Macedonian Journal of Ecology and Environment
Vol. 25, issue 2
pp. 135-151
Skopje (2023)
ISSN 1857 - 8330 (on-line)
ISSN 0354-2491 (print)
Original scientific paper
Available online at www.mjee.org.mk
DOI: https://doi.org/10.59194/MJEE23252135b

Tracking soil particle deposition using bio-indication evidence and nondestructive FESEM and EDS analyses: A preliminary (pilot) study

Biljana Balabanova*[1], Ivan Boev[2], Maja Lazarova[2], Lucian Barbu-Tudoran[3], Maria Suciu[4]

[1]*Faculty of Agriculture, Goce Delcev University, Krste Misirkov No. 10-A, 2000 Štip, Republic of North Macedonia*

[2]*Faculty of Natural and Technical Sciences, Goce Delcev University, Krste Misirkov No. 10-A, 2000 Štip, Republic of North Macedonia*

[3]*National Institute for R&D of Isotopic and Molecular Technology, 67-103 Donat St., 400293 Cluj-Napoca, Romania*

[4]*Biology and Geology Faculty, Babes-Bolyai University, 5-7 Clinicilor Str., Cluj-Napoca, Romania*

Abstract

The present study present chemometric bioindication methodology for fast and effective monitoring in long distant distribution of top soil pollution and evidence for element soil dust deposition. Both varieties of Briophytes, *Hypnum cupressiforme* (Hedw.) and *Homalothecium lutescens* (Hedw.) has been used for improving the effective nondestructive bio-indication. Although mosses do not have a root system, influence from soil dusting cannot be disregarded, in praticular in areas with windblown mineral dust from local soil. As far as the surface bound fraction is concerned, little is known about the binding mechanisms, but the fact that different metals show rather large differences in their retention capacities, indicates that both simple cation exchange on negative surface charges and complex formation with ligands on the moss surface are involved. Laboratory analysis, using field emission scanning electron microscopy (FESEM) and energy dispersive X-ray spectrometer (EDS) has been involved for determination of the dry deposition occurred within moss biomonitors. The bioindication chemometric model was improved for nondestuctive evidence effectively for biogenic elements carbon and oxygen, macroelements Mg, Al, Si, K, Ca, and microelements Fe, Cu and Zn. Both moss species can be used interchangeable for dust deposition investigation.

Key words: Soil particle deposition, bioindication, moss, FESEM, X-ray, North Macedonia

Introduction

In the last decade there has been a research expansion of methodologies that establish fast and effective methodological approaches for monitoring soil surface dust deposits. Furthermore, heavy metals pollution has been alarming in past decades globally. The distribution of microparticles originating from different emission sources poses a huge threat to the health of the human population (Finlayson-Pitts & Pitts 1999). The number of researches done so far is huge, but the most common problem is to find a fast, effective and transparent research model. The distribution of microparticles originating from different emission sources poses a huge threat to the health of the human population. The number of researches done so far is huge, but the most common problem is to find a fast, effective and transparent research model.

Heavy metals in the environment come from natural and anthropogenic sources (Beelen et al. 1997; Neff et al. 2008). Natural sources of these elements are volcanic eruptions, dust, salts and fires. Various

Submitted: 27.09.2023
Accepted: 18.11.2023

* Author for correspondence: biljana.balabanova@ugd.edu.mk

human activities such as mining, industrial production (smelting, oil refineries, petrochemical plants, and the chemical industry), untreated sewage sludge, coal combustion, and traffic produce far more heavy metals than come from natural sources (Fordyce et al. 2005; Goodarzi 2006). Anthropogenic and non-anthropogenic activities have increased throughout the world (Ajmone-Marsan et al. 2008; Berg & Steinnes 1997). The consequence is air pollution. Environmental pollution with heavy metals has created a lot of problems which have affected living and non-living things. To avoid or reduce these problems, constant monitoring of the environment should be ensured (Leblanc & Rao 1974; Martin & Coughtrey 1982; Gjengedal & Steinnes 1990; Mackay 2001).

Long-term lithogenic degradation effectively disrupts a number of natural conditions in the environment (White 2013). The presence of heavy metals in the soil is a consequence of natural and anthropogenic processes. There are natural pedogenetic processes by which the soil inherits heavy metals from the parent substrate, and anthropogenic processes include urbanization, industrialization, trade and agricultural production (He et al. 2005; Basile et al. 2012). In remote areas with low anthropogenic impact on atmospheric deposition, heavy metals in soils are mostly derived from the parent substrate, while in urban and agricultural areas the concentrations of heavy metals in soils are higher than concentrations in parent substrates due to continuous input into the ecosystem. The geogenic origin of the most ecologically interesting heavy metals, Cu, Zn, Cd and Pb, is most often associated with sulfur minerals, which oxidize relatively quickly in the environment, and the metal cation separates from sulfur at an early stage of mineral depletion (He et al. 2005). In the later stages of pedogenesis, Cu, Zn and Cd are more often in the composition of Mn oxide, and Pb in the composition of Fe oxide and hydroxide. Natural geological processes on different parent rocks can result in multiple concentrations of heavy metals compared to the soil average, affecting flora and fauna (Järup 2003; Bourennane et al. 2010; Boquete et al. 2014). Natural sources of heavy metals in the soil are, in addition to parent rocks, volcanic eruptions, marine aerosols, and forest fires (Ceburnis & Valiulis 1999; Fernandez et al. 2012; Keller et al. 2015).

Sources of heavy metals can be of anthropogenic origin and contribute to their spread to rural areas and agricultural land (metal processing industry, electroplating industry, ore smelters, as well as burning of fossil fuels, landfills of urban and industrial waste, sewage sludge). Toxic metals can contaminate agricultural land in the process of fertilization with sewage sludge (Cd, Ni, Cu, Pb, Zn), as well as the use of phosphate and organic fertilizers (Cd, Cr, Mo, Pb, U, V, Zn, etc.), and pesticides (Cu, As, Pb) which is why the application of these agents must be under strict control, because heavy metals are found in them as impurities (De Miguel et al. 1999; He et al. 2005; Bourennane et al. 2010). A significant part of pesticides, fungicides and herbicides also contain Cu, Zn, Fe, Mn, and even As, and some heavy metals such as Cd and Pb are introduced into the soil as impurities present in fertilizers (Bourennane et al. 2010). Thus, the agricultural land with longlasting pesticide treatment are considered as potentially affected with toxic elements as well. The soil dust deposition and distribution within the potential polluted agricultural sites should be considered in monitoring programs as well.

Lately, numerous available data reveal many methodologies for monitoring of the lithogenic and anthropogenic distribution of toxic elements in different ecosystems. The conventional techniques is costly because it requires a lot of money and time consuming. Biomonitoring has been the alternative method (Markert et al. 2003). Moss, lichens and plants are biomonitors available to entrap air pollutants. However, different species of mosses have been used for biomonitoring in a number of different ways which may lead to rather different results, and some kind of classification seems necessary at this point. *Epigeic* mosses (growing on the ground) are preferred in the regional surveys in Europe (Onianwa 2001; Fernandez et al. 2007; Harmens et al. 2010). Whereas uptake efficiencies for particulate-bound trace elements are generally poorly known, ions may be subject to active uptake into cells or attached on the moss surface by physical and chemical forces. Methods are available to distinguish between intracellular and surface-bound fractions of elements. Main problem with issue moss-biomonitoring are reveal as: a) transport of soluble compounds from the soil into moss tissue, particularly during periods with excessive soil/water contact (Onianwa 2001). Although mosses have a non developed root system, influence from this source cannot be disregarded, in praticular in areas with low atmospheric deposition and b) windblown mineral dust from local soil (Aboal et al. 2010).

As far as the surface bound fraction is concerned, little is known about *the **binding mechanisms***, but the fact that different metals show rather large differences in their ***retention capacities***, indicates that both simple ***cation exchange*** on negative surface charges and ***complex formation with ligands*** on the moss surface are involved (Nagajyoti et al. 2010). Furthermore, the last two decades in developed countries mosses has been increasingly tested as bio-filters for airborne toxins, with emphasis for the xenobiotic's molecules and heavy metals.

Moss bio-indication

Bryophytes are non-vascular plants (without conducting vessels) that include moss, liver, and antiseroses. At the organizational level, bryophytes

are found among green algae (of which their offspring are very likely) and among simpler lower vascular plants such as lycopods. Unlike higher plants, the gametophyte (sexual form) is the dominant generation. The sporophyte (asexual form) develops above the gametophyte and remains almost entirely dependent on it. Bryophytes do not have true conductive tissues because they are found in ferns and taller plants (Mägdefrau 1982). Some species of bryophytes are aquatic and others are able to survive in warm and dry regions. Although its size varies from microscopic to 30 cm, the average bryophyte measures approximately 1.2 to 5 cm, with color ranging from green to black to almost colorless. Hepatic are the most primitive bryophytes and have a flat shape, sometimes their thickness is only one cell (Mägdefrau 1982; Salemaa et al. 2004).

Bryophytes accumulate heavy metals by several mechanisms, but the initial and frequently limiting step is reversible adsorption on the cell surface (González & Pokrovsky 2014). Adsorbed metals can be trapped as particulate matter within the surface layer, dissolved in liquids or deposits surrounding cells (intercellular fraction), bound in exchangeable form to exchange or chelating sites on the cell wall and outer surface of the plasma membrane (extracellular fraction) or transported inside the cells and held in soluble or insoluble form (intracellular fraction) (González & Pokrovsky 2014). The extracellular accumulation of heavy metals is mediated by the ion exchange process and the formation of complexes between the metals and the organic functional groups in the cell walls of bryophytes (Shakya et al. 2008). The great binding capacities of mosses for some heavy metals are often attributed to the functional groups of polygalacturonic acid and related polymers in the cell walls (Shakya et al. 2008). Experiments exploring the acid-base properties of the mosses resulted in the detection of several possible functional groups involved in the binding of heavy metals. These include phosphodiester, carboxyl, phosphoryl and amine groups, as well as polyphenols. Considering the organic composition of the cell walls of mosses, carboxyl and phosphoryl groups could be regarded the dominant metal-binding groups forming the complexes with heavy metals at the surface of moss cells. Other groups, such as sulfhydryl and amine, could be determinants in the presence of small amounts of heavy metals or under extreme pH conditions (González & Pokrovsky 2014).

The focus of this research is on the uses of the two moss species *Hypnum cupressiforme* (Hedw.) and *Homalothecium lutescens* (Hedw.) for monitoring atmospheric heavy metal deposition in lead-zinc mine environ. Sharing the same common name "fernmoss" with other monitoring mosses, this species similarly has extensive branching, allowing for a large exposed surface area for ion exchange. These features make *H. cupressiforme* and *H. lutescens* a likely candidate for use as a biomonitor (Wolterbeek 2002; Stankovic et al. 2018). The primary objective of this study was to evaluate the suitability of two moss species as a biomonitor of heavy metals on a regional landscape scale in the highly polluted area. To accomplish this objective, we tested the availability of *H. cupressiforme* and *H. lutescens* and possibility of applying two types of moss interchangeably to cover denser sampling network. Mosses as pollution biomonitors give only an overview of the areas where we found the presence of high content of toxic metals in atmospheric dust, but not a real measurement of the content in the ambient air. Because of that, the expressiveness of moss species to the metals content was monitored also.

Certain surveys, especially in larger areas, require quick and easy screening of a given area. Analytical techniques are absolutely dominant when it comes to quantification of chemical elements. But usually before setting up the monitoring experiment, it is necessary to scan a larger area in order to obtain semi-quantitative information about the condition of the selected area. Rapid screening will aim to provide information on where the most effective sub-areas are, where careful sampling network will need to be carefully generated later. Therefore, the aim of this research is to propose a fast hemometric model for scanning contaminated areas with heavy metals. This model is particularly applicable to large areas or areas where certain incidents have occurred, for which the effect of pollution has yet to be determined. To better understand the factors leading to metal binding in complex moss matrix and trace metal fate, electron microscopic characterization of samples will be conducted. Scanning electron microscopy can have revealed the presence of trace metal specific incorporation in organic matrix in close association with biological structures with morphologies consistent. Furthermore, another approach will be introduced for semi-quantitative screening of moss surface. Preliminary investigation has been implemented for improving this technique for monitoring of soil surface dust physical transport.

Material and methods

Moss sampling

Mosses are suitable biomonitor for the detection of long-term pollution with heavy metals because it accumulates elements and retains them for many years after pollution stopped (Rühling & Tyler 1968, 1970; Harmens et al. 2010; Barandovski et al. 2015; Balabanova et al. 2010, 2016; Bačeva et al. 2012; Barandovski et al. 2015; Urošević et al. 2023).

Moss samples of *Hypnum cupressiforme* (Hedw.) and *Homalothecium lutescens* (Hedw.) were collected in the critical mining areas in the Eastern part of the Republic of North Macedonia (copper mine and Pb-Zn mine). These samples were used in similar investigation

as given by Balabanova et al. (2010, 2012, 2014, 2017, 2019). Three sampling point were selected, two critically affected and one control site. Depending on the conditions and the accessibility of the locations, the species that is available and typical for the region was collected. The moss sampling protocol was performed according to set standard rules for collection of such samples. The whole procedure was done in this order: one sampling spot is formed by collecting five sub-spots in the area of 50x50 m^2. Every spot of sampling network must be in a distance of minimum 300 m from main roads, 100 m from local roads, and 200 m from villages. Moss samples were collected using polyethylene gloves, to prevent contamination of any further samples. The collected material was stored in paper bags. After it was cleaned from other plant species, soil individual plant samples are separated and air dried for several days. Dry samples were again placed in paper bags until analyses were performed.

FESEM analysis

Field Emission Scanning Electron Microscope (FESEM) is used for observation of specimen surfaces. When the specimen is irradiated with a fine electron beam (called an electron probe) secondary electrons are emitted from the specimen surface. Topography of the surface can be observed by two-dimensional scanning of the electron probe over the surface and acquisition of an image from the detected secondary electrons.

For this research, double-sided adhesive carbon tape is applied to the sample stub, to which a small amount of the sample is attached. The sample is made of gold, the coating is up to 20nm thick. The sample thus prepared is placed in the chamber of a scanning electron microscope and the necessary analyzes are performed by achieving a high vacuum. The analyzes were performed using SE (secondary detector) at 30kV voltage. FESEM analysis were conducted in National institute for R&D of isotopic and molecular technologies, Integrated electron microscopy laboratory, in Cluj, Romania. For the present investigation, scanning electron microscope model Hitachi (model SU8320), will be used in order of characterization of the morphology, structure, and metal binding mechanisms.

The energy dispersive X-ray spectrometer (EDS) is used to analize characteristic X-ray spectra by measuring the energies of the X-rays. When the X-ray emitted from the specimen enter the semiconductor detector, electron-hole pairs are generated whose quantities correspond to the X-ray energy. Measuring these quantities (electric current) enables to obtain the values of X-ray energy. The detector is cooled by liquid nitrogen, in order to reduce the electric noise. The advantage of the EDS is that the X-rays from a wide range of elements from B to U are analysed simultaneously.

Results and Discussion

Moss sample FESEM analysis

Moss surface micrographs, can give us very useful information of the soil particles composition, and moss tissue accumulation of potentially toxic elements. A total of 16 squares were monitored on each sample. Representative plots were extracted for the best visualization of the collected data.

Figures 1 to 5 represents the FESEM micrographs of the surface of *Hypnum cupressiforme* moss samples. The data given in Figures 1 and 2 represent the analysis of the control sample of the *Hypnum* species (from non-polluted area). Figure 1 presents single EDS spectrum view for elemental mapping, while Figure 2 represents the comparison of two spectrums of the surface, spectrum 3 (yellow) and spectrum 4 (red) lines and the differences of the elementary composition into the samples. Spectrum of gold is visible in every analysis but it's not counts, because the sample is coated with gold so the quantitative analysis of gold it's not showed into the figure (settings conducted before each analysis). Surface micrographs and surface elemental mapping for the *Hypnum* sample from polluted site are given on Figures 3 to 5. Figure 3 represents the single point EDS spectra with elemental mapping of the surface of *Hypnum* sample from polluted site, showing for Fe, Cu, Zn and Al elemental ranging from: <0,1%, <0.1%-0.2%, <0.1%-0.2%, and <0,1%, respectively. The visualized data given in Figure 5 within the comparison of the two EDS spectra (Spectrum 13-yellow and Spectrum 14-red), reveals a comparison of elemental enrichments in moss tissue versus soil particles trapped in moss tissue. Significant enrichment of Fe, Cu and Zn was detected in the soil particles of 3.2%, 0.7% and 0.6%, respectively.

The FESEM micrographs given on Figures 6 to 10 represented corresponding top-view FESEM images of the moss samples (the above images). Furthermore, the same figures (the below parts) represents the surface EDS spectra and elemental mapping of the *Homalotecium lutescens* moss sample. Figure 6 represent the data of the FESEM analysis of the *Homalotecium* specie form control site. Compared between the two moss species, the mineral composition is less expressed (contained) in the *Homalotecium* specie. Figures 7 to 10 present the FESEM micrographs and EDS spectra of the *Homalotecium lutescens* sample from the contaminated site. The visualized data given in Figure 7 within the comparison of the two EDS spectra (Spectrum 9-yellow and Spectrum 10-red), reveals a comparison of elemental enrichments in moss tissue versus soil particles trapped in moss tissue. Figures 8 to 10 represents the single point EDS spectra with elemental mapping of the surface of *Homalothecium lutescens* moss samples from polluted site, showing for Fe, Cu, Zn and Al elemental ranging from: <0.1%-0.2%; 0.1%-0.4%, 0.1%-0.3% and 0.1%-0.2% respectively.

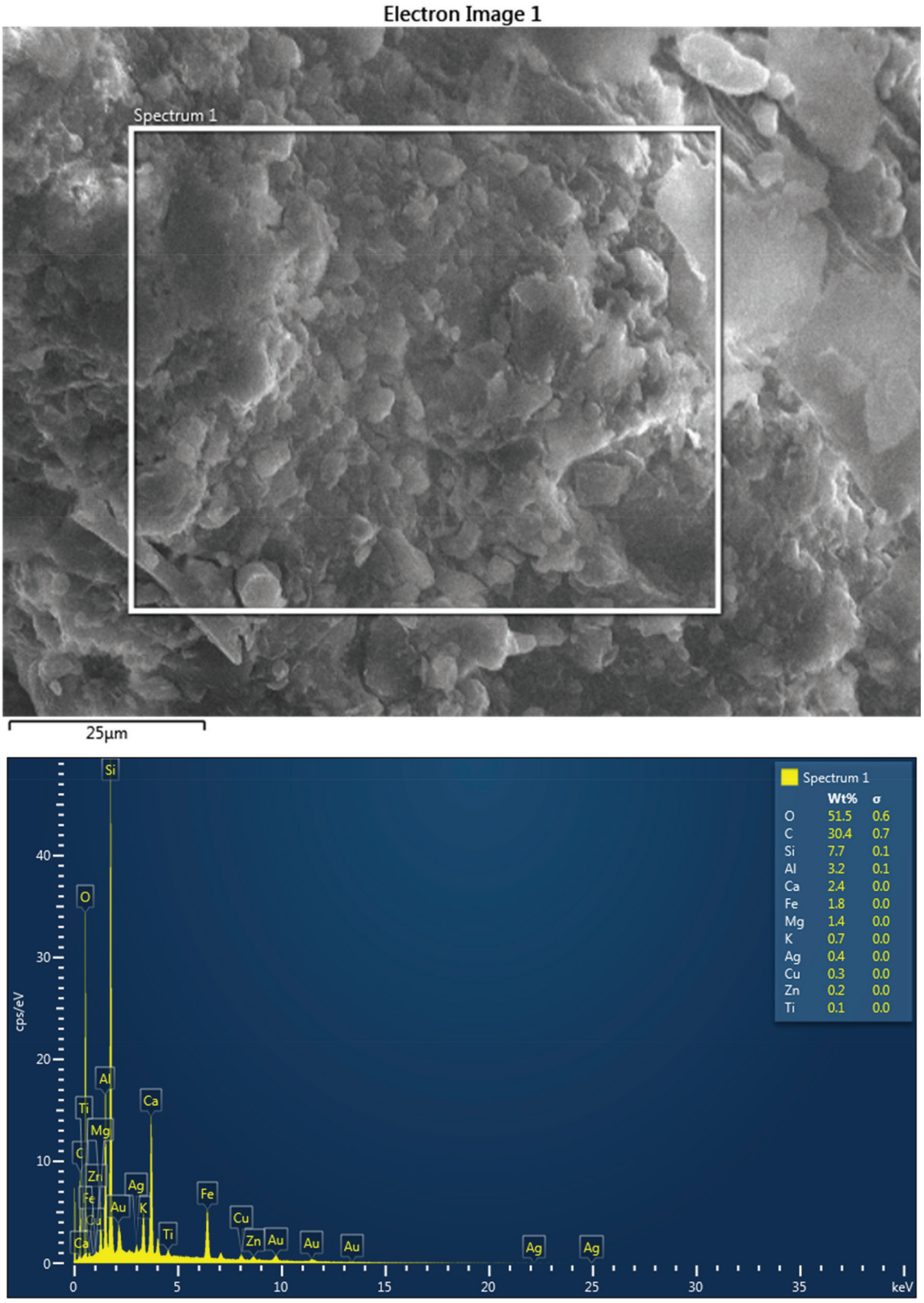

Figure 1. FESEM micrographs, (above) and EDS spectrum with elemental mapping of the surface of *Hypnum cupressiforme* - control sample (below)

Comparative analysis of two surface screening, spectrum 15 (yellow) and spectrum 16 (red), is presented on Figure 4, for *Hypnum cupresiforme* sample from polluted site. The samples were scanned and surface absorption was monitored. Advanced scans help to better identify the particles and how they are retained in the moss tissue. For each FESEM analysis, a chemical characterization was performed for the given scan. The

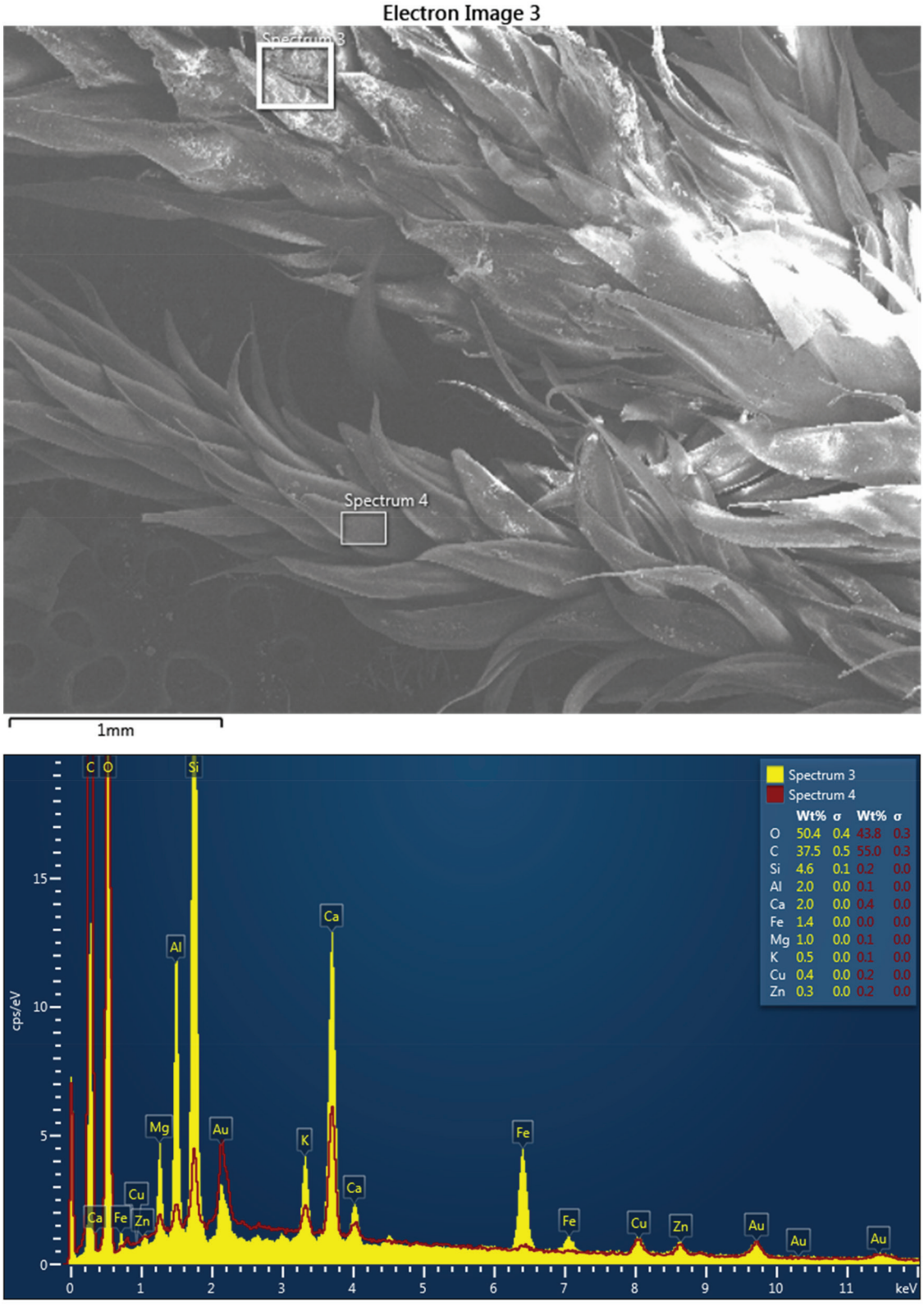

Figure 2. FESEM micrographs, (above) and comparison of two EDS spectra with elemental mapping of the surface of *Hypnum cupressiforme* - control sample (below)

analysis of the moss surface compared to the substance / (absorbed substance) on the moss surface is very important for this research. Such comparisons are made for the control sample (Figure 2), Spectrum 4 detects the tissue surface of the moss sample, while Spectrum 3 is the absorbed dust. The chemical characterization is dominant for the biogenic elements, while the deposited matter is dominated by iron, aluminium and silicon.

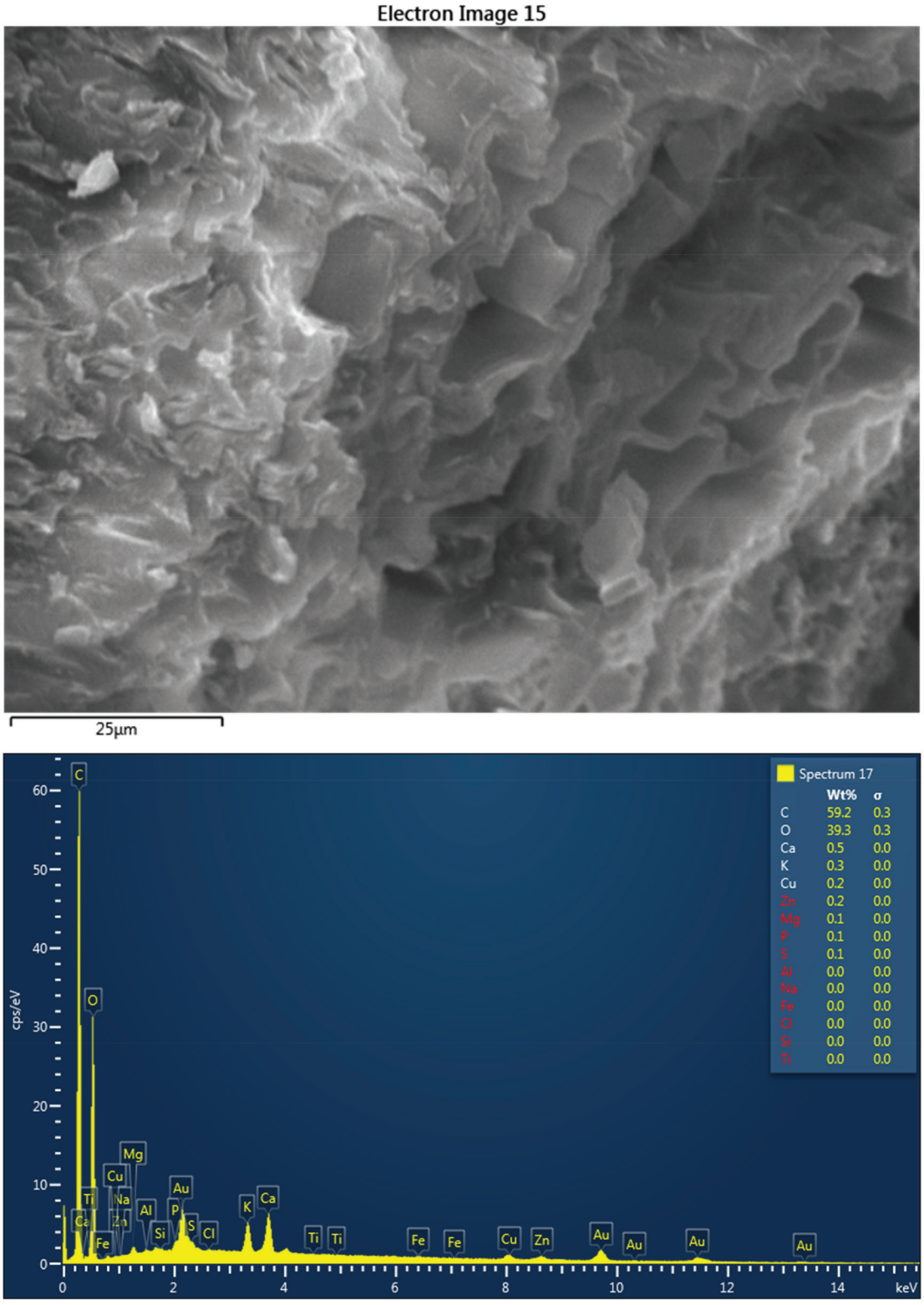

Figure 3. FESEM micrographs, (above) and EDS spectrum with elemental mapping of the surface of *Hypnum cupressiforme* from polluted site (below)

The other two specimens are from a potentially contaminated area, but are different species. Therefore, the analysis was aimed at comparing the two types as well as the possibility of expression of anthropogenic elements in the deposited dust. The dual surface analysis of *Homalothecium* moss is presented in Figure 7. The scan shows two spectra, one from the surface of the tissue (spectrum 10) and a part where the presence of a

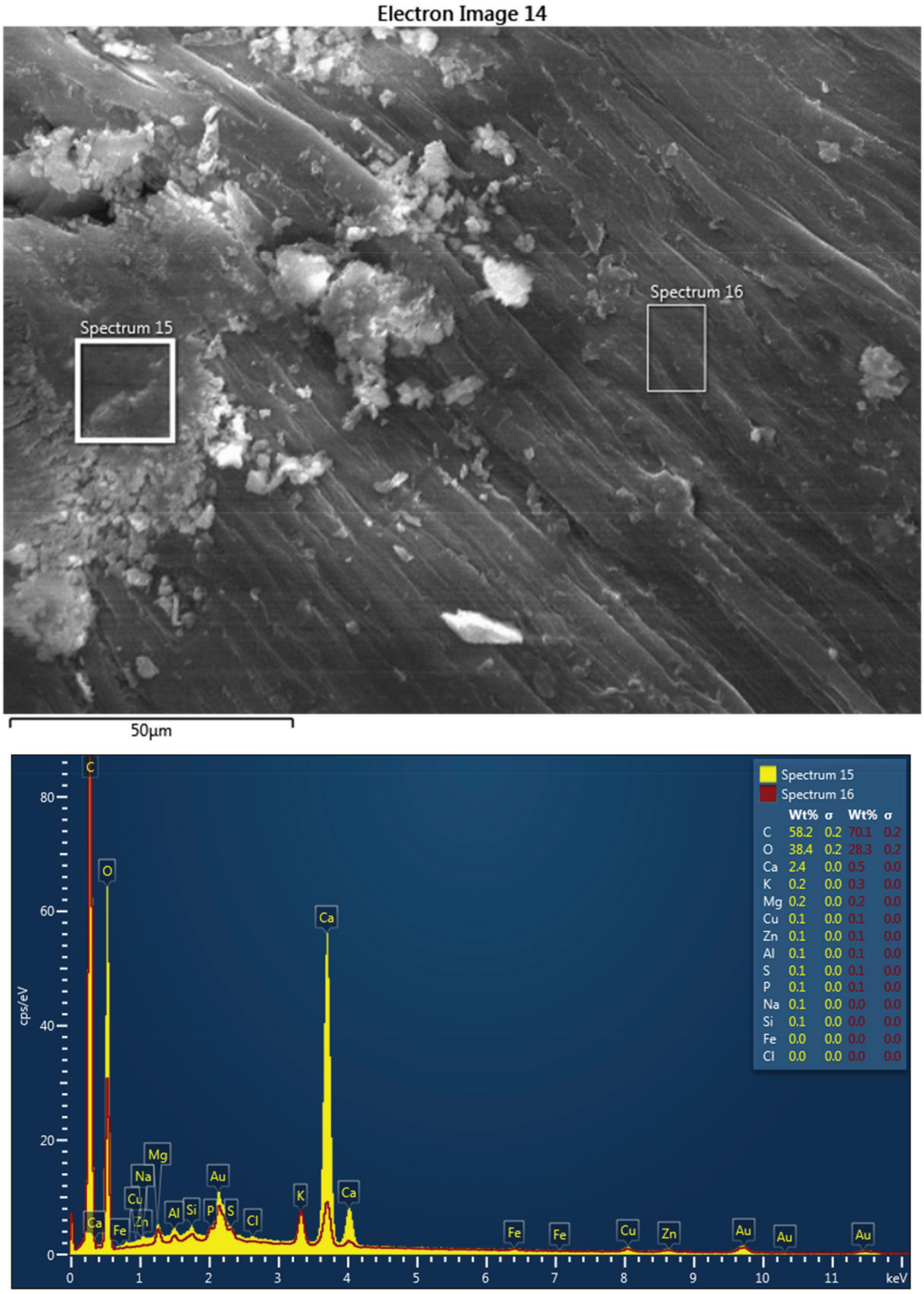

Figure 4. FESEM micrographs, (above) and comparison of two EDS spectra and elemental mapping of the surface of *Hypnum cupressiforme* from polluted site (below)

deposit has been determined (spectrum 9). Biogenic elements dominate the chemical characterization (C, O, and Si), while dust shows a charge for several anthropogenic elements. Analysis of *Hypnum cupressiforme* showed more significant expression in the identification of the elements contained in the deposited dust. Two identifications were made, presented in Figure 4, where only a difference in biogenic elements is observed

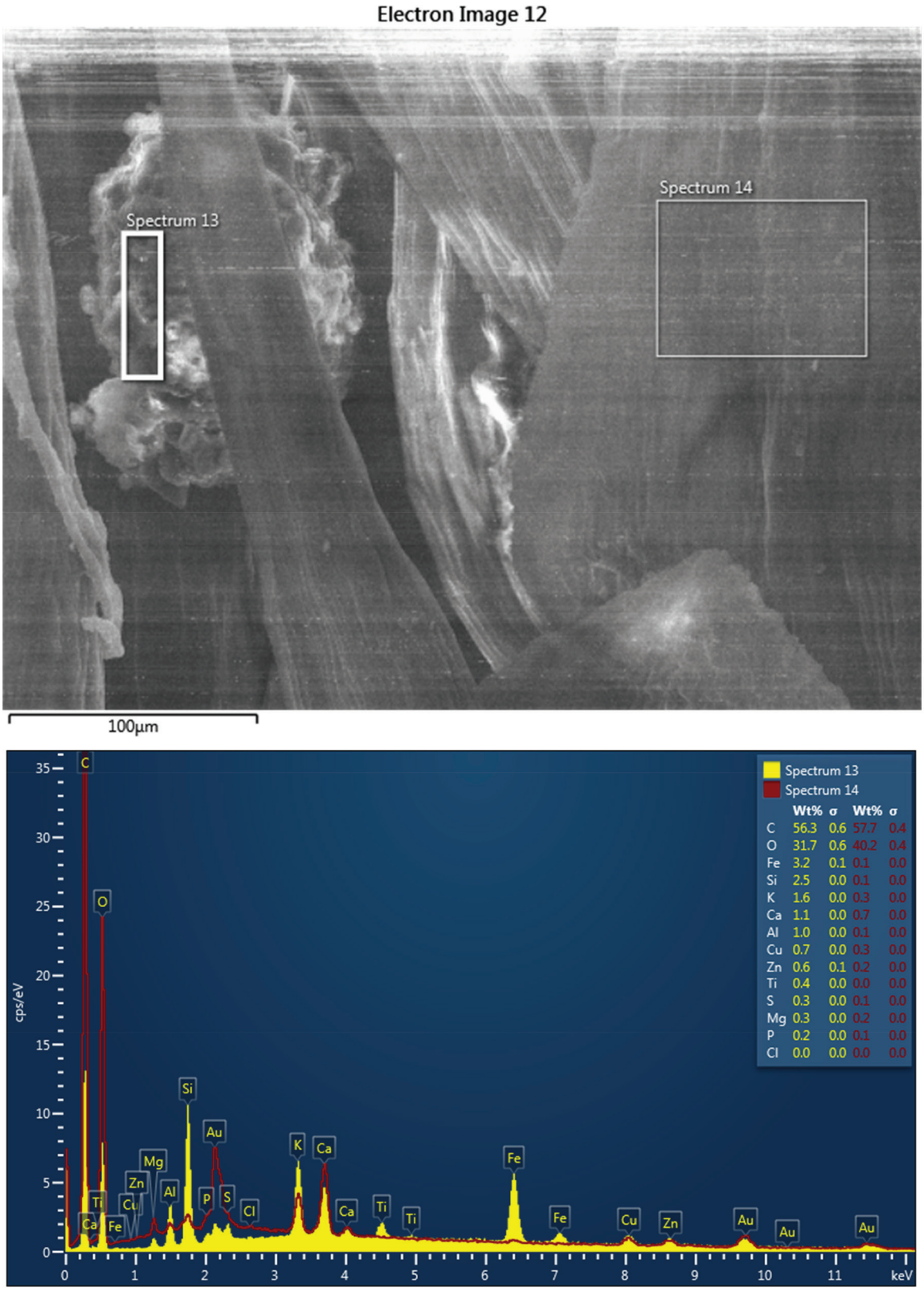

Figure 5. FESEM micrographs, (above) and comparison of two EDS spectra with elemental mapping of the surface of *Hypnum cupressiforme* from polluted site

between the moss tissue (spectrum 16) and the deposited matter that is not enriched with anthropogenic elements (spectrum 15). The analysis of both spectra 13 and 14 (Figure 5) identified a deposited particle with a significant difference in the content of Cu, Fe and Zn, which is characteristic of the mine environment for the exploitation of copper minerals.

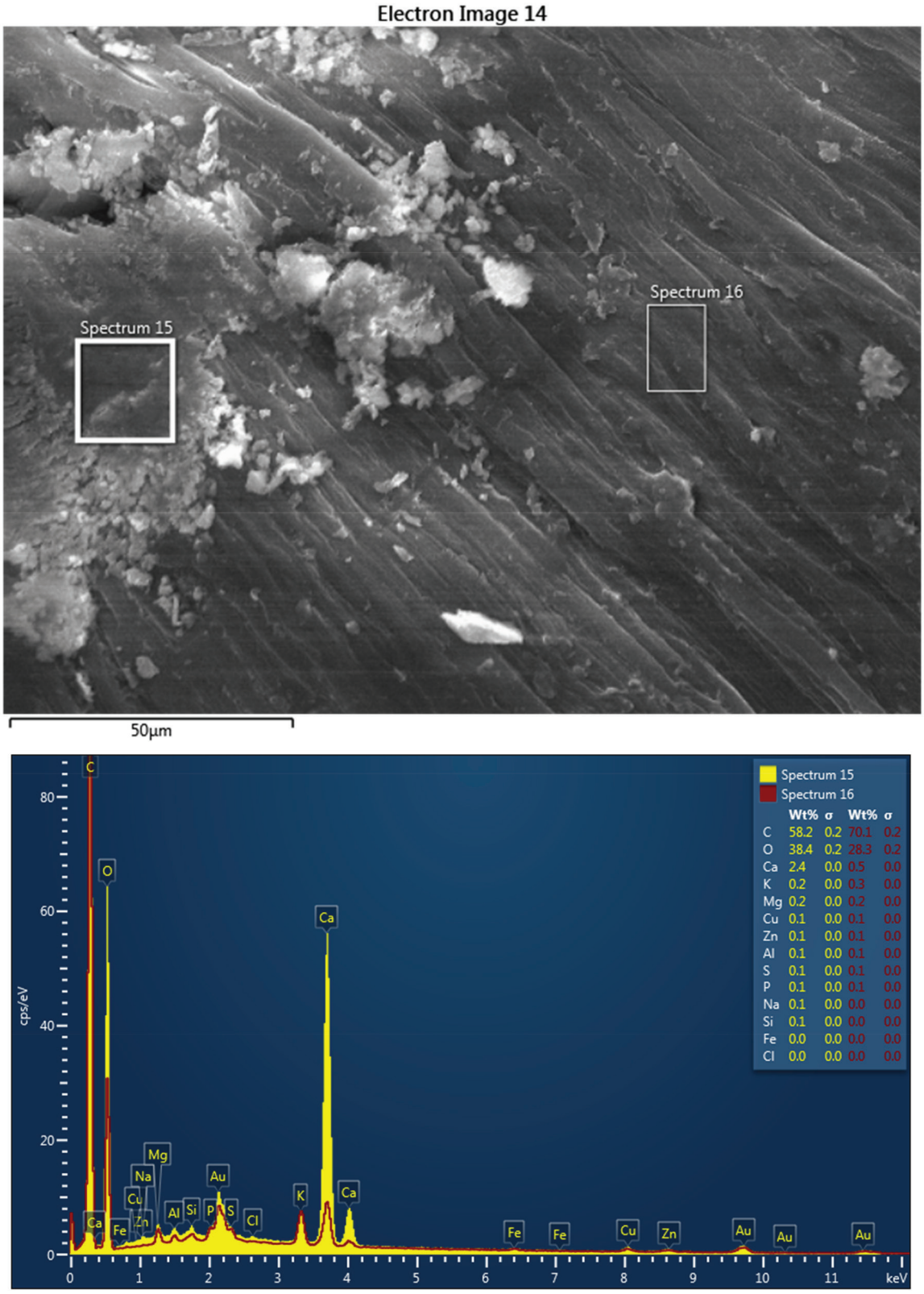

Figure 6. FESEM micrographs, (above) and EDS spectrum with elemental mapping of the surface of *Homalothecium lutescens* - control site (below)

X-ray mapping

X-ray mapping was used to obtain the distributions of specific elements. Thus, the electron probe is scanned over a specified area and characteristic X-ray with specific energies are acquired. It should be noted that if the P-B ratio is extremely low (the peak intensity is very small compared to the background); X-ray maps show the

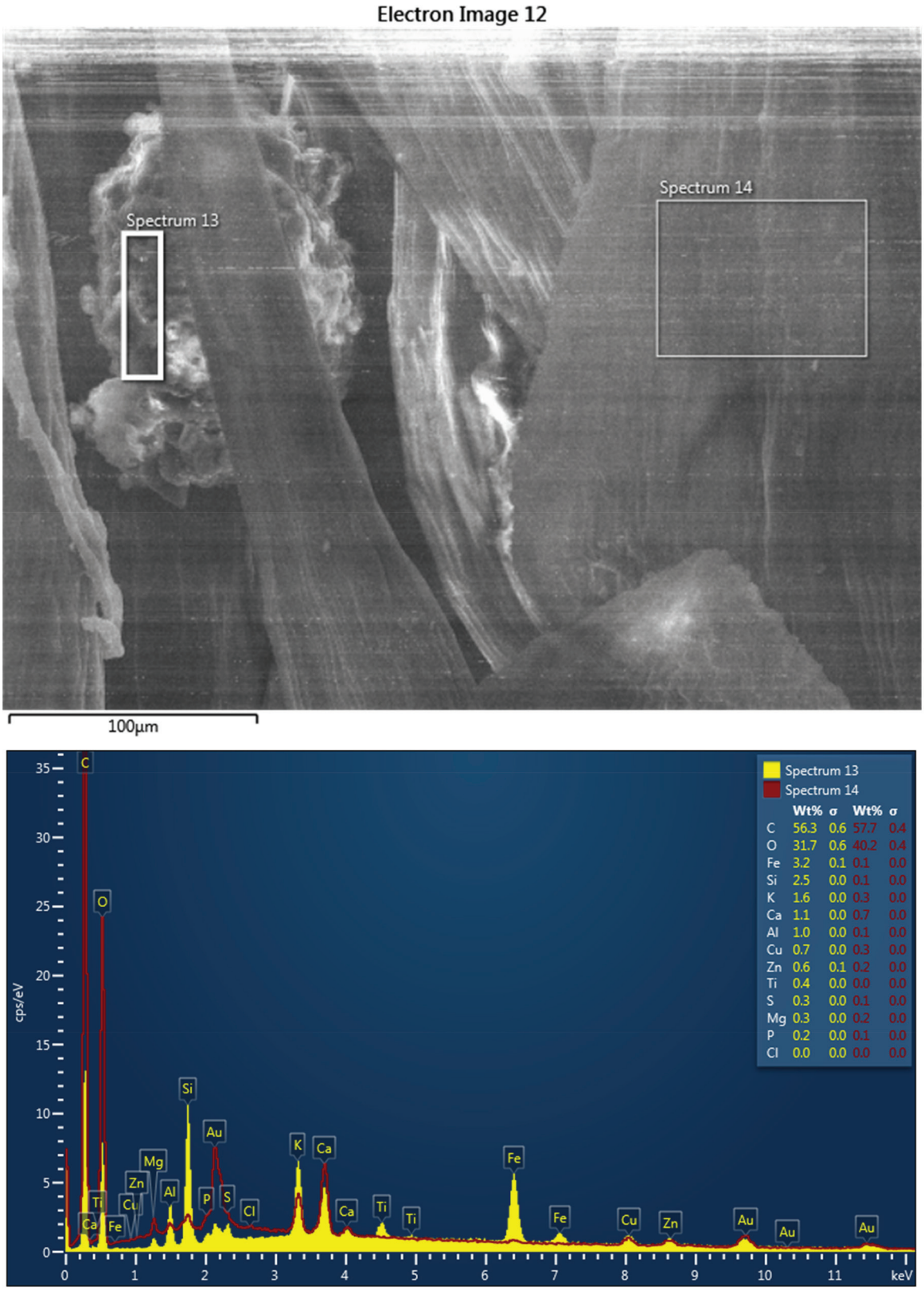

Figure 7. FESEM micrographs, (above) and comparison of two EDS spectra with elemental mapping of the surface of *Homalothecium lutescens* from polluted site (below)

distribution of continuous X-rays (not the distributions of elements of interest). Energies of characteristic X-ray elements not of interest are very close to those of the elements of interest, X-ray maps might show the distributions of the elements that are not of interest. This occurs when the energy difference between the elements not of interest and elements of interest is equal to the energy resolution of the spectrometer. Figures 11

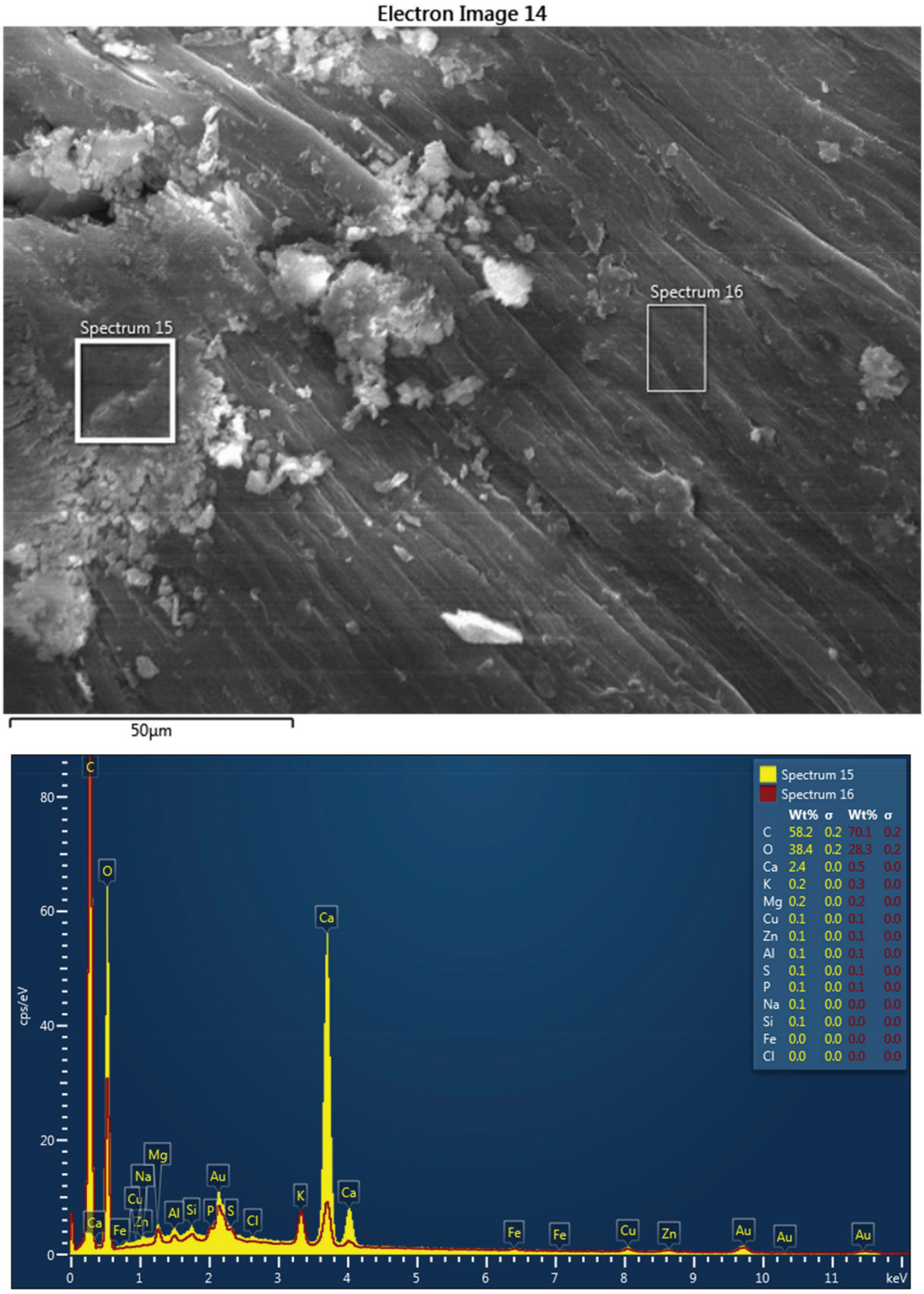

Figure 8. FESEM micrographs, (above) and EDS spectrum with elemental mapping of the surface of *Homalothecium lutescens* from polluted site (below)

and 12 present X-ray mapping of the both moss species. The resolution of X-ray mapping is limited by "analysis area". But even when a particle smaller than the analysis area is present on the specimen surface, sometimes this particle can be recognized. On the other hand, a method that quantitatively analyses on the specimen one by one while scanning the electron probe is called 'quantitative mapping". Using this method, even if the

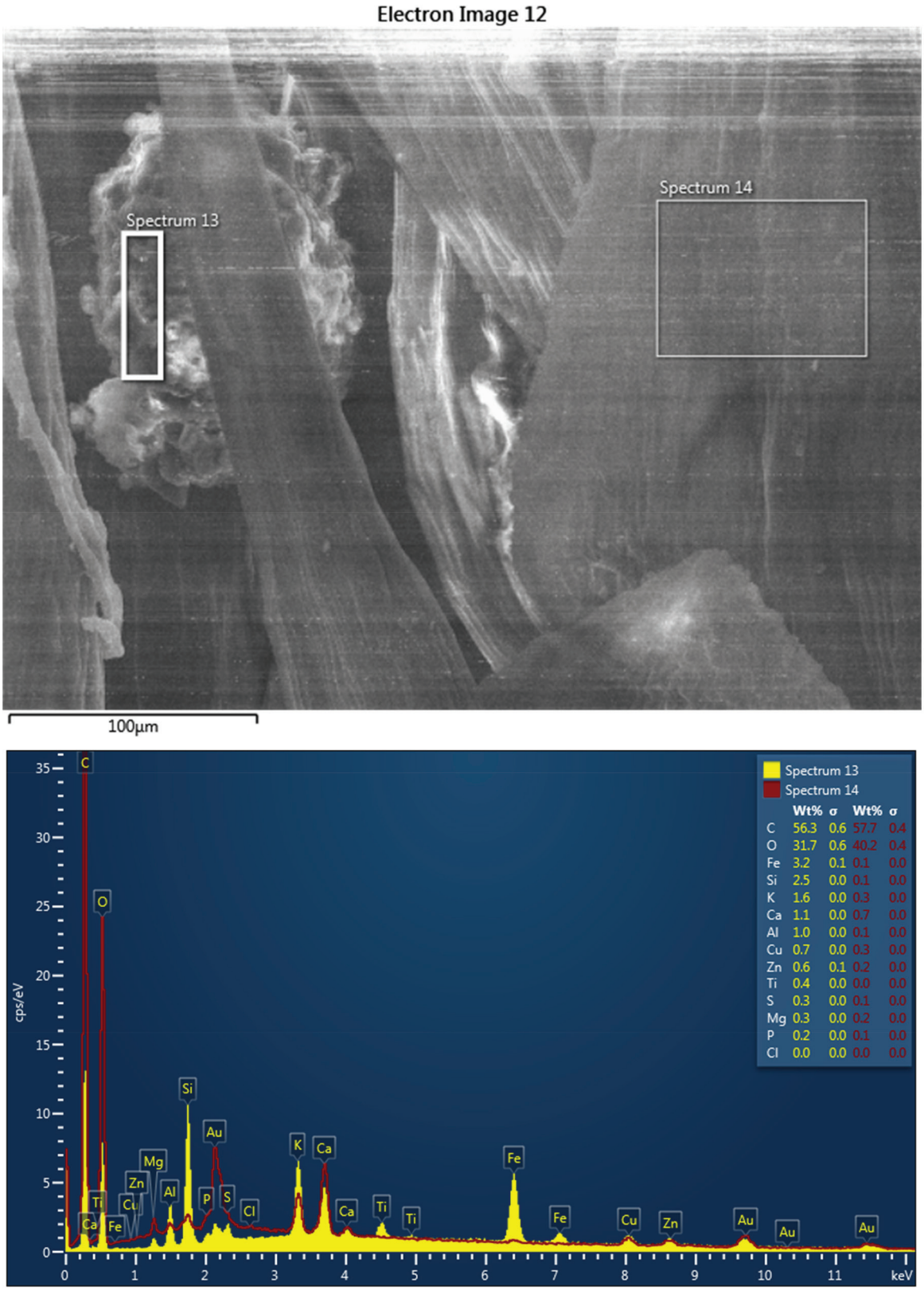

Figure 9. FESEM micrographs, (above) and EDS spectrum with elemental mapping of the surface of *Homalothecium lutescens* from polluted site (below)

P-B ratio is low, accurate distribution of elements can be obtained. This is an advantage of quantitative mapping, which cannot be achieved using simple X-ray mapping (qualitative mapping). Figures 11 and 12 presents the mapping, which is used to coloured image depend of the elementary composition. We choose different colors manual for each detected element. Duration time for this kind of analysis is minimum 15 min, longer period

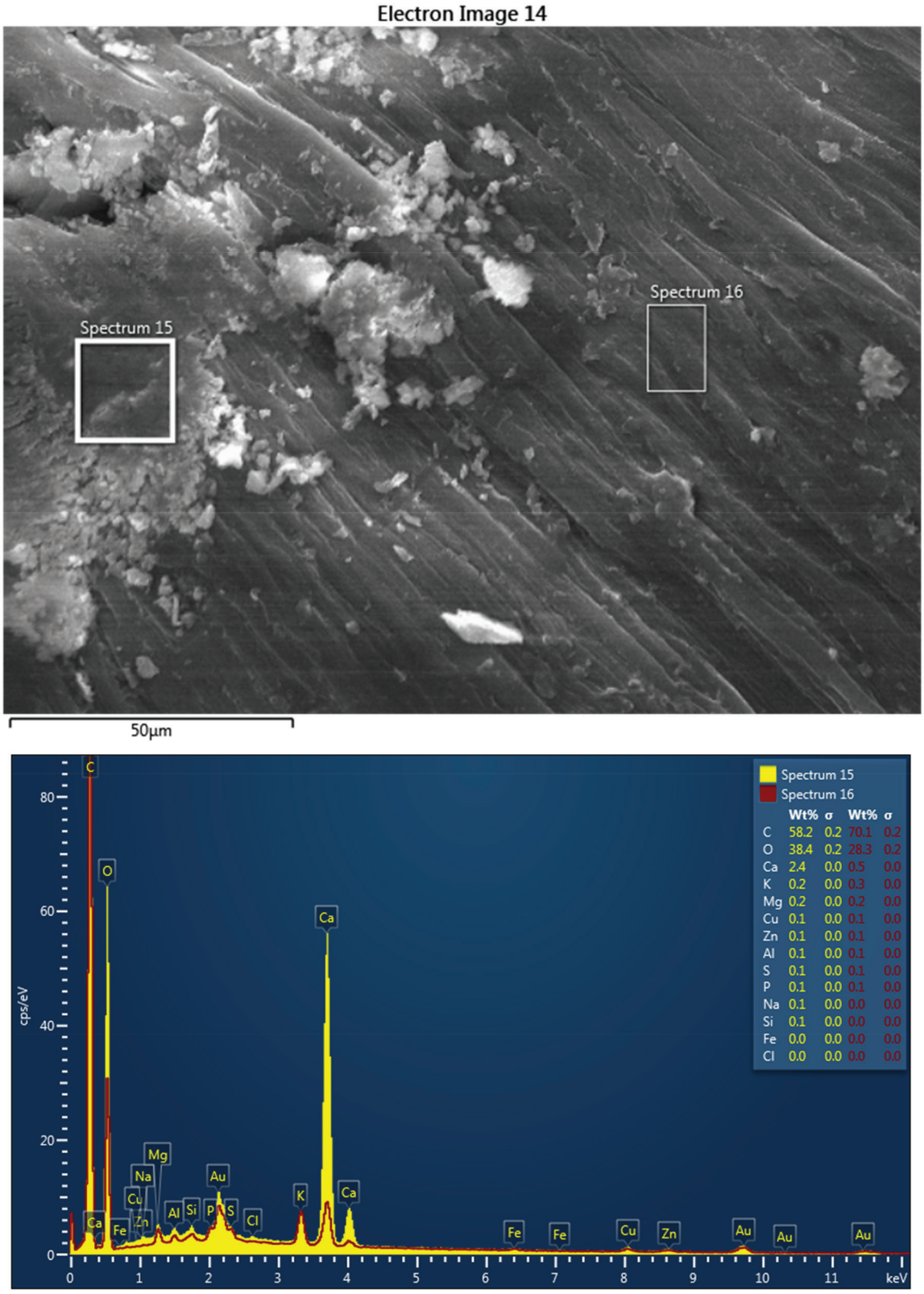

Figure 10. FESEM micrographs, (above) and EDS spectrum with elemental mapping of the surface of *Homalothecium lutescens* from polluted site (below)

it's better for better colorized image. Characterization of 13 elements (Si, Al, Zn, Cu, Fe, Mg, Ca, K, O, P, Ti, S and C) were spotified at the moss surface and visual comparative analysis between both moss species has been evaluated (control samples of moss has been used for this analysis). The surface deposition has been extracted the most dominant elements on the moss tissue surface. For *Hypnum cumpressiforme* as most

Figure 11. Mapping for elementary composition of *Hypnum cumpressiforme* sample

Figure 12. Mapping for elementary composition of *Homolathecium lutescens* sample

dominant elements were extracted: Si, Al, Zn, Cu, Fe, Mg, Ca, K, O and C. While for *Homolathecium lutescens* control sample this evidence has extract the folowing elements: S, P, Si, Al, Zn, Cu, Fe, Ti, Mg, Ca, K, O and C. This correlation is important due to the posibility of using both species interchangeable. The dominant identified element weight for *Hypnum cumpressiforme* (in percent) are given as following: C=50.5%, O=46.1%, Si=1.05%, Mg=0.61%, Al=0.92%, K=0.12%, Ca=0.10%, Fe=0.63%, while Zn and Cu were <0.05%. The dominant identified element weight for *Homolathecium lutescens* are given as following: C=37.7%, O=50.06%, Si=0.43%, Mg=1.12%, Al=1.79%, Ti=1.04%, K=0.47%, S=0.50%, P=0.087%, Ca=0.24%, Fe=0.59%, while Zn and Cu were <0.01%.

Conclusion

The proposed chemometric model can serve as an initial research for subsequent screening models. This chemometric model, unlike the typical analysis with existing chemical instrumentation techniques, does not require chemical destruction of the sample and additional costs for chemical agents. But on the other hand this model is suitable for screening for large areas in order to identify critical areas. Quantitative chemical analysis still remains an irreplaceable identification method for determining the multi-element distribution in the environment.

This study also introduced the ability to monitor isolated atmospheric deposition on the surface of these plant species. Their unique structure and physiology allow dust particles to remain trapped on their surface. With the help of electron microscopy, the composition of anthropogenic elements in the dust particles trapped on the surface of these structures can be monitored. Chemical characterization is possible but additional research is needed to compare analytical methods with these non-destructive analyzes. Both types of moss, which have been proven to be effective biomonitors, were used in this study. But when examining large areas, it is necessary to use them alternately given their specific geographical distribution. Analyzes have shown that they have similar surface adsorption to dust particles, as well as insignificant variability in the chemical composition plant surface: extracted for the biogenic elements carbon and oxygen, macroelements Mg, Al, Si, K, Ca, and microelements Fe, Cu and Zn. Both moss species can be used interchangeable for dust deposition investigation.

References

Aboal, J. R., Fernández, J. A., Boquete, T., Carballeira, A. (2010). Is it possible to estimate atmospheric deposition of heavy metals by analysis of terrestrial mosses? Science of the Total Environment, **408**(24): 6291–6297.

Ajmone-Marsan, F., Biasioli, M., Kralj, T., Grčman, H., Davidson, C. M., Hursthouse, A. S., Madrid, L., Rodrigues, S. (2008). Metals in particle-size fractions of the soils of five European cities. Environmental Pollution, **152**(1): 73-81.

Bačeva, K., Stafilov, T., Šajn, R (2012). Monitoring of air pollution with heavy metals in the vicinity of ferronickel smelter plant by deposited dust. Macedonian Journal of Ecology and Environment, **1**(1-2): 17-24.

Balabanova, B., Stafilov, T., Bačeva, K., Šajn, R. (2010). Biomonitoring of atmospheric pollution with heavy metals in the copper mine vicinity located near Radovиš, Republic of Macedonia. Journal of Environmental Science and Health Part A, **45**: 1504–1518.

Balabanova, B., Stafilov, T., Šajn, R. (2019). Enchasing anthropogenic element trackers for evidence of long-term atmospheric depositions in mine environs. Journal of Environmental Science and Health Part A, **54**(10): 988-998.

Balabanova, B., Stafilov, T., Šajn, R., Bačeva, K. (2012). Characterisation of heavy metals in lichen species *Hypogymnia physodes* and *Evernia prunastri* due to biomonitoring of air pollution in the vicinity of copper mine. International Journal of Environmental Research, **6**(3):779-794.

Balabanova, B., Stafilov, T., Šajn, R., Bačeva, K. (2014). Comparison of response of moss, lichens and attic dust to geology and atmospheric pollution from copper mine. International Journal of Environmental Science and Technology, **11**: 517-528.

Balabanova, B., Stafilov, T., Šajn, R., Tănăselia, C. (2016). Geochemical hunting of lithogenic and anthropogenic impacts on polymetallic distribution (Bregalnica river basin, Republic of Macedonia) Journal of Environmental Science and Health Part A, **51**(13): 1180-1194.

Balabanova, B., Stafilov, T., Šajn, R., Baceva, K. (2017). Quantitative assessment of metal elements using moss species as biomonitors in downwind area of lead-zinc mine. Journal of Environmental Science and Health Part A, **52**(3): 290-301.

Barandovski, L., Frontasyeva, M. V., Stafilov, T., Šajn, R., Ostrovnaya, T. M. (2015). Multi-element atmospheric deposition in Macedonia studied by the moss biomonitoring technique. Environmental Science and Pollution Research, **22**(20): 6077-16097.

Basile, A., Sorbo, S., Pisani, T., Paoli, L., Munzi, S., Loppi, S. (2012). Bioaccumulation and ultrastructural effects of Cd, Cu, Pb and Zn in the moss *Scorpiurum circinatum* (Brid.) Fleisch. & Loeske. Environmental Pollution, **166**: 208-211.

Beelen, R., Hoek, G., Pebesma, E., Vienneau, D., de Hoogh, K, Briggs, D. J. (2009). Mapping of background air pollution at a fine spatial scale across the European Union. Science of the Total Environment, **407**(6): 1852-1867.

Berg, T. & Steinnes, E. (1997). Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as biomonitors of heavy metal deposition: from relative to absolute deposition values. Environmental Pollution, **98**(1): 61-71.

Boquete, M. T., Bermudez-Crespo, J., Aboal, J. R., Carballeira, A., Fernandez, J. A. (2014). Assessing the effects of heavy metal contamination on the proteome of the moss Pseudoscleropodium purum cross-transplanted between dif-

ferent areas. Environmental Science and Pollution Research, **21**: 2191-2200.

Bourennane, H., Douay, F., Sterckeman, T., Villanneau, E., Ciesielski, H., King, D., Baize, D. (2010). Mapping of anthropogenic trace elements inputs in agricultural topsoil from Northern France using enrichment factors. Geoderma, **157**(3):.165-174.

Ceburnis, U. D. & Valiulis, D. (1999). Investigation of absolute metal uptake efficiency from precipitation in moss. Science of the Total Environment, **226**: 247–25.

De Miguel, E., Llamas, J. F., Chacon, E., Mazadiego, L. F. (1999). Sources and pathways of trace elements in urban environments: a multi-elemental qualitative approach. Science of the Total Environment, **235**(1): 355-357.

Fernandez, J. A., Aboal, J. A., Real, C. (2007). A new moss biomonitoring method for detecting sources of small scale pollution. Atmospheric Environment, **41**: 2098–2110.

Fernandez, J. A., Ares, A., Rey-Asensio, A., Carballeira, A., Aboal, J. R. (2012). Effect of growth on active biomonitoring with terrestrial mosses. Journal of Atmospheric Chemistry, **63**(1): 1-11.

Finlayson-Pitts, B. J. & Pitts, J. J. N. (1999). Chemistry of the upper and lower atmosphere: theory, experiments, and applications. Academic press.

Fordyce, F. M., Brown, S. E., Ander, E. L., Rawlins, B. G., O'Donnell, K. E., Lister, T. R. Breward, N., Johnson, C. C. (2005). GSUE: urban geochemical mapping in Great Britain. Geochemistry: Exploration Environment Analysis, **5**(4): 325-336.

Gjengedal, E. & Steinnes, E. (1990). Uptake of metal ions in moss from artificial precipitation. Environmental Monitoring and Assessment, **14**: 77-87.

González, G. & Pokrovsky, O. S. (2014). Metal adsorption on mosses: Toward a universal adsorption model. Journal of Colloid and Interface Science, **415**: 169-178.

Goodarzi, F. (2006). Assessment of elemental content of feed coal, combustion residues, and stack emitted materials for a Canadian pulverized coal fired power plant, and their possible environmental effect. International Journal of Coal Geology, **65**: 17-25.

Harmens, H., Norris, D. A., Steinnes, E., Kubin, E., Piispanen, J., Alber, R., Aleksiayenak, Y., Blum, O., Coşkun, M., Dam, M., De Temmerman, L., Fernández, J. A., Frolova, M., Frontasyeva, M., González-Miqueo, L., Grodzińska, K., Jeran, Z., Korzekwa, S., Krmar, M., Kvietkus, K., Leblond, S., Liiv, S., Magnússon, S. H., Maňkovská, B., Pesch, R., Rühling, Å., Santamaria, J. M., Schröder, W., Spiric, Z., Suchara, I., Thöni, L., Urumov, V., Yurukova, L., Zechmeister, H. G. (2010). Mosses as biomonitors of atmospheric heavy metal deposition: Spatial patterns and temporal trends in Europe. Environmental Pollution, **158**: 3144-3156.

He, Z., Yang, X. E., Stoffella, P. J. (2005). Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology, **19**(2-3): 125-40.

Järup, L. (2003). Hazards of heavy metal contamination. British Medical Bulletin, 68: 167-182.

Keller, C. B., Schoene, B., Barboni, M., Samperton, K. M., Husson, J. M. (2015). Volcanic-plutonic parity and the differentiation of the continental crust. Nature, **523**(7560): 301-307.

Leblanc, F. & Rao, D. N. (1974). A review of the literature on bryophytes with respect to air pollution. Bulletin de la Société Botanique de France, **121**(2): 237-255.

Mackay, D. (2001). Multimedia Environmental Models: The Fugacity Approach, CRC Press, Taylor & Francis Group.

Mägdefrau, K. (1982). Life-forms of Bryophytes. In: Smith AJE (eds) Bryophyte Ecology. Springer Academic Press.

Markert, B. A., Breure, A. M., Zechmeister, H. G. (2003). Definitions, strategies, and principles for bioindication/biomonitoring of the environment. Elsevier Press.

Martin, M. H. & Coughtrey, P. J. (1982). Biological monitoring of heavy metal pollution: land and air. Applied Science Publishers.

Nagajyoti, P. C., Lee, K. D., Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. Environmental Chemistry Letters, **8**: 199-216.

Neff, J. C., Ballantyne, A. P., Farmer, G. L., Mahowald, N. M., Conroy, J. L., Landry, C. C. Overpeck, J. T., Painter, T. H., Lawrence, C. R. and Reynolds, R. L. (2008). Increasing eolian dust deposition in the western United States linked to human activity. Nature Geoscience, **1**(3): 189-195.

Onianwa, P. C. (2001). Monitoring atmospheric metal pollution: a review of the use of mosses as indicators. Environmental Monitoring and Assessment, 71(1): 13-50.

Rühling, Å. & Tyler, G. (1968). An ecological approach to the lead problem. Botaniska Notiser, 122: 248-342.

Rühling, Å. & Tyler, G. (1970). Sorption and retention of heavy metals in the woodland moss Hylocomium splendens (Hedw.) Br. et Sch. Oikos, **21**(1): 92-97.

Salemaa, M., Derome, J., Helmisaari, H. S., Nieminem, T., Vanha-Majamaa, I. (2004). Element accumulation in boreal bryophytes, lichens and vascular plants exposed to heavy metal and sulfur deposition in Finland. Science of the Total Environment, **324**(1-3): 141-160.

Shakya, K., Chetti, M. K., Sawidis, T. (2008). Impact of heavy metals (copper, zinc, and lead) on the chlorophyll content of some mosses. Archives of Environmental Contamination and Toxicology, **54**(3): 412-421.

Stankovic, J., Sabovljevic, A., Sabovljevic, M. S. (2018). Bryophytes and heavy metals: A review. *Acta Botanica Croatica*, **77**(2): 109-118.

Urošević, M. A., Lazo, P., Stafilov, T., Nečemer, M., Andonovska, K. B., Balabanova, B., Hristozova, G., Papagiannis, S., Stihi, C., Suljkanović, M., Špirić, Z. (2023). Active biomonitoring of potentially toxic elements in urban air by two distinct moss species and two analytical techniques: a pan-Southeastern European study. *Air Quality Atmosphere* and *Health*, **16**(3): 595-612.

White, W. M. (2013). Geochemistry. John Wiley & Sons.

Wolterbeek, B, (2002), Biomonitoring of trace element air pollution: principles, possibilities and perspectives. Environmental Pollution, **120**(1): 11-21.