Multivariate assessment of atmospheric deposition studies in Bulgaria based on moss biomonitors: trends between the 2005/2006 and 2015/2016 surveys

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Abstract

This study aims to investigate the changes in atmospheric deposition trends in Bulgaria, studied using the moss biomonitoring technique since 1995. For the first time, a paired (site-wise) comparison was performed after a critical review of the sampling networks and adjusting for location, the distance between the sampling points, and moss species. Data from the 2005/2006 and 2015/2016 moss surveys were chosen as instrumental neutron activation analysis was employed in both to determine the content of 34 elements (Al, As, Ba, Br, Ca, Ce, Cl, Co, Cr, Cs, Fe, Hf, I, K, La, Mn, Na, Nd, Ni, Rb, Sb, Sc, Se, Sr, Ta, Tb, Th, Ti, Tm, U, V, W, Yb, Zn). In addition, Cd, Cu, and Pb were determined using complementary analytical methods: inductively coupled plasma atomic emission spectroscopy in 2015/2016 and atomic absorption spectroscopy in 2005/2006. For the subset of 57 routinely sampled locations in Bulgaria, hierarchical clustering on principal components and multiple factor analysis (MFA) were applied to assess the spatial trends in the 10 years elapsed between the surveys, as well as to characterise the origin of the determined elements. Elevation and distance between the sampling points were used as additional variables in the multiple factor analysis plane to ascertain their effect on the overall variance in the datasets. Distribution maps were constructed to illustrate the deposition patterns for the pollutant Pb. The results were consistent with decreased industrial output in the country, increased coal combustion and transport pollution, and construction of roads.

Keywords Multiple factor analysis · Metals · Pollution · Atmospheric deposition · Moss · Biomonitoring · Bulgaria · GIS

Introduction

Moss biomonitoring, ICP vegetation

Mosses have been used as passive bioindicators for metal accumulation from atmospheric deposition in terrestrial ecosystems since the late 1960s (Rühling and Tyler 1968). Their widespread application in biomonitoring surveys owes to the fact that essential nutrients are primarily obtained through wet and dry deposition. The lack of advanced root system warrants negligible substrate uptake, and the absence of vascular tissue ensures that nutrients are not redistributed

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internally between organs of the plant (Smith 1982). The high cation exchange capacity of the moss cell walls is an important advantage over conventional bulk precipitation collectors, where the low concentrations of precipitated elements are associated with problems related to sample contamination and measurement (Harmens et al. 2007).

Wide geographical distribution, abundance, and characteristic morphological and physiological properties of mosses facilitate large-scale monitoring of element depositions from the atmosphere at high-density networks (González and Pokrovsky 2014; Vanderpoorten and Goffinet 2009). Since 1990, European-scale moss surveys have been conducted at 5-year intervals under the auspices of the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation). The programme aims to assess transboundary air pollution by investigating the spatial and temporal trends of atmospheric deposition of Al, As, Cd, Cr, Cu, Fe, Ni, Pb, Sb, V, and Zn to naturally growing moss bioindicators (Harmens et al. 2014, 2015a). The data are reported to the Working Group on Effects (WGE) of the United Nations Economic Commission for Europe (UNECE n.d.) Convention on Long-range Transboundary Air Pollution and are used to validate air pollution models by the International Cooperative Programme on Modelling and Mapping of Critical Levels and Loads and Air Pollution Effects, Risks, and Trends – ICP Modelling and Mapping (UNECE Air Convention).

To ensure comparability of the data, the programme's standardised sampling protocol defines a list of appropriate moss biomonitoring species; specifies minimum distances between sampling sites and local emission sources; recommends the sampling locations for subsequent surveys, the conditions for storage, and transportation of the samples; and appoints preferred analytical methods, and standard reference materials (Fernández et al. 2015; Frontasyeva and Harmens 2014; Harmens et al. 2015b).

Moss surveys in Bulgaria

The country first joined the European moss survey programme for the 1995/1996 sampling campaign (Yurukova 2000). During the five ICP Vegetation moss surveys conducted in Bulgaria to date, the approved biomonitoring species *Hypnum cupressiforme*(Hedw) was collected predominantly and not exclusively. Sampling was performed at the same time for each survey. Sampling density was corrected several times over to suit the determined deposition patterns and to avoid local point pollution sources, as well as due to financial and time constraints. The mountainous terrains, land use, climate conditions, and different groups partaking in sampling campaigns led to additional variation in the locations of the sampling sites.

The protocol recommendation for sampling location, namely, collecting moss either from the same points as in the preceding survey or no more than 2 km away, was not always abided. Information concerning the type of neighbouring vegetation, distance to the nearest canopy projection, land use, slope, and the exact number of subsamples (always between 5 and 10) was either not recorded or was lost; therefore, the relevant uncertainties cannot be estimated.

Comparability of the data could have been compromised owing to the non-uniform and inconsistent sampling design—e.g. hardly any samples were collected in northern Bulgaria for the 2010/2011 and 2015/2016 campaigns (Fig. 1).

Between 1995/1996 and 2010/2011, the primary analytical technique used was inductively coupled plasma atomic emission spectroscopy (ICP-AES)(Yurukova 2000, 2006, 2010; Yurukova et al. 2014). Neutron activation analysis (NAA) was employed in 2000/2001 and once more in 2005/2006 to provide supplementary independent data (Marinova et al. 2010; Stamenov et al. 2002). For the 2015/2016 campaign, NAA was the main analytical technique used (Hristozova et al. 2020). The concentrations of the elements Cu, Cd, and Pb were determined additionally by atomic absorption spectroscopy (AAS) in 2005/2006 and by ICP-AES in 2015/2016. The number of sampling sites in each survey was as follows: 215 in 1995/1996, 217 in 2000/2001, 213 (by Yurukova 2010) and 97 (by Marinova et al. 2010) in 2005/2006, 129 in 2010/2011, and 115 in 2015/2016. The elements Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn were determined in the first survey. The same elements were determined in the second survey and As was added. For the third survey, new results for Al were presented and the same set of 10 elements were determined once more during the fourth moss survey. Stamenov et al. (2002) presented data for 35 elements: Al, As, Au, Ba, Br, Ca, Ce, Cl, Co, Cr, Cs, Fe, Hf, I, K, La, Mg, Mn, Mo, Na, Ni, Rb, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, U, V, W, Yb, and Zn. Marinova et al. (2010) ascertained the content of 38 elements: Al, As, Au, Ba, Br, Ca, Ce, Cl, Co, Cr, Cs, Dy, Fe, Hf, I, K, La, Mn, Mo, Na, Nd, Ni, Rb, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, Ti, Tm, U, V, W, Yb, and Zn. Hristozova et al. (2020) provided data for 40 elements: Al, As, Ba, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Eu, Fe, Hf, I, K, La, Lu, Mg, Mn, Na, Nd, Ni, Pb, Rb, Sb, Sc, Se, Sr, Ta, Tb, Th, Ti, Tm, U, V, W, Yb, and Zn. Yurukova (2007) and Gribacheva et al. (2016) summarised the available data for Bulgaria reported to the ICP Vegetation programme and concluded that "hot spots" of local sources of pollution emissions were related to the non-ferrous metal industry, open-pit mining, quarries, historic and active mines, as well as secondary pollution with windblown dust.

Objectives

The aim of this study was to perform a retrospective comparison of suitable data available for Bulgaria and to ascertain the temporal and spatial trends of atmospheric deposition by
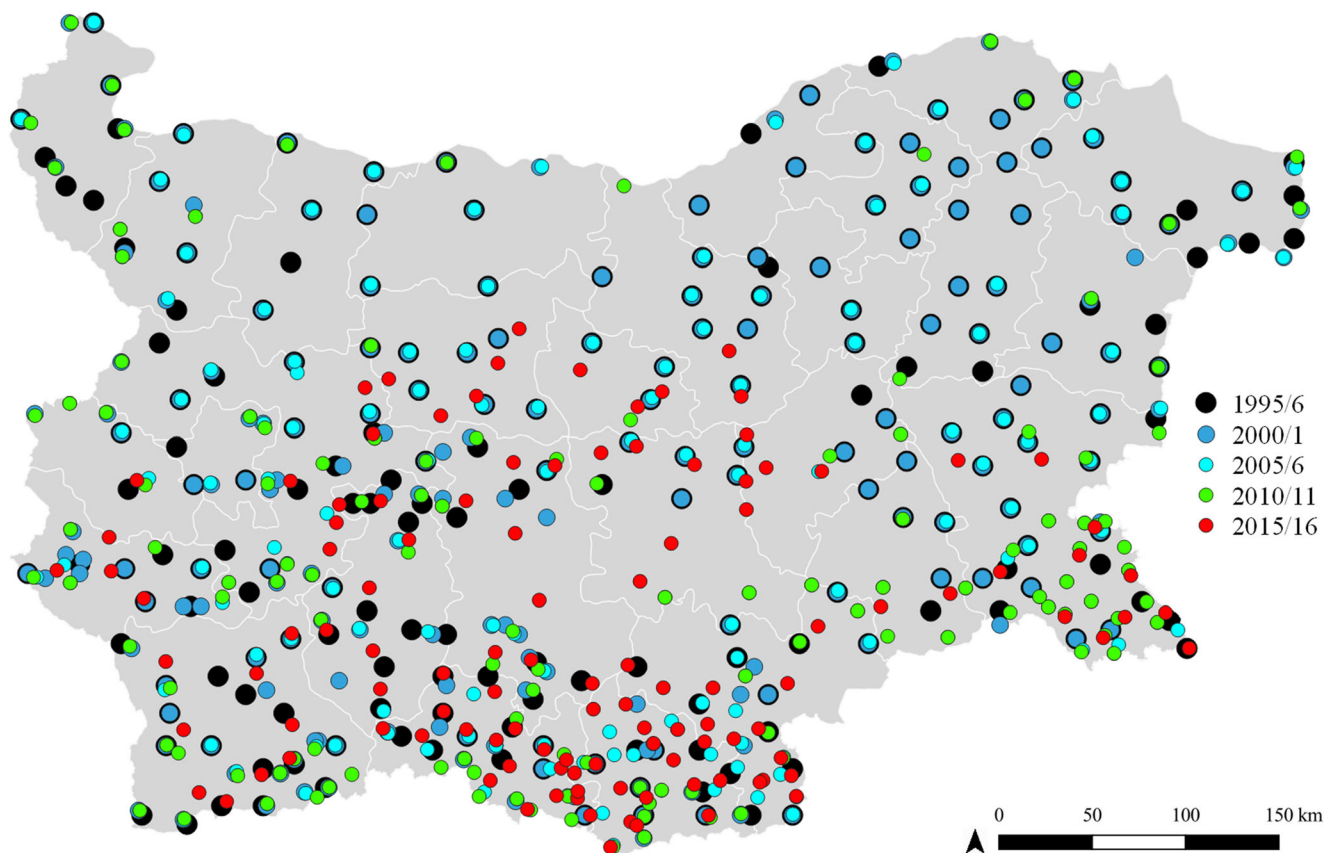
Fig. 1 Location of all sampling sites for the five ICP Vegetation moss biomonitoring surveys conducted in Bulgaria to date

overcoming possible shortcomings of the data. The most recent 2015/2016 moss survey results were preferred, and the 2005/2006 additional data were selected since the design and density of the sampling networks were similar, serving to ensure some level of comparability. Moreover, the same main analytical technique was used (NAA), and the concentrations of large sets of elements were determined. To appoint the origin of the determined elements and to study how they characterised the routinely sampled points, element-wise and site-wise multivariate analyses were performed. Using GIS technology, element distribution maps for the toxicant Pb were constructed to illustrate the regional extent of the atmospheric depositions determined during the two surveys.

Materials and methods

Study area

Bulgaria is situated in the central-east part of the Balkan Peninsula in South-east Europe. The area of about 111,000 km² covers mostly mountainous terrains. For the selected data points, elevation ranged from 2 to 1600 m. The climate is temperate continental and transitions to subtropical and Mediterranean to the south, near the border with Greece.

Summers are hot and dry, with temperatures often exceeding 40 °C, presenting conditions hampering the growth of mosses in arid regions (Marinova et al. 2017).

According to the ICP Vegetation recommendations, samples should be collected in forest clearings and at least 5 m away from the projection of the nearest trees and bushes (Frontasyeva and Harmens 2014). However, given the high density of trees in mountain forests in Bulgaria, moss samples collected in such regions often fell inside the crown projections of trees and were, therefore, inevitably subjected to the effects of canopy drip.

The production processes of many large Soviet legacy industrial enterprises from the heavy industry and energy sectors were gradually phased-out and shutdown since the first moss survey in 1995/1996(Yurukova 2000, 2010). Still, the industry remains the second largest sector of the economy after services and includes energy production, machine building, food industry, chemical industry, building materials production, and metallurgy. Bulgaria has abundant deposits of lignite coal, non-metalliferous minerals, ferrous and non-ferrous metals. Among the still operational large facilities are the "Mini Maritza Iztok complex" with coal-fired thermal power stations and coal mines, the non-ferrous complexes: "Elatsite", "Asarel-Medet", "Dundee Precious Metals Chelopech", "Aurubis Bulgaria", and "KCM 2000 group";

and the crude oil refinery "LUKOIL Neftochim Burgas" (Bulgarian Chamber of Mining and Geology 2014).

Sampling and analytical procedures

The sampling procedure, conditions for storage, transportation, and sample preparation are described in detail in the Moss Survey Protocol (Frontasyeva and Harmens 2014). For the two surveys selected, the samples were collected during the same season and at sites representative of non-urban areas: at least 300 m away from main roads, railroad tracks, agricultural fields, and settlements; 100 m away from smaller roads and farming units; and about 10 km away from industries with chimneys. At each site, 5–10 subsamples were collected within a 50×50 m area and were combined into a compound sample in a paper bag. At the laboratory, the samples remained unwashed and were cleaned and sorted by hand, so that only the green shoots were subjected to analysis.

Data points from non-overlapping geographical regions in the selected moss surveys were dismissed. Additionally, all data points for moss species other than *Hypnum cupressiforme*(Hedw) sps. were excluded. To aid statistical treatment, the number of remaining observations (sites) was to be greater than the number of variables (element concentrations) (Henry et al. 1984). For that reason, sites from the two surveys were paired if their location fell within a radius of 8 km, despite the protocol recommendation for the locations of the sampling sites (which states that between surveys, the points should not shift by more than 2 km). Four of the sites from the 2015/2016 datasets were matched two times, to different data points within the same radius (from the 2005/2006 dataset), and were designated the same numbers with the letters "a" and "b". These criteria resulted in 57 matched pairs of sites from the 2005/2006 and 2015/2016 campaigns.

For the two moss surveys selected in this study, neutron activation analysis was performed in the radioanalytical laboratory at the fast pulsed reactor IBR-2 of the Frank Laboratory of Neutron Physics (Pavlov et al. 2016), Joint Institute for Nuclear Research (FLNP JINR), Dubna, Russia.

Details for the analytical procedures and quality control for the 2005/2006 survey are available elsewhere (Marinova et al. 2010).

In 2015/2016, inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used as a supplementary technique for the determination of Cd, Cu, and Pb. The measurements were carried out in the Agricultural University, Plovdiv, Bulgaria. The procedures and quality control were the same as described by Hristozova et al. (2020).

Statistical treatment

All statistical analyses were performed in R (R Core Team 2018). No instances of concentrations below detection limit occurred in the data points remaining in the datasets; no outliers were detected.

The concentrations of all the determined elements were transformed using the centred log-ratio(clr) transformation to tackle the closure problem characteristic of compositional data (Aitchison and Egozcue 2005). A considerable body of work dedicated to compositional data analyses indicates that the spurious relationships arising from the closed nature of the data takes priority over the non-normal frequency of distribution, commonly occurring in environmental monitoring studies (Filzmoser and Hron 2009; Filzmoser et al. 2009, 2010; Reimann and Filzmoser 1999).

Two multivariate statistical techniques were used to analyse the data structure:

Hierarchical clustering on principal components Observations can be grouped or classified on the basis of the similarity or dissimilarity of the measured variables, and in this case, hierarchical clustering using Ward's method was applied. Principal component analysis (PCA) was first performed as a pre-treatment of the data to render the analysis more robust. The number of principal components with eigenvalues exceeding 1 was determined, and the clustering was performed only on these dimensions, kept for analysis, and the rest was considered noise. Cluster analysis is aimed at optimising homogeneity within groups of observations simultaneously with heterogeneity between said groups. HCPC was performed for site-wise testing of the similarities between the datasets based on the trends in the elapsed time between the surveys, with respect to all variables.

Multiple factor analysis This analysis could be viewed as an extended principal component analysis on a weighted table, constructed by weighting each variable by the first eigenvalue of the group to which it belongs. The two groups of data were treated as active, i.e. the principal components constructed from one of the datasets were compared with the principal components from the other, all in the shared frame of reference provided by the multiple factor analysis (MFA). Additionally, 'elevation' and 'distance' were projected as supplementary variables, meaning they did not contribute to the construction of the factor planes.

Results and discussion

Median values of the determined elemental concentrations in moss bioindicator species are the preferred statistics reported by the ICP Vegetation moss surveys. A rough comparison between the results from different surveys and between different countries could be made on the basis of these values. The median values of the results from the 2005/2006 and 2015/2016 moss surveys conducted in Bulgaria are shown in

Table 1, alongside the medians for the adjusted datasets, comprised of 57 sites each. For the pairs of the two original and two adjusted datasets, relative differences were calculated. A strong decrease in the concentrations was observed for most elements determined in mosses, except for Cu and K. Possible emission sources and interpretations of these trends in Bulgaria were the main topics of discussion of previous publications (Gribacheva et al. 2016; Hristozova et al. 2020; Marinova et al. 2010; Stamenov et al. 2002; Yurukova 2000, 2006, 2007, 2010; Yurukova et al. 2014). It could be hypothesised that since the decrease in elemental deposition reported for Bulgaria remains quite similar after the exclusion of nearly half of the original sampling sites (from $N = 97$ and $N = 115$, to $N = 57$), the sampling design and density could be revised and optimised (the number of sampling sites could be reduced).

Table 1 Median values of the determined elements in the 2005/2006 and 2015/2016 moss surveys in Bulgaria and relative differences in %: as reported and after adjusting for location

Elements determined	2005/2006 survey (mg/kg) $N = 97$	2015/2016 survey (mg/kg) $N = 115$	Relative difference	Adjusted 2005/2006 survey (mg/kg) $N = 57$	Adjusted 2015/2016 (mg/kg) $N = 57$	Relative difference
Al	6872	2310	− 66%	7861	2310	− 71%
As	0.97	0.447	− 54%	1.04	0.447	− 57%
Ba	78	46	− 41%	92	45.3	− 51%
Br	4.4	2.76	− 37%	4.8	2.76	− 43%
Ca	8891	6630	− 25%	8819	5920	− 33%
Cd	0.3	0.1	− 67%	0.37	0.08	− 78%
Ce	6.8	2.41	− 65%	7.4	2.28	− 69%
Cl	232	78.8	− 66%	242	74.9	− 69%
Co	1.5	0.585	− 61%	1.34	0.525	− 61%
Cr	5	2.73	− 45%	5.7	2.58	− 55%
Cs	0.52	0.207	− 60%	0.59	0.196	− 67%
Cu	7.1	7.36	4%	7.09	7.4	4%
Fe	2994.5	1190	− 60%	2998	1090	− 64%
Hf	0.45	0.158	− 65%	0.49	0.159	− 68%
I	2.6	1.28	− 51%	2.6	1.32	− 49%
K	5997.5	5670	− 5%	6016	5520	− 8%
La	3.3	1.35	− 59%	4	1.35	− 66%
Mn	243	180	− 26%	243	180	− 26%
Na	709	225	− 68%	782	169	− 78%
Nd	3.125	1.33	− 57%	3.3	1.24	− 62%
Ni	5	2.11	− 58%	5.1	2.1	− 59%
Pb	12.5	10.72	− 14%	15.65	10.12	− 35%
Rb	15	7.38	− 51%	15	7.38	− 51%
Sb	0.29	0.113	− 61%	0.39	0.107	− 73%
Sc	0.92	0.408	− 56%	0.99	0.393	− 60%
Se	1.305	0.2	− 85%	1.3	0.206	− 84%
Sr	35.5	25	− 30%	38	24.7	− 35%
Ta	0.127	0.0355	− 72%	0.14	0.033	− 76%
Tb	0.08	0.0258	− 68%	0.08	0.0255	− 68%
Th	0.84	0.39	− 54%	1	0.361	− 64%
Ti	340	143	− 58%	366	136	− 63%
Tm	0.06	0.0137	− 77%	0.06	0.0137	− 77%
U	0.3	0.124	− 59%	0.36	0.109	− 70%
V	8.5	3.89	− 54%	9.00	3.8	− 58%
W	1.21	0.0994	− 92%	1.43	0.0936	− 93%
Yb	0.215	0.094	− 56%	0.26	0.0838	− 68%
Zn	45	27.8	− 38%	49	25.4	− 48%

Lead deposition maps

Differences in the elemental deposition patterns are best visualised using spatial distribution maps. Yurukova (2010), Yurukova et al. (2014), and Hristozova et al. (2020) presented such maps for elements regularly reported to the ICP Vegetation programme. In the present study, maps for the element Pb were constructed using the adjusted datasets of 57 paired sites (Fig. 2).

Lead does not have a role in the normal processes of cell metabolism and is considered a toxicant. Lead was targeted in the 1998 Aarhus Protocol on Heavy Metals as it is known to be both persistent and harmful to the environment and human health. In Bulgaria, leaded petrol was phased out in 2004 and metal production has been the major source contributing to atmospheric Pb emissions ever since (Thöni et al. 2011). In previous moss surveys, samples collected in Bulgaria had the highest reported Pb content among all ICP Vegetation participants (both median and maximal values) (Yurukova 2007).

The constructed Pb distribution maps (Fig. 2) illustrated the deposition patterns determined during the two selected moss surveys at the sites paired for this study. Interpolations were performed in ArcGIS/ArcMap using the spline interpolation method with tension. The ICP Vegetation Thematic reports were referred for concentration classifications and respective colouring (Harmens et al. 2014).

The maps illustrated a great reduction in the anthropogenic impact by several known industrial "hot spots" in the 10 years elapsed between the two surveys.

The diagonal concentration pattern observed during the 2005/2006 moss survey can be explained by the location of the open-pit copper mines and tailings in the regions of Chelopech, Zlatitsa, Elatsite, Pirdop, and Panagiyrishte (to the northwest of the studied area), a non-ferrous plant near Plovdiv (the "KCM Group" denoted as a "factory" in the maps), as well as the Kardzhali Pb-Zn plant (also denoted as with "factory") and adjacent mines and tailings in the southeast of the studied area.

After the shutdown of the Kardzhali Pb-Zn plant in 2011, it is evident in the map for the 2015/2016 moss survey that the environmental situation in the region vastly improved, even though it is still affected by the tailings and quarries. The highest Pb concentrations for the sites paired in this study were determined in moss samples collected to the north of the Kardzhali Pb-Zn plant, to the east of Madan (Pb-Zn-Ag mining complex and ore minerals) and the southwest of the "KCM" non-ferrous plant.

The use of unleaded gasoline since 2004 could also partially explain the decrease of Pb emissions, as the most impacted regions in the 2005/2006 survey were located parallel to the International E-road network in Bulgaria: routes E871, E80, and E85.

Fig. 2 Deposition maps for the elements Pb determined in the 2005/2006 ▶ (top) and 2015/2016 ICP Vegetation moss surveys (bottom). Only sites paired for this study illustrated. The locations of known industrial Pb-emission sources are shown (the "KCM Group" non-ferrous plant and the Kardzhali Pb-Zn plant) along with wind rose diagrams for the regions
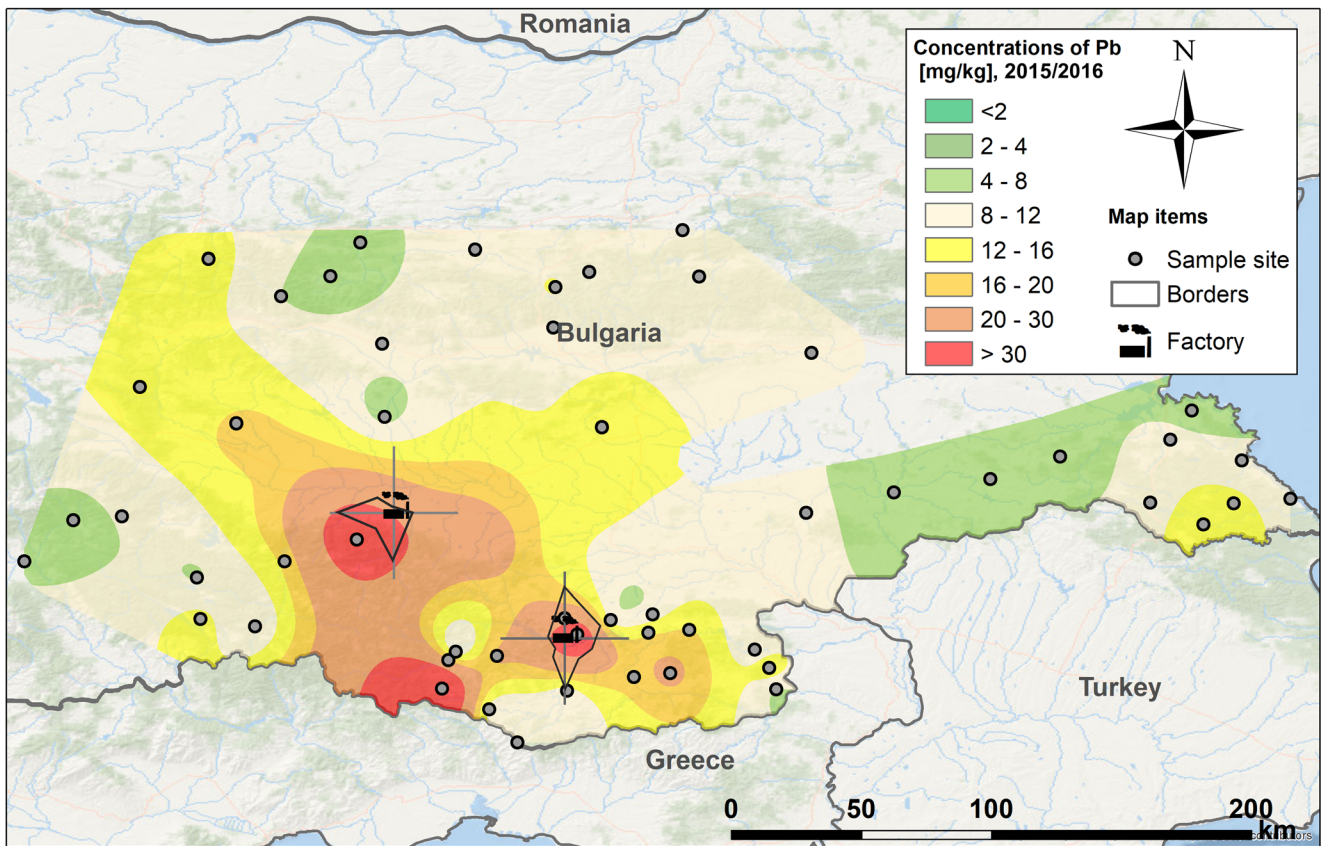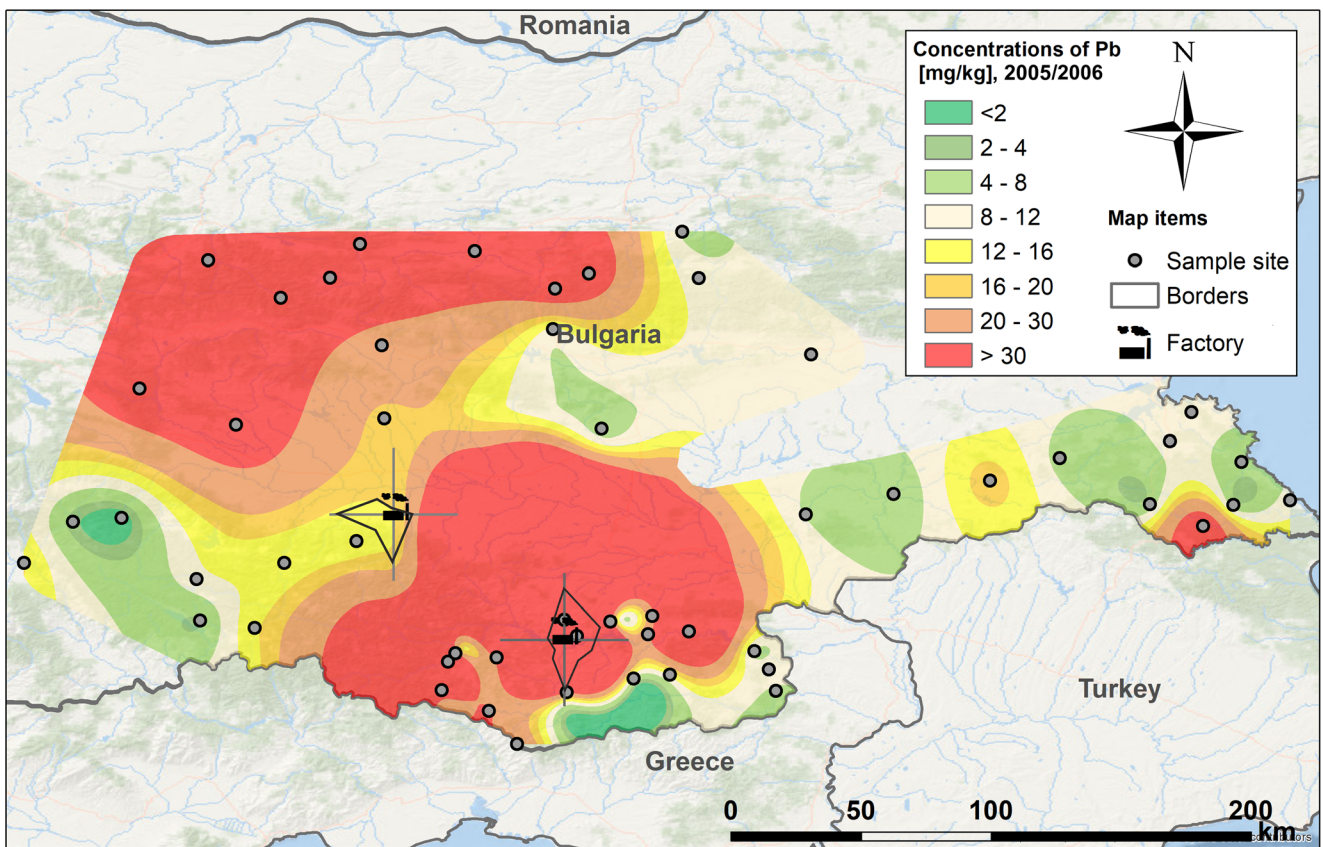
Site-wise comparison

For both datasets, the number of principal components used for the clustering procedure was 10. Four clusters were present for the 2005/2006 data, and only 3 appeared in the data from the most recent survey (Fig. 3). Similar types of sources of atmospheric deposition would explain identical grouping of paired sites at the same clusters for both datasets.

The first cluster (cluster a) for the 2005/2006 dataset shown in Fig. 3 consisted of sites with high loadings for rare earth elements (REEs: Ce, La, Nd, Sc, Tb, Tm, Yb), as well as Al, Ti, and Fe, implying crustal origin. Most of the data points were located in the mountains and their outskirts, where the sloped terrain and erosion processes have a predominant effect. Soil particles are easily retained by moss tissues and should be cleaned carefully without resorting to washing for the purposes of the routine moss biomonitoring technique (Fernández et al. 2015).

The second cluster (cluster b) for the 2005/2006 dataset was characterised by elements Pb, Zn, Cd, Sb, U, Th, and Cs. It consisted of points located in proximity to the Kardzhali lead-zinc plant, a site to the north from it, and a site close to a quarry. The prevailing wind direction in the Kardzhali region is namely North; therefore, dust particles originating from the lead-zinc plant production processes and the open tailings, quarries, and open-pit mines in the region were expected to have impacted these sites. The Kardzhali lead-zinc plant was one of the main sources of contamination with Pb, Cd, Zn, and SO_2 in the country, recognised as a hot spot in 1994 by the Bulgarian council of ministers (Yurukova et al. 2014).

The geographical distribution of the sites in the third cluster (cluster c) in Fig. 3 could not be attributed to any specific local point sources of pollution, and the elements which formed it were Na, Ba, Cd, and W. Possible emission sources are road surface abrasion and vehicles or contamination from fertilisers (Lazo et al. 2012; Monaci et al. 2000).

The fourth cluster in Fig. 3 (cluster d) was characterised by a set of elements that could be interpreted in groups and not as a whole. The presence of the halogens I, Br, and Cl in moss tissues characterises a contribution by marine aerosols (Frontasyeva and Steinnes 2004). This interpretation is in agreement with the location of most sampling sites comprising this cluster—they were collected in proximity to the Black Sea coastline and in the south-east of the country, where the climate is Mediterranean. Five additional data points from this cluster were located at a relatively high altitude, in dense
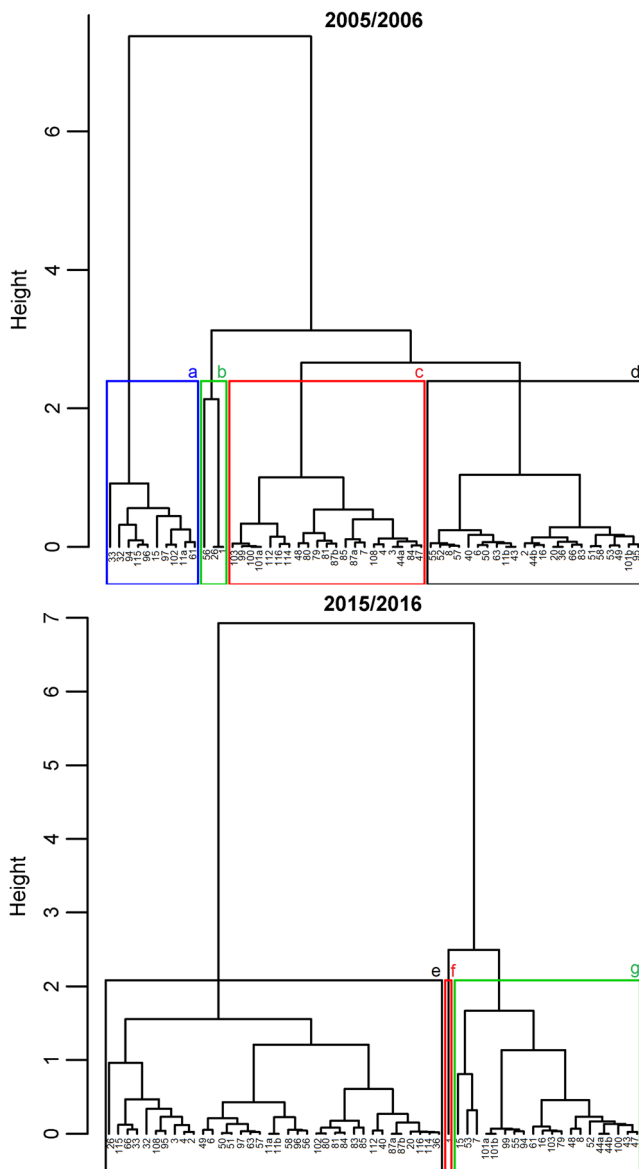
Fig. 3 Hierarchical clustering on principal components: 2005/2006 dataset (top) and 2015/2016 (bottom) dataset

most of the sites from the dataset. The set of the elements Cu, Zn, Pb, Sb, and Cd, all characterising the heavy industry, played an equal role in the formation of this cluster as the elements Ba, Na, Ca, K, Mn, and also the halogens I and Br. The sites were clustered differently in the 2005/2006 dataset, among all four clusters. Such grouping of sites and elements in a single cluster in the 2015/2016 dataset could indicate an overall decrease in heavy industrial output and related pollution in the country and improved implementation of air pollution control systems.

The second cluster (cluster f) in Fig. 3 corresponded to cluster b and was related to the production processes of the Kardzhali lead-zinc plant. The plant was shut down in 2011, which explained the differences in the number of sites grouped in the corresponding clusters (Hristozova et al. 2014). The single-point cluster for the 2015/2016 dataset was characterised by the elements Cd, U, and Th, all present in the polymetallic ores from the region. These elements likely reflected windblown dust from the open tailings. The geographic location of this single data point in cluster f confirmed such an interpretation: the paired moss samples were collected to the North of the open tailings. Moreover, this data point was the closest one of all to the chimneys of the smelter.

The third cluster for the 2015/2016 dataset in Fig. 3 (cluster g) was similar to the first cluster in the 2005/2006 dataset (cluster a). The elements which characterised it were almost the same: REEs, Fe, Al, V, Ni, Cr, Co, U, and Th. The majority of the sites were similarly located on sloped mountainous terrains, and some of the data points were the same as in the first cluster in Fig. 3 (cluster a). The rest were located in proximity to newly opened border checkpoints with Greece and Turkey, newly built roads, and new quarries supplying building materials for construction. These sites were previously grouped in the third cluster in Fig. 3 (cluster c), and few were contained in the fourth cluster (cluster d). Therefore, the third cluster in the 2015/2016 dataset (cluster g) characterised a geogenic origin of the elements: dust particles and soil erosion; in combination with influences by traffic pollution, oil combustion, and the emergence of new quarries in the regions close to the border with Greece.

Effects by varying distances between paired sampling sites

No statistically significant relationships (Pearson correlation or linear regression) between the overall distance and the multivariable profile of the matched sites were observed. Therefore, it could be hypothesised that the natural variance characterising each sampling site was represented well enough by the compound moss samples.
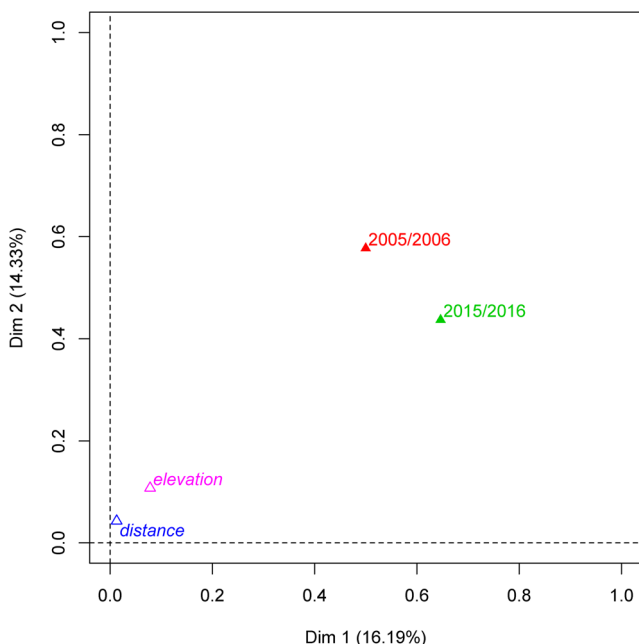
mountain forests, which coincides with the second group of elements characterising this cluster: Ca, K, and Mn. They are essential to plants and may hint at canopy drip from higher vegetation in the forests or possible differences in the moss metabolism at higher altitudes and under the effect of marine aerosols (Frontasyeva and Steinnes 2004). The element Sr also characterised this cluster, and this could be explained by the similarity of the chemical properties of Sr to those of the essential element Ca. The last group of elements characterising this cluster was Ni and V reflecting crude oil production at the refinery in Burgas (at the Black Sea coastline), whose operating licence was briefly revoked in 2013.

The first cluster for the 2015/2016 dataset (cluster e) in Fig. 3 was formed by the largest number of elements and contained

Fig. 4 MFA: Only two first principal components were retained (eigenvalue over 1). Elevation and distance were projected as supplementary variables. The elemental composition in the sampling years and the supplementary variables were rather independent

Element-wise comparison

To further test the similarities between the two selected surveys, MFA was applied. Two first principle components were retained for the datasets (eigenvalue > 1).

The association between the datasets was illustrated by the group representations shown in Fig. 4, in which each survey was depicted by a single point. The two groups (moss surveys) could be deemed similar if they were projected close on all dimensions of the MFA. Indeed, the two points were relatively close to each other in the first two dimensions. However, the association was rather weak: in the first factor plane, the 2015/2016 observations had higher scores on Dimension 1, while those from 2005/2006 had higher scores on Dimension 2. In the same figure, the supplementary variables "elevation" and "distance" were projected close to the origin and were rather independent on the elemental composition determined during the sampling years. Still, elevation was of slightly higher importance than distance. The groups (and, thus,

sampling years) as a whole had a major role in explaining the differences between the datasets.

The matrix shown in Table 2 contains MFA-specific values indicating the mean configuration of the analysis. The RV coefficients are normalised using the dimensionality of each of the groups and denote the similarity between the datasets, meaning they are analogous to correlation coefficients. The values obtained were not equal or close to 1; however, after testing, it was confirmed that the two datasets displayed significant similarity between each other. Additionally, the relationship between the transformed supplementary variable "elevation" and the 2015/2016 moss survey data was significant ($p = 0.01$), even though the effect was weak (RV = 0.2).

The representation of each of the groups' factors treated as separate quantitative variables is shown in Fig. 5, in a correlation circle. For each survey, a PCA was performed and the resulting dimensions were projected as supplementary information onto the MFA dimensions. None of the axes of the correlation circle was strongly correlated to the components of the groups. However, the first dimensions for both datasets were both well represented and projected. The second dimension did not separate the data on any specific criterion since Dim2.2015/16 and Dim2.2005/6 were not well projected and were rotated at a small angle relative to the second axis.

Not all available variables were very well represented onto the first MFA plane. This is illustrated in Fig. 6 with the "degree" of correlation between them. The overall rotation of the datasets (survey campaigns) would imply which of the variables were independent of the factor plane and of each other. The quadrants containing distance and elevation arrows, and the ones opposing them, delineate the elements most affected and whether their correlation with the additional variables was positive or negative. Even though it was shown previously that the elevation variable did not account for most of the explainable variance in the data, the direction of the arrow in the correlation circle coincided with the direction of distinct separation of the active variables into two groups. It would seem that elevation was only associated with the 2015/2016 survey, meaning that this variable could explain variance in one of the surveys better than the other.

It was verified that the amount of annual precipitation during the 2015/2016 survey was lower than that in 2005/2006: about 650 mm and 920 mm, respectively (Bulgarian

Table 2 Multiple factor analysis: Rv and MFA coefficients. Statistically significant values shown in italics

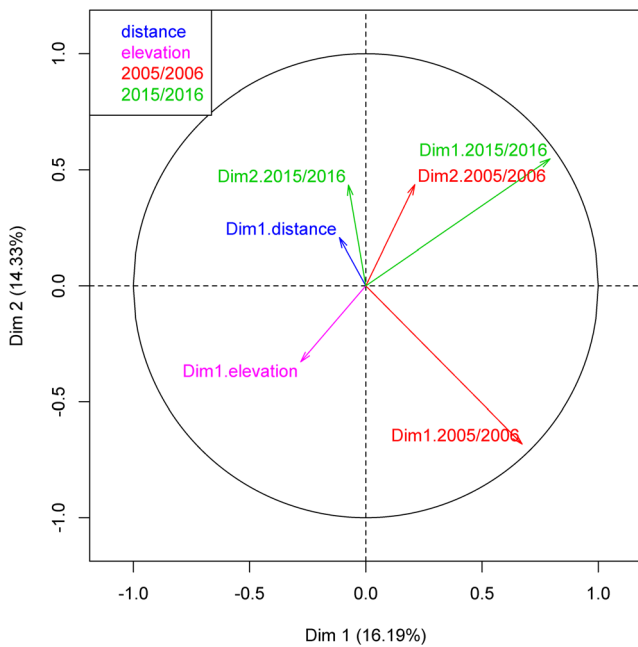| RV	Distance	Elevation	2005/2006	2015/2016	MFA
Distance	1				0.0667
Elevation	0.0037	1			0.1713
2005/2006	0.0633	0.0531	1		0.7630
2015/2016	0.0410	*0.2090*	*0.2062*	1	0.7898
MFA	0.0667	0.1713	0.7630	0.7898	1

Fig. 5 The first dimensions of the datasets were well-projected but uncorrelated with the first dimension of the MFA. The second dimensions were correlated better with the second MFA dimension but not represented as well

Executive Environmental Agency 2017). As a variable, elevation hints at differences in local microclimate conditions, the effect of the surrounding vegetation (i.e. canopy drip), and other confounding variables discussed by Fernández et al. (2015), Nickel et al. (2014, 2017), and Økland et al.
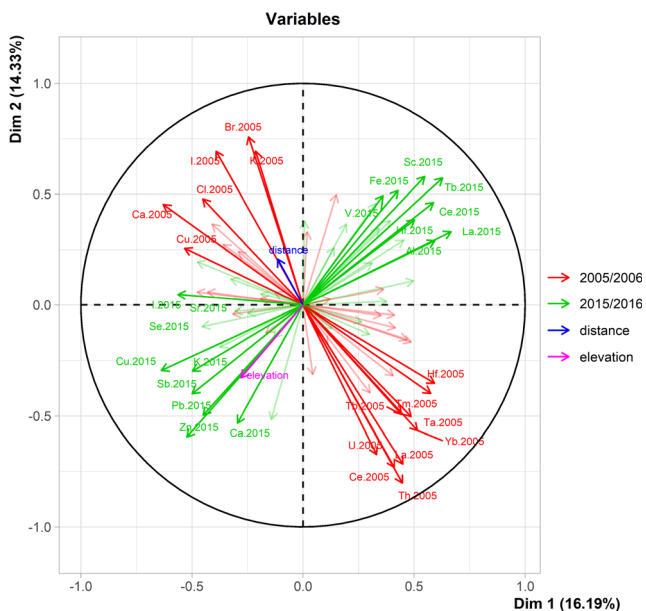
Fig. 6 Correlations between the elements: the quadrants with the "distance" and "elevation" arrows (and the ones opposing them) define the elements most affected by the two supplementary variables. Only the variables with the highest scores on the two first dimensions were labelled (for the sake of legibility)

(1999). It could be hypothesised that the differences in climate conditions in the typically dry lowlands versus the relatively high humidity of the forests of the mountains were amplified during the dryer period of the 2015/2016 survey, which caused an increased variance in the dataset.

The concentrations of elements associated with a typically geogenic origin were the predominant sources of variability in both surveys, even though their measured concentrations remained relatively constant at each separate sampling site. Following the mathematical convention for a counter-clockwise numbering of the quadrants, most of the REEs determined in the 2005/2006 moss survey were projected in the fourth quadrant in Fig. 6, while the REEs from the 2015/2016 campaign were in the first quadrant. REEs have low mobility and are rarely emitted from atmospheric anthropogenic sources (Allajbeu et al. 2016; Lee Jr and Von Lehmden 1973; Pais et al. 1997). The moss biomonitoring species *Hypnum cupressiforme*(Hedw) sps. was used in Albania for the determination of spatial distributions of REEs and it was confirmed that their deposition originated from soil and dust particles and that the lithological effects masked any atmospheric anthropogenic influence (Lazo et al. 2019). The most common sources of REE enrichment in soils are phosphate fertilisers (Allajbeu et al. 2016). It has been suggested that the elevated heavy metal load commonly determined in Eastern Europe might be partially explained by the presence of natural sources for environmental metals (e.g. serpentinites), or overall drier mineral soils (Harmens et al. 2014; Mposkos et al. 2013). The contribution of soil and rock particles in the dry deposition fraction could be reduced only by modifying the recommended sample preparation procedures in the ICP Vegetation protocols.

All the clr-transformed concentrations of elements positively correlated with "elevation" were represented in the third quadrant in Fig. 6. In one active moss biomonitoring study, it was demonstrated that for most of these elements (As, Co, Ca, Sr, Se, W, and Zn) humid conditions improve the capacity of retention potential by the moss tissue (Aničić et al. 2009). Negative correlations with this variable were determined for the elements in the first quadrant: predominantly REEs from the 2015/2016 dataset, hinting at more pronounced soil erosion and windblown dust affecting the samples during drier seasons. Distance, as a supplementary variable, was projected in the second quadrant in Fig. 6, along with the transformed concentrations for the halogens Br, Cl, and I from both datasets, as well as K, Ca, and Cu from the 2005/2006 dataset.

A superimposed representation of each sampling site from the "point of view" of the two groups allowed to further investigate the changes occurring between the two surveys. The resulting points, called partial points, are shown in Fig. 7 connected with a line, at the centre of which lies the projection of the data point as represented by the mean configuration of the MFA. For the majority of the sites, the changes (represented by the length of the lines) were apparent. A horizontal shift
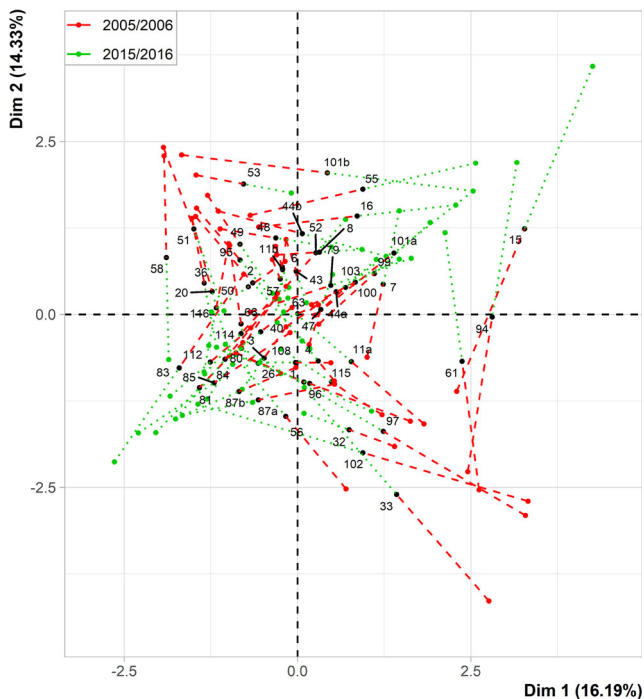
Fig. 7 Mean configuration of the MFA with plotted differences between the observations for the surveys projected to the first factor plane

implied an association with the variables correlated with the first dimension, and a vertical direction of the shift was associated mainly with variables correlating with the second dimension. Information about the elements correlated with the two axes and their respective correlation coefficients and *p*-values can be found in the supplementary material.

Dimension 1 was positively correlated with the REEs and Al from the 2015/2016 dataset, and negatively correlated with the halogens from the 2015/2016 dataset, as well as Pb, Zn, and Cu from both datasets; and Ca, and Cd from the 2005/2006 dataset. A horizontal direction of the shift was observed for sites 101, 53, 55, and 16: their 2005/2006 partial points of fell within the second quadrant and, with the exception of site 53, the 2015/2016 partial points were projected within the first quadrant (quadrants are labelled in counter-clockwise order). Site 53 was located near the town of Ahtopol, very close to the Black Sea coastline. Both of the partial points fell within the second quadrant, defining a rather short "shift". Site 55 was located 16 km to the west of site 53, within the territory of Strandzha Nature Park. Site 15 was located close to the Makaza–Nymfaia cross-border, which connects Bulgaria and Greece since 2013. Site 16 was located near the town of Zlatograd, where, in 2010, a border checkpoint with Greece was inaugurated (Zlatograd-Thermes). Therefore, it could be concluded that the 2015/2016 partial points projected in the first quadrant reflected increased traffic volume and construction of roads near the border with Greece.

Sites 63 and 47 were projected very close to the origin of the coordinate plane. Their locations were 25 km to the north and

17 km to the southeast of the Maritsa Iztok Complex, respectively. The Maritsa Iztok Complex is the largest opencast lignite field mined in the Balkans (the Maritsa Iztok coal basin) with three adjacent lignite-fired thermal power stations. According to the wind rose for the region, North, Northwest, and South are the most frequent directions of the wind. In 2014, the European Environment Agency ranked one of the Maritsa Iztok lignite-fired power stations as the industrial facility causing the highest damage costs to health and the environment in the European Union (European Environment Agency 2014). Still, in the case of industries with chimneys, the industrial output should not be the main source of atmospheric emissions in radii over 10 km; therefore, sites 63 and 47 were likely, not representative of atmospheric pollution caused by the Maritsa Iztok Complex. It was determined that site 63 was located about 1.5 km away from the Alamura open-pit quarry, while site 47 was located in the vicinity of croplands.

Dimension 2 was positively correlated with the halogens Br, I, and Cl from the 2005/2006 dataset, also K, Ca, and Ni from the 2005/2006 survey, and the metals Fe, Co, and V from the 2015/2016 dataset. REEs from the 2015/2016 dataset were positively correlated with this dimension, too. The strongest negative correlation coefficients with Dimension 2 were observed for the elements U, Th, La, and Ce (REEs) from the 2005/2006 dataset, and the elements Ca, Zn, Pb, Cd, and Sb from the 2015/2016 survey.

Sites 61, 94, and 15 were projected close to each other (first and fourth quadrants) and the direction of their shifting was mostly parallel to the secondary axis. Site 94 was located between Zlatna Panega, one of Bulgaria's largest cement factories, the Elatsite copper mine, the Asarel copper extracting and processing factory, and the Chelopech copper and gold mine. The region is rich in copper sulfides, porphyry copper deposits, and polymetallic ores. Site 61 is located in the region of Sliven, near a farmyard. On the opposite side relative to the origin the sites 51, 58, 36, 20, 66, and 116 were projected. Sites 51 and 58 were both located in the vicinity of quarries. Site 116 was located near Sopot, where an explosion of an ordnance plant took place in 2012. Sites 20, 36, and 66 were located in different rural parts of the country and their grouping may reflect the input of gravel road dust.

The fourth quadrant contained the sites 32, 33, 97, and 102, located in different regions of the country, yet all of them were collected along the peripheries of croplands and had a similar direction of the shift, with the partial points for the 2015/2016 dataset projected close to the secondary axis.

Conclusions

After correcting for moss biomonitor species and geographic location, the results from the 2005/2006 and 2015/2016 moss surveys in Bulgaria were compared. Hierarchical clustering on

principal components of the clr-transformed data illustrated the similarities between the datasets and proved to be effective in determining the decreased atmospheric pollution loads in the area of the Kardzhali lead-zinc plant, where the production processes ceased in 2011. Multiple factor analysis was used to assess that distances between the paired sampling points, and elevation had no strong effect on the overall variance in the data. The transformed concentrations of rare earth elements along with other elements of typically edaphic and lithogenic origin had a major role in constructing the MFA factor plane. This confirmed the strong influence of secondary pollution by dust and soil particles in the moss samples, as well as the increased number of quarries used for road construction, and hinted at the decreased number of environmental hot spots in Bulgaria related to the heavy industry. In the 10 years elapsed between the two surveys, the effect of the shutdown of several major industrial facilities and the implementation of improved air pollution control systems is evident and illustrated by deposition maps for the toxicant Pb, regularly reported to the ICP Vegetation programme.

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