7.3.3, Appendix 3**). Therefore, most of the elements (Ba, Cu, Zn, and V) probably indicate a major influence of traffic-related sources, which have been already considered in earlier studies (e.g., Zechmeister et al., 2005; Viana et al., 2008). Aluminium and Fe are considered typical geological marker elements (Sternbeck et al. 2002). However, in urban areas these elements often originate from the metallic wear parts of vehicles and from the road dust resuspension.

In accordance to higher traffic intensity, significantly higher concentration of the elements was pronounced in the moss bags exposed in the tunnel compared to the street canyons. The obtained concentrations of the majority of elements in SC_{ped} were significantly lower than in the moss bags exposed in the other studied street canyons. The exceptions were the concentrations of Al, Ba, and Zn, which were close to the concentrations in the other studied street canyons. Note that SC_{ped} is unique due to an alley in the middle. Namely, several studies suggest that trees in street canyons reduce the air circulation and lead to higher local PM concentration (e.g., Gromke and Ruck, 2007) and consequently, lead to higher concentration of trace elements. However, among the other street canyons, the highest concentrations of elements were not found in the street with the highest traffic intensity. This contradiction leads to the conclusion that traffic flow is not the only factor affecting the presence and latter deployment of elements in the air. The street geometry, as well as air movement (e.g., direction of the primary vortex) is of great importance to this process.

4.2. **Experiment 2:** Ability of moss bags for monitoring of trace element air pollution in enclosed spaces such as parking garages

4.2.1. Horizontal distribution of moss element concentrations in parking garages

The concentrations of the elements determined in the *S. girgensohnii* moss bags exposed in four parking garages are given in **Table 7.3.4** (**Appendix 3**). The results showed higher post-exposure element concentrations in the moss bags than initial values (**Figure 4.2.1**). An order of element enrichment in the moss was Ca > Al > Fe > Zn > Ba > Sr > Cu > Pb > Ni > Cr > Cd > Co. The concentrations of physiologically active elements – K, Mg, Mn and Na in the exposed moss bags were close to their initial values. This is in accordance with the previous research (Aničić et al., 2009b; Aničić et al., 2009c). In general, relative element content in the moss bags exposed in semi-enclosed space of parking garages was lower than in the moss bags exposed previously at open space — street canyons in the Experiment 1 (Vuković et al., 2013) where the parking garages are situated. In addition, the relative moss element content was higher in the city tunnel described in the above-mentioned Experiment 1. The moss may take up elements not only as particles, but also in ionic form, lower relative element moss enrichment in garage probably is a consequence of the indoor environment dry conditions limiting moss physiological activity and further element uptake.

ANOVA showed that concentration of the most elements measured in the moss, exposed in four parking garages, were significantly different depending on diversity in the garage design: enclosed and open one (**Figure 4.2.1**). Furthermore, moss exposed at the garage entrance showed significantly higher element concentrations than moss exposed in the interior, indicating that the moss bags reflect changes in small-scale spatial variations in the content of particle-bound elements.

Aluminium, Ba, Cd, Cr, Pb, Sr, and Zn were more abundant in the moss exposed near the tollgate in comparison with moss suspended inside the following garages: PG_{SE1}, PG_{U}, and PG_{SE2} (**Figure 4.2.1**). The exception was the moss content of Al, Ba, Fe, and Sr in PG_{O}, where the element enrichment was lower near tollgate than in the interior. As above-mentioned, PG_{U}, PG_{SE1}, and PG_{SE2} are classified as underground and semi-enclosed, while PG_{O} is a completely open garage. Thus, the impact of airflow and consequently dilution of pollutant concentrations could be decisive parameters for the garage air quality.

Therefore, the moss *S. girgensohnii* is moderated to intercept elements in enclosed spaces due to the absence of direct atmospheric deposition. The garage environment is usually characterized by stagnant ambient air because of poor airflow. In these conditions, moss element enrichment is a result of weak dust resuspension and/or movements caused by vehicle activities, which are possibly predominant in above ground level (up to ≈ 1 m). Since, in this experiment, moss bags were exposed at about 2.5 m, further moss biomonitoring surveys are necessary in order to get information about the most representative height of moss bags exposure in indoor area.

4.2.2. Instrumental vs. moss bag monitoring

In two garages – PG_U and PG_{SE1}, instrumental and moss bag monitoring were simultaneously performed. The element abundances in the PM10 sampled during the experimental period are given in **Table 7.3.5** (**Appendix 3**). The comparison of the moss biomonitoring measurements with the data obtained by instrumental monitoring pointed out similar order of element abundances in the PM10 and moss samples. Exception was concentrations of the elements usually depleted from moss tissue (K, Mg, Mn, and Na). However, despite the fact that instrumental measurements recorded extremely elevated indoor concentration of brake dust-related elements – Ba, Cu, and Zn, the moss did not reflect such increased element content in comparison with open space (Experiment 1, Vuković et al., 2013). In our opinion, this suggests the height of moss bag exposure to be possibly inappropriate.

Different air pollution level was expected due to diverse garage design: underground (PG_U) and aboveground (PG_{SE1}). Significant difference between the ambient element concentrations was not obtained by both the instrumental measurements and moss bag technique. In particular, it was observed that in the case of higher/lower concentrations of certain element in the moss bags exposed near the tollgate in one of the garages, the concentration of the same element was also higher/lower in the PM10 sampled from that garage (**Figure 4.2.2**). The results need further justifications and future research could be clarified representativeness of the height of the moss bags indoor exposure.

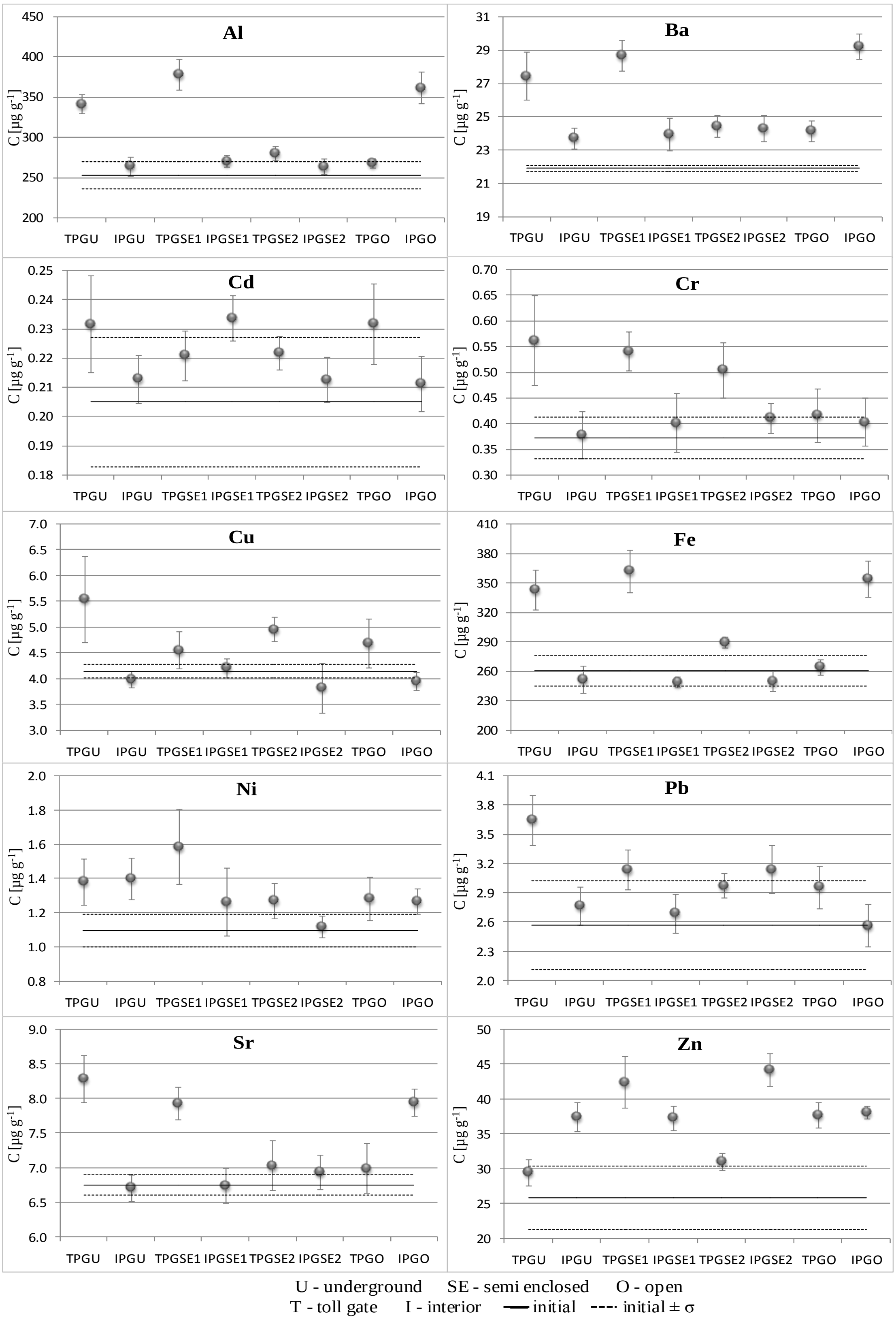

Figure 4.2.1. Average element concentrations (µg g-1) in the *S. girgensohnii* moss with standard deviation bars after ten-week exposure in the parking garages (PG) during the winter of 2012; initial – the element concentrations in the unexposed moss.

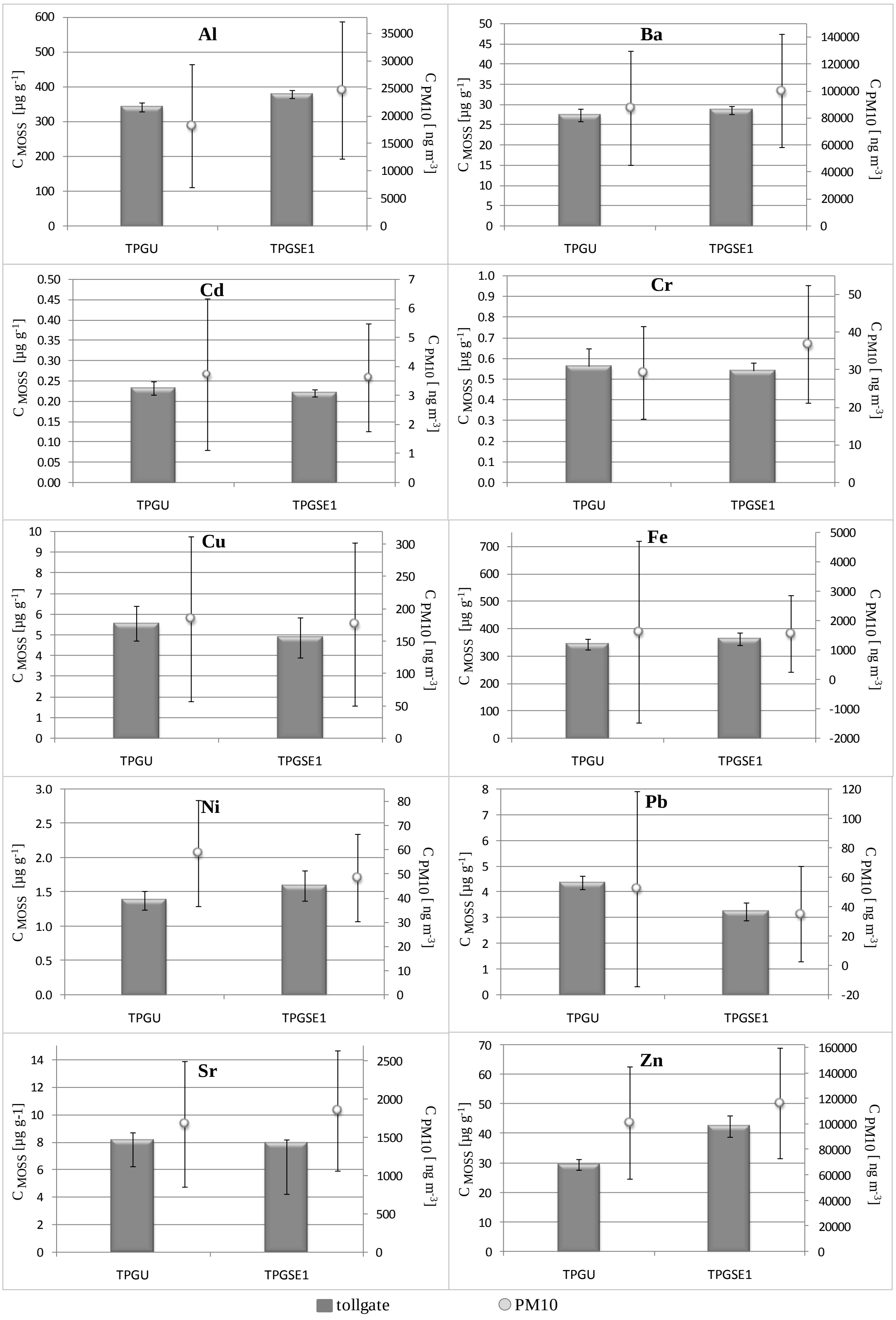

Figure 4.2.2. Average element concentrations with standard deviation bars (µg g^{-1}) in the *S. girgensohnii* moss bags exposed for ten weeks during the winter of 2012 near the tollgate (T) in the parking gareges (PG); dots – element concentrations (ng m^{-3}) in the PM10 sampled in two parking garages; U – underground parking garage, SE1 – semi enclosed parking garage.

4.3. **Experiment 3:** Ability of moss bags for biomagnetic monitoring through measurement of saturation isothermal remanent magnetization (SIRM)

4.3.1. Spatial variations of moss SIRM in urban microenvironments

The moss SIRM enrichment in comparison to the initial moss SIRM was observed in all samples after the exposure period (**Table 4.3.1**). The moss SIRM values measured at the three considered types of urban microenvironments – street canyons, parking garages, and city tunnel, were significantly different ($p < 0.05$).

The results of cluster analysis for the moss SIRM are given as a dendogram with two main clusters (**Figure 4.3.1**). The first cluster (IT_{10}, ET_{10}, OT_{10}, IT_5) represents the exposure positions of the moss bags in the tunnel where the highest SIRM values were observed. The second cluster contains two smaller ones: a group of the moss exposure positions in the street canyons (ET_5, OT_5, SC_2 I, SC_2 III, SC_2 II, SC_4 I, SC_3 II, TPG_{SE2}, SC_4 II, SC_1 II, SC_1 I, SC_4 III SC_1 II, SC_1 III, SC_3 I, SC_{ped} II, SC_1 II, SC_3 III SC_1 II, SC_{ped} III SC_1 II, SC_{ped} I), and another group of the moss exposure positions (TPG_{SE1}, TPG_U, TPG_O, IPG_{SE2}, IPG_{SE1}, IPG_U, IPG_O) in the parking garages with the lowest moss SIRM enrichement.

A correlation between ambient PM concentrations and SIRM values has been well documented (Rai, 2013), just as a correlation between traffic intensity and SIRM values (Kardel et al., 2012). The city tunnel and street canyons were characterised by intense traffic flows, giving rise to an increased ambient PM concentration. The highest moss SIRM values were observed for the tunnel samples suggesting the highest atmospheric PM concentrations will overall be found in the tunnel. In general, atmospheric deposition (e.g., sedimentation) represents the main mechanism of the moss enrichment with PM, and the ventilation effect – depending on both airflow speed and direction – could be considered as the main factor influencing PM interception.

The high SIRM values observed in the tunnel will be due to high ambient PM concentrations in the tunnel caused by the intense traffic flow combined with poor ventilation, as well as a lack of removal of particulates from the air through wet deposition. Steady moving of vehicles provides the main aerodynamic drag force that causes the drafting ventilation of airflow inside the tunnel. The consequence of this phenomenon is a “piston-type effect” in a tunnel (Hung-Lung and Yao-Sheng, 2009). In open air, when a vehicle travels along, air pushed aside can move in any direction except into the ground.

However, inside the tunnel, air is confined by the tunnel walls to move along the tunnel. For the considered street canyons, mostly regular or deep with a height/width (H/W) ratio > 1.3 (**Table 4.1.1**), their geometry will have aided to the built up of atmospheric PM concentrations. Due to their open character, natural ventilation is less disturbed in the street canyons in relation to the tunnel and dry and wet atmospheric particle deposition is enabled as well.

In the parking garages, the moss SIRM values were the lowest. Comparison to the tunnel and street canyons, traffic flow in the garages was significantly ($p < 0.05$) lower. Slow, almost stagnant movements of the vehicles inside the parking garages create only poor turbulence and limited dust resuspension. These processes are dominant in the ground air layer (up to ≈ 1 m) whereas the moss bags were exposed higher (at ≈ 2.5 m). In combination with the above-mentioned, natural ventilation depending on the garage structure (underground, semi-enclosed, and open) as well as effectiveness of the available ventilation system resulted in moderated moss interception of particles.

For biomagnetic monitoring of air pollution using moss bags it is crucial that the exposition of the moss bag is representative for the particulate load at the sites of interest which depends on the structure of the site and its specific air ventilation.

Table 4.3.1. Average moss SIRM values and standard deviation – σ **(×** 10^{-5} A m² kg^{-1}**),** SIRM enrichment ratio ($ER = SIRM_{exposed\ moss} / SIRM_{initial\ moss}$) and element concentrations (μg g^{-1}) in the *S. girgensohnii* moss bags exposed for ten weeks in the street canyons (SC), city tunnel (T) and parking garages (PG)

	Moss	SIRM ± σ	SIRM	Element concentrations in the moss samples (μg g^{-1})									
	exposition site	(× 10^{-5} A m^2 kg^{-1})	enrichment ratio	Al	Ba	Co	Cr	Cu	Fe	Ni	Pb	V	Zn
[a]	SC_1 I	134 ± 3	2.14	397	36.8	0.36	1.4	12.83	555	2.02	16.4	1.26	64.8
	SC_1 II	139 ± 7	2.23	469	37	0.39	1.59	11.47	619	1.95	9.2	3.81	55.4
	SC_1 III	127 ± 7	2.04	398	37.8	0.34	1.1	7.9	507	1.59	5.4	1.11	46.9
	SC_2 I	191 ± 3	3.07	541	58.4	0.45	2.1	15.04	834	2.61	9.9	2.08	234.3
	SC_2 II	149 ± 4	2.39	477	39.6	0.38	1.61	11.43	635	1.88	5.6	1.41	98.9
	SC_2 III	153 ± 7	2.45	393	36	0.37	1.56	11.4	575	1.87	4.7	1.48	110.6
	SC_3 I	130 ± 14	2.08	344	29.8	0.38	1.28	8.33	464	1.34	6.6	0.92	66.4
	SC_3 II	152 ± 3	2.43	417	35.2	0.51	1.65	10.07	558	1.92	17.9	1.09	61.5
	SC_3 III	112 ± 7	1.8	356	31.1	0.45	1.19	8.06	471	1.64	7.9	0.92	56
	SC_4 I	153 ± 2	2.45	421	32.5	0.36	1.58	13.7	579	2.05	6.7	1.32	69.7
	SC_4 II	136 ± 10	2.19	345	30.2	0.31	1.03	9.71	463	1.78	3.7	1.05	47.1
	SC_4 III	123 ± 4	1.97	320	20.7	0.31	0.96	8.1	424	1.73	5.2	1.2	44.7
	SC_{ped} I	105 ± 5	1.68	344	41.3	0.34	0.96	8.8	457	1.45	5.8	1.01	67.5
	SC_{ped} II	136 ± 18	2.18	439	60.2	0.39	0.97	9.79	594	1.77	5.9	1.21	91.8
	SC_{ped} III	113 ± 9	1.81	387	33.3	0.33	1.24	7.4	506	1.45	3.8	1.02	53.2
[b]	IT_5	533 ± 21	8.55	595	49.8	0.58	4.65	34.5	1379	3.34	4.7	0.39	71.3
	IT_{10}	936 ± 56	15.02	915	65.6	0.81	8.17	66.3	2254	5.3	16.3	1.76	154.2
	ET_5	205 ± 24	3.33	398	35.2	0.43	1.81	16.3	635	2.93	7.4	1.06	57.8
	ET_{10}	364 ± 13	4.95	563	44.1	0.7	3.35	38.4	1061	5.1	8.2	2.32	111.7
	OT_5	208 ± 16	3.29	429	37.8	0.43	2.07	19.3	727	3.52	7	2.01	109.5
	OT_{10}	309 ± 70	5.84	531	42.3	0.62	2.96	33.3	961	5.16	10.3	1.41	80
[c]	TPG_{SE1}	81 ± 3	1.3	280	24.4	0.08	0.5	5	290	1.27	3	0.51	31.1
	IPG_{SE1}	52 ± 2	0.83	264	24.3	0.07	0.41	3.8	250	1.12	3.1	0.4	44.3
	TPG_{SE2}	99 ± 3	1.59	378	28.7	0.14	0.54	4.6	362	1.59	3.2	0.56	42.5
	IPG_{SE2}	50 ±51	0.8	271	24	0.11	0.4	4.2	249	1.26	2.7	0.41	37.3
	TPG_O	63 ± 4	1.01	268	24.2	0.06	0.42	4.7	264	1.28	3	0.41	37.7
	IPG_O	50 ± 9	0.81	362	29.2	0.11	0.4	3.9	355	1.27	2.6	0.46	38.2
	TPG_U	69 ± 1	1.11	342	27.5	0.16	0.56	4	343	1.38	3.6	0.61	29.5
	IPG_U	51 ± 1	0.82	264	23.7	0.09	0.38	5.5	252	1.18	2.8	0.41	37.5
	initial	62 ± 1	~	226	23.5	0.27	0.53	3.5	275	1.03	2.6	0.55	27

[a] Exposure heights: I – 4 m; II – 8 m and III – 16 m, in four trafficked street canyons (SC_{1-4}) and pedestrian one (SC_{ped})

[b] Exposure positions in the tunnel: inside - IT, entrance - ET, outside - OT; five- and ten-week exposure duration

[c] Exposure positions: T – tollgate and I – interior, in different types of parking garages (PG_{SE} - semi-enclosed; PG_O – open; PG_U – underground)

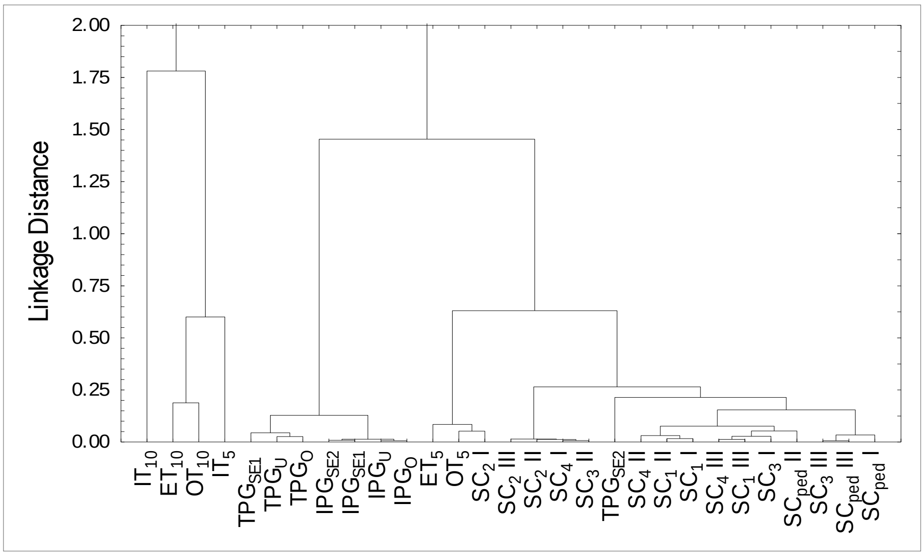

Figure 4.3.1. Dendrogram resulting from the Ward's method of hierarchical cluster analysis of SIRM values in the *S. girgensohnii* moss. The exposure heights: I – 4 m; II – 8 m, and III – 16 m, in five street canyons ($SC_{1\text{-}4}$, SC_{ped}); the exposure positions in the tunnel: inside – IT, entrance – ET, outside – OT; exposure duration – five and ten weeks; exposure positions in different types of parking garages: T – tollgate and I – interior (PG_{SE} – semi-enclosed; PG_O – open; PG_U – underground).

4.3.2. Vertical moss SIRM variation in street canyons

Among the studied street canyons, the lowest SIRM value was observed at the first exposure height (4 m) in the pedestrian street. This confirms the validity of this street as a background site. A significant ($p < 0.05$) decreasing trend of the moss SIRM, from the lowest (4 m) to the highest (16 m) sampling height, was observed in the two deep street canyons, SC_2 and SC_4 (**Table 4.3.1**), whereas in the other street canyons (SC_1, SC_3, and SC_{ped}), the highest SIRM values were observed at the second exposure height (8 m).

Similar vertical distribution patterns for the concentrations of Al, Ba, Co, Cr, Cu, Fe, Ni, Pb and Zn accumulated in the same moss bags were observed previously (Experiment 1, Vuković et al., 2013). Variation in the vertical gradient of the moss SIRM values and the element concentrations in urban street canyons presumably depend on the street canyon characteristics. Vertical dispersion of pollutants is influenced by the street canyon geometry (e.g., height/width – H/W and length/height – L/H ratio) affecting the airflow and turbulence inside the street canyons. Besides the street geometry different directions of the dynamically and thermals induced vortices, depending on meteorological conditions, may have an effect on the distribution pattern of pollutants. However, when average wind speed is below 1.5 m s^{-1}, as during the study period, the main wind vortex within the street canyons tends to disappear and the air stagnates in the street (De Paul and Sheih, 1986). As the wind speed was below 1.5 m s^{-1} we can expect air to stagnate in these streets, while wind speed was up to 2 m s^{-1} in SC_4. This latter street is a typical deep street canyon in which weak secondary and tertiary vortices may be observed at street level (Vardoulakis et al., 2003). These vortices may contribute to increased PM concentrations at the first height of moss bag exposure, and consequently the highest moss SIRM enrichment.

According to Weber et al. (2006), the vertical distribution of PM2.5 and PM1.0 is similar under different meteorological conditions in symmetric and regular street canyons. Thus, the above-mentioned PM fractions decrease in the upper part of the canyon due to enhanced mixing near the rooftop-level while a decrease of particles is observed at the near-surface level, which is believed to be due to enhanced turbulent mixing produced by vehicle movement.

In this experiment, in three symmetric and regular street canyons − SC_1, SC_3, and SC_{ped}, vertical distribution of the moss SIRM followed the above-mentioned vertical profiles of PM2.5 and PM1.0 – increasing from the first to the second height and then slightly decreased towards the third one. Note that a moss bag entraps the particles classes <2.5 µm predominantly (79%) over the coarser (2.5-10 µm) fraction (Tretiach et al., 2011).

Comparing the moss SIRM values with the element concentrations in the same moss samples in the street canyons, it was found that maximum SIRM at a particular height was accompanied by maximum concentrations of Al, Ba, Cr, Cu, and Fe. However, at the first height, moss SIRM values showed the highest correlation ($p < 0.05$) with the moss element concentrations in comparison with the other heights. Such results probably are due to fact that abrasion of tyres and brakes results in larger particles (>PM2.5) of which the concentration clearly decreases with height as well as leaf SIRM values starting from 3 m to 12 m (Hofman et al., 2013; 2014). Based on the results from this experiment, between three tested heights, the moss SIRM at 4 m could be chosen as the most representative for biomonitoring of traffic related PM air pollution.

4.3.3. Moss SIRM distribution in parking garages

The moss SIRM was significantly ($p < 0.05$) higher at the tollgate (TPG) than at the interior (IPG) of the garages situated in each of the studied street canyons (**Table 4.3.1**). Higher SIRM values at the tollgates might be a consequence of the slow-moving and breaking vehicle regime at this place. The moss SIRM values were significantly lower in the parking garages than in the street canyons and in or in front of the tunnel. Since *S. girgensohnii* has proven to be an efficient biomonitor of atmospheric deposition of particles, the significantly lower SIRM in the parking garages can be attributed to the absence of direct atmospheric deposition. Low traffic intensities and weak dust resuspension caused by slow vehicle movements also led to the reduction of PM capture by the moss bags inside the garages. In combination with the above-mentioned, natural ventilation depending on the garage structure (underground, semi-enclosed, and open) as well as effectiveness of the available ventilation system resulted in moderated moss particle interception.

Amongst the considered parking garages the highest moss SIRM values were observed for the semi-enclosed garages (PG_{SE1} and PG_{SE2}), although lower traffic flows were observed at these parking garages than in the closed (PG_U) and open one (PG_O). The highest traffic flows were measured in the street canyons where PG_{SE1} and PG_{SE2} are situated (SC_1 and SC_3, respectively). Thus, due to the half-open character of the parking garages, the SIRM values were directly influenced by the traffic intensity in the neighbouring street canyon.

The highest traffic flow was recorded together with the lowest moss SIRM in PG_O in comparison to the other garages, which demonstrates the effect of the garage structure on the atmospheric PM concentrations. Similar observations were made for the distribution of the moss element concentrations at the studied parking garages (Vuković et al., 2014).

4.3.4. Horizontal and temporal moss SIRM variations in city tunnel

Just as in previously described experiments, higher SIRM values were observed in the moss exposed for five and ten weeks in the city tunnel compared to the unexposed moss. Along the tunnel, a significantly ($p < 0.05$) higher SIRM enrichment was confirmed for the moss exposed inside the tunnel (IT), then at the entrance (ET) and outside of the tunnel (OT) (**Table 4.3.1**). The observed differences along the tunnel probably are a consequence of the 'piston-type effect' inside the tunnel(s) as described above. The high SIRM values in the tunnel are in agreement with other studies that reported on higher concentrations of PM10 and/or PM2.5 inside road tunnels than outside or in the neighbouring/surrounding urban environment (Cheng et al., 2010; Hung-Lung and Yao-Sheng, 2009; Pio et al., 2013). Thus, the horizontal SIRM distribution, which was obtained in the tunnel from this experiment, confirmed the possibility to use moss magnetic properties for biomonitoring of small-scale spatial variation of air pollution, just as tree leaves (Hofman et al. 2013).

A longer moss exposure (ten weeks versus five weeks) leads to significantly ($p < 0.05$) higher moss SIRM values. However, the decreasing trend of moss SIRM from the inside, via the entrance to the outside of the tunnel was evident in both shorter and longer exposure periods. As the spatial patterns do not change, and as proposed previously (Ares et al., 2012), an exposure period of about 5 weeks should suffice to get prompt insight into the spatial pollutant distribution. Longer exposure periods did not change the spatial pollution pattern of the SIRM values of urban tree leaves (Kardel et al., 2012), but could enhance differences between sites. In addition, the SIRM results show a similar spatio-temporal trend as the results of trace element analyses in the Experiment 1 (Vuković et al., 2013).

This experiment demonstrates that the *S. girgensohnii* moss bags can be used for magnetic urban biomonitoring studies, since they entrap airborne particles effectively containing metals and other elements, and they can be applied to monitor spatial and temporal variations in atmospheric pollution.

4.3.5. Correlation between moss SIRM and element concentration

Very high correlation coefficients ($r \geq 0.95$) between the moss SIRM and concentrations of Cr, Cu, Fe, and Ni were demonstrated (**Table 4.3.2**). High correlation coefficients ($r > 0.90$) were observed among the concentrations of listed element.

The above-mentioned elements are those with the highest relative accumulation factor obtained in moss samples as described in the Experiments 1 and 2 (Vuković et al. 2013; 2014). At a height of 4 m, the majority of moss-entrapped particles (up to 98%) belong to the inhalable, small size classes (≤ PM10) (Tretiach et al., 2011). Furthermore, it has been shown that SIRM represents especially the deposited fraction in the size range of 2.5 μm or even smaller (Matzka and Maher, 1999; Maher et al., 2008), although Hofman et al. (2014) recently showed that the majority of the leaf SIRM signal is attributed to particles larger than PM10. As the fine (PM2.5) and ultra-fine (PM<0.1) particles were reported to contain more anthropogenic, traffic-related metals (Ni, Cr, V, Pb and Ba) than larger particles (Singh et al., 2002), the results suggest that the impact of traffic could be a dominant pollution source in the studied sites. In addition, Kardel et al. (2012) showed that leaf particulate deposition estimated by SIRM was a good traffic indicator. In agreement with these authors, moss SIRM values exhibited a relatively high linear correlation (r = 0.72) with the average traffic intensity counted at the studied urban microenvironments (**Figure 4.3.2**).

A strong relationship between Fe concentration and SIRM values was obtained in the moss samples (**Table 4.3.2**). Iron usually occurs as an impurity in fossil fuels and it is released during combustion in the exhaust emissions. Another source of Fe-rich particles is the abrasion/corrosion of the engine and vehicle body materials (Lu et al., 2008). The magnetic fraction of Fe in vehicle-derived particulate pollution is estimated at 89% (Maher et al., 2008), predominantly in the form of magnetite (Kim et al., 2009, Chaparro et al., 2010; Chaparro et al., 2013). In agreement with previous studies (Gautam et al., 2005; Lu et al., 2005; Maher et al., 2008), the concentrations of Cr, Cu, and Ni exhibited a significant correlation with SIRM values.

Table 4.3.2. Spearman`s correlation coefficients between the moss SIRM values (× 10^{-5}A m^2 kg^{-1}) and the element concentrations (µg g^{-1}) in the moss bags; correlations marked in bold are significant at $p < 0.05$; italic bold values indicate correlation coefficients > 0.9

SIRM	Al	Ba	Co	Cr	Cu	Fe	Mn	Ni	Pb	Sr	V	Zn	Traffic flow
1													
0.877	1												
0.779	**0.892**	1											
0.886	**0.873**	**0.817**	1										
0.963	**0.893**	0.793	***0.924***	1									
0.957	**0.855**	0.785	**0.873**	***0.932***	1								
0.954	***0.964***	**0.883**	***0.921***	***0.959***	***0.936***	1							
0.723	0.584	0.549	0.735	0.692	0.654	0.669	1						
0.955	**0.892**	0.758	**0.871**	***0.935***	***0.951***	***0.939***	0.638	1					
0.742	0.700	0.676	**0.841**	**0.805**	0.781	0.759	0.518	0.793	1				
0.725	0.795	**0.824**	0.751	0.744	0.733	0.784	0.437	0.768	**0.812**	1			
0.715	0.712	0.648	0.661	0.699	0.721	0.740	0.400	0.759	0.763	**0.857**	1		
0.855	**0.816**	**0.856**	**0.842**	**0.842**	**0.857**	**0.880**	0.685	0.796	0.729	0.751	0.731	1	
0.806	0.696	0.519	0.723	**0.833**	0.795	0.779	0.358	**0.812**	0.571	0.460	0.505	0.521	1

The results of this experiment suggested that some trace elements, from e.g., abrasion of brakes as Cu (Hildemann et al., 1991), are possibly associated and co-precipitate with the magnetic Fe particulates. Metals and other elements could be incorporated into the lattice structure of the ferromagnetic particles during the combustion processes or could be adsorbed onto the surface of the pre-present ferromagnetic particles in the environments (Lu et al., 2008).

Finally, the results showed that, in urban microenvironments, *S. girgensohnii* is capable to reflect moss SIRM enrichment depending on traffic intensity, as well as its relation to the ambient element concentrations.

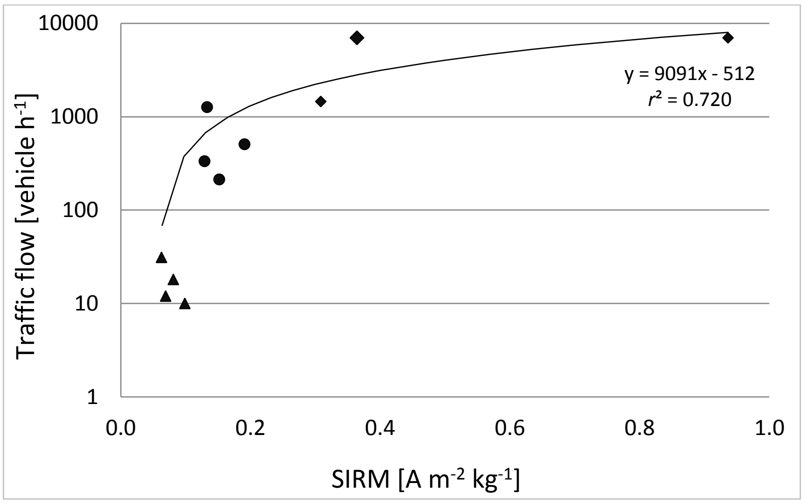

Figure 4.3.2. Correlation ($p < 0.05$) shown on logarithmic scale between traffic flow (vehicles per hour) and the SIRM (× 10^{-5} A m^{-2} kg^{-1}) measured in the *S. girgensohnii* moss, observed at ≈ 4 m in the street canyons (●) and a city tunnel (◆), and at ≈ 2.5 m in the parking garages (▲); n = 8 per studied position.

4.4. **Experiment 4:** Ability of moss bags for extensive screening of air pollution across urban area

4.4.1. Biomagnetic monitoring of urban air quality

Before chemical analysis of the moss samples, SIRM was determined as a proxy for particle enrichment of the exposed *S. girgensohnii* and *H. cupressiforme* bags. A significantly higher ($p < 0.05$) moss SIRM enrichment was recorded for *S. girgensohnii* in comparison with *H. cupressiforme*. The SIRM values exhibited ranges of 49 to 475 × 10^{-5} × A m^2 kg^{-1} for *S. girgensohnii* and 92 to 478 × 10^{-5} × A m^2 kg^{-1} for *H. cupressiforme* (**Table 4.4.1**). According to the available literature, only one study that uses magnetic measurements with respect to moss bags has been performed. That study involved 22 sampling points in an urban area in SW Finland (Salo et al., 2012), and moss SIRM data were observed within a range of 112 mA m^2 kg^{-1} for an urban forest to 917 mA m^2 kg^{-1} for a major urban road. A direct comparison between the SIRM data obtained in the above-mentioned study and the present experiment is not directly possible due to differences in exposure time, i.e., 88 versus 60 days, respectively, and the design of the moss bags, i.e., spherical versus flat, respectively.

Although the majority of the moss SIRM data were distributed uniformly over the study area, a noticeable distinction between different urban land use classes was observed (**Figure 4.4.1**). The highest SIRM values, an indicator of PM pollution according to Hofman et al. (2014), were registered at sampling sites near local industries, followed by those characterized by high-intensity traffic flows. For both moss species, the median moss SIRM values were approximately 60% higher for streets with public transportation (buses, trams, and trolleys) compared to residential zones, city parks and forests (**Figure 4.4.1**). Moreover, 60 − 70% higher SIRM values were recorded in streets occupied by tram traffic compared to streets with only buses and cars. Similar findings were reported for a tree leaf SIRM survey in the urban area of Gent (Belgium) (Kardel et al., 2012). Additionally, Revuelta et al. (2014) showed that the origin of vehicular-sourced magnetic particles is more related to abrasion/friction emissions, particularly brakes, rather than exhaust emissions. Finally, moss SIRM data suggest that higher PM concentrations could be expected near streets with higher traffic intensity (Weijers et al., 2004).

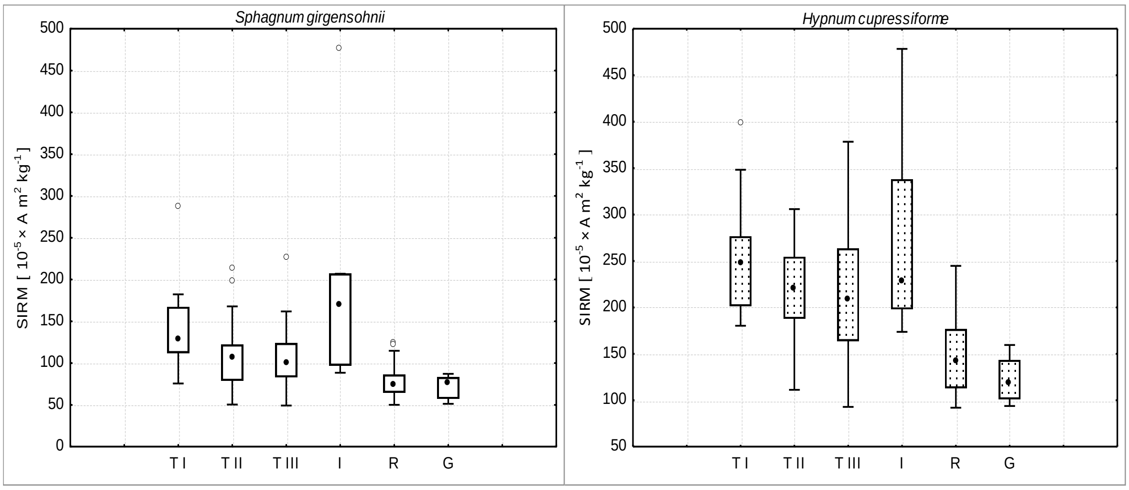

Figure 4.4.1. Median SIRM values (10^{-5} × A m^2 kg^{-1}) of the mosses *S. girgensohnii* (blank box) and *H. cupressiforme* (dotted box) according to different land use classes within the city of Belgrade: urban central – public transport I (T I) and II (T II); suburban – public transport III (T III) and residential (R); industrial – local industry (I); urban green – parks and forests (G).

Significant correlation coefficients ($p < 0.05$) were observed between moss SIRM values and concentrations of Cr, Cu, and Fe (**Table 4.4.1**), indicating that elements originating from both vehicle exhaust and abrasion processes could be incorporated into the lattice structure of ferromagnetic particles (Lu et al., 2008). The coefficients are lower than those reported between moss SIRM and Cr ($r = 0.963$), Cu ($r = 0.957$), and Fe ($r = 0.957$) in the Experiment 3 (Vuković et al., 2015b), in which the moss bags were exposed in specific urban microenvironments, such as street canyons, a city tunnel and parking garages, which were only or mostly influenced by vehicular emissions. In the present experiment, the relation between moss SIRM and elements was possibly weakened due to the mixed impact of different pollution sources over the large urban area. The composition of magnetic particulates from vehicle- and industry-derived sources is known to differ (Hansard et al., 2011).

Despite moderate correlation coefficients between moss SIRM values and element concentrations, noticeably lower SIRM values were observed for the residential zones and parks than for the public transportation zones. The results indicate that biomagnetic monitoring could be a valuable technique for the first assessment of particulate matter air pollution before more complex and expensive trace elemental analyses.

Table 4.4.1. Descriptive statistics of SIRM ($\times 10^{-5}$ A m^2 kg^{-1}) and element concentrations (μg g^{-1}) for two moss species, *S. girgensohnii* and *H. cupressiforme*, exposed over the city of Belgrade; significant ($p < 0.05$) Spearman's correlation coefficients (r) between moss SIRM and element concentrations are bold and italicised.

Sphagnum girgensohnii

*N = 153	SIRM ($10^{-5} \times$ A m^2 kg^{-1})	Al	Ba	Cd	Co	Cr	Cu (μg g^{-1})	Fe	Ni	Pb	Sr	V	Zn
Initial	34	328	28	0.17	0.36	0.40	2.61	300	1.72	2.51	6.52	0.48	24
Min	49	328	21	0.09	0.25	0.21	2.48	163	1.36	1.85	3.33	0.47	36
Max	475	754	48	0.32	0.77	2.56	14.58	904	7.85	11.11	18.19	2.07	353
Mean	105	459	32	0.20	0.41	1.11	5.81	495	2.34	4.34	9.03	1.02	86
Median	90	448	31	0.20	0.39	1.03	5.39	475	2.17	3.90	8.70	0.87	74
SD	54	76	4	0.05	0.07	0.45	2.35	117	0.77	1.70	3.27	0.39	49
r ($p < 0.05$)		***0.513***	***0.510***	0.012	***0.326***	***0.550***	***0.788***	***0.632***	***0.460***	***0.335***	***0.178***	***0.383***	***0.109***

Hypnum cupressiforme

*N = 153	SIRM ($10^{-5} \times$ A m^2 kg^{-1})	Al	Ba	Cd	Co	Cr	Cu (μg g^{-1})	Fe	Ni	Pb	Sr	V	Zn
Initial	125	934	19	0.46	0.27	1.23	7.55	657	2.42	5.77	13.54	1.63	40
Min	92	623	12	0.30	0.18	0.42	7.10	474	1.73	3.97	9.12	1.13	40
Max	478	1584	34	0.69	0.59	3.56	22.33	1375	5.87	14.39	20.39	3.19	578
Mean	197	1013	23	0.48	0.34	1.79	11.30	823	3.18	6.47	14.03	1.87	97
Median	188	998	23	0.44	0.34	1.69	10.70	819	3.06	6.18	13.99	1.85	87
SD	73	205	6	0.11	0.09	0.53	2.77	179	0.80	1.62	2.81	0.43	59
r ($p < 0.05$)		***0.564***	***0.642***	***-0.200***	***0.596***	***0.820***	***0.783***	***0.798***	***0.413***	***0.627***	-0.021	***0.500***	0.037

* N - total number of sampling sites; Initial - the data obtained in an unexposed moss, Min – minimum value, Max – maximum value, Mean – average value, SD standard deviation

4.4.2. Moss element content – Interspecies comparison

For the majority of the elements, the initial concentrations in the mosses were relatively low and in the range of those obtained in the previous studies (**Table 4.4.1**) (Aničić et al., 2009c; Frontasyeva et al., 2004; Vuković et al., 2013), which indicates that the moss collection sites are appropriate background areas. The orders of mosses' elemental RAF values were Zn > Cr > Cu > Pb > Ni > V ≥ Fe > Sr > Al > Co > Cd > Ba and Zn > Cu ≥ Cr > Pb > Ni > Co > Fe > V > Ba > Al > Sr > Cd for *S. girgensohnii* and *H. cupressiforme*, respectively. Higher RAF values were obtained for *S. girgensohnii* than for *H. cupressiforme*.

The highest abundance of Zn, Cr, and Cu was observed in the central land use class, characterized by the intense flow of public transport along with heavy and light vehicles (**Figure 4.4.2** and **4.4.3**). The elements are emphasized by EMEP/EEA (2013) as trace metals sourced from exhaust emissions of road transport. In contrast, the lowest concentrations of the selected elements were obtained in residential zones and city parks/forests, confirming these sampling sites as urban background areas.

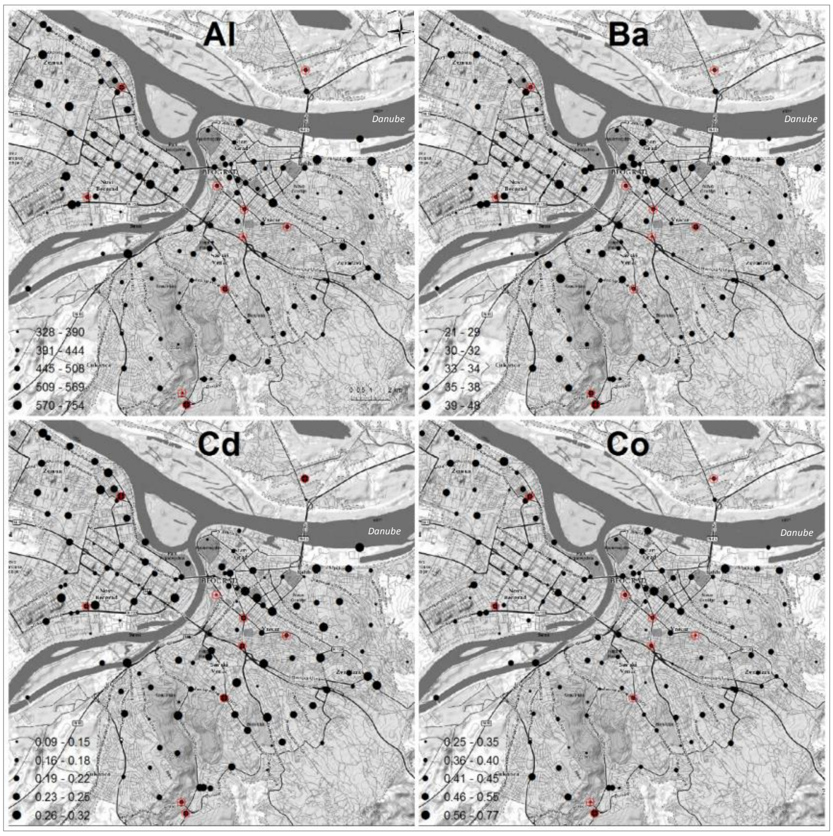

Figure 4.4.2. Distribution maps of Al, Ba, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sr, V, and Zn concentrations (μg g^{-1}), classified in five ranges, in the *S. girgensohnii* moss bags exposed for ten weeks during the summer of 2013 over the city of Belgrade.

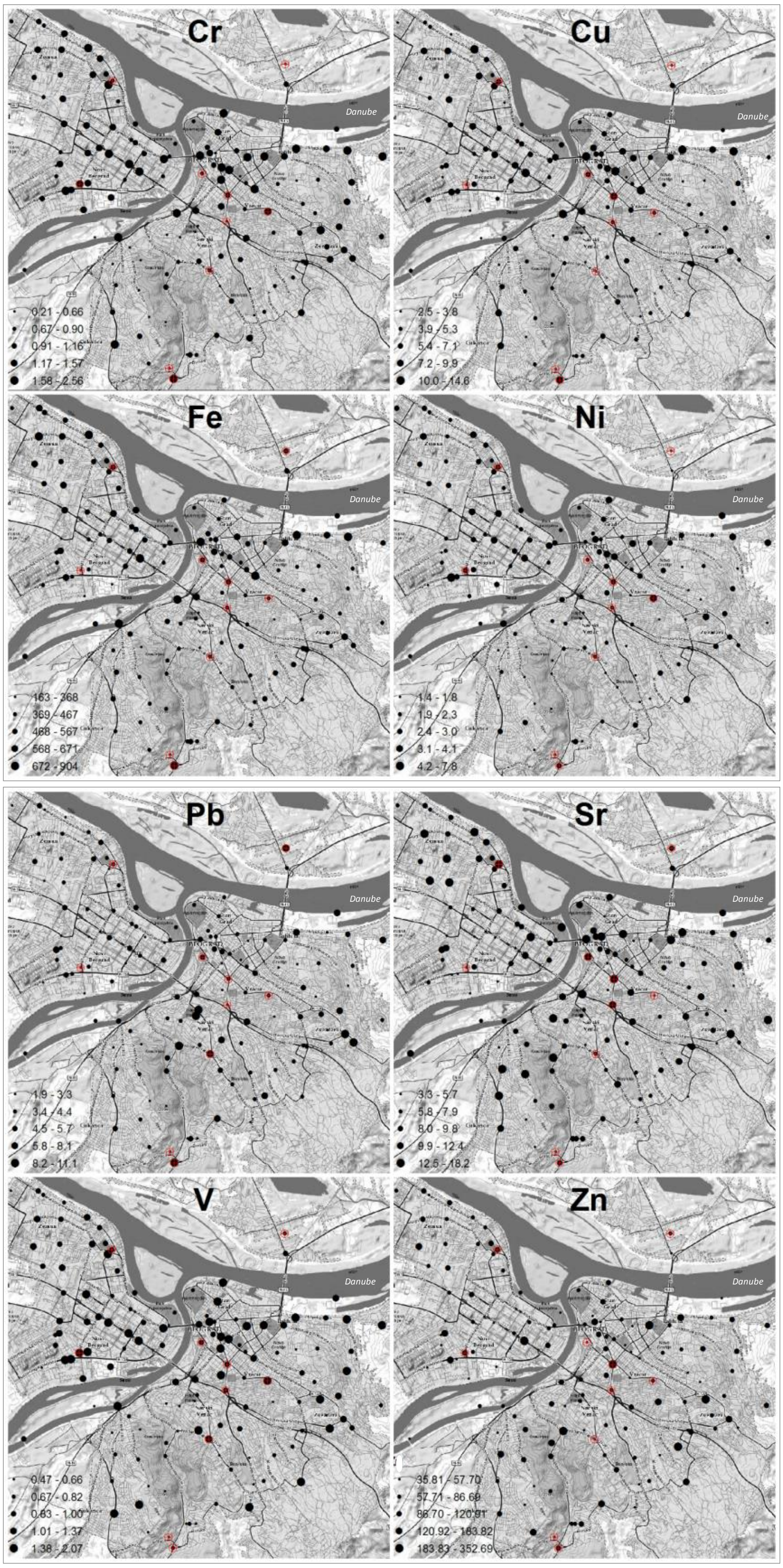

Figure 4.4.2. (continued).

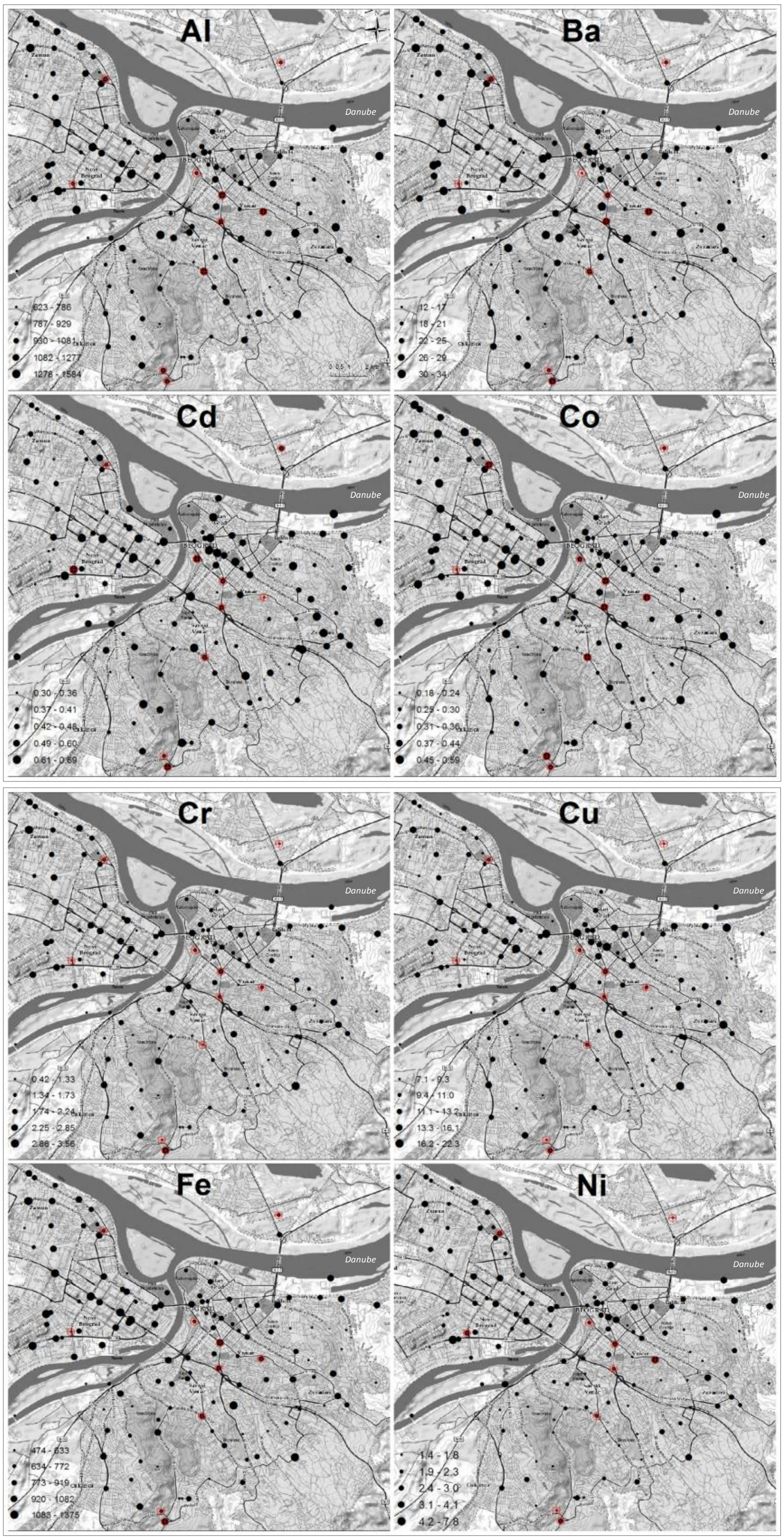

Figure 4.4.3. Distribution maps of Al, Ba, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sr, V, and Zn concentrations (μg g^{-1}), classified in five ranges, in the *H. cupressiforme* moss bags exposed for ten weeks during the summer of 2013 over the city of Belgrade; regulatory monitoring stations are represented by red circles.

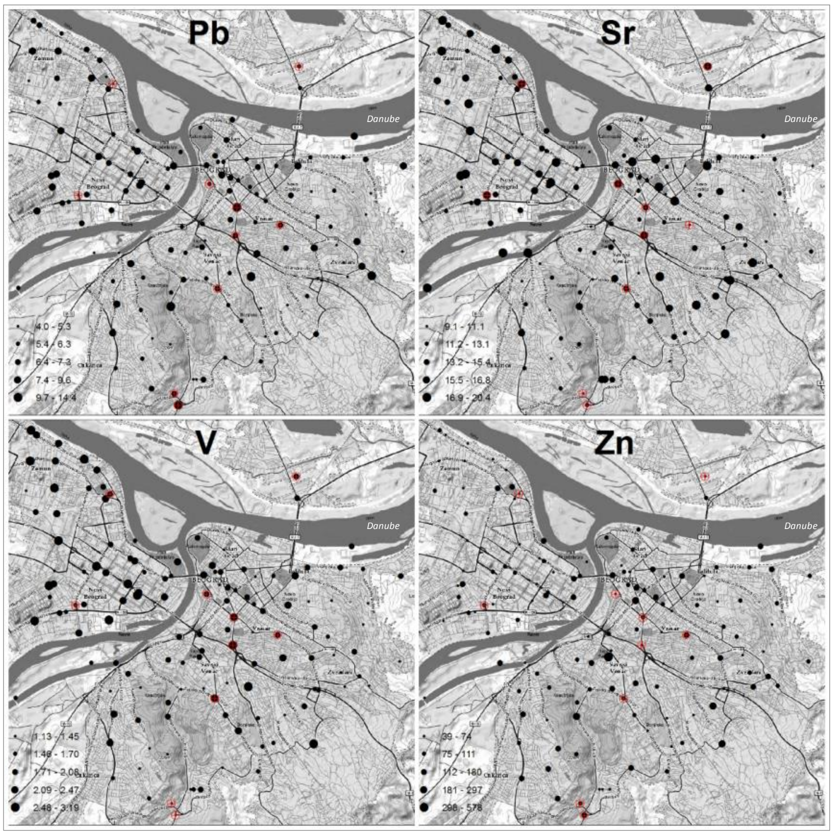

Figure 4.4.3. (continued).

Thus far, inter-calibration between moss species used for active moss biomonitoring purposes has been performed in a few studies. Specifically, Culicov and Yurukova (2006) conducted active moss biomonitoring using *S. girgensohnii* and *H. cupressiforme* moss bags at three industrial sites; Castello (2007) compared the element enrichment abilities of the mosses *H. cupressiforme* and *P. purum* at ten sampling sites; and Ares et al. (2014) conducted a survey using *S. denticulatum* and *P. purum* at thirteen sampling sites. The genus *Sphagnum* has been found to accumulate the highest quantities of pollutants, but these conclusions have been based on limited datasets. In our experiment, an extensive dataset (153 sampling sites in highly polluted to relatively unpolluted areas) was created with a wide range of major and trace element concentrations.

Type II linear regression was applied for interspecies comparison (**Figure 4.4.4**). All the regression lines were significant, except those for Co, Ni and V; however, low determination coefficients were obtained for the majority of elements ($r^2 \leq 0.10$), with the exception of Cr ($r^2 = 0.24$) and Cu ($r^2 = 0.50$). Although there are no conventional criteria for the use of regression lines for inter-species calibration, Berg and Steinnes (1997) suggested that regression equations with $r^2 \geq 0.5$ could be used to correct data for the interchangeable use of terrestrial mosses *H. splendens* and *P. schreberi* in large-scale surveys. Correlation coefficients close to one have also been recommended as suitable for inter-calibration of terrestrial mosses (Carbaleira et al., 2008). From our perspective, based on the results for the majority of the elements obtained by inter-species comparison ($r^2 \leq 0.10$, Figure 3), the *S. girgensohnii* and *H. cupressiforme* moss bags could not be interchangeably used in urban areas with high traffic emissions except for the biomonitoring of Cu atmospheric pollution.

4.4.3. PAH diagnostic ratios for mosses

The total PAH content varied between 42 and 178 ng g^{-1}, and 80 and 249 ng g^{-1} for *S. girgensohnii* and *H. cupressiforme*, respectively. The PAH concentrations are given in **Table 10** (Section: *4.5.3. Moss PAH variations*). In both moss species, the most abundant low-molecular-weight PAHs were Nap, Phen, Fla, Pyr, and Chr, and the most abundant high-molecular-weight PAHs were B[b]F, B[k]F, B[a]P, and I[cd]P. These PAHs were reported as dominant in the other active biomonitoring studies in which mosses were exposed to intense traffic pollution along major highways (Viskari et al., 1997), main streets (Orlinski et al., 2002) and in the street canyon (De Nicola et al., 2013).

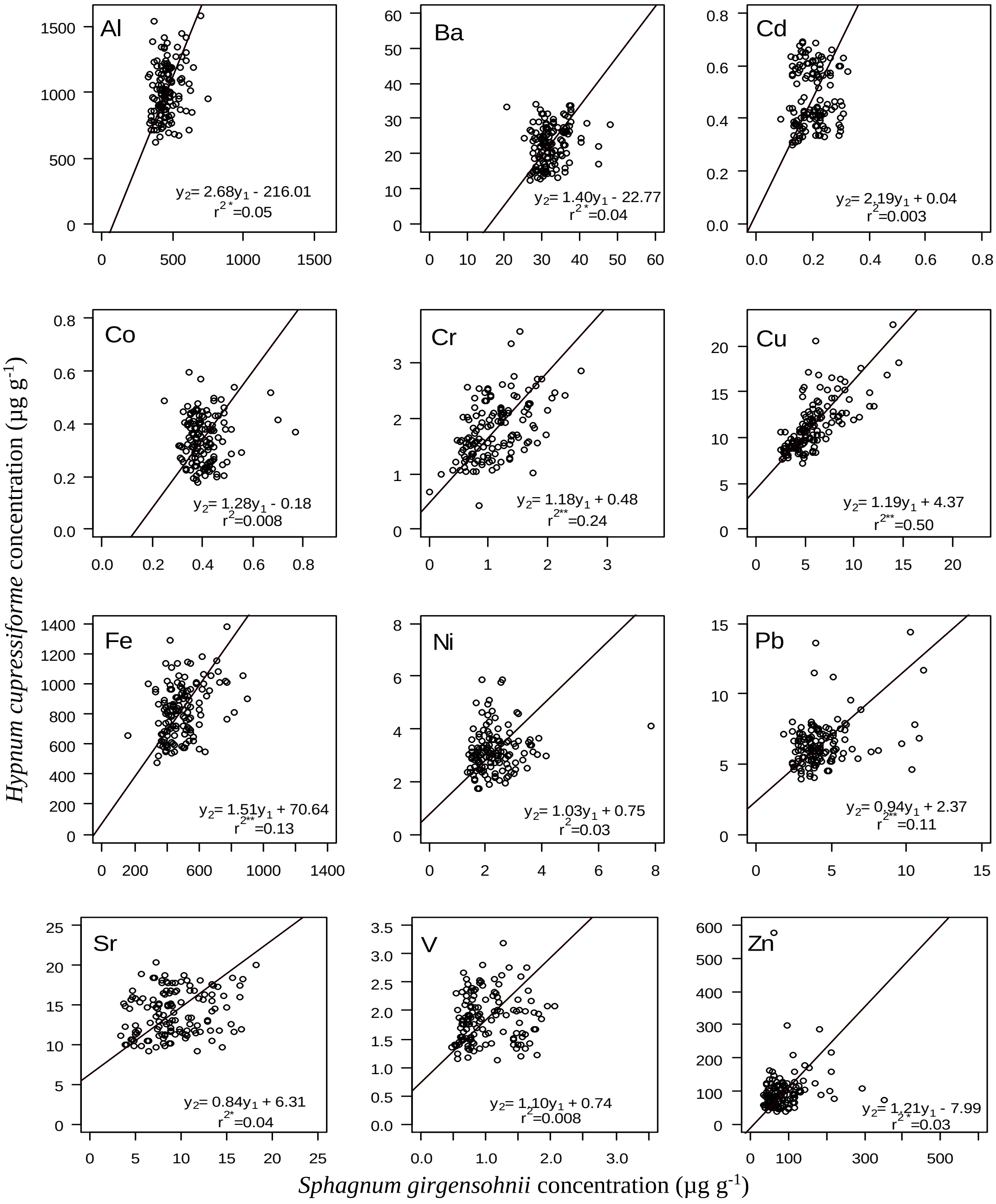

Figure 4.4.4. Type II regression lines presented for Al, Ba, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sr, V, and Zn concentrations (µg g^{-1}) in the mosses *S. girgensohnii* and *H. cupressiforme*. The black line is obtained using the standardised major axis regression method; the coefficients of determination that are significant at the $p < 0.05$ and $p < 0.001$ level are indicated by * and **, respectively.

In general, the low-molecular-weight PAHs are known to be indicators of both diesel and gasoline vehicle exhaust. Medium- and high-molecular-weight PAHs, B[b]F, Chr, Fla, Pyr and I[cd]P, are primarily identified as indicators of the diesel exhaust contributions (Ho et al., 2002; He et al., 2014).

The PAH diagnostic ratios represent a worthy indication of PAH pollution sources but should not be considered a definitive confirmation (Tobiszewski and Namieśnik, 2012). Additionally, source identification is usually used as a relative determinant based on a combination of more than one diagnostic ratio. However, diagnostic ratios have been proposed according only to particle-bound PAH concentrations assessed according to standardized procedures (Ravindra et al., 2008; Oliveira et al., 2011). The ratios calculated for both moss species, *S. girgensohnii* and *H. cupressiforme*, indicated traffic-sourced emissions, particularly diesel exhaust emissions (**Table 4.4.2**). The diagnostic ratios did not fluctuate significantly ($p < 0.05$) between the moss species.

The obtained results comply with magnetic measurements and element compositions of *S. girgensohnii* and *H. cupressiforme*, implying that traffic is the primary source of urban air pollution over the city of Belgrade during the summer season.

Table 4.4.2. PAH diagnostic ratios (minimum, maximum, mean and median values) in the *S. girgensohnii* and *H. cupressiforme* moss bags exposed for ten weeks during the summer of 2013 over the city of Belgrade.

Moss PAH diagnostic ratios							
		Min	Max	Mean	Median	Pollution sources	References
Fla/(Fla+Pyr)	*S. girgensohnii*	0.49	0.63	0.54	0.54	Vehicular emissions: 0.4 - 0.5	Yunker et al., 2002
	H. cupressiforme	0.49	0.61	0.55	0.55		
I[cd]P/ (I[cd]P+B[g,h,i]P)	*S. girgensohnii*	0.43	1.00	0.90	1.00	Grass, wood and coal combustion: > 0.5	Tobiszewski and Namieśnik, 2012
	H. cupressiforme	0.62	1.00	0.91	1.00		
B[a]A/ (B[a]A+Chr)	*S. girgensohnii*	0.12	0.27	0.19	0.18	Petrogenic: < 0.2	Yunker et al., 2002
	H. cupressiforme	0.10	0.24	0.20	0.21		
B[b]F/B[k]F	*S. girgensohnii*	1.39	2.91	2.24	2.28	Diesel emissions: > 0.5	Ravindra et al., 2008; Oliveira et al., 2011
	H. cupressiforme	1.81	4.23	2.40	2.33		
Pyr/B[a]P	*S. girgensohnii*	0.87	2.39	1.33	1.28	Gasoline emissions: ~1	Ravindra et al., 2008
	H. cupressiforme	0.65	2.37	1.09	0.96		

4.4.4. Moss bag technique as an air pollution monitoring approach

The moss bag technique provides information about the spatial and temporal deposition of air pollutants. In addition, mosses show how pollutant deposition interacts with living organisms through processes that depend on the environmental conditions in a particular study area (Aboal et al., 2010).

The evaluation of air quality parameters, such as particulates, trace metals and PAHs, by *S. girgensohnii* and *H. cupressiforme* moss bags over the urban area of Belgrade indicated different air pollution zones. Among the defined land use classes, both moss species clearly identified areas with high, intermediate, and low levels of air pollution (**Figures 4.4.2, 4.4.3, 4.4.5 and 4.4.6**). The highest pollutant levels were confirmed for the urban central land use class. Furthermore, both *S. girgensohnii* and *H. cupressiforme* revealed new pollution hotspots over the land use classes where regulatory monitoring stations are absent (**Figures 4.4.2 and 4.4.3**), in particular in industrial (local stationary emission sources) and urban central zones (public transportation zone I). As expected, urban green zones, such as city parks and forests, were characterized by the lowest moss pollutant levels. Based on the magnetic and chemical trace metal and PAH analyses, both moss species indicated that traffic exhaust emissions were the dominant source of air pollution within the city of Belgrade.

According to the results, *S. girgensohnii* proved a more sensitive indicator of small-scale variations in air pollutant levels, i.e., between neighbouring sampling sites within the same land use class. Neighbouring sites are often arranged into classes that were different by 2 - 3 orders of magnitude (**Figures 4.4.2 and 4.4.3**). *Sphagnum* sp. has been already recommended as the most suitable species for the moss bag technique (Ares et al., 2012). However, this genus is uncommon in the territory of Serbia; thus, the moss *H. cupressiforme* was chosen as the most recommended alternative species (ICP Vegetation, 2005) and tested for interchangeable use in our experiment. Our findings show that *H. cupressiforme* can also reflect spatial pollutant variations, but contrasting pollution levels between different land use classes exist at a large-scale.

The results indicate that moss bags represent a means to screen extensively air pollution over large urban areas to obtain information on the time-integrated spatial distributions of particle-bound trace metals and PAHs, demonstrating the moss bags' potential for epidemiological studies (Gailey & Lloyd, 1993; Hellström et al., 2004; Wolterbeek et al., 2004).

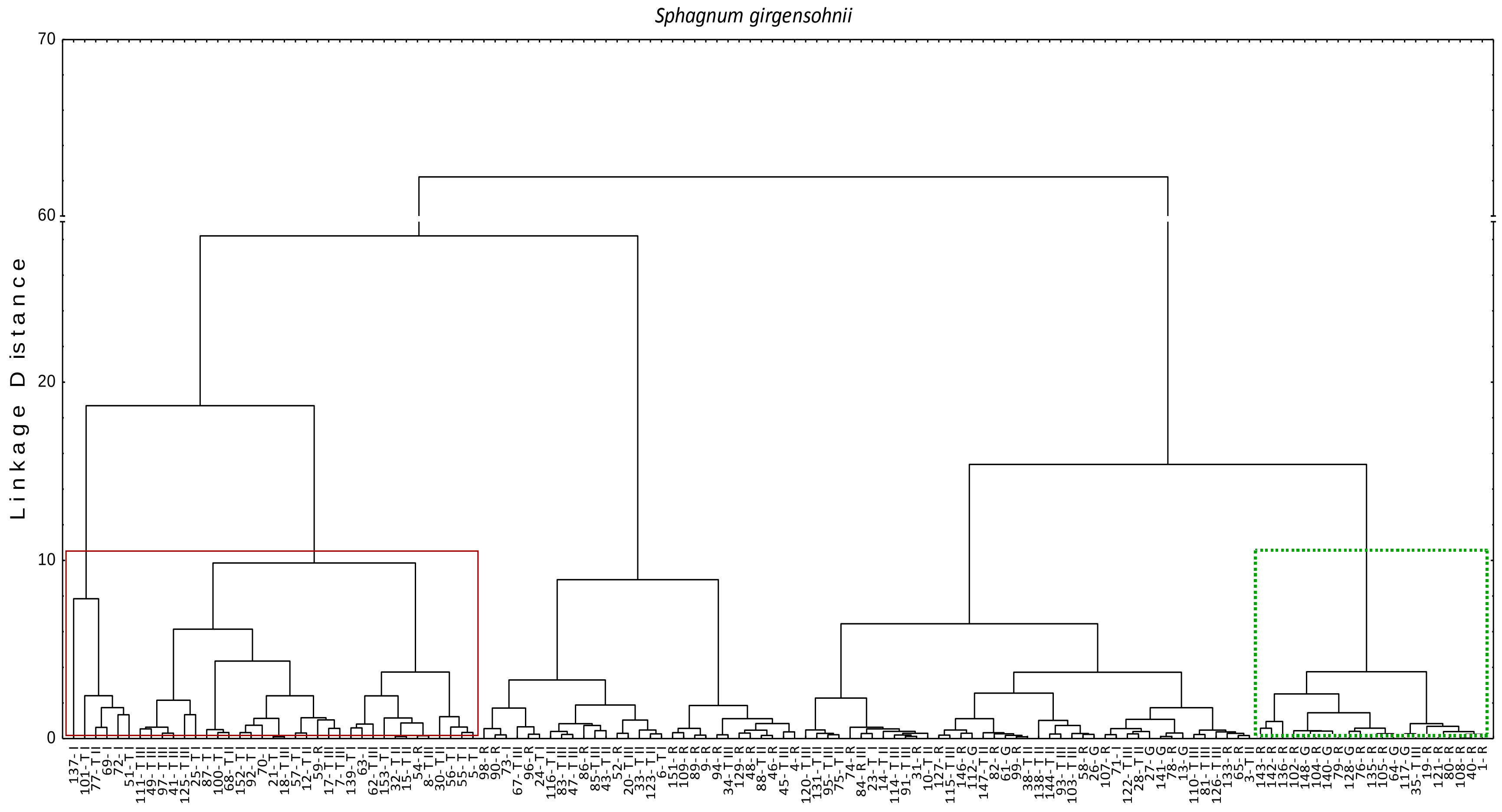

Figure 4.4.5. Dendrogram resulting from the Ward's method of hierarchical cluster analysis of moss SIRM and trace element (Cu and Cr) concentrations in the *S. girgensohnii* moss bags. Land use classes: urban central – public transport I (T I) and II (T II); suburban – public transport III (T III) and residential (R); industrial (I); urban green – parks and forests; red triangles represent the sampling sites with the highest pollutant concentrations (public transport zones); green triangles represent the sampling sites with the lowest pollutant concentrations (residential and green zones).

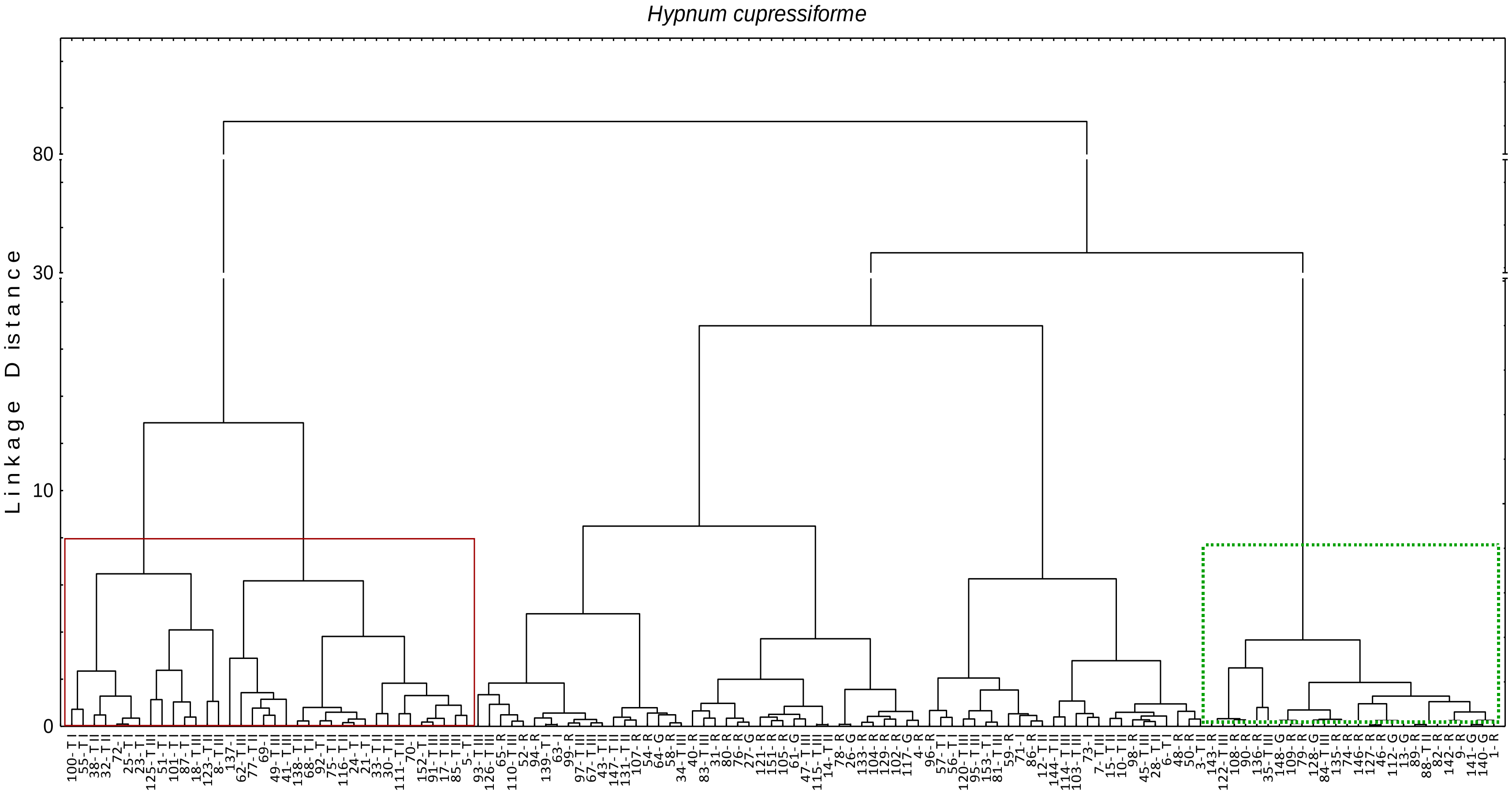

Figure 4.4.6. Dendrogram resulting from the Ward's method of hierarchical cluster analysis of moss SIRM and trace element (Cu and Cr) concentrations in the *H. cupressiforme* moss bags. Land use classes: urban central – public transport I (T I) and II (T II); suburban – public transport III (T III) and residential (R); industrial (I); urban green – parks and forests; red triangles represent the sampling sites with the highest pollutant concentrations (public transport zones); green triangles represent the sampling sites with the lowest pollutant concentrations

4.5. **Experiment 5:** Ability of moss bags to reflect seasonal variations of air pollutants

4.5.1. Seasonal variations of moss element concentrations

Descriptive statistics for major, trace and rare element concentrations in the moss *S. girgensohnii*, after exposure during the winter, are shown in **Table 4.5.1**. Concentrations of the majority of elements determined in the moss samples were significantly higher than the initial values, which indicate that the study area was polluted by the elements in question. Except for Al, Ba and Cr, the concentrations of all determined elements were significantly higher ($p < 0.05$) in the winter compared to the summer study period.

The highest moss RAF values were obtained for Zn (up to 150), Na (up to 80), Sb (up to 70) and, V (up to 20), and Cu and Ni (up to 10). Negative RAF values were mainly observed for physiologically active elements such as K, Mg, and Mn, and for Rb, just as it was previously pronounced (Aničić et al., 2009a). This confirms that physico-chemical characteristics of elements, i.e., their binding properties, define the moss elements uptake (Varela et al., 2015). Manganese could be also involved with caution in moss biomonitoring surveys because increased Mn concentrations will be only observed in the moss tissues in the surroundings of industries, which emitted a high concentration of this metal (Boquete et al., 2011).

The lowest concentration of all elements in the moss samples was observed within the green zones of the study area, confirming city parks and forests as an urban pollution background. In the urbanized parts of the study area, the moss samples were markedly enriched with the elements, especially Sb, Cu, V, Ni, Zn, and Na for which RAF_{max} values were above ten (**Figure 4.5.1**). In addition, very high correlation coefficient between the pairs of these elements: V and Ni ($r = 0.97$) along with Cu and Sb ($r = 0.95$) indicated their similar origin.

Vanadium and Ni are considered as typical tracers of oil combustion processes and could be emitted in large amounts by the heat-producing units (Pacyna and Pacyna, 2001). In this experiment, moss concentrations of V and Ni were markedly higher than in the summer (Experiment 4, Vuković et al., 2015a) (**Figure 4.5.1**). For the same study area, seasonal variability of Ni and V was also observed by the multi-year bulk deposition monitoring (Mijić et al., 2010) and the moss bags (Aničić et al., 2009a). In addition, significant correlation coefficients between concentrations of these elements in the moss and bulk deposit samples were also observed (Aničić et al., 2009a). Above-mentioned clearly suggest the contribution of residential heating, based on oil fuel combustion, to the content of V and Ni in ambient air during the cold, i.e., heating season.

Table 4.5.1. Descriptive statistics of the element concentrations (μg g^{-1}) determined in the *S. girgensohnii* moss bags exposed for ten weeks during the winter of 2013/2014 over the city of Belgrade

Element	Initial	Min	Max	Mean	Median	RAF$_{min}$	RAF$_{max}$	RAF$_{mean}$	RAF$_{median}$
				N = 22; n = 3					
Al	300	330	1600	530	450	0.1	4.3	0.8	0.5
Ba	27	26	46	32	30	0	0.7	0.2	0.1
Be	0.03	0.01	0.13	0.02	0.02	-0.7	3.5	-0.2	-0.4
Ca	3600	3900	12300	5900	5100	0.1	2.4	0.6	0.4
Cd	0.18	0.23	0.51	0.33	0.32	0.3	1.8	0.8	0.8
Ce	0.321	0.382	3.123	0.934	0.73	0.2	9	2	1.3
Co	0.32	0.35	2.71	0.69	0.46	0.1	7.5	1.2	0.4
Cr	0.28	0.48	5.34	1.49	0.88	0.7	18	4.3	2.1
Cu	3.8	5.4	44.4	16.4	11.1	0.4	11	3.3	1.9
Dy	0.019	0.024	0.195	0.054	0.042	0.3	9.5	1.9	1.3
Er	0.009	0.012	0.095	0.026	0.021	0.3	9.1	1.8	1.2
Eu	0.0058	0.0074	0.0596	0.017	0.0142	0.3	9.2	1.9	1.4
Fe	330	400	2600	800	580	0.2	6.9	1.4	0.8
Ga	0.07	0.1	0.92	0.25	0.21	0.3	12	2.4	1.8
Gd	0.022	0.029	0.249	0.069	0.054	0.3	10	2.1	1.4
Ho	0.0038	0.0045	0.0367	0.0101	0.0079	0.2	8.8	1.7	1.1
K	9400	2800	9700	6800	7200	-0.7	0	-0.3	-0.2
La	0.16	0.2	1.56	0.49	0.39	0.3	8.7	2	1.4
Li	0.11	0.171	1.329	0.392	0.291	0.6	11	2.6	1.6
Lu	0.0011	0.0013	0.0113	0.0031	0.0024	0.2	9.4	1.8	1.2
Mg	1220	1160	1460	1280	1290	0	0.2	0	0.1
Mn	230	180	250	230	230	-0.2	0.1	0	0
Mo	0.21	0.21	1.37	0.5	0.36	0	5.5	1.4	0.7
Na	160	280	12700	2900	1350	0.8	78	17	7.4
Nd	0.13	0.16	1.49	0.41	0.33	0.3	11	2.2	1.5
Ni	1.6	2	16.9	5.5	3.5	0.3	9.9	2.5	1.2
Pb	3.5	4.6	19.36	7.5	7.1	0.3	4.5	1.2	1.1
Pr	0.035	0.043	0.375	0.107	0.086	0.3	9.9	2.1	1.5
Rb	65	23	77	49	52	-0.6	0.2	-0.2	-0.2
Sb	0.04	0.19	3.06	0.94	0.46	3.9	78	23	11
Sc	0.048	0.06	0.625	0.155	0.114	0.2	12	2.2	1.3
Sm	0.025	0.033	0.292	0.082	0.064	0.3	11	2.3	1.5
Sr	10	8	30	15	15	-0.2	2	0.5	0.4
Tb	0.0034	0.0042	0.0359	0.0099	0.0077	0.2	9.5	1.9	1.3
Th	0.019	0.026	0.358	0.086	0.06	0.4	18	3.5	2.2
Tl	0.022	0.025	0.044	0.032	0.031	0.1	1	0.4	0.4
Tm	0.0019	0.0017	0.0707	0.0077	0.0037	-0.1	36	3	0.9
V	0.45	1.15	13.96	4.25	2.66	1.6	30	8.5	4.9
Yb	0.007	0.0091	0.0779	0.0208	0.016	0.3	10	2	1.3
Zn	25	65	3920	860	570	1.6	155	33	22

* N – total number of sampling sites, n – number of subsamples per exposure site; Initial – the data obtained in the unexposed moss, Min – minimum value, Max – maximum value, Mean – average value, RAF – relative accumulation factor

Antimony and Cu in the moss tissue are pronounced as tracers of traffic emissions (e.g., Zechmeister et al., 2005). This statement was also supported in the Experiment 4 (Vuković et al., 2015a) conducted during the summer when the residential heating system does not operate. In those conditions, the highest moss Cu content was observed at the traffic-oriented sampling sites. Based on this, we assume that the high correlation coefficients between concentrations of Cu and Sb determined in the winter indicate their traffic emission origin.

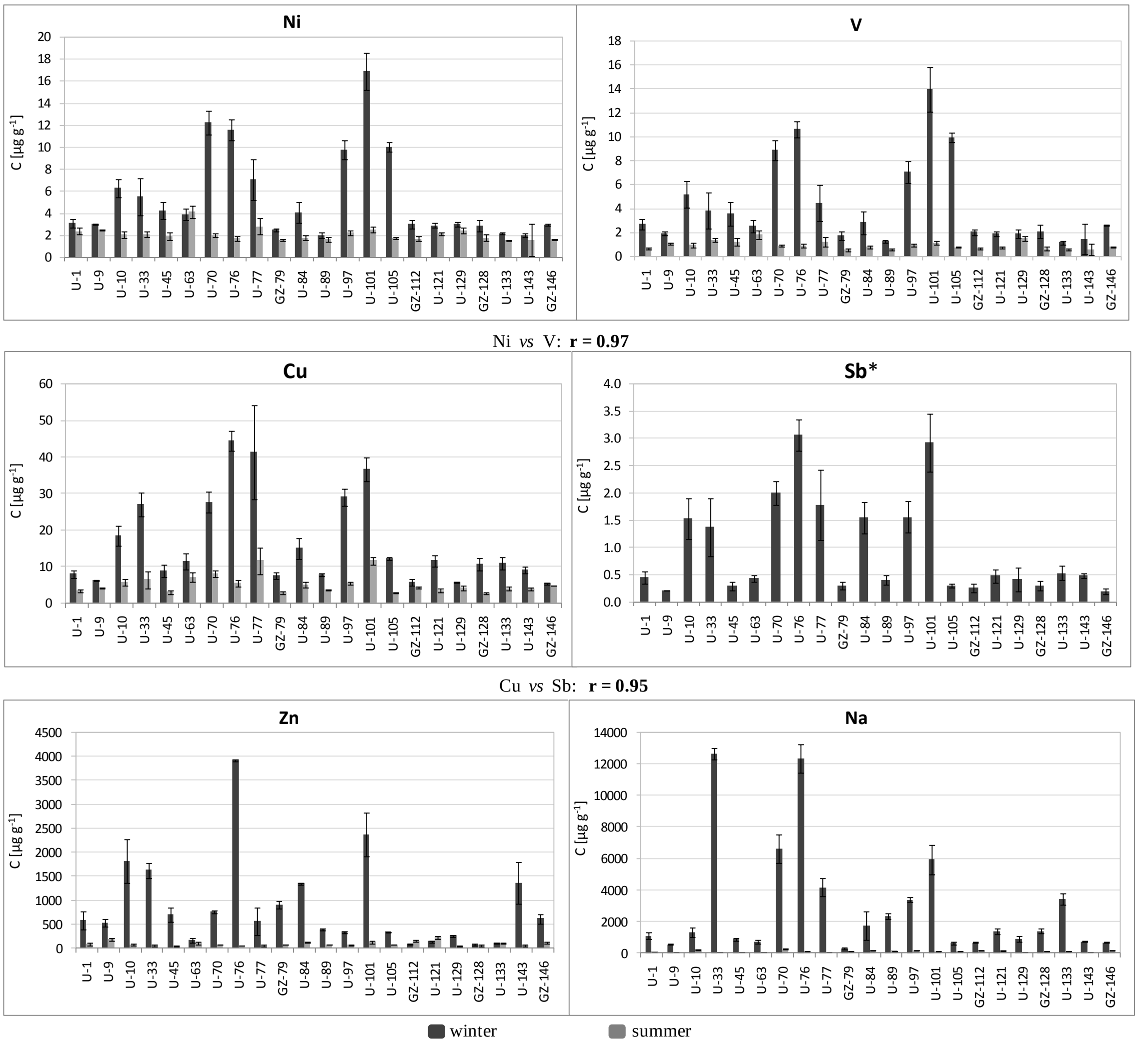

Figure 4.5.1. Average concentrations (µg g^{-1}) of the most enriched elements (Ni, V, Cu, Sb, Zn, and Na) in the *S. girgensohnii* moss bags exposed for ten weeks during the winter of 2013/2014 over the city of Belgrade. Standard deviation bars (n=3) and significant correlation coefficients (*r*) for the winter are indicated; data for the summer were taken from the Experiment 4 (Vuković et al., 2015a); * moss Sb concentrations were not determined in the summer.

No significant (*p < 0.05*) correlation coefficients were found between Na and Zn concentrations, and other elements implying the presence of specific Na and Zn source. Remarkably higher Na concentrations during the winter compared to the summer could be explained by the use of de-icing agents to prevent ice forming on the road surface, which is confirmed in other studies (e.g., Lawrence et al., 2013). Thus far, increased levels of Zn was also observed in the study area during the winter which was attributed to coal combustion, emissions from the local industry and traffic (Aničić et al., 2009a; Mijić et al., 2010).

4.5.2. REE concentrations in moss

The RAF values calculated for the majority of REEs were up to ten (**Table 4.5.1**). The shale-normalised patterns of the REEs (**Figure 4.5.2**) in the moss bags are similar to their abundances in the Earth's crust (US EPA, 2012). A significant fractionation between light (Ce, Eu, La, Nd, Pr, and Sm) and heavy (Dy, Er, Gd, Ho, Lu, Tb, Tm, and Yb) REEs was not revealed. However, positive Eu anomaly was observed for both NASC and PAAS normalized concentrations of the REEs. The occurrence of the Eu anomaly may be linked to different oxidation states of Eu (II and III) which can lead to both depletion and enrichment of Eu. The positive Eu anomaly was also shown for the native biomonitors, mosses and lichens (Dołęgowska and Migaszewski, 2013; Agnan et al., 2014).

The majority of the REEs were markedly enriched in the moss bags exposed in highly urbanised zones (**Figure 4.5.2**). In contrast, the moss REE concentrations were the lowest at the sites located within the green zones. This result indicates that the moss REE entrapment is mainly induced by anthropogenic activities. We suppose that crustal dust is a dominant component of road dust. Thus, the moss enrichment with road dust is primarily dependent on resuspension process induced by vehicles movements, traffic intensity and certainly duration of the moss bag exposure. At urbanized sites of the study area, a gradual increase of the moss REEs enrichment was previously observed from 15-days to 5-months (Aničić et al., 2009a). In addition, Rühling and Tyler (2004) reported that reduction in anthropogenic dust emissions reflected in decreased deposition of REEs in the moss tissue.

Along with a worldwide increase in the use of REEs in new-technology and possible detrimental health effects (US EPA, 2012), it is of importance to analyse and evaluate the level and distribution of REEs in the air. The *S. girgensohnii* moss bags could be suggested as a tool for monitoring of the REEs in urban area, which spatial dispersion depends on windblown and vehicle-induced resuspension of the continental dust, based on: 1) the distinct enrichment of the moss with REEs, and 2) the wide concentration range obtained among the sampling sites within the study area.

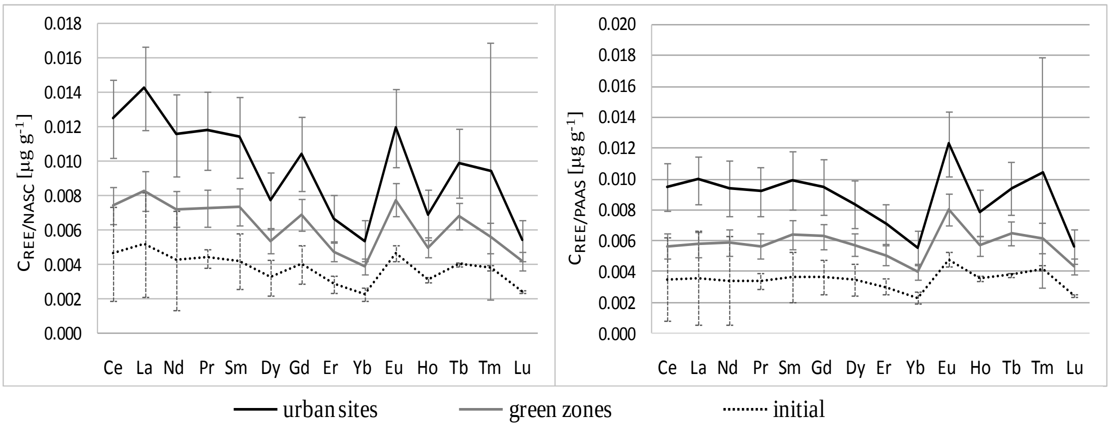

Figure 4.5.2. The median concentrations (µg g^{-1}) of the REEs normalised to NASC and PAAS in the *S. girgensohnii* moss bags exposed for ten weeks during the winter of 2013/2014; initial – element concentrations in the unexposed moss; the REEs order on the x-axis is chosen according to their abundances in the NASC and PAAS.

4.5.3. Seasonal variations of moss PAH concentrations

The PAH concentrations determined in the *S. girgensohnii* moss bags after the winter exposure period are presented in **Table 4.5.2**. The concentrations of all PAHs were significantly higher ($p < 0.05$) in the exposed moss bags than in the unexposed ones. Comparing to the corresponding LOQ, the moss concentration of medium- (3-ring) and high- (5- and 6-ring) molecular-weight PAHs were higher up to 150 and 80 times, respectively. The most abundant PAHs in the moss samples were Phen, Fla, Pyr, Chr, B[b]F, B[ghi]P and I[cd]P.

Total moss PAH content varied between 42 to 178 ng g^{-1} during the summer whereas it exhibited significantly ($p < 0.05$) higher range from 158 and 1158 ng g^{-1} in the winter. (**Table 4.5.2**). Significantly higher moss PAH concentrations were measured during the winter than in the summer by the regulatory monitoring across the same study area (Cvetković et al., 2015). Markedly higher moss concentrations of medium- and high-molecular-weight PAHs in the winter could be attributable to both, meteorological conditions, and increased emissions from residential heating (Ravindra et al., 2006). Regarding to meteorological conditions, decrease of air temperature and solar radiation enhanced sorption of PAHs to particles (Ravindra et al., 2008) which then may be entrapped by moss. Thus far, relation between the PAH concentrations and meteorological variables, such as temperature, atmospheric pressure, relative humidity and wind speed, was confirmed in lichens by Augusto et al. (2013).

In terms of identification of PAH pollution sources, the moss PAH diagnostic ratios were calculated (**Table 4.5.3**). The seasonal variation of Phen / (Phen / Ant), Pyr / B[a]P, B[a]P / B[ghi]P, I[cd]P / B[ghi]P, three + four ring PAHs / ΣPAHs and ΣCOMPAHs / ΣPAHs was observed. Values of ΣCOMPAHs / ΣPAHs close to 1 indicated combustion processes (Ravindra et al., 2008) as dominant pollution source during the winter. Furthermore, two possible pollution sources were distinguished: oil fuel/coal combustion and vehicular emissions. According to Katsoyiannis et al. (2011), B[a]P / B[ghi]P values lower than 0.6 indicate non-traffic emissions. The I[cd]P / B[ghi]P ratio higher than 1.25 implies the use of brown coal for residential heating (Caricchia et al., 1999). Moreover, the three + four ring/total PAHs ratio value higher than 0.9 indicates wood combustion sources, whereas about 0.7 points up to coal combustion sources (Migaszewski et al., 2009). On the contrary, values of Pyr / B[a]P close to one and ten represent gasoline and diesel exhaust emissions, respectively (Ravindra et al., 2008) while values of Phen / (Phen / Ant) higher than 0.7 indicate presence of both lubricant oils and fossil fuels (Alves et al., 2001). Our findings are in agreement with the results of Cvetković et al. (2015) obtained by instrumental monitoring conducted for one-year period over metropolitan area of the city of Belgrade. Gasoline, diesel, coal, and wood combustion have been proposed as the principal emission of PAHs associated with PM10 during the winter (Cvetković et al., 2015). However, PAHs are susceptible to photoreactions and/or oxidation, and can be affected by solar radiation and temperature. Thus, the PAH diagnostic ratios could only be used as indicative markers of PAH pollution sources if measurements are made near well-characterized pollution sources.

Since no data about PAH seasonal variation have been reported for moss bags thus far, this experiment could be seen as an upgrade of the moss bag application for PAH biomonitoring. Our results denote a possibility of *S. girgensohnii* moss bags to: 1) reflect PAH seasonal variations between summer and winter; and 2) recognize different fossil fuel combustions as significant contributor to total PAHs content.

Table 4.5.2. Average PAH concentrations (ng g^{-1}) in the *S. girgensohnii* moss samples from the winter and summer experiments; data for the summer were obtained in the Experiment 4 (Vuković et al., 2015a), *<0.01* – below detection limit

Sampling site*	Season	Nap	Acy	Acp	Fl	Phen	Ant	Fla	Pyr	B[a]A	Chr	B[b]F	B[k]F	B[a]P	I[cd]P	D[ah]A	B[ghi]P	Σ PAH
U-1	*winter*	14.1	3.4	*<0.01*	4.6	37.9	3.64	78.2	60.2	5.9	35.1	23.1	12.5	11.9	28.6	5.1	20.9	345
	summer	3.2	0.9	*<0.01*	1.8	3.7	0.89	4.1	3.9	0.7	3.2	5.2	2.4	3.1	12.6	*<0.01*	*<0.01*	46
U-10	*winter*	9.9	2.1	*<0.01*	4.9	30.1	3.04	57.2	47.9	8.1	31.5	26.9	10.4	12.2	27.5	4	24.2	300
	summer	13.7	0.9	8.61	3.2	13	2.22	16.3	15.8	3	11.6	12.1	4.3	6.6	12.9	*<0.01*	*<0.01*	124
U-33	*winter*	8.3	2.7	*<0.01*	5.4	29.3	2.96	42.5	35	6	22.5	20.4	10.2	11.9	33	4.9	25.8	261
	summer	14	1.4	*<0.01*	4.1	8.5	0.94	6.1	6	1.2	4.7	6.3	3.4	4.4	17.8	*<0.01*	*<0.01*	79
U-63	*winter*	8.3	1.9	*<0.01*	3.1	22.9	2.82	54.3	43	6.2	42.9	30.3	12.7	13.8	30.4	6.2	21.5	300
	summer	13.1	0.9	*<0.01*	1.8	4.1	0.57	3	2.4	0.4	2.1	4.4	1.8	2.5	9.6	*<0.01*	*<0.01*	47
U-70	*winter*	10.5	3	*<0.01*	2.4	35.5	4.23	75.5	63.7	12.2	44.4	29.2	15.5	17.6	34.7	3.7	33.5	386
	summer	22.1	0.9	*<0.01*	4	10.2	0.87	6.5	6.9	1.6	6.9	6.7	4.6	5.2	7.8	*<0.01*	*<0.01*	84
U-76	*winter*	19.6	10.4	*<0.01*	14.6	90.4	10.22	206	175.9	42.3	181.5	92.4	49.8	52.8	110.3	3.1	98.9	1158
	summer	27.7	0.9	*<0.01*	3.3	7.9	1.14	10	9.4	1.8	8	10.1	4.1	7.4	13.3	*<0.01*	17.9	123
GZ-79	*winter*	8.7	1.1	0.6	2.7	25.4	2.89	48.1	33.7	3.6	20.3	13.6	5.4	7.4	20.2	4.5	13.7	212
	summer	9.2	1	*<0.01*	2.8	4.8	0.87	2.3	2.3	0.4	2	3.9	1.4	2.4	16.2	*<0.01*	*<0.01*	50
U-84	*winter*	13.2	2.2	*<0.01*	6.6	25.1	1.34	44.6	34.6	7.9	32.3	22	13.9	10.7	31.8	9.4	25.1	281
	summer	8.2	0.7	*<0.01*	1.8	3.5	0.66	3.4	3.2	0.8	3.1	4.8	1.9	2.5	12.5	*<0.01*	*<0.01*	47
U-89	*winter*	11.3	4.6	*<0.01*	6	43.1	4.66	111.4	89.2	12.5	69.2	43.6	16.7	19.7	42.4	11.1	30.8	516
	summer	14.3	0.9	*<0.01*	2.4	6.3	0.69	4.3	3.4	0.9	3	5.5	2.9	3.9	23.3	*<0.01*	*<0.01*	72
U-97	*winter*	10.8	3.4	*<0.01*	3.3	23.9	4.52	57.1	48.7	13.5	54.5	40	23.3	16.7	106.9	67.9	65.3	540
	summer	15.2	1.1	*<0.01*	2.1	7.7	1.03	16.4	12.1	1.8	13.4	14.3	7.1	8.6	58.7	*<0.01*	18.5	178
U-101	*winter*	7.8	3.5	*<0.01*	4.3	29.9	2.61	75.9	61.8	8	41.5	29.1	10.1	12.8	29.4	5.9	22.3	345
	summer	27.5	0	4.36	4.9	20.3	2.54	29.8	22	3	17	16.5	6.1	9.9	*<0.01*	*<0.01*	*<0.01*	164
GZ-112	*winter*	11.1	1.6	*<0.01*	3.7	19.9	1.78	29.4	20.1	3.5	13.7	9.8	5.5	5.7	15.9	4	12.1	158
	summer	19.6	1	9.89	3.3	8.9	1	7.5	4.4	0.5	2.1	2.7	1.9	2.8	3.4	*<0.01*	*<0.01*	69
U-121	*winter*	13	2.6	*<0.01*	4	32.5	4.12	79.6	64.4	10.1	44.3	27.5	19.5	17.4	45.7	10.5	36.1	411
	summer	10.1	0.6	0.67	2.3	4.1	0.85	2.6	2.1	0.8	2.5	3.2	1.7	2	8.4	*<0.01*	*<0.01*	42
GZ-128	*winter*	7.5	1.5	*<0.01*	3.5	26.4	2.16	44.8	32.9	4.6	19.9	20.7	8.8	9.7	35.7	10.6	24.9	253
	summer	16	0.6	3.1	2.9	7.2	0.82	3.1	2.3	0	0	3.4	1.2	2.1	*<0.01*	*<0.01*	*<0.01*	43
U-133	*winter*	12.6	2.7	*<0.01*	3.8	30.6	2.97	40.2	30.2	3.7	19.7	14.6	8.3	9.4	23.2	4	16.9	223
	summer	23.8	0	3.97	3.1	9.1	1.4	5.7	4.5	0.6	2.8	4.4	1.9	2.9	*<0.01*	*<0.01*	*<0.01*	64
GZ-146	*winter*	11.8	1.7	*<0.01*	2.6	21.6	2.07	44.9	32.2	4.8	24.8	19.3	6.2	8.4	22.8	5.2	17.2	226
	summer	23.8	0.9	3.38	3.6	6.4	1.03	4.3	3.8	1	2.7	5.7	3.1	3.5	*<0.01*	*<0.01*	*<0.01*	63

* After exposure, the moss bags were missing at six sampling sites

Table 4.5.3. PAH diagnostic ratios (minimum, maximum, mean and median values) in the *S. girgensohnii* moss bags exposed for ten weeks during the winter 2013/2014 over the city of Belgrade and the summer; data for the summer were obtained in the Experiment 4 (Vuković et al., 2015a)

Moss PAH diagnostic ratio					
	Season	Min	Max	Mean	Median
Fla / (Fla+Pyr)	*winter*	0.54	0.59	0.56	0.56
	summer	0.49	0.63	0.54	0.52
Phen / (Phen/Ant)	*winter*	1.34	10.22	3.50	2.93
	summer	0.66	2.54	1.09	0.97
B[a]A / (B[a]A+Chr)	*winter*	0.14	0.22	0.18	0.19
	summer	0.12	0.27	0.19	0.19
B[b]F / B[k]F	*winter*	1.58	3.13	2.14	1.94
	summer	1.39	2.91	2.23	2.31
Pyr / B[a]P	*winter*	2.92	5.07	3.73	3.57
	summer	0.93	2.39	1.35	1.30
B[a]P / B[ghi]P	*winter*	0.26	0.58	0.50	0.50
	summer	/	/	/	/
I[cd]P / (I[cd]P+B[ghi]P	*winter*	0.51	0.62	0.57	0.57
	summer	0.43	1.00	0.93	1.00
I[cd]P / B[ghi]P	*winter*	1.03	1.64	1.32	1.32
	summer	/	/	/	/
three + four ring PAHs/ ΣPAHs	*winter*	0.39	0.68	0.61	0.60
	summer	0.31	0.63	0.42	0.42
ΣCOMPAHs* / ΣPAHs	*winter*	0.73	0.87	0.81	0.80
	summer	0.28	0.85	0.58	0.63

*ΣCOMPAHs – combustion-related PAHs: Fla, Pyr, B[a]A, Chr, B[k]F, B[b]F, B[a]P, I[cd]P and B[ghi]P

4.6. **Experiment 6:** Ability of moss bags to reflect distance-decrease of trace element concentrations depending on different traffic burden across crossroads, two- and one-lane street

4.6.1. On-road concentrations of airborne elements

Moss bag technique enables a longer sampling period of airborne pollutants such as trace elements and better spatio-temporal resolution of sampling sites. These advantages provide characterization of long-term human exposure to trace elements, which is more accurate and relevant in the assessment of chronic health effects than daily instrumental measurements. In this experiment, markedly high RAF values of Sb, Sn, Sm, Cu, Cr, and Zn (**Table 4.6.1 and 4.6.2**) in the moss samples of the studied species, *S. girgensohnii* and *H. cupressiforme*, imply significantly elevated concentrations of these elements within on-road microenvironments. In addition, at the crossroads, Pt, which naturally occurs in very low concentrations in the mosses, was markedly enriched. Thus far, concerning the moss bag technique application, Pt has been only observed in the moss bags exposed within a road tunnel (Zechmeister et al., 2006a), and very scarcely in outdoor air in proximity of bus lines (Rivera et al., 2011).

Due to practical constraints, an interchangeable use of the studied moss species was tested. *S. girgensohnii* is a species of the most recommended biomonitoring moss genus while *H. cupressiforme* is a common moss in the territory of Serbia. Thus, an interchangeable use of the studied moss species could be helpful. For all elements, a significantly higher ($p < 0.05$) RAF values were obtained for *S. girgensohnii* than *H. cupressiforme*. The results of type II linear regression model are presented for the elements among which significant correlation ($r > 0.80$) was observed (**Figure 4.6.1**). The obtained regressions were significant ($p < 0.05$) for As, Ba, Cr, Cu, Fe, Sb, and V, but the highest determination coefficients were observed for Sb along with Cu and Cr. High determination coefficients ($r^2 \geq 0.65$, **Figure 4.6.1**) obtained for Sb, Cu, and Cr suggest the interchangeable use of *S. girgensohnii* and *H. cupressiforme* in biomonitoring of these elements. In the Experiment 4 (Vuković et al., 2015a), the interchangeable use of *S. girgensohnii* and *H. cupressiforme* was recommended for only Cu based on the determination coefficient ($r^2 = 0.50$). However, in that study, sampling sites were chosen to be representative for the wider areas, and not only the on-road microenvironment.

Table 4.6.1. Descriptive statistics of the element concentrations (µg g^{-1}) determined in the *S. girgensohnii* moss bags exposed for ten weeks during the summer 2014 over the crossroads, two- and one-lane street in the city of Belgrade

Element	Initial	Min	Max	Mean	Median	RAF$_{min}$	RAF$_{max}$	RAF$_{mean}$	RAF$_{median}$
					N = 48; n = 3				
Al	290	420	1180	730	710	0.5	3.1	1.5	1.5
As	0.07	0.13	0.58	0.31	0.28	0.8	7.2	3.4	3
Ba	29	32	92	53	50	0.1	2.2	0.8	0.7
Ca	3520	5000	10560	7030	6970	0.4	2	1	1
Cd	0.17	0.19	1.69	0.36	0.26	0.1	8.8	1.1	0.5
Ce	0.212	0.001	1.547	0.809	0.794	-1	6.3	2.8	2.7
Co	0.4	0.46	1.25	0.72	0.68	0.1	2.1	0.8	0.7
Cr	0.25	0.73	7.43	2.85	2.59	1.9	28	10	9.3
Cu	3.1	5.6	124.5	27.8	20	0.8	40	8.1	5.5
Dy	0.024	0.033	0.117	0.068	0.066	0.4	4	1.9	1.8
Er	0.01	0.016	0.061	0.034	0.033	0.7	5.4	2.5	2.4
Eu	0.006	0.01	0.0377	0.0215	0.0207	0.7	5.3	2.6	2.4
Fe	360	580	2570	1320	1210	0.6	6.2	2.7	2.4
Ga	0.08	0	0.58	0.32	0.31	-1.1	6	2.9	2.8
Gd	0.024	0.041	0.164	0.089	0.087	0.7	5.9	2.8	2.7
Ho	0.0035	0.0064	0.0223	0.0128	0.0125	0.8	5.4	2.7	2.6
K	10000	270	4300	1400	1080	-1	-0.6	-0.9	-0.9
La	0.18	0.01	1.21	0.66	0.63	-1.1	5.7	2.6	2.5
Lu	0.0008	0.0017	0.0069	0.0038	0.0037	1.1	7.4	3.7	3.5
Mg	1620	2030	7720	4390	4340	0.3	3.8	1.7	1.7
Mn	246	141	320	248	251	-0.4	0.3	0	0
Na	110	50	1960	154	96	-0.6	17	0.4	-0.1
Nd	0.14	0	1.06	0.55	0.54	-1	6.7	3	2.9
Ni	1.1	1.3	8.1	3.2	2.9	0.2	6.7	2	1.8
Pb	3.9	4.8	28	8.3	7.5	0.2	6.1	1.1	0.9
Pr	0.0386	0.0001	0.2663	0.1417	0.1401	-1	5.9	2.7	2.6
Pt	0.0001	0	0.0028	0.0005	0.0003	-1.3	24	3	1.8
Rb	70	0	30	11	8	-1	-0.6	-0.8	-0.9
Sb	0.09	0.2	3.87	1.38	1.21	4.4	102	35	31
Sc	0.032	0.066	0.268	0.137	0.129	1	7.3	3.3	3
Sm	0.009	0	0.197	0.104	0.103	-1.2	41	21	21
Sr	5	0	23	16	17	-2.4	3.7	2.3	2.4
Tb	0.0036	0.0056	0.0244	0.0129	0.0119	0.5	5.8	2.6	2.3
Th	0.022	0	0.256	0.124	0.116	-1.3	11	4.7	4.3
Ti	0.93	1.59	8.98	4.59	4.41	0.7	8.7	3.9	3.7
Tl	0.027	0.012	0.065	0.031	0.027	-0.6	1.4	0.1	0
Tm	0.0012	0.0026	0.0111	0.0061	0.0057	1.2	8.4	4.2	3.8
V	0.5	0.96	6.82	2.29	2.16	0.9	13	3.6	3.3
Y	0.11	0	0.6	0.35	0.35	-1	4.4	2.2	2.2
Yb	0.002	0.013	0.05	0.028	0.027	1.8	9.6	4.9	4.7
Zn	24	73	700	250	210	2.1	28	9.2	7.9

*N – total number of sampling sites, n – number of subsamples per exposure site; Initial – the data obtained in the unexposed moss, Min – minimum value, Max – maximum value, Mean – average value, RAF – relative accumulation factor

Table 4.6.2. Descriptive statistics of the element concentrations (μg g^{-1}) determined in the *H. cupressiforme* moss bags exposed for ten weeks during the summer 2014 over the crossroads, two- and one-lane street in the city of Belgrade

Element	Initial	Min	Max	Mean	Median	RAF_{min}	RAF_{max}	RAF_{mean}	RAF_{median}
					N = 48; n = 3				
Al	730	650	2370	1260	1193	-0.1	2.2	0.7	0.6
As	0.35	0.31	1.12	0.62	0.57	-0.1	2.2	0.8	0.7
Ba	16	13	73	32	29	-0.2	3.5	1.0	0.8
Ca	5630	3130	9380	7300	7440	-0.4	0.7	0.3	0.3
Cd	0.32	0.16	0.76	0.44	0.43	-0.5	1.4	0.4	0.3
Ce	1.084	1.043	3.064	1.804	1.678	0.0	1.8	0.7	0.6
Co	0.4	0.3	0.9	0.6	0.6	-0.3	1.5	0.6	0.6
Cr	0.83	0.9	6.01	2.74	2.57	0.1	6.3	2.3	2.1
Cu	8	6	82.1	21.2	18.5	-0.3	9.3	1.7	1.3
Dy	0.119	0.104	0.357	0.192	0.183	-0.1	2.0	0.6	0.5
Er	0.06	0.05	0.17	0.09	0.09	-0.1	1.9	0.6	0.6
Eu	0.022	0.022	0.06	0.039	0.036	0.0	1.7	0.7	0.6
Fe	650	530	3170	1450	1330	-0.2	3.9	1.3	1.1
Ga	0.39	0.4	1.25	0.73	0.68	0.0	2.2	0.9	0.7
Gd	0.131	0.125	0.383	0.217	0.206	0.0	1.9	0.7	0.6
Ho	0.0225	0.0189	0.0677	0.0358	0.0343	-0.2	2.0	0.6	0.5
K	4980	1930	4510	4040	4100	-0.6	-0.1	-0.2	-0.2
La	0.85	0.86	2.5	1.44	1.39	0.0	1.9	0.7	0.6
Lu	0.0056	0.0052	0.0161	0.0093	0.0091	-0.1	1.9	0.7	0.6
Mg	1670	820	2050	1750	1790	-0.5	0.2	0.1	0.1
Mn	109	52	138	116	117	-0.5	0.3	0.1	0.1
Na	35	0	600	53	40	-1.0	16.0	0.5	0.1
Nd	0.7	0.7	2.03	1.18	1.08	0.0	1.9	0.7	0.6
Ni	1.5	1	6.5	3.4	3.1	-0.4	3.2	1.2	1.0
Pb	6.2	4.9	27.3	12.3	11.9	-0.2	3.4	1.0	0.9
Pr	0.1892	0.1837	0.5514	0.3174	0.2958	0.0	1.9	0.7	0.6
Pt	0.0002	0.0002	0.0017	0.0005	0.0004	0.0	9.0	1.8	1.2
Rb	6	3	8	6	6	-0.5	0.4	0.1	0.1
Sb	0.04	0.08	2.52	0.82	0.71	1	66	21	18
Sc	0.094	0.046	0.372	0.215	0.211	-0.5	3.0	1.3	1.3
Sm	0.143	0.136	0.415	0.237	0.22	-0.1	1.9	0.7	0.5
Sr	18	7	25	21	21	-0.6	0.4	0.2	0.2
Tb	0.0206	0.0187	0.0615	0.0336	0.0322	-0.1	2.0	0.6	0.6
Th	0.167	0.099	1.416	0.34	0.286	-0.4	7.5	1.0	0.7
Ti	3.85	1.07	18.34	8.51	8.5	-0.7	3.8	1.2	1.2
Tl	0.012	0.014	0.052	0.026	0.023	0.1	3.2	1.1	0.9
Tm	0.0076	0.0068	0.0227	0.0124	0.0122	-0.1	2.0	0.6	0.6
V	1.3	1.1	6.3	2.9	2.7	-0.1	3.8	1.2	1.1
Y	0.62	0.55	1.86	1.02	0.99	-0.1	2.0	0.6	0.6
Yb	0.044	0.038	0.124	0.071	0.069	-0.1	1.8	0.6	0.6
Zn	29	48	726	155	136	0.7	24	4.4	3.7

*N – total number of sampling sites, n – number of subsamples per exposure site; Initial – the data obtained in the unexposed moss, Min – minimum value, Max – maximum value, Mean – average value, RAF – relative accumulation factor

Finally, based on the results from this experiment, *S. girgensohnii* and *H. cupressiforme* could be interchangeably used for biomonitoring of Sb, Cu, and Cr in studies regarding studied on-road microenvironments.

Comparison of the element concentrations between oven-dried and live moss bags of both moss species indicated no significant difference ($p < 0.05$). The same pattern of abundance and spatial decline of the element concentrations was observed for oven-dried and live bags of both species.

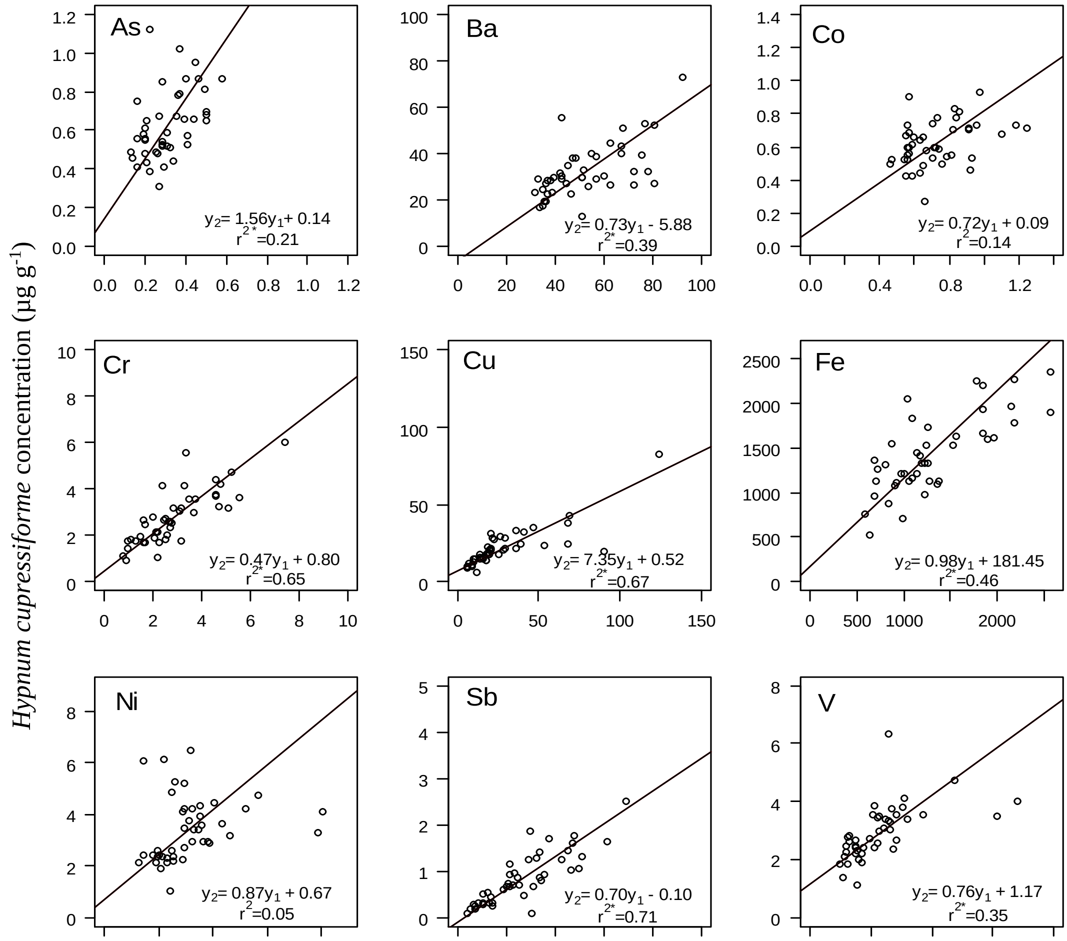

Figure 4.6.1. Type II regression lines present for As, Ba, Co, Cr, Cu, Fe, Ni, Sb, and V concentrations (µg g^{-1}) in the *S. girgensohnii* and *H. cupressifrome* moss samples. The black line represent the standardised major axis regression method; * the coefficients of determination that are significant at $p < 0.05$ level.

4.6.2. Spatial variability of moss element content: crossroad, two- and one-lane street

The moss species, *S. girgensohnii* and *H. cupressiforme,* indicated spatial variability for the majority of determined element concentrations within sets of 3 sampling sites with markedly different traffic flows. Particularly, concentration gradient of As, Ba, Co, Cu, Cr, Ni, Sb, and V in the moss samples (**Figure 4.6.2**) clearly emphasizes crossroads as pollution hotspots where pedestrians and commuters were exposed to highly increased level of trace elements. At crossroads, a pattern of braking and accelerating driving mode, so called "stop and go", is specially increased. In comparison to constant free flowing traffic mode, which characterised two- and one-lane streets, "stop and go" mode leads to elevated pollutant emissions from both, exhaust and non-exhaust traffic source (Tsai et al., 2003).

The decrease in the moss shale-normalized concentration of the REEs (**Figure 4.6.3**) confirmed spatial dependence of these elements on anthropogenic activities, i.e., vehicle movements. Both moss species, *S. girgensohnii* and *H. cupressiforme*, identified similar order of abundance of the REEs, just as it has been pointed out in the Experiment 5 (Vuković et al., 2015c). This confirms consistent signature of the REEs in the study urban area.

The results draw attention for better spatial resolution of regulatory monitoring sites from which data are used in epidemiological studies. Note that permitted levels of emission of Cu, Sb, Cr, Pt, and REEs such as Sm, in the air has not been regulated by legislation (Directive 2004/107/EC) yet, although these elements are recognized as toxic by WHO (2013).

4.6.3. Correlation between moss element concentrations and traffic flows

A relation among the element concentrations in the samples of *S. girgensohnii* and *H. cupressiforme* can be used as a possible indicator of the traffic-related air pollutants. Based on the high correlation coefficients ($r > 0.80$) among Ba, Cr, Cu, Fe, Ni, Sb, and V (**Table 4.6.3 and 4.6.4**) in the moss samples of both species, the common sources of these elements can be assumed. The vehicle movements cause: (i) exhaust emissions from tailpipe; (ii) non-exhaust emissions due to wear and tear of vehicle parts such as brakes, tyres and clutches; and (iii) re-suspension of road dust.

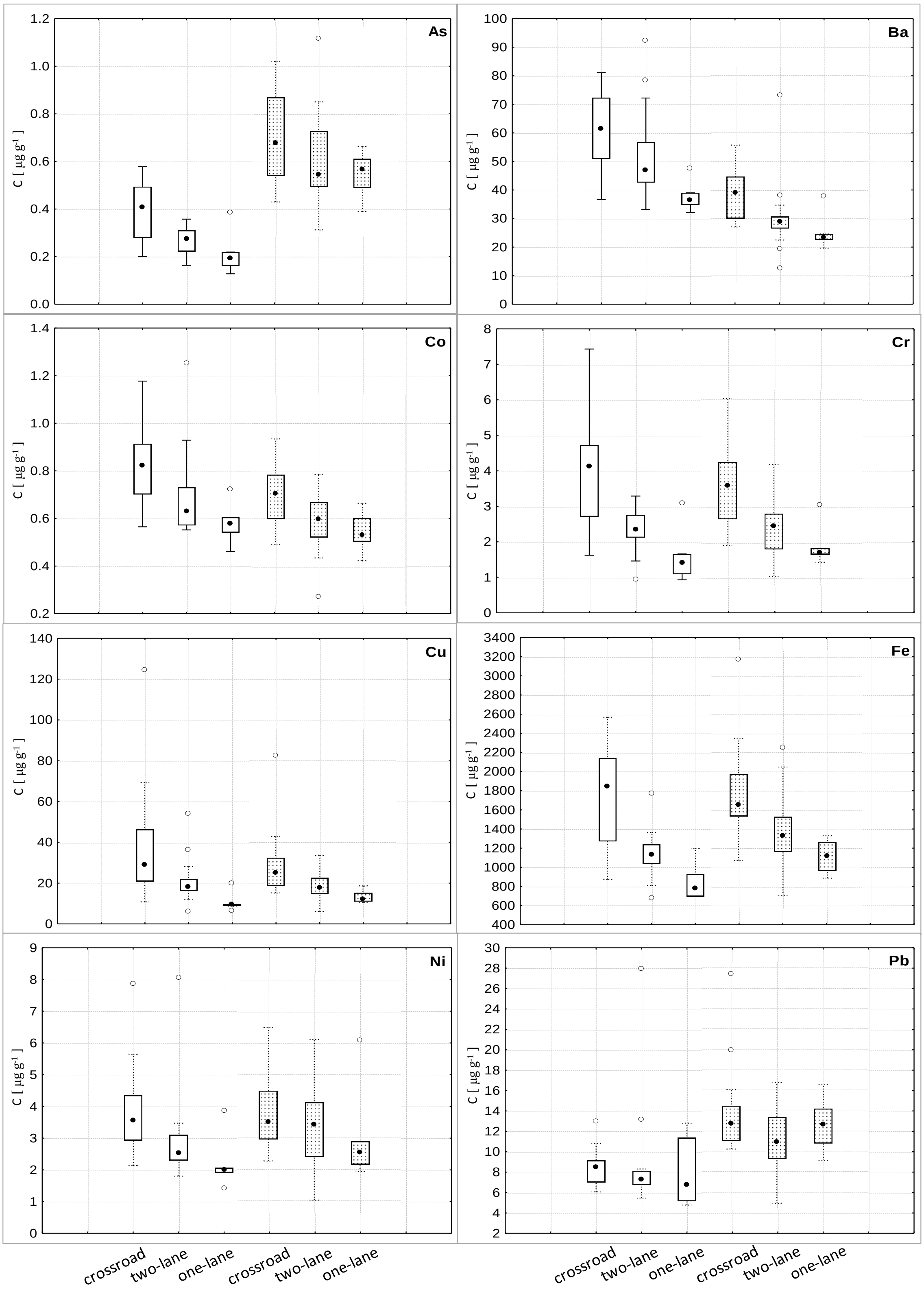

Figure 4.6.2. Concentrations (µg g^{-1}) of As, Ba, Co, Cr, Cu, Fe, Ni, Pb, Pt, Sb, V, and Zn in the *S. girgensohnii* (blank box) and *H. cupressiforme* (dotted box) moss bags exposed for ten weeks during the summer of 2014 over the crossroads, two- and one-lane streets in the city of Belgrade; * platinum was only detected at the crossroads.

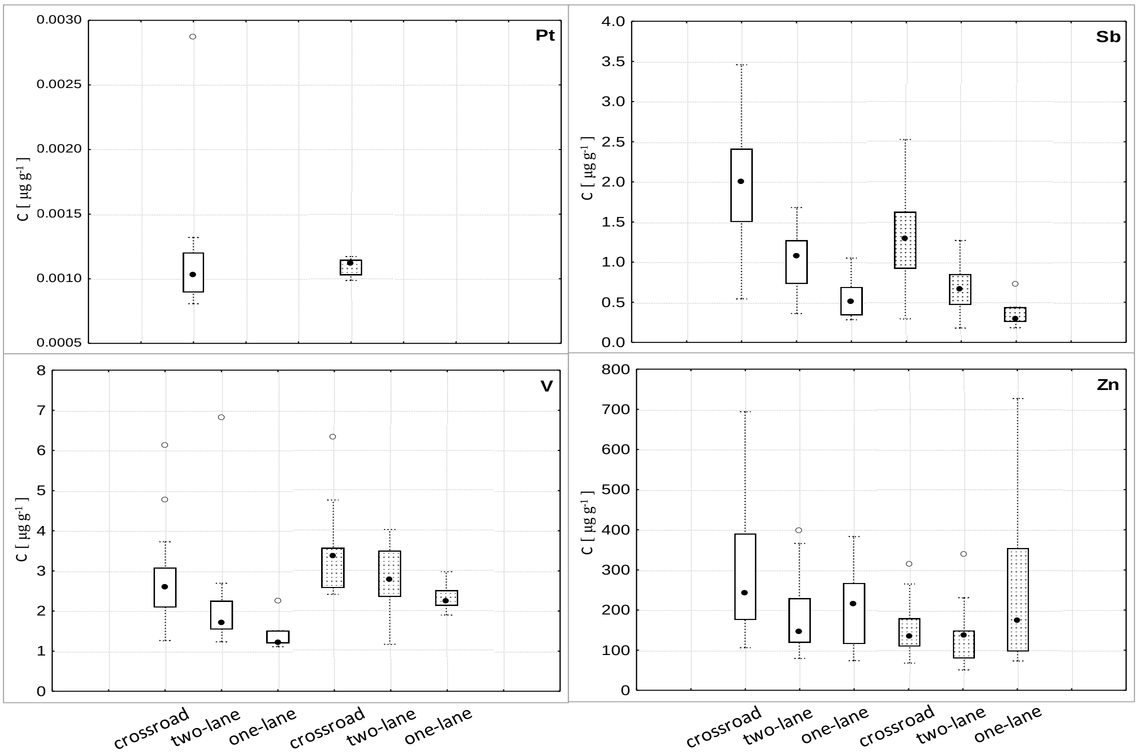

Figure 4.6.2. (continued).

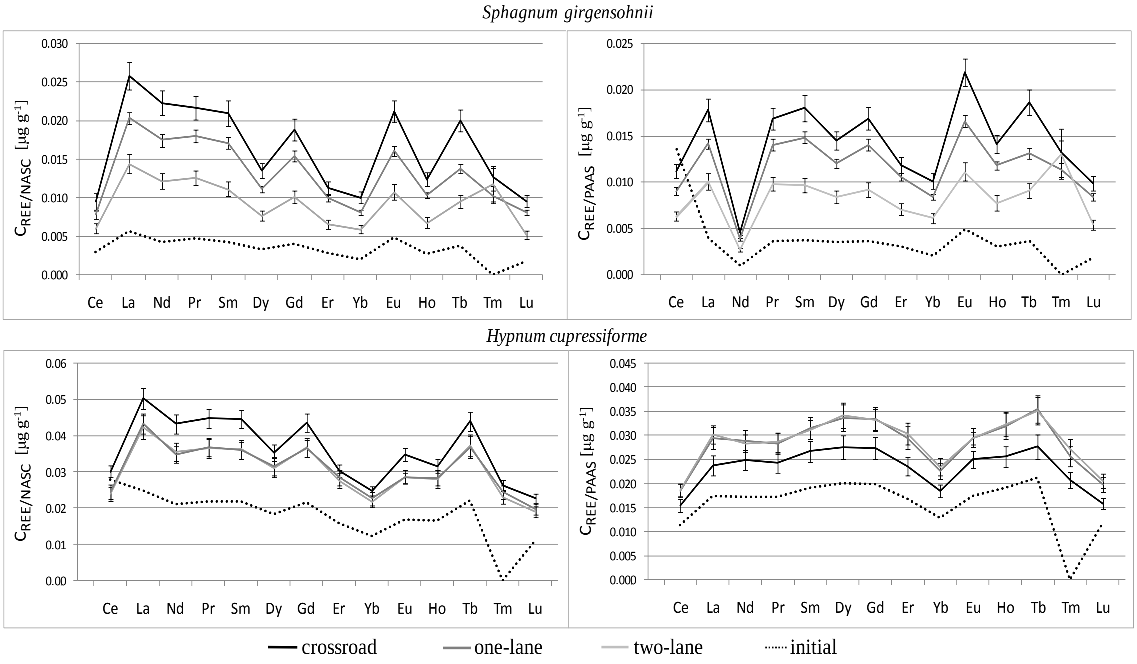

Figure 4.6.3. Median concentrations (µg g^{-1}) of REEs in the *S. girgensohnii* and *H. cupressiforme* moss bags exposed for ten weeks during the summer of 2014 over the crossroads, two- and one-lane streets in the city of Belgrade; initial – element concentrations in the unexposed moss; the REEs order on the x-axis is chosen according to their abundances in the NASC and PAAS.

Non-exhaust emissions mainly contribute to increased concentrations of Ba, Fe, Cu, and Sb (Thorpe and Harrison, 2008; Gietl et al., 2010) while fuel combustion is the major source of Cr, Ni, V, and Cu (Song and Gao, 2010; EMEP/EEA, 2013).

Given the moderate, but significant, correlation coefficient (r = 0.65 – 0.70, $p < 0.05$) (**Table 4.6.3 and 4.6.4**) between the total traffic flows and the concentrations of Cr, Cu, Fe, and Sb in the *S. girgensohnii* and *H. cupressiforme* samples, these elements could be considered as reliable tracers of traffic-related emissions. Our results support previous findings from the road traffic studies using both terrestrial mosses and moss bags biomonitors (Zechmeister et al., 2005; Zechmeister et al., 2006b) in which Cr, Cu, Fe, and Sb have been identified to be traffic-originated. Regarding the categories of vehicles, the significant correlation coefficient ($r > 0.60$, $p < 0.05$), implies tight relation between the flows of passenger cars and light duty vehicles, and the moss element concentrations of Cr, Cu, Fe, and Sb (**Table 4.6.3 and 4.6.4**). This is in agreement with the results previously pronounced for the correlation between the flows of light duty vehicles and concentrations of Cu and Fe in the moss samples (Adamo et al., 2011).

Zinc has often been reported as one of the tracers for non-exhaust traffic emissions, i.e., tyre wear (e.g., Pant and Harrison, 2013). In this experiment, very low correlation coefficients ($r < 0.20$) were obtained between the moss concentrations of Zn and traffic flows (**Table 4.6.3 and 4.6.4**). A distinct spatial variability, which is dependent on the traffic flow, was not observed (**Figure 4.6.3**). Moreover, in the Experiments 1 and 4 (Vuković et al., 2013; Vuković et al., 2015a), there was no any regularity in the Zn spatial distribution, related to traffic flow at the study sites. Therefore, Zn could not be considered as a reliable tracer of traffic emissions.

4.6.4. Pedestrian zones as an urban background?

It was hypothesized that significantly lower moss element content would be obtained at the control sites in comparison with the remaining on-road sampling sites. However, the elevated concentrations of the majority of elements were determined in the moss bags exposed in the central pedestrian zone (**Table 4.6.5**). Specifically, markedly high concentration of Cu was found in this pedestrian zone, which is in accordance with the results of previous studies (Vuković et al., 2015a; Aničić et al., 2009a). Elevated Cu concentrations was also previously pronounced for the same pedestrian zone in the research related to the usage of the tree leaf biomonitors (Aničić et al., 2011) and the bulk deposition collectors (Mijić et al, 2010). Thus, the marked Cu moss enrichment in this study could not be considered as an accidental case.

The moss element content indicated that the second pedestrian zone, situated in peri-urban area, was less polluted than the central pedestrian zone. Finally, the third control site was in the biggest urban forest zone, and since the lowest element content was obtained in the moss bags exposed there, it proved to be the cleanest ambient within the city (**Table 4.6.5**).

According to our findings, control sites should be chosen with caution in experimental set-up in urban areas regarding its position. The sampling sites situated in green urban niches could be recommended as appropriate for the assessment of urban background levels of air pollutants.

Table 4.6.3. Spearman's correlation coefficients between the element concentrations (µg g^{-1}) in the *S. girgensohnii* moss samples and the traffic flows per vehicle category, and the total traffic flows (vehicles h^{-1}); correlations marked in bold are significant ($p < 0.05$); LDV – light duty vehicles, HDV – heavy duty vehicles

	Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	Ni	Pb	Pt	Sb	Sn	Sr	V	Zn
Al	1																
As	**0.83**	1															
Ba	0.50	0.53	1														
Ca	0.74	0.78	0.58	1													
Cd	0.62	0.56	0.49	0.74	1												
Co	0.68	0.62	0.69	0.71	0.69	1											
Cr	**0.82**	**0.94**	0.53	0.78	0.61	0.63	1										
Cu	0.65	**0.84**	0.54	0.60	0.55	0.60	**0.88**	1									
Fe	**0.81**	**0.95**	0.58	0.77	0.56	0.64	**0.97**	**0.87**	1								
Ni	0.76	0.79	0.54	0.74	0.63	0.69	**0.80**	0.77	0.77	1							
Pb	0.65	0.70	0.55	0.64	0.62	0.74	0.64	0.57	0.65	0.65	1						
Pt	0.69	0.69	0.44	0.60	0.46	0.45	0.68	0.53	0.75	0.54	0.55	1					
Sb	0.72	**0.89**	0.48	0.69	0.47	0.58	**0.94**	**0.83**	**0.94**	0.69	0.61	0.68	1				
Sn	0.71	0.69	0.48	0.57	0.64	0.48	0.78	0.68	0.75	0.55	0.48	0.59	0.67	1			
Sr	0.56	0.49	0.50	0.74	0.68	0.60	0.48	0.37	0.42	0.68	0.45	0.31	0.30	0.39	1		
V	**0.82**	**0.82**	0.49	0.66	0.58	0.55	**0.84**	0.74	**0.82**	**0.90**	0.60	0.64	0.70	0.69	0.59	1	
Zn	0.54	0.42	0.45	0.55	0.57	0.43	0.46	0.41	0.36	0.60	0.41	0.20	0.29	0.45	0.70	0.57	1
Car	0.49	0.57	0.46	0.46	0.36	0.48	***0.64***	***0.65***	***0.65***	0.32	0.39	0.47	***0.64***	0.55	0.25	0.35	0.11
Bus	0.49	0.57	0.31	0.44	0.21	0.47	0.58	0.45	***0.60***	0.44	0.40	0.48	***0.72***	0.24	0.17	0.41	0.03
Tram	0.40	0.36	0.16	0.22	0.11	0.16	0.30	0.24	0.31	0.26	0.19	0.24	0.33	0.14	0.11	0.22	0.01
Trolley	0.04	0.14	0.03	-0.03	0.24	0.19	0.17	0.47	0.18	0.13	0.09	0.03	0.10	0.23	-0.09	0.11	0.05
LDV	0.47	***0.61***	0.44	0.47	0.30	0.49	***0.64***	***0.62***	***0.64***	0.34	0.43	0.47	***0.65***	0.42	0.24	0.34	0.01
HDV	0.39	0.44	0.06	0.27	0.25	0.31	0.39	0.30	0.40	0.27	0.26	0.29	0.40	0.34	0.21	0.35	0.02
Motorcycle	0.36	0.49	0.54	0.50	0.50	0.47	0.56	0.55	0.57	0.30	0.42	0.38	0.54	0.61	0.28	0.35	0.22
Σ traffic flow	0.50	0.59	0.46	0.48	0.36	0.49	***0.65***	***0.66***	***0.67***	0.33	0.40	0.48	***0.66***	0.56	0.26	0.36	0.11

Table 4.6.4. Spearman's correlation coefficients between the element concentrations (µg g^{-1}) in the *H. cupressiforme* moss samples and the traffic flows per vehicle category, and the total traffic flows (vehicles h^{-1}); correlations marked in bold are significant (*$p < 0.05$*); LDV – light duty vehicles, HDV – heavy duty vehicles

	Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	Ni	Pb	Pt	Sb	Sn	Sr	V	Zn
Al	1																
As	**0.83**	1															
Ba	0.56	0.78	1														
Ca	0.36	0.63	0.73	1													
Cd	0.30	0.41	0.43	0.27	1												
Co	0.72	**0.91**	**0.84**	0.69	0.49	1											
Cr	0.72	**0.85**	**0.88**	0.73	0.39	**0.90**	1										
Cu	0.49	0.70	**0.81**	0.62	0.44	0.76	**0.86**	1									
Fe	**0.84**	**0.91**	**0.82**	0.68	0.34	**0.90**	**0.94**	0.78	1								
Ni	0.57	0.71	0.69	0.48	0.52	0.72	0.62	0.60	0.63	1							
Pb	0.63	0.74	0.53	0.38	0.60	0.68	0.56	0.43	0.62	0.56	1						
Pt	0.21	0.33	0.28	0.23	0.00	0.26	0.24	0.31	0.30	0.21	0.28	1					
Sb	0.48	0.66	**0.83**	0.70	0.31	0.74	**0.89**	**0.80**	0.77	0.53	0.44	0.23	1				
Sn	0.75	0.65	0.67	0.41	0.26	0.58	0.74	0.62	0.71	0.46	0.43	0.10	0.66	1			
Sr	0.10	0.32	0.46	0.60	0.08	0.39	0.34	0.29	0.29	0.40	0.19	0.20	0.35	0.18	1		
V	0.76	0.76	0.73	0.56	0.46	0.74	0.81	0.75	**0.84**	0.73	0.54	0.14	0.65	0.69	0.26	1	
Zn	-0.05	0.08	0.33	0.12	0.33	0.17	0.11	0.17	0.06	0.16	0.20	0.05	0.07	0.08	0.20	0.06	1
Car	0.35	0.50	***0.64***	0.43	0.24	0.57	***0.66***	***0.70***	***0.61***	0.34	0.24	0.35	***0.70***	0.44	0.16	0.40	0.03
Bus	0.28	0.44	0.51	0.50	0.04	0.45	0.54	0.42	0.52	0.28	0.27	0.42	***0.70***	0.32	0.17	0.33	-0.15
Tram	0.10	0.07	0.25	0.04	0.02	0.21	0.19	0.17	0.27	0.04	-0.03	0.04	0.11	-0.07	-0.11	0.07	0.17
Trolley	0.01	0.12	0.10	0.14	0.25	0.15	0.14	0.52	0.10	0.19	0.02	0.12	0.12	0.17	0.07	0.24	-0.01
LDV	0.32	0.50	0.59	0.47	0.26	***0.60***	***0.64***	***0.65***	0.57	0.28	0.25	0.29	***0.66***	0.37	0.09	0.34	-0.08
HDV	0.18	0.27	0.23	0.41	-0.02	0.35	0.37	0.33	0.41	0.09	0.19	0.20	0.36	0.21	-0.07	0.19	-0.18
Motorcycle	0.26	0.36	0.60	0.37	0.39	0.44	***0.64***	***0.61***	0.49	0.39	0.25	0.22	***0.67***	0.45	0.26	0.46	0.19
Σ traffic flow	0.36	0.51	0.65	0.44	0.25	0.58	***0.68***	***0.70***	***0.62***	0.34	0.25	0.35	***0.71***	0.45	0.16	0.41	0.03

Table 4.6.5. Average concentrations (µg g^{-1}, n=3) of pronounced traffic-related elements (r = 0.65 – 0.70) in the *S.girgensohnii* and *H. cupressiforme* moss bags exposed in the pedestrian zones and the nearest on-road sampling sites over the city of Belgrade; pedestrian zone are italicized

	Sphagnum girgensohnii			
Sampling site	Cr	Cu	Fe	Sb
Crossroad	4.56	22.8	1852	1.68
Central pedestrian zone	2.55	90.6	1374	0.54
Crossroad	2.44	16.0	998	1.50
Peri-urban pedestrian zone	0.90	5.6	645	1.52
Crossroad	2.01	13.5	910	0.54
Urban forest	0.73	5.8	584	0.20
	Hypnum cupressiforme			
Sampling site	Cr	Cu	Fe	Sb
Crossroad	3.76	27.1	1672	1.42
Central pedestrian zone	2.01	19.9	1129	0.29
Crossroad	2.65	15.7	1214	1.87
Peri-urban pedestrian zone	0.90	9.6	528	0.08
Crossroad	1.92	15.2	1072	0.30
Urban forest	1.10	9.1	756	0.08

5. Conclusion

Thus far, the use of the moss bag technique has been limited only to scientific research. Despite the advantages associated with the technique, it has not been implemented by public authorities to monitor levels of air pollutants. This doctoral dissertation demonstrates possibilities of practical application of the moss bag technique in the field emphasizing its usefulness. Through the series of experiments, aspects concerning the application of the technique in urban areas were investigated.

Based on the results of the Experiment 1, the following conclusions could be drawn:

- The *S. girgensohnii* moss bags are sensitive to the small-scale changes in vertical and horizontal spatial distribution of airborne major and trace element content in the street canyons and city tunnel.
- Besides the sources of pollutant emissions, the specific spatial distribution of major and trace elements is a consequence of the competing influence of the geometry of sampling site, disturbance of the natural ventilation, different directions of the dynamic, and thermal induced vortices.
- The obtained vertical variations in the street canyons emphasized that residents in upper parts of some street canyons may be exposed to higher concentration of trace and carcinogenic elements than those in pedestrian breathing zones. Thus, it is necessary to evaluate the exposure of human population in both high-level apartments and the ground level.
- An exposure height of 4 m can be recommended for the moss bag exposure in urban environment, since a decrease in the vertical distribution of elements was revealed from 4 m to 16 m.

The results obtained in the Experiment 2 demonstrate that:

- The *S. girgensohnii* moss bags are moderated to intercept elements in the enclosed spaces, such as parking garages, due to stagnant ambient conditions. Indoor environment is characterized by dry conditions, which limit moss physiological activity and further element uptake.
- Further research should focus on clarifying influence of dry indoor air conditions on the moss element enrichment. Representativeness of the height for exposure of the moss bags indoor area should be investigated.

The measurements of magnetic parameter of the exposed moss bag (Experiment 3), saturation isothermal remanent magnetisation (SIRM), pointed out that:

- The *S. girgensohnii* moss bags could be effectively applied for biomagnetic monitoring of the spatio-temporal distribution of road traffic- and vehicle-derived pollutants in urban areas.
- Some trace elements are possibly associated and co-precipitate with the Fe-magnetic particles during the combustion processes or could be adsorbed onto the surface of the pre-present ferromagnetic particles in the environments. Very high correlation coefficients ($r \geq 0.95$, $p < 0.05$) were found between moss SIRM values and the moss concentration of Cr, Cu, Fe, and Ni.

Based on the Experiment 4, the following results were emphasized:

- The moss bags of *S. girgensohnii* and *H. cupressiforme* revealed new pollution hotspots in addition to the sites previously recognized by the instrumental monitoring. Clear distinctions among high-, moderate- and low-pollution urban land use classes were observed by both species. The highest moss elemental concentrations were recorded at sampling sites affected by the local industry emissions and at the sampling sites situated on streets with high-intensity traffic. The lowest moss elemental concentrations were obtained in the residential zones and city parks/forests.
- Significantly higher levels of all measured pollutants were recorded by *S. girgensohnii* in comparison with *H. cupressiforme*. However, linear regression analysis type II showed that the studied moss species could not be interchangeably used for assessment of trace elements, except for Cu.
- Significantly lower values for the magnetic parameter SIRM in the residential zones and parks relative to the public transportation zones confirmed that moss magnetic analysis represents an effective first step tool for screening of particulate air pollution prior to more complex and expensive chemical analyses.

Biomonitoring performed during the winter, i.e., heating season (Experiment 4), demonstrate that:

- Residential heating system is a significant contributor to the air pollution during the winter season. *S. girgensohnii* is very efficient in reflecting a seasonal variability of emerging air pollutants, especially PAHs. Markedly higher concentration of all PAHs was observed during the winter in comparison to the summer season, but also high-molecular-weight combustion PAHs, DB[ah]A, and B[ghi]P, were only determined in the winter. As in the current literature is a lack of data on moss bags as PAH biomonitors, the results of this study could be a step forward to the moss bag application for the airborne PAH monitoring of seasonal variation.

- During the heating season, the study area was polluted by major and trace elements far more than in the preceding summer. Among the most of determined elements, the moss samples were especially enriched with V, Ni, Sb, Cu, and Zn. After normalization against the NASC and PAAS, the REEs in the moss samples showed consistent lithological signature, but with enhanced RAF values of emerging elements – Ce, Ga, La, Nd, Pr, and Sm, at the urban sites.
- Regarding health issues, it is of importance to assess the airborne levels of broadened set of elements, such as trace and REEs. Emission of Cu, Sb, V, and any of REEs into the air has not been regulated by EU Directive yet, although they represent well-known carcinogenic/toxic compounds.

Based on the results of the Experiment 6:

- The *S. girgensohnii* and *H. cupressiforme* moss bags have outlined distinct spatial decline in the airborne trace element content along the crossroads, two- and one-lane streets.
- According to the results of linear regression analysis type II, it could be concluded that the studied moss species could only be interchangeably used for the assessment of Cr, Cu, and Sb within on-road microenvironments.
- The both moss species marked Sb, Cu, and Cr as reliable traffic tracers regarding the high correlation coefficients ($r > 0.65$) between the moss element concentrations and the counted traffic flows.
- The live and oven-dried moss bags of *S. girgensohnii* and *H. cupressiforme* showed no significant differences in abundance and spatial pattern of the majority of 44 determined elements. Under the conditions of the continental climate zone, which is characteristic for the study area, the elements entrapment is mainly passive uptake. Thus, devitalizing pre-treatment should not be considered as a necessary step in further application of moss bag biomonitoring in the study area (Serbia). However, devitalized moss bags should be appropriate if objective is to compare results from biomonitoring surveys performed in regions with diverse climatic conditions.

Finally, the higher concentrations of the airborne pollutants were found in the moss samples of *S. girgensohnii* than *H. cupressiforme*. According to the data regarding the content of moss SIRM, major and trace elements, and PAHs, *S. girgensohnii* can be considered to be a more sensitive species than *H. cupressiforme* for monitoring of air pollutants in urban areas. It was shown that *S. girgensohnii* exhibited the potential to indicate small-scale variations in air pollutants levels, i.e., between neighbouring urban microenvironments within the same land use class. Moreover, *H. cupressiforme* reflects spatial pollutant variations between different land use classes, but only at a large-scale.

Variable determination coefficients were obtained by the interspecies comparison of *S. girgensohnii* (a species of the most recommended biomonitoring moss genus) to *H. cupressiforme* (a common moss in the study area). The results suggested interchangeable use of the studied mosses for biomonitoring of Sb, Cu, and Cr in specific ambience such as on-road microenvironment. However, when sampling sites are chosen to be representative for different land use classes, interchangeable use of the studied moss species could be recommended only for Cu. Thus, conversion the data of one studied species to the other should be considered with caution in active moss biomonitoring applied in urban areas.

The results presented in this dissertation represent an upgrade to the application of the moss bag technique in urban areas. Considering magnetic measurements, the moss bags are proved useful in biomagnetic monitoring of the PM fraction associated with trace elements that derives from different sources of air pollution. The moss bag technique is promoted as a complementary method to standardized measurements by filling gaps in the regulatory monitoring network and optimizing its representativeness. In addition, the results point out elevated concentrations of emerging pollutants in urban areas and highlighted a need for better characterization of micro-scale siting for monitoring of trace element distribution.

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7. Appendix

7.1. Appendix 1

Table 7.1.1. Major and trace elements associated with particles originated from natural and anthropogenic sources

Source type	Associated elements	References
Exhaust traffic		
Diesel emissions	Al, Cu,Cr, Fe, Mn, V, Zn	EMEP/EEA, 2013
Gasoline emissions	Br, Ba, Sb, V	Pulles et al., 2012
Lubricating oil combustion	Cd, Cr, Cu, Ni, Pb, Zn	Pulles et al., 2012
Non-exhaust traffic		
Brakes	Fe, Cu, Sn, Sb, Zn	Gietl et al., 2010
Tyres	Co, Cr, Fe, Mn, Pb	Thorpe and Harisson, 2009
Road dust	Al, Si, Ca, Fe, K	Viana et al., 2008
Industrial		
Metal working	Fe, K, Na, Pb, Zn	Sanderson et al., 2014
Power generation	Ce, Fe, La, Na, K, V	
Incinerators	Cd, Pb, Sb, Zn	
Natural		
Marine aerosol	Cl, Na, Mg	Viana et al., 2008

Table 7.1.2. Abundances of the REEs in the Earth's crust (Wedepohl, 1995)

Element	Crustal Abundance (ppm)
Ce	60.0
La	30.0
Nd	27.0
Y	24.0
Sc	16.0
Pr	6.7
Sm	5.3
Gd	4.0
Dy	3.8
Er	2.1
Yb	2.0
Eu	1.3
Ho	0.8
Tb	0.7
Lu	0.4
Tm	0.3
Pm	10^{-18}

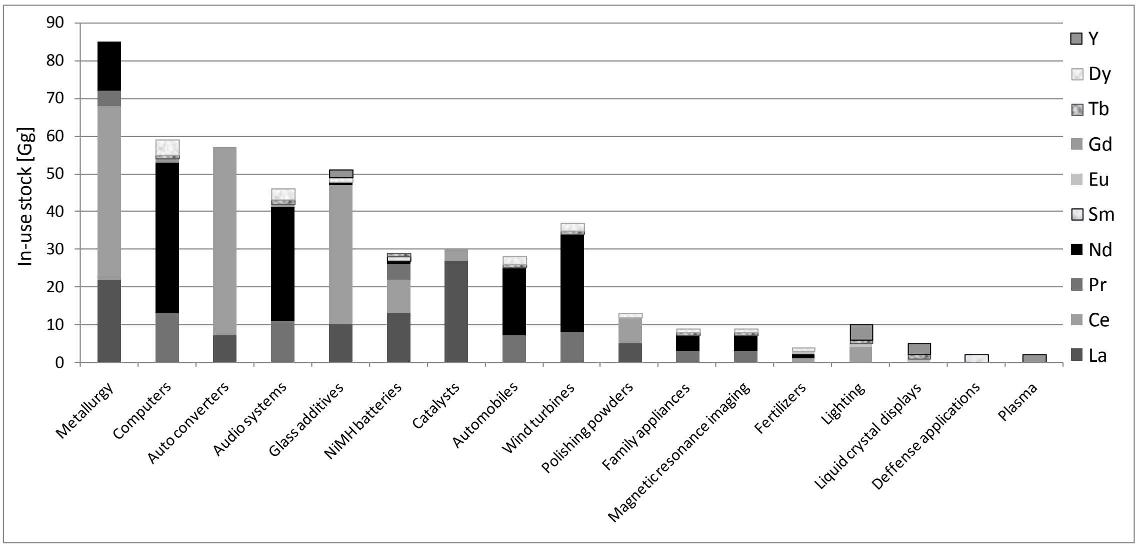

Figure 7.1.1. In-use stocks (Gg) of selected REEs by specific application or industry (Du and Graedel, 2011).

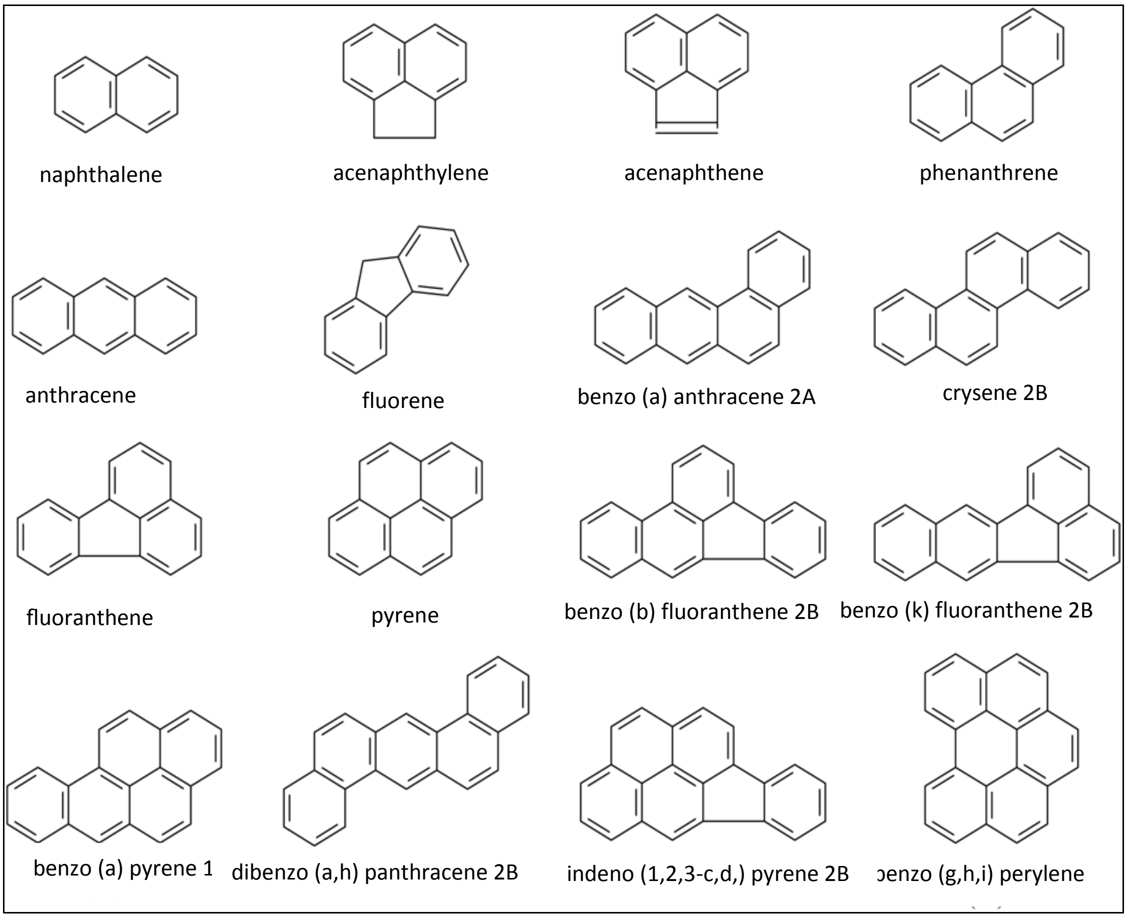

Figure 7.1.2. US EPA priority PAHs; IARC classification (2013) is indicated: Group 1 – carcinogenic to humans, Group 2A – probable carcinogenic to humans, Group 2B – possibly carcinogenic to humans.

Table 7.1.3. The PAH emission rates estimated from antrophogenic sources – domestic, mobile, industrial and agricultural (Ravindra et al., 2008)

Source type	Emission rate	Remarks	References
Domestic			
Natural gas home appliances	1–2000 pg kg^{-1}	pg kg^{-1} of natural gas burned	Rogge et al., 1993
Coal	0.95 mg kg^{-1}	Average of 4 coal ranks	Oros and Simoneit, 2000
Wood fuel	24–114 mg kg^{-1}	12 different cook stoves were used	Oanh et al., 2005
Anthracite coal	2 mg kg^{-1}		
Domestic burning	100 mg kg^{-1}	Controlled burning experiments for space heating	Lee et al., 2005
Fire wood	1.6–8.2	B[a]P	Kakareka et al., 2005
Peat Briquette	0.8	B[a]P	
Mobile			
Gasoline engine	350 or 26 μg km^{-1}	Catalytic convertor equipped light vehicles (average of 7)	
Gasoline engine	72.5 μg kg^{-1}	Light vehicles	Miguel et al., 1998
Gasoline engine	8.1. or 0.7 μg km^{-1}	Catalytic onvertor equipped light vehicles (average of 9)	Schauer et al., 2002
Light-duty gasoline	21 μg kg^{-1}	For benzo[ghi]perylene, for pyrene (particle phase)	Marr et al., 1999
Heavy-duty diesel	1000 μg kg^{-1}		
Diesel engine	240 or 60.2 μg km^{-1}	Heavy-duty trucks	Rogge et al., 1993
Diesel engine	14.3 μg km^{-1}	Heavy-duty trucks	Miguel et al., 1998
Break dust	16 mg kg^{-1}	Emitted from the hydraulic break system	Rogge et al., 1993
Industrial			
Industrial stacks	77–3970 μg kg^{-1}	9 different stacks	Yang et al., 1988
	2–16 μg kg^{-1}	For B[a]P	
Industrial boiler	13,300 μgkg^{-1}	Lower molecular weight PAHs dominated in the stack flue gas	Li et al., 1999
Heavy oil	2900 μg kg^{-1}		
Diesel	2880 μg kg^{-1}		
Heavy oil + natural gas coke + oven gas	208 μg kg^{-1}		
Water tube boiler	85–320 μg kg^{-1}	Only 3 PAHs were detected	Pisupati et al. , 2000
Waste tire pyrolysis	4000 μg kg^{-1}	Total emissions gaseous, particulate, and residual PAHs	Chen et al., 2007
	3.400 μg kg^{-1}		
	200 μg kg^{-1}		
	400 μg kg^{-1}		
Agricultural			
Open burning	5–683 mg kg^{-1}	Wind tunnel simulations of agricultural and forest biomass fuels burning	Jenkins et al., 1996
Open burning	240–571 mg kg^{-1}	Simulation of agricultural debris	Kakareka and Kukharchyk, 2003

7.2. Appendix 2

Table 7.2.1. Average values of the meteorological parameters: temperature, relative air humidity, amount of precipitation and wind speed, during the experiments performed over the city of Belgrade

	Temperature (ºC)	Relative air humidity (%)	Precipitation (mm)	Wind speed (m s^{-1})
Experiment 1,2,3	24	58	40	2
Experiment 4	22	69	50	3
Experiment 5	6	77	25	6
Experiment 6	22	68	225	2

Table 7.2.2. The results of the quality control performed for the element determination by ICP-OES and ICP-MS using the certified reference materials, lichen-336 (IAEA) and moss *Pleurozium schreberi*, M2 and M3. The recoveries were calculated for: Al, Ba, Cd, Co, Cr, Cu, Fe, K, Mn, Na, Pb, Sr, V, and Zn in the lichen-336, and for: Al, Ba, Be, Cd, Ce, Co, Cr, Cu, Fe, Ga, K, La, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Sc, Sr, Th, Tl, Y, V, and Zn in the M2 and M3

	Recovery range (%)			Exceptions (%)		
	lichen-336	M2	M3	lichen-336	M2	M3
Experiment 1, 3	70 - 120	/	/	Al, Co, K, V (60)	/	/
Experiment 2, 3	70 - 113	/	/	Cr, Mn (60); K (55), V(50)	/	/
Experiment 4	70 - 100	85 - 115	80 - 105	Co, K, Na (67)	/	Co (127)
Experiment 5	/	80 - 113	80 - 110	/	Mo (65)	Ga (120); Th (74); Na, Sc (70); Cr, Mo, Sb (50)
Experiment 6	/	75 - 111	80 - 112	/	Ni (126); Na (67); Ce (56), Sb (47)	Sm (76); Th (69); Sc (67); Cu (63); Ce (57); Na (18)

Table 7.2.3. Method detection limit and method quantification limit (μg g^{-1}) of the elements determined by ICP-OES in the *S. girgensohnii* moss bags exposed for ten weeks during the summer and autumn 2013 in the street canyons, the city tunnel, and parking garages (Experiment 1, 2, and 3)

Element	Limit of detection (μg g^{-1})	Limit of quantification (μg g^{-1})
Al	0.02	0.07
Ba	0.005	0.017
Ca	0.0005	0.0017
Cd	0.012	0.039
Co	0.085	0.283
Cr	0.035	0.117
Cu	0.065	0.216
Fe	0.04	0.14
K	0.1	0.3
Mg	0.002	0.006
Mn	0.01	0.04
Na	0.06	0.21
Ni	0.06	0.20
Pb	0.18	0.59
V	0.04	0.13
Zn	0.03	0.11

Table 7.2.4. Method detection limit and method quantification limit (μg g^{-1}) of the elements determined by ICP-OES in the moss bags of *S. girgensohnii* and *H. cupressiforme* exposed for ten weeks over the city of Belgrade (Experiments 4 – 6)

Element	Limit of detection (μg g^{-1})	Limit of quantification (μg g^{-1})
Al	0.4	1.3
Ba	0.01	0.04
Ca	0.4	1.4
Cd	0.01	0.04
Co	0.05	0.18
Cr	0.05	0.17
Cu	0.09	0.31
Fe	0.1	0.4
K	0.2	0.6
Li	0.002	0.007
Mg	0.1	0.3
Mn	0.02	0.07
Na	3.1	10.3
Ni	0.43	1.45
Pb	0.18	0.60
Sr	0.005	0.016
V	0.08	0.26
Zn	0.01	0.05

Table 7.2.5. Method detection limit and method quantification limit (μg g^{-1}) of the elements determined by ICP-MS in the *S. girgensohnii* moss bags exposed for ten weeks over the city of Belgrade during the winter 2013 (Experiment 5)

Element	Limit of detection (μg g^{-1})	Limit of quantification (μg g^{-1})
Ce	0.00038	0.00127
Dy	0.00006	0.00020
Er	0.00006	0.00019
Eu	0.00004	0.00013
Ga	0.00138	0.00460
Gd	0.00008	0.00025
Ho	0.00001	0.00004
La	0.00044	0.00148
Lu	0.00002	0.00008
Mo	0.00192	0.00639
Nd	0.00030	0.00100
Pd	0.00039	0.00130
Pr	0.00006	0.00021
Rb	0.00416	0.01384
Sb	0.00183	0.00610
Sc	0.00109	0.00361
Sm	0.00013	0.00044
Tb	0.00002	0.00005
Tl	0.00067	0.00223
Th	0.00009	0.00029
Tm	0.00001	0.00003
Y	0.00035	0.00116
Yb	0.00006	0.00021

Table 7.2.6. Method detection limit and method quantification limit (μg g^{-1}) of the elements determined by ICP-MS in the *S. girgensohnii* and *H. cupressiforme* moss bags exposed for ten weeks during the summer 2014 over the crossroads, two- and one-lane streets during the summer of 2014 (Experiment 6)

Element	Limit of detection (μg g^{-1})	Limit of quantification (μg g^{-1})
As	0.005	0.015
Ce	0.0005	0.0015
Cd	0.002	0.007
Co	0.001	0.003
Cr	0.004	0.013
Dy	0.0002	0.0005
Ga	0.0016	0.0053
Gd	0.0002	0.0006
Er	0.0001	0.0005
Eu	0.0001	0.0003

Table 7.2.6. (continued).

Element	Limit of detection (μg g^{-1})	Limit of quantification (μg g^{-1})
Ho	0.0001	0.0002
La	0.0005	0.0016
Lu	0.0001	0.0002
Ni	0.027	0.088
Nd	0.0005	0.002
Pb	0.0159	0.0529
Pr	0.0001	0.0004
Pt	0.0002	0.0007
Rb	0.005	0.02
Sb	0.001	0.004
Sc	0.0028	0.0095
Sm	0.0002	0.0007
Sn	0.0012	0.0041
Sr	0.001	0.03
Tb	0.0001	0.0002
Th	0.0003	0.001
Ti	0.0093	0.0309
Tl	0.0003	0.001
Tm	0.00005	0.00016
Y	0.0005	0.0016
Yb	0.0002	0.0008
V	0.001	0.005

Table 7.2.7. Concentration (mg kg^{-1}) of the rare earth elements (REEs) in the North American Shale Composite (NASC) and Post-Archean Australian Shales (PAAS) (Dołęgowska and Migaszewski, 2013), used for normalization of the REEs concentrations in the mosses *S. girgensohnii* and *H. cupressiforme*

Element	NASC (mg kg^{-1})	PAAS (mg kg^{-1})
La	31.1000	44.5600
Ce	67.0330	88.2500
Pr	7.9000	10.1500
Nd	30.4000	37.3200
Sm	5.9800	6.8840
Eu	1.2533	1.2150
Gd	5.5000	6.0430
Tb	0.8500	0.8914
Dy	5.7500	5.3250
Ho	1.2000	1.0530
Er	3.2750	3.0750
Tm	0.5000	0.4510
Yb	3.1133	3.0120
Lu	0.4560	0.4386

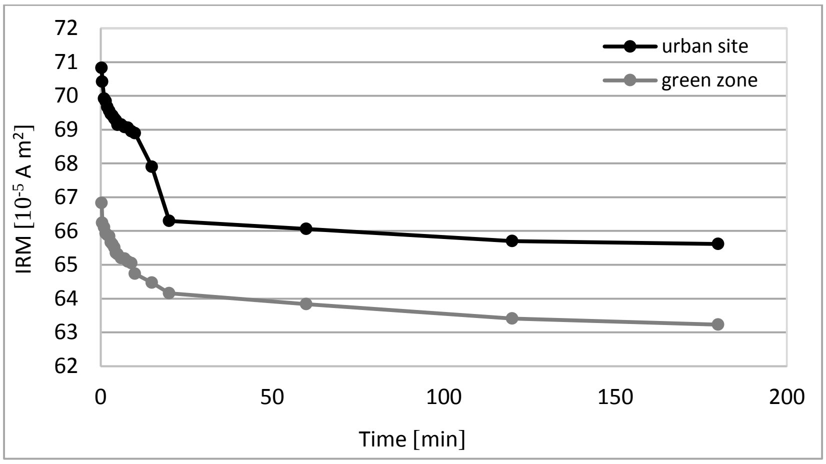

Figure 7.2.1. Time-dependence of the isothermal remanent magnetisation, IRM (10^5 A m^2) measured in the *S. girgensohnii* moss bags exposed for ten weeks during the summer 20104 at the urban site and green zone.

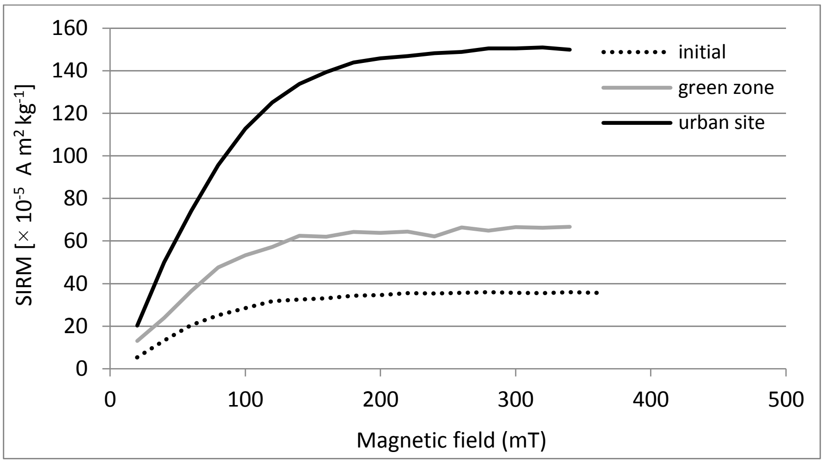

Figure 7.2.2. Changes of the moss saturation isothermal remanent magnetisation, SIRM ($\times$ 10^{-5} A m^2 kg^{-1}), with increase of the applied magnetic field (mT); initial – moss SIRM values in the unexposed moss bags; urban site and green zone – exposure sites of the moss bags.

7.3. Appendix 3

Table 7.3.1. Element concentrations and standard deviations (μg g^{-1}) for the *S. girgensohnii* moss bags exposed for ten weeks (summer 2011) in four traffic-burden (SC$_1$, SC$_2$, SC$_3$, and SC$_4$) and pedestrian (SC$_{ped}$) street canyons in the centre of Belgrade; I, II and III – height of the moss bag exposure, 4 m, 8 m and 16 m, respectively

	Concentration ± σ (μg g^{-1})							
*n = 8	Al	Ba	Ca	Cd	Co	Cr	Cu	Fe
Initial	226±20	23.5±0.5	3130±200	0.22±0.02	0.28±0.03	0.53±0.08	3.5±0.1	275±30
SC$_1$ I	397±50	36.8±5.0	5757±900	0.31±0.06	0.36±0.05	1.41±0.20	12±2.4	555±90
SC$_1$ II	469±80	37.0±3.0	5612±400	0.29±0.02	0.39±0.04	1.59±0.20	11.5±1.8	619±80
SC$_1$ III	398±50	37.8±3.0	5123±400	0.28±0.02	0.34±0.04	1.10±0.06	7.9±1.3	507±60
SC$_2$ I	541±70	58.4±5.0	5589±500	0.32±0.04	0.45±0.05	2.10±0.30	15.0±1.7	834±100
SC$_2$ II	477±80	39.6±4.0	4395±400	0.28±0.02	0.38±0.04	1.61±0.30	11.4±1.2	635±50
SC$_2$ III	393±40	36.0±3.0	4657±400	0.30±0.03	0.37±0.05	1.55±0.30	11.4±2.0	574±60
SC$_3$ I	421±100	32.4±4.0	4463±400	0.33±0.03	0.36±0.06	1.58±0.50	13.7±2.2	579±100
SC$_3$ II	345±80	30.2±5.0	4303±400	0.27±0.03	0.31±0.05	1.03±0.20	10.0±1.5	462±90
SC$_3$ III	320±30	20.7±1.0	4035±300	0.29±0.02	0.31±0.01	0.95±0.10	8.1±1.6	424±20
SC$_4$ I	344±40	29.8±1.0	4298±200	0.27±0.02	0.38±0.03	1.28±0.20	8.3±2.1	464±40
SC$_4$ II	417±30	35.2±2.0	5113±400	0.30±0.03	0.51±0.05	1.65±0.30	10.1±1.9	558±30
SC$_4$ III	356±40	31.1±3.0	4175±200	0.29±0.03	0.45±0.06	1.19±0.20	8.3±1.2	471±50
SC$_{ped}$ I	344±40	41.3±4.0	4383±300	0.27±0.02	0.34±0.02	0.97±0.20	8.8±1.4	456±40
SC$_{ped}$ II	439±90	60.2±10.0	4758±500	0.32±0.03	0.39±0.07	0.97±0.20	9.8±1.8	594±100
SC$_{ped}$ III	387±50	33.3±3.0	4479±500	0.29±0.03	0.33±0.04	1.24±0.30	7.4±1.4	507±60

*n – number of the analysed subsamples

Table 7.3.1. (continued).

	Concentration ± σ (μg g^{-1})								
*n = 8	K	Mg	Mn	Na	Ni	Pb	Sr	V	Zn
Initial	7329±200	1268±90	264±25	100±8	1.03±0.20	1.6±1.4	8.2±0.3	0.55±0.06	27±2
SC_1 I	3076±200	1330±70	43±10	129±40	2.02±0.30	16.3±2.0	14.2±2.5	1.26±0.20	65±11
SC_1 II	3819±600	1458±50	61±10	165±30	1.95±0.30	9.2±3.0	15.0±2.0	3.81±0.20	55±8
SC_1 III	3692±300	1483±50	52±10	124±20	1.59±0.20	5.4±1.0	14.1±2.0	1.11±0.20	46±7
SC_2 I	3096±400	1438±60	266±20	197±30	2.61±0.70	9.9±1.0	16.6±2.30	2.08±0.20	234±46
SC_2 II	3706±400	1341±80	250±30	168±20	1.88±0.20	5.6±1.0	12.2±1.2	1.41±0.30	99±26
SC_2 III	3180±200	1396±30	258±20	143±10	1.87±0.30	4.7±2.0	11.4±1.0	1.48±0.20	111±20
SC_3 I	3548±200	1405±120	260±20	178±30	2.04±0.40	6.7±2.0	11.9±1.1	1.32±0.30	70±8
SC_3 II	3450±400	1354±80	260±20	165±30	1.77±0.50	3.7±0.8	12.4±1.4	1.05±0.20	47±6
SC_3 III	3547±400	1406±60	253±20	139±20	1.73±0.30	5.2±3.0	10.9±1.9	1.20±0.03	45±5
SC_4 I	3908±400	1349±70	262±20	138±20	1.34±0.07	6.6±2.0	10.2±0.6	0.92±0.10	66±6
SC_4 II	3480±400	1459±20	285±20	147±30	1.92±0.40	17.9±13.0	13.4±2.3	1.09±0.06	62±11
SC_4 III	3646±400	1373±60	245±20	140±30	1.64±0.40	7.9±3.0	11.2±1.7	0.92±0.20	56±13
SC_{ped} I	3507±500	1429±40	259±30	128±20	1.45±0.20	5.8±1.0	11.9±1.3	1.01±0.20	68±16
SC_{ped} II	3134±500	1462±60	277±20	159±30	1.77±0.40	5.9±2.0	13.9±1.7	1.21±0.20	92±28
SC_{ped} III	3662±600	1452±60	261±20	130±40	1.45±0.20	3.8±1.0	11.5±0.8	1.02±0.20	53±14

*n – number of the analysed subsamples

Table 7.3.2. Element concentrations and standard deviations (μg g^{-1}) for the *S. girgensohnii* moss bags exposed inside (IT), at the entrance (ET) and outside the city tunnel (OT); 5 and 10 – exposure periods of five and ten weeks during the autumn of 2011

	Concentration ± σ (μg g^{-1})						
*n = 8	Initial	IT_5	IT_{10}	ET_5	ET_{10}	OT_5	OT_{10}
Al	226±20	360±150	878±100	392±10	650±50	424±20	541±40
Ba	23.5±0.5	31.0±11.0	55.3±4.1	31.1±0.7	40.0±1.0	32.1±0.5	36.2±0.9
Ca	3130±200	3240±400	5346±500	3181±200	6226±300	4132±400	5639±400
Cd	0.22±0.02	0.30±0.03	0.37±0.02	0.34±0.06	0.36±0.04	0.28±0.01	0.34±0.02
Co	0.28±0.03	0.07±0.09	0.54±0.03	0.20±0.04	0.65±0.30	0.72±0.05	0.88±0.05
Cr	0.53±0.08	1.81±0.60	5.53±0.60	1.09±0.10	2.85±0.50	1.22±0.20	2.00±0.40
Cu	3.5±0.1	21.4±7.4	66.4±3.9	16.4±1.0	42.3±5.6	17.8±1.2	31.0±5.0
Fe	275±30	783±300	1946±60	554±20	1098±80	618±40	822±90
K	7329±200	7316±200	7357±200	6850±200	7130±100	6175±200	6612±300
Mg	1268±90	1515±40	1745±50	1484±20	1615±20	1526±30	1631±40
Mn	264±25	270±10	279±10	282±10	287±10	280±10	295±10
Na	100±8	768±80	/	485±30	3060±200	570±50	3430±400
Ni	1.03±0.20	1.84±0.80	4.33±0.20	2.48±0.20	4.63±0.30	2.65±0.20	4.12±0.80
Pb	1.6±1.4	4.7±2.3	16.3±3.1	7.4±1.6	8.2±1.1	7.0±1.1	10.3±0.7
Sr	8.2±0.3	11.5±0.6	15.1±1.6	8.2±0.5	15.9±0.6	12.4±1.3	16.0±2.0
V	0.55±0.06	0.39±0.40	1.76±0.20	1.06±0.10	2.32±0.40	2.01±0.20	1.41±0.30

*n – number of the analysed subsamples

Table 7.3.3. Spearman's **c**orrelation coefficients between the element concentrations in the *S. girgensohnii* moss bags exposed for ten weeks in the five street canyons and the city tunnel

	Al	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sr	V	Zn
Al	1																
Ba	**0.76**	1															
Ca	0.48	**0.63**	1														
Cd	0.38	0.34	0.39	1													
Co	**0.53**	0.45	0.37	0.37	1												
Cr	0.19	0.18	0.32	0.25	0.15	1											
Cu	**0.53**	**0.60**	**0.57**	**0.61**	0.31	0.21	1										
Fe	**0.89**	**0.88**	**0.58**	0.43	0.56	0.21	**0.73**	1									
K	-0.10	-0.29	-0.30	-0.28	-0.14	-0.13	-0.52	-0.33	1								
Mg	0.34	0.35	0.48	0.44	0.38	0.06	0.23	0.32	0.04	1							
Mn	0.34	0.32	**0.55**	0.35	0.35	0.08	0.14	0.28	0.11	0.76	1						
Na	0.27	0.35	0.17	0.21	0.09	0.00	0.54	0.36	0.07	0.18	0.00	1					
Ni	0.14	0.32	0.38	0.15	0.07	0.07	0.39	0.25	-0.24	0.26	0.10	0.28	1				
Pb	0.11	0.15	0.35	0.27	0.32	0.05	0.20	0.19	-0.07	0.08	0.12	0.07	0.08	1			
Sr	0.13	0.21	0.25	0.12	0.00	0.05	0.26	0.19	-0.04	0.24	0.07	**0.57**	0.23	0.08	1		
V	**0.80**	**0.84**	0.48	0.45	0.30	0.20	**0.72**	**0.89**	-0.36	0.30	0.21	0.38	0.25	0.01	0.16	1	
Zn	**0.58**	**0.87**	0.39	0.35	0.41	0.16	**0.67**	**0.80**	-0.43	0.17	0.06	0.43	0.30	0.08	0.14	**0.83**	1

Table 7.3.4. Element concentrations and standard deviations (µg g^{-1}) for the *S. girgensohnii* moss bags exposed for ten weeks (winter 2011/2012) in four parking garages (PG) in the centre of Belgrade; T and I – the exposure positions of the moss bags near the tollgate and interior, respectively; U – underground, SE – semi-enclosed, and O – open

	Concentration ± σ (µg g^{-1})																
*n = 8	Al	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sr	V	Zn
Initial	253 ± 20	21.9 ± 0.2	3253 ± 700	0.21 ± 0.03	0.09 ± 0.05	0.37 ± 0.04	4.2 ± 0.1	260 ± 20	5450 ± 400	1251 ± 100	171 ± 40	97 ± 50	1.09 ± 0.09	2.8 ± 0.4	6.8 ± 0.2	0.47 ± 0.02	26 ± 5
TPG$_U$	342 ± 20	27.5 ± 1.4	3549 ± 300	0.23 ± 0.02	0.16 ± 0.04	0.56 ± 0.09	5.5 ± 0.8	343 ± 20	6184 ± 500	1335 ± 80	192 ± 20	138 ± 50	1.38 ± 0.20	3.6 ± 0.3	8.3 ± 0.3	0.61 ± 0.08	30 ± 2
IPG$_U$	264 ± 20	23.7 ± 0.6	3132 ± 100	0.21 ± 0.01	0.09 ± 0.02	0.38 ± 0.04	4.0 ± 0.2	252 ± 20	5784 ± 200	1221 ± 30	173 ± 10	88 ± 40	1.18 ± 0.20	2.8 ± 0.2	6.7 ± 0.2	0.41 ± 0.05	38 ± 2
TPG$_{SE1}$	378 ± 20	28.7 ± 0.9	3550 ± 200	0.22 ± 0.01	0.14 ± 0.03	0.54 ± 0.04	4.6 ± 0.4	362 ± 20	5438 ± 200	1212 ± 40	178 ± 10	104 ± 30	1.59 ± 0.30	3.2 ± 0.2	7.9 ± 0.2	0.56 ± 0.04	43 ± 4
IPG$_{SE1}$	270 ± 10	24.0 ± 1.0	3109 ± 100	0.23 ± 0.01	0.11 ± 0.04	0.40 ± 0.06	4.2 ± 0.2	249 ± 10	5779 ± 200	1205 ± 40	169 ± 10	85 ± 10	1.26 ± 0.20	2.7 ± 0.2	6.7 ± 0.2	0.41 ± 0.04	37 ± 2
TPG$_{SE2}$	280 ± 10	24.4 ± 0.7	3157 ± 100	0.22 ± 0.01	0.08 ± 0.03	0.50 ± 0.05	5.0 ± 0.2	290 ± 10	5622 ± 200	1245 ± 50	177 ± 10	261 ± 60	1.27 ± 0.10	3.0 ± 0.1	7.0 ± 0.4	0.51 ± 0.04	31 ± 1
IPG$_{SE2}$	264 ± 10	24.3 ± 0.8	3282 ± 200	0.21 ± 0.01	0.08 ± 0.04	0.41 ± 0.03	3.8 ± 0.5	250 ± 10	5694 ± 100	1240 ± 30	179 ± 10	459 ± 100	1.12 ± 0.10	3.1 ± 0.2	6.9 ± 0.2	0.40 ± 0.03	44 ± 3
TPG$_O$	268 ± 10	24.2 ± 0.6	3180 ± 100	0.23 ± 0.01	0.06 ± 0.06	0.42 ± 0.05	4.7 ± 0.5	264 ± 10	5475 ± 200	1239 ± 50	168 ± 10	94 ± 40	1.28 ± 0.20	3.0 ± 0.2	7.0 ± 0.4	0.41 ± 0.03	38 ± 2
IPG$_O$	362 ± 20	29.2 ± 0.8	3426 ± 80	0.21 ± 0.01	0.11 ± 0.04	0.40 ± 0.05	3.9 ± 0.2	354 ± 20	5747 ± 200	1223 ± 30	183 ± 10	68 ± 10	1.27 ± 0.10	2.6 ± 0.2	7.9 ± 0.2	0.46 ± 0.05	38 ± 1

*n – number of the analysed subsamples

Table 7.3.5. Average daily element abundances (ng m^{-3}) determined in PM10 sampled for ten weeks durng the autumn 2013 in two parking garages (PG) in the centre of Belgrade; U – underground and SE – semi-enclosed

PG_U																
Day	Al	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sr	Zn
Mon	18428	71072	124347	3.02	4.82	36	249	6304	51706	8495	100	166463	54	64	1722	76687
Tue	14686	69263	152192	4.5	3.92	24	247	2104	55190	8761	111	171029	56	80	1696	79117
Wed	14947	63915	131764	4.63	6.04	28	221	2118	49692	8769	134	164088	53	89	1516	73628
Thu	32285	123338	142617	4.01	4.03	42	264	2046	94874	10670	119	274015	67	76	2442	134115
Fri	17183	80652	127483	3	4.53	25	149	1334	64313	8365	98	198890	42	101	1832	93430
Sat	17679	76065	181425	6.81	5.08	31	281	1326	63271	10569	106	195204	70	119	1751	88034
Sun	11568	61764	102907	2.78	2.43	21	132	1607	46982	7172	81	139418	51	34	1475	65860

PG_{SE1}																
	Al	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Sr	Zn
Mon	21209	86530	141938	3.23	5.33	32	220	1636	49171	7809	102	163019	43	46	1516	72957
Tue	23393	93533	123372	4.24	3.68	37	264	2930	71285	9240	127	216330	66	42	2031	101960
Wed	23735	93197	159757	4.45	5.54	41	236	2345	89341	10467	128	270894	69	39	2294	129801
Thu	28951	108352	141294	3.67	6.96	47	261	2366	82010	9226	123	250730	42	46	2160	118520
Fri	23313	87984	129094	2.22	4.54	27	132	928	67233	8515	90	207398	57	14	1888	96201
Sat	24612	92965	131867	4.23	3.3	37	232	1091	74868	8713	96	217698	48	75	1952	104045
Sun	23026	99939	165315	3.62	4.19	31	170	2562	82013	11221	108	254311	61	33	2217	115446

Biography

Gordana Vuković was born on 16 November 1988, in Cetinje, the Republic of Montenegro. She enrolled in the Faculty of Chemistry, University of Belgrade (study programme Environmental Chemistry) in the academic year 2007/2008. She graduated from the faculty on 19 September 2011, with GPA score of 9.26 and Bachelor Thesis graded with 10.00. She started Master academic studies at the Department of Applied Chemistry, Faculty of Chemistry, University of Belgrade in the academic year 2011/12. She finished her Master studies with GPA score of 10.00, and successfully defended Master Thesis "Aktivni biomonitoring elemenata u tragovima u vazduhu gradskih ulica kanjonskog tipa i tunelu korišcenjem mahovine *Sphagnum girgensohnii* Russow" (in Serbian) on 24 September 2012, with a score of 10. She enrolled Doctoracal academic studies at the same department in 2012/2013.

Since 2013, Gordana Vuković has been employed in the Environmental Physics Laboratory, Institute of Physics Belgrade in the project: "*Investigation of climate change and its influences on environment – monitoring the influences, adaptations, and offsets*", *No III43007, Ministry of Education and Science of the Republic of Serbia*. In 2013, Gordana was engaged in the project: "*Active biomonitoring of airborne major and trace elements in the Belgrade urban area using moss bag technique*", supported by the Secretariat for Environmental Protection of the City of Belgrade (project leader: Dr Mira Aničić Urošević). In the following year, she was involved in the project: "*Biomonitoring of heavy metals in the air along the main roads of the city of Belgrade*", supported by the Oil Company "NIS -Novi Sad" and the City of Belgrade (project leader: Dr Mira Aničić Urošević).

In 2012, Gordana was awarded with a special recognition for outstanding achievement during her Bachelor studies by Serbian Chemical Society.

Изјава о ауторству

Потписани-а Гордана Вуковић

број уписа 05/2012

Изјављујем

да је докторска дисертација под насловом

„Биомониторинг загађености ваздуха градске средине суспендованим честицама, елементима у траговима и полицикличним ароматичним угљоводоницима коришћењем маховина *Sphagnum girgensohnii* Russow и *Hypnum cupressiforme* Hedw.“

резултат сопственог истраживачког рада,

- да предложена дисертација у целини ни у деловима није била предложена за добијање било које дипломе према студијским програмима других високошколских установа,
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио интелектуалну својину других лица.

Потпис докторанда

У Београду, 31. јул, 2015. године

Гордана Вуковић

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора Гордана Вуковић

Број уписа 05/2012

Студијски програм доктор хемијских наука

Наслов рада „Биомониторинг загађености ваздуха градске средине суспендованим честицама, елементима у траговима и полицикличним ароматичним угљоводоницима коришћењем маховина *Sphagnum girgensohnii* Russow и *Hypnum cupressiforme* Hedw.“

Ментор др Александар Поповић и др Мира Аничић Урошевић

Потписана Гордана Вуковић

изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла за објављивање на порталу **Дигиталног репозиторијума Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског звања доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

Потпис докторанда

У Београду, 31. јул, 2015. године

Гордана Вуковић

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић" да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

„Биомониторинг загађености ваздуха градске средине суспендованим честицама, елементима у траговима и полицикличним ароматичним угљоводоницима коришћењем маховина: *Sphagnum girgensohnii* Russow и *Hypnum cupressiforme* Hedw."

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигитални репозиторијум Универзитета у Београду могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

1. Ауторство

2. Ауторство - некомерцијално

3. Ауторство – некомерцијално – без прераде

4. Ауторство – некомерцијално – делити под истим условима

5. Ауторство – без прераде

6. Ауторство – делити под истим условима

(Молимо да заокружите само једну од шест понуђених лиценци, кратак опис лиценци дат је на полеђини листа).

Потпис докторанда

У Београду, 31. јул, 2015. године

Гордана Вуковић

1. ɸ ɮlstsʦʨfɲʝɘʦ - ɼ ʦʟɘʦˀ ʘɚʘlsʝ ɮʤʣʥsʞ ʘɚʘˁ ʝ, ʜɕʃfɲʝsʨɕʃʙɮʮɕʃˁɮ ɕʃ ˁʘɚʣʥs fɲʘsʧʰ lsʘɚʘˁ ʝ ʜʝʣʘ, ɕʃ ʧʨʝ ʨʘʜʝ, ʘʢts fɲʝ ʣʘɚʝʜʝ ɕʃʤʝ ʘɮlstsʨʘ ʣʘ ʣʘʮɕʃʣʐ ʦʜʨʝ ʹ ʝʣʐ ʦʜ fɲʝsʨʘʣʝ ʘɮlstsʨʘ ɕʃʣɕʃ ʜʘɚʘsʮʘ ʣɕʃʮʝʣʝ, ʮʘʢ ɕʃ ɮ ʡtsʤʝʨʮɕʃˁʘʣʝ fɲɮʨ=ʝ. ʆ ɘʦ ˁʝ ʣʘfɲʣʦʙʦʜʣɕʃˁʘ ʦʜ fɲɕʃ=ʣɕʃʮʝʣʝɕʃ.

2. ɸ ɮlstsʦʨfɲʝɘʦ ï ʣʝ ʡtsʤʝʨʮɕʃˁʘʣʣs. ɼ ʦʟɘʦˀ ʘɚʘlsʝ ɮʤʣʥsʞ ʘɚʘˁ ʝ, ʜɕʃfɲʝsʨɕʃʙɮʮɕʃˁɮ ɕʃ ˁʘɚʣʥs fɲʘsʧʰ lsʘɚʘˁ ʝ ʜʝʣʘ, ɕʃ ʧʨʝ ʨʘʜʝ, ʘʢts fɲʝ ʣʘɚʝʜʝ ɕʃʤʝ ʘɮlstsʨʘ ʣʘ ʣʘʮɕʃʣʐ ʦʜʨʝ ʹ ʝʣʐ ʦʜ fɲʝsʨʘʣʝ ʘɮlstsʨʘ ɕʃʣɕʃ ʜʘɚʘsʮʘ ʣɕʃʮʝʣʝ. ʆ ɘʘ ʣɕʃʮʝʣʝʘ ʣʝ ʜʦʟɘʦˀ ʘɚʘ ʡtsʤʝʨʮɕʃˁʘʣʣɮ ɮʧtslsʨʝʙɮ ʜʝʣʘ

3. ɸ ɮlstsʦʨfɲʝɘʦ - ʣʝ ʡtsʤʝʨʮɕʃˁʘʣʣs ï ʙʝʟ ʧʨʝ ʨʘʜʝ. ɼ ʦʟɘʦˀ ʘɚʘlsʝ ɮʤʣʥsʞ ʘɚʘˁ ʝ, ʜɕʃfɲʝsʨɕʃʙɮʮɕʃˁɮ ɕʃ ˁʘɚʣʥs fɲʘsʧʰ lsʘɚʘˁ ʝ ʜʝʣʘ, ʙʝʟ ʧʨʦʤʝʣʘ, ʧʨʝʦʙʣɕʃʡtsɘʘˁ ʘ ɕʃʣɕʃ ɮʧtslsʨʝʙʝ ʜʝʣʘ ɮ fɲɘtsʒ ʜʝʣɮ, ʘʢts fɲʝ ʣʘɚʝʜʝ ɕʃʤʝ ʘɮlstsʨʘ ʣʘ ʣʘʮɕʃʣʐ ʦʜʨʝ ʹ ʝʣʐ ʦʜ fɲʝsʨʘʣʝ ʘɮlstsʨʘ ɕʃʣɕʃ ʜʘɚʘsʮʘ ʣɕʃʮʝʣʝ. ʆ ɘʘ ʣɕʃʮʝʣʝʘ ʣʝ ʜʦʟɘʦˀ ʘɚʘ ʡtsʤʝʨʮɕʃˁʘʣʣɮ ɮʧtslsʨʝʙɮ ʜʝʣʘ ʋ ʦʜʣʦfɲɮ ʣʘ fɲɚʝ ʦfɲʝʘʣʝ ʣɕʃʮʝʣʝ, ʦɘʦʒ ʣɕʃʮʝʣʝʦʒ fɲʝ ʦɢʨʘʣɕʃʮʘɚʘʣʘɘʝ <ɕʃ ʦʙɕʃʒ ʧʨʘɚʘʡtsʨɕʃʰ <ʝˁ ʘʜʝʣʘ

4. ɸ ɮlstsʦʨfɲʝɘʦ - ʣʝ ʡtsʤʝʨʮɕʃˁʘʣʣs ï ʜʝʣɕʃlsɕʃ ʧʦʜ ɕʃfɲʝsɕʃʒ ɮfɲʣʦɘɕʃʒʘ ɼ ʦʟɘʦˀ ʘɚʘlsʝ ɮʤʣʥsʞ ʘɚʘˁ ʝ, ʜɕʃfɲʝsʨɕʃʙɮʮɕʃˁɮ ɕʃ ˁʘɚʣʥs fɲʘsʧʰ lsʘɚʘˁ ʝ ʜʝʣʘ, ɕʃ ʧʨʝ ʨʘʜʝ, ʘʢts fɲʝ ʣʘɚʝʜʝ ɕʃʤʝ ʘɮlstsʨʘ ʣʘ ʣʘʮɕʃʣʐ ʦʜʨʝ ʹ ʝʣʐ ʦʜ fɲʝsʨʘʣʝ ʘɮlstsʨʘ ɕʃʣɕʃ ʜʘɚʘsʮʘ ʣɕʃʮʝʣʝ ɕʃ ʘʢts fɲʝ ʧʨʝ ʨʘʜʘ ʜɕʃfɲʝsʨɕʃʙɮɕʃʨʘ ʧʦʜ ɕʃfɲʝsʒ ɕʃʣɕʃ fɲʣɕʃʮʣʦʒ ʣɕʃʮʝʣʝʦʒ ʆ ɘʘ ʣɕʃʮʝʣʝʘ ʣʝ ʜʦʟɘʦˀ ʘɚʘ ʡtsʤʝʨʮɕʃˁʘʣʣɮ ɮʧtslsʨʝʙɮ ʜʝʣʘɕʃ ʧʨʝ ʨʘʜʘ

5. ɸ ɮlstsʦʨfɲʝɘʦ ï ʙʝʟ ʧʨʝ ʨʘʜʝ. ɼ ʦʟɘʦˀ ʘɚʘlsʝ ɮʤʣʥsʞ ʘɚʘˁ ʝ, ʜɕʃfɲʝsʨɕʃʙɮʮɕʃˁɮ ɕʃ ˁʘɚʣʥs fɲʘsʧʰ lsʘɚʘˁ ʝ ʜʝʣʘ, ʙʝʟ ʧʨʦʤʝʣʘ, ʧʨʝʦʙʣɕʃʡtsɘʘˁ ʘ ɕʃʣɕʃ ɮʧtslsʨʝʙʝ ʜʝʣʘ ɮ fɲɘtsʒ ʜʝʣɮ, ʘʢts fɲʝ ʣʘɚʝʜʝ ɕʃʤʝ ʘɮlstsʨʘ ʣʘ ʣʘʮɕʃʣʐ ʦʜʨʝ ʹ ʝʣʐ ʦʜ fɲʝsʨʘʣʝ ʘɮlstsʨʘ ɕʃʣɕʃ ʜʘɚʘsʮʘ ʣɕʃʮʝʣʝ. ʆ ɘʘ ʣɕʃʮʝʣʝʘʜʦʟɘʦˀ ʘɚʘ ʡtsʤʝʨʮɕʃˁʘʣʣɮ ɮʧtslsʨʝʙɮ ʜʝʣʘ

6. ɸ ɮlstsʦʨfɲʝɘʦ - ʜʝʣɕʃlsɕʃ ʧʦʜ ɕʃfɲʝsɕʃʒ ɮfɲʣʦɘɕʃʒʘ ɼ ʦʟɘʦˀ ʘɚʘlsʝ ɮʤʣʥsʞ ʘɚʘˁ ʝ, ʜɕʃfɲʝsʨɕʃʙɮʮɕʃˁɮ ɕʃ ˁʘɚʣʥs fɲʘsʧʰ lsʘɚʘˁ ʝ ʜʝʣʘ, ɕʃ ʧʨʝ ʨʘʜʝ, ʘʢts fɲʝ ʣʘɚʝʜʝ ɕʃʤʝ ʘɮlstsʨʘ ʣʘ ʣʘʮɕʃʣʐ ʦʜʨʝ ʹ ʝʣʐ ʦʜ fɲʝsʨʘʣʝ ʘɮlstsʨʘ ɕʃʣɕʃ ʜʘɚʘsʮʘ ʣɕʃʮʝʣʝ ɕʃ ʘʢts fɲʝ ʧʨʝ ʨʘʜʘ ʜɕʃfɲʝsʨɕʃʙɮɕʃʨʘ ʧʦʜ ɕʃfɲʝsʒ ɕʃʣɕʃ fɲʣɕʃʮʣʦʒ ʣɕʃʮʝʣʝʦʒ ʆ ɘʘ ʣɕʃʮʝʣʝʘ ʜʦʟɘʦˀ ʘɚʘ ʡtsʤʝʨʮɕʃˁʘʣʣɮ ɮʧtslsʨʝʙɮ ʜʝʣʘ ɕʃ ʧʨʝ ʨʘʜʘ ʉ ʣɕʃʮʣʘ ˁʝ fɲʝsʷ lsɘʝ ʨfɲʡɕʃʒ ʣɕʃʮʝʣʝʘʒʘ, ʦʜʣʦfɲʣʦ ʣɕʃʮʝʣʝʘʒʘʦlsɘʦʨʝʣʦɢ ʡʦʜʘ