The use of passive lichen biomonitoring in combination with positive matrix factor analysis and stable isotopic ratios to assess the metal pollution sources in throughfall deposition of Bolu plain, Turkey

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A B S T R A C T

The main objective of this study was to determine the elemental content of (51 elements) and stable isotope (δ13C, δ15N) content of lichen, Xanthoria parietina. The results were evaluated according to seasonal changes, pollution source, and altitudes. The data indicated that there is no significant impact of seasonal variation in concentrations of most of the elements and isotopes. The uptake efficiency of Xanthoria parietina and pine needle was compared and it was found out that significantly higher amounts of metals were accumulated by Xanthoria parietina than by pine needles. The lowest δ13C and the highest δ15N values were obtained in pine needle in both seasons. Both δ13C and δ15N showed increasing (for summer) and decreasing (for winter) trends with a rise in altitude.

Source apportionment of elements was performed by using positive matrix factorization (PMF) and supported by G-score maps. As a result, six sources were determined for the area namely urban environment, lichen nutrient elements, contaminated soil, traffic and mixed soil, lichen metabolism and coal combustion. The concentration pollution maps and G-score maps of Pb and Ba were similar and this was an indication of the vehicle emissions. The elements As, Bi and Tl signed the coal combustion.

1. Introduction

Atmospheric pollution has become a major environmental concern due to its adverse effects on human health and the ecosystem (Desauziers, 2004; Goienaga et al., 2013). There are many methods used to obtain reliable information on the air quality and characteristics of the environment. Compared with the conventional air sampling methods, biomonitoring is an easy, cheap and indirect method of determining pollutants and their distributions over large areas (Sloof, 1993; Yenisoy-Karakas and Tuncel, 2004a).

In biomonitoring, information about the air quality of certain area is obtained by using the properties of an organism or a part of it (Sloof, 1993). Various organisms such as lichens (Conti and Cecchetti, 2001; Gür and Yaprak, 2011; Beck and Mayr, 2012; Demiray et al., 2012; Paoli et al., 2014), mosses (Mendil et al., 2009; Agnan et al., 2015), ferns (Ho and Tai, 1985), tree barks (Loppi et al., 1997; Boltersdorf et al., 2014), tree rings (Cocozza et al., 2016) and pine needles (Rudawksa and Leski, 2005; Al-Alawi and Mandiwana, 2007; Kwak et al., 2009) can be used as biomonitor organisms. In this study, the most common epiphytic lichen, Xanthoria parietina (L.) Th. Fr. (XP) and pine needles were chosen as biomonitor organisms. Lichens are commonly stated as the best suitable bioaccumulators of trace elements because their metabolism is dependent on pollutant atmospheric exchanges and they are metal tolerant (Bargagli and Nimis, 2002). The concentrations of the trace elements in the lichen tissues were found to be directly correlated with the environmental levels of these elements (Yenisoy-Karakas, 2000; Bari et al., 2001). Bari et al. (2001) It was stated that the correlation between the transplant and the air metal concentrations was positive and significant for Fe, Ni, Zn, Cd, Pb and Cu, and positive but not significant for Cr. Yenisoy-Karakas (2000) also expressed that there were positive and significant relationship between the transplanted values and the cumulative total deposition values of Cr, K, Mn, Na, Cd, Zn, Al, Fe, Mg and V elements. Pine needles are also effective biomonitor due to the excellent uptake properties of their waxy layer (Holoucek et al., 2000; Xu et al., 2004; Al-Alawi and Mandiwana, 2007).

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Biomonitoring of lichens and pine needles was applied to determine elemental pollution in many studies (Nimis et al., 2000; Scerbo et al., 2002; Brunialti and Frati, 2007; Agnan et al., 2013; Yenisoy-Karakoş and Tuncel, 2004a; Al-Alawi and Mandiwana, 2007; Demiray et al., 2012; Cocozza et al., 2016; Boamponsem et al., 2017) but there are relatively few studies focused on stable carbon and nitrogen isotopes examined in lichen and/or pine needles (Lakatos et al., 2007; Batts et al., 2004; Kwak et al., 2009; Cipro et al., 2011; Beck and Mayr, 2012). With the help of isotope research, it is possible to identify potential carbon and nitrogen sources, to which the biomonitor organisms are exposed (Robinson, 2001; Kwak et al., 2009; Boltersdorf et al., 2014). This is the first study in Turkey that determines stable carbon and nitrogen isotope ratios in the lichen and pine needles to monitor the air quality. Besides, biomonitoring methodology was applied for the first time in Bolu plain and it will be a baseline for future studies in Bolu.

The objectives of this study are as follows: (1) to determine the elemental content, carbon, and nitrogen isotopic ratios; (2) to determine the levels of pollution in Bolu plain; (3) to evaluate the variability between seasons; (4) to determine the seasonal change of isotopes with altitude and determine if they could be used as proxies for atmospheric pollution; (5) to assess the spatial distribution of 51 elements and C-N isotopes; (6) to identify pollution sources; (7) to compare the accumulation capacities of 51 elements and C-N isotopes in lichens and pine needles; (8) to compare the levels of air pollution with other studies.

2. Material and methods

2.1. Sampling area

The study was conducted in the city of Bolu which is in the Western Black Sea Region of Turkey. It has a population of around 300,000 and the total area of the city is 8323.9 km². 15% of the total area is used for agriculture (Bolu Environmental Status Report, 2016). Despite the small percentage of the area used for agriculture, this city is important in agriculture for Turkey. Moreover, Bolu city center hosts a small number of industrial sectors including cement, poultry, woodworking, metal and glass (Bolu Environmental Status Report, 2016). The average altitude of the province is 1000 m while that of the city center is 725 m. The height of mountains increases from four sides of the city center and it causes the lowest mixing height especially in the winter season (~150 m) (Bayındır et al., 2010). The climate of the city is like the Black Sea Region and generally is warm in summer, cold in winter and rainy in all seasons. Over the past 89 years, the mean annual precipitation was 388.1 mm and the mean annual temperature was 12.0 °C (Turkish State Meteorological Service, 2019). Generally, West South West winds dominate within a year and transport the emissions of the most industrialized cities of Turkey, like Istanbul, Kocaeli, Gebze, Adapazarı, Düzce and countries from Eastern Europe.

The location of the sampling points for both winter and summer campaigns is illustrated in Fig. 1. The sampling area can be categorized into 5 zones according to the sources of pollutants. The first zone has the highest altitude forested areas where the small villages are located. The second zone is near to sand quarries and a coal mine and there are hen houses around the villages. The highly populated area in Bolu is in the third zone (the city center). The two highways, TEM and D-100 (parallel to TEM) connecting to two big cities Istanbul and Ankara are passing through the city center. There are agricultural and industrial areas in the city center. The city center is encircled with high altitude mountains. The fourth zone includes the road reaching to Kartalkaya ski center which is the second important ski center in Turkey. There are hen houses in the fourth zones. The very popular tourism center such as Abant Lake is located in the fifth zone. The campus of Bolu Abant Izzet Baysal University is also in the fifth zone.

2.2. Sampling, preparation of the samples and analyses

Sampling of lichen species and pine needles were performed in November 2012 and in June 2013 within 0.12175 km² area of Bolu. Both sampling campaigns lasted in five days. The study was conducted in 1 × 1 km grids in the winter campaign in the city center, it was changed to 3 × 3 km grids in the summer campaign due to absence of lichen species in every 1 × 1 km grid. Furthermore, no samples could be collected in some of the grids because there were no trees or because the trees had no lichens. In winter sampling, 115 samples of XP (n = 102) and pine needles (n = 13) were collected. Totally 113 samples (XP (n = 64) and pine needles (n = 49)) were sampled during summer sampling.

Lichen thalli with different sizes were collected from 1 and 2 m height from the ground and around the tree by using a steel knife. If enough lichen samples on the branches or trunks of the trees could not be found, they were collected from the tree base. Thus, the soil suspension was dominated and should be avoided by washing the lichens (Yenisoy-Karakoş, 2000). Species from the same tree and from 3 to 7 nearby trees were combined in a grid to obtain a representative sample of the point (Yenisoy-Karakoş and Tuncel, 2004a; Agnan et al., 2013; Paoli et al., 2014). Yenisoy-Karakoş (2000) stated that different size ranges (< 2.5 cm and > 2.5 cm) of lichens collected at the same sampling points did not show any statistically significant difference (p = 0.05) based on the collection capacities. To avoid contamination, samples were handled only with polyethylene gloves and carried in acid-washed polyethylene bags.

The lichen species were separated from the debris and soil particles by putting in a beaker filled with deionized water and rinsing few seconds. Then, the cleaned thalli were put in folded filter papers and air-dried in a clean glass box in the cleanroom for approximately one day at room temperature (Yenisoy-Karakoş and Tuncel, 2004a). The pine needle samples were also rinsed in deionized water for a few seconds. They were dried using the same method as the lichens. The dried lichen and pine needle samples were kept in the labelled acid-washed polyethylene bags.

All digestion apparata were waited in 30% (v/v) HNO₃ (65%-Merck, Germany) bath for one day. Samples (0.4 g) were placed into Teflon digestion vessels with a clean Teflon forceps. 0.5 mL suprapure 48% HF (Merck, Germany) and 5 mL 60% ultrapure HNO₃ (distilled by subboiling) and 1 mL 31% H₂O₂ (Merck, Germany) were added into the digestion vessels. They were digested in the microwave oven (Berghof, Germany). After the microwave digestion process, the vessels were cooled to room temperature. The content of the vessels was transferred into polypropylene centrifuge tubes and diluted to 50 mL with 18.3 μs ultra-pure deionized water (SG-Labostar, Germany) (Yenisoy-Karakoş and Tuncel, 2004a; Agnan et al., 2013; Paoli et al., 2014). Following the digestion, all samples were kept in the refrigerator until analysis. Analysis of lichen and pine needle samples were carried out by using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin Elmer, USA). Samples were analyzed for a total of 51 elements (Li, Be, Na, K, Mg, Al, Ca, Ba, Ti, V, Cr, Sc, Mn, Fe, Co, Ni, Cu, Zn, Ge, As, Se, Rb, Sr, Y, Mo, Cd, Sn, Sb, Cs, La, Ce, Pr, Nd, Eu, Sm, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, W, Pb, Hg, Bi, Th, U and e). In order to check the accuracy of ICP-MS analysis, acid blank samples and certified reference materials (SRM 1648a-Urban Particulate Matter (NIST), SRM 1575a-Trace Elements in Pine Needles (NIST) and Reference Material IAEA-336 Trace and Minor Elements in Lichen (IAEA)) were prepared and analyzed as samples.

Before isotopic composition analysis, dried lichen and pine needle samples were powdered by agate mortar and pestle and kept in the labelled Eppendorf tubes. The contents of stable carbon and nitrogen isotopes were specified with the Thermo Finnigan DeltaPlus Xp Continuous Flow Isotope Ratio Mass Spectrometer which is connected to a Thermo-Electron Flash EA 1112 Model Elemental Analyzer with Thermo Finnigan ConFlo IV interface (EA-IRMS, Thermo, USA) in Turkey Atomic Energy Agency Sarayköy Nuclear Research Center. In
order to solve the repeatability problem, especially during the stable nitrogen isotope measuring process, samples were freeze-dried in a liquid nitrogen tank and so, they were homogenized through a process of pulverization. Then, 0.1 mg was used for the stable nitrogen isotope determination. Moreover, 2.0 mg sample for the determination of carbon isotope was analyzed.

2.3. Data analysis

2.3.1. Calculation of isotope fractionation

Both carbon and nitrogen have two stable isotopes, $^{12}\text{C}$, and $^{13}\text{C}$ for carbon and $^{14}\text{N}$ and $^{15}\text{N}$ for nitrogen. The ratio of one isotope relative to the other varies slightly with chemical structure and the environment (Batts et al., 2004). Such small changes in the isotope ratio are referred to as fractionation. The extent of this isotope fractionation is expressed in terms of a ‘$\delta$’ value. For carbon, for example, $\delta^{13}\text{C}$ is a measure of the change in the proportion of $^{13}\text{C}$ to $^{12}\text{C}$ in the sample of interest, relative to that in a standard substance. The unit of this measurement is ‘per mil’ ($‰$) (Batts et al., 2004).

$\delta^{13}\text{C}=\left(\frac{\text{nitrogen}}{\text{carbon}}\right)_{\text{lichen}}-\left(\frac{\text{nitrogen}}{\text{carbon}}\right)_{\text{standard}}\times 1000\%$

2.3.2. Mann Whitney (Wilcoxon) $W$ test

The lichen data showed lognormal distributions and tend to be influenced by outliers. To overcome this situation, the nonparametric test can be performed to compare the medians rather than the means. In the examination of differences in seasonal ratios of elements and isotopes in lichen samples, the comparison of the median values was performed using the Mann-Whitney (Wilcoxon) $W$ test. This test was carried out in STATGRAPHICS® Centurion XV. In this test, the combined data of the two samples are ranked from 1 to $n_1 + n_2$, and the original data values are replaced by their respective ranks. A test statistic $W$ is then constructed comparing the average ranks of the observations in the two samples (The User’s Guide to STATGRAPHICS® Centurion XV, 2005).

2.3.3. Positive matrix factorization (PMF)

Source apportionment helps giving information about pollution sources (Lage, 2016) and their contributions to air pollution. There are three types of approaches for source apportionment: Emission inventories, source-oriented models and receptor-oriented models. These models classify receptors, the measured mass of an atmospheric pollutant at a given site, to its emission sources by using multivariate analysis to solve a mass balance equation. The receptor-oriented models are among the source apportionment studies which focus on the properties of the ambient environment at the receptor site (Almeida et al., 2015). In addition, the application of receptor modeling to chemical compositions of biomonitor like lichens is a method that helps to give information about pollution sources in large areas (Lage et al., 2016). In this study, PMF version 5.0 was used as a receptor-oriented model to analyze source apportionment since nonnegative constraints are applied in PMF (Manousakas et al., 2015). The method is a multivariate factor analysis tool that uses a matrix of speciated sample data into two matrices (Norris and Duvall, 2014). These are factor contributions ($G$) and factor profiles ($F$). The results should be interpreted by the analyst to determine the pollution sources. It allows each data point to be individually weighed and it helps to adjust the influence of each data point, depending on the confidence in the measurement (Norris and Duvall, 2014). In addition, it uses sample concentration and associated with the sample data uncertainty which is determined by the user for individual points.

Two matrices are derived by using the minimized objective function, $Q$, in PMF:

$Q = \sum_{i=1}^{n_1}\sum_{j=1}^{n_2} \left[ x_{ij} - \sum_{k=1}^{P} u_{ik} F_{kj} \right] \quad \text{(Norris and Duvall, 2014)}$

where $x$ is the data matrix of $i$ number of samples by $j$ chemical species which were measured, and $u$ is the uncertainties. PMF solves the problem iteratively and this minimizes the $Q$. Factor profiles can be affected by each PMF run. Each run must be evaluated by the user. The user must also evaluate all error estimates.

In this study, the mass concentrations of 51 elements determined in lichen samples in both seasons were included in the PMF analysis. The concentration file of summer and winter samples were combined for the PMF analysis due to the need for the extensive data (Norris and Duvall, 2014). The concentration file had no missing values. The equation-based uncertainty file was prepared by using the equation (Norris and Duvall, 2014):

$u = \sqrt{(0.1 \times \text{Concentration})^2 + (0.5 \times \text{MDL})^2}$

If the signal to noise ratio (S/N) was less than 0.5, the species was categorized as ‘Bad’ and if the (S/N) ratio was greater than 0.5 but less than 1 species was categorized as ‘Weak’ (Norris and Duvall, 2014). Copper, Zn, As, Cd, Bi were categorized as Weak and Ca, Se, Sn, Sb, W, Mo, Hg were selected as Bad. Nine samples were discarded due to not having residuals between + 3 and − 3 (Norris and Duvall, 2014). The PMF model was tried with five to seven source factors. $Q_{\text{robust}}$ and $Q_{\text{true}}$
values show how the model fits input data (Norris and Duvall, 2014). $Q_{\text{robust}}/Q_{\text{expected}}$ was found to be 1.02 for six factors. If the values are greater than 1.5, we increased the number of factors. Another parameter to check the quality of the run is to calculate $Q_{\text{robust}}/Q_{\text{expected}}$ ratio. $Q_{\text{expected}}$ was calculated by

$$Q_{\text{expected}} = nm - p(n + m)$$

where $n$ is a number of non-weak data points, $m$ is a number of species and $p$ is a number of factors which is used for PMF analysis (Norris and Duvall, 2014). $Q_{\text{robust}}/Q_{\text{expected}}$ value was lower than 1.5 for six factors. Error estimation by Displacement method (DISP), which explores the rotational ambiguity in a PMF solution by assessing the largest range of effects of a small set of observations on the solution and effects of rotational ambiguity to the solution (Norris and Duvall, 2014). The minimum number of BS runs, 20, was performed and 100%, 98%, 87%, 97%, 100%, and 100% mapping were observed for Factor 1, Factor 2, Factor 3, Factor 4, Factor 5 and Factor 6, respectively. Eighty percent mapping of each factor represents that the PMF solution is acceptable (Norris and Duvall, 2014). GA is the amount of mass contributed by each factor to individual sample (Gupta et al., 2012) and therefore the G scores of resulting solutions for each factor were mapped by using MapInfo Professional 7.5 for visualizing each source.

3. Results and discussion

3.1. Bioaccumulation in lichen samples with seasonal aspect

The summary statistics including the count of quantified samples, arithmetic mean, median, geometric mean, standard deviation, minimum and maximum concentrations of the elements investigated in lichen species collected in the winter and the summer field campaigns are given in Tables S1 and S2, respectively. The concentrations were expressed in terms of $\mu$g g$^{-1}$ except for $\delta^{13}$N and $\delta^{13}$C which were represented as ‰.

In evaluation, median concentrations were used since the elements measured in this study were log-normally distributed. Among major elements (K, Na, Mg, Fe, Ca, Al and Ti), the lowest median lichen concentration was found for Ti (93 $\mu$g g$^{-1}$) and the highest median lichen concentration was found for K for the summer season (2465 $\mu$g g$^{-1}$). Major elements showed a similar trend in the winter season. Titanium had the lowest median lichen concentration (98 $\mu$g g$^{-1}$) and K had the highest median lichen concentration (2465 $\mu$g g$^{-1}$) in the winter season. The abundance of K in lichen species can be a result of their intake both from the atmosphere and from the leachate of other dead foliage (Steinnes et al., 1992). In addition to major elements, trace elements were investigated. The lowest median lichen concentration was found for Lu for the summer season (0.0055 $\mu$g g$^{-1}$) and the winter season (0.0057 $\mu$g g$^{-1}$). Manganese was the trace element with the highest median concentration for both in the summer (25 $\mu$g g$^{-1}$) and the winter (27 $\mu$g g$^{-1}$) in lichens.

The elemental concentrations of lichen species in the winter and the summer were similar for most of the elements. Except for Hg (the ratio was 3.95), the summer to winter ratios were found between 0.80 and 1.80. To compare seasonal concentrations, the ratios of medians of the elements were compared by using Mann-Whitney (Wilcoxon) W test. According to the Mann-Whitney (Wilcoxon) W test, most of the elements did not show a statistically significant difference between the seasons at the 95% confidence level ($p = 0.05$). The elements which had significant differences between seasons were found to be Hg, K, Rb, Mo, Mg, Hg, Cu, Ca, As, and Cd with the summer to winter ratios of 3.95, 1.79, 1.36, 1.16, 0.88, 0.85, 0.81, 0.80, respectively. The coal combustion derived elements such as As and Cd showed an increase in the winter season whereas the lichen metabolism elements such as K, Mg and Rb showed an increase in the summer season. The presence of natural sources such as outgassing of the crustal material, water bodies, vegetation surfaces, wildfires, and geothermal sources can lead to strikingly higher Hg concentration in the summer season (Schröder and Munthe, 1998).

In the summer season, the median values of $\delta^{15}$N and $\delta^{13}$C for lichen samples were found as $-10.24$ and $-22.47‰$, respectively. On the other hand, they were obtained as $-9.64$ and $-22.41‰$, respectively, in the winter season (Fig. 2). Thus, isotope signatures of Xanthoria parietina did not show great differences between seasons (Mann-Whitney (Wilcoxon) W test, $p = 0.05$).

3.2. Comparison with literature

The element contents measured in XP were compared with data reported in the literature (Table S3). The concentrations of the majority of the measured elements in XP were very similar to those reported in the literature. However, several factors such as site characteristics, meteorological conditions, and sample handling technique must be also considered in the comparison of the data. Among the literature studies presented in Table S3, in Yeniso-Karakaş (2000) and the present study, lichens were washed before analysis because, without moisturing, lichen thallus could not be separated from the tree bark (Yeniso-Karakaş, 2000). This washing technique is used to separate lichens from the tree barks and remove accumulated dust. Yeniso-Karakaş (2000) and Saiki et al. (1997) stated that washing procedure has no significant effect on Xanthoria parietina for most of the elements such as Mn, Zn, Cr, V, and Cd while concentrations of soil-derived elements such as Al,

![Fig. 2. Comparison of stable carbon isotope ($\delta^{13}$C) values and stable nitrogen isotope ($\delta^{15}$N) values in summer and winter seasons. X denotes the arithmetic mean of the isotopes measured at various sites in Bolu, Turkey.](image-url)
Mg, K, Na, and Fe generally decreased as a result of washing process. Hence, the washing procedure may lead to losses on XP for soil-derived elements. When the concentrations of the elements were compared, the elements As, Cu, V, and Ni were found to be higher in Kocaeli industrial area than our sampling area. These elements were also determined as associated with industrial areas by Lage et al. (2014). Arsenic is a coal-burning indicator (Yenisoy-Karakas and Tuncel, 2008) was the highest level (14.2 µg g⁻¹) in Soma where coal-fired power plant has operated (Gür and Yaprac, 2011). It was followed by İzmir (3 µg g⁻¹) and Kocaeli (1.63 µg g⁻¹) where many industrial districts were located (Demiray et al.; 2012; Yenisoy-Karakas, 2000). The two latter followed also by Albi (1.29 µg g⁻¹) where there is a power plant disused since 1988 (Ağan et al., 2013). Arsenic concentrations of this study (1.19–1.47 µg g⁻¹) was lower than the previously mentioned cities. However, the usage of Turkish coal including the highest proportion of As led to having higher concentrations in Bolu than le Séquestre (0.53 µg g⁻¹) (Yenisoy-Karakas and Tuncel, 2004a). Moreover, the waste incineration indicators Cd, Ni, and Zn were found in higher concentrations in other studies except le Séquestre than this study (Paoli et al., 2012). Higher concentrations of Pb and Ba were drawn attention in urban cities. These elements were mostly related to vehicular emission (Paoli et al., 2013). In cities that contain industrial districts, the concentrations of elements like Se, Sr, Zn, Cs, Rb, Ba, and Co were found to be higher than in this study (Frati et al., 2005).

Stable isotopes measured in this study were compared with the other isotope studies performed on XP (Table S4). The lowest (more negative) δ15N values (−1.8 to 1.44‰; −15 to −0.4‰) were found in Bolu which is a semi-urban region with dense agricultural activities. It was followed by the study carried out in Bavaria (-1 to 1.2‰) that include urban, semi-urban and rural locations (Beck and Mayr, 2012). Forestry dominated area, Sweden, had less negative δ15N (-4.2 to −2.4‰) values than the urban and highly agriculture affected areas, and more negative than the coastal areas of France (−1.9 to 0.3‰) (Boltersdorf and Werner, 2013). The results demonstrated a similar pattern with the results of Boltersdorf et al. (2014). Moreover, the effects of substrate on δ15N values of lichens could be considered. Laurand and Riera (2006) collected XP from the minerogenic (rocky) surface whereas the other studies used XP growing on an organic substrate (wood or tree). In Table S4, lichens that were growing on the minerogenic substrate had significantly different δ15N values. The extra depleted δ15N values observed on the organic substrate could be caused by the presence of additional or different N sources that are not available to rock lichens (Beck and Mayr, 2012).

δ13C values of this study (−24 to −21; −24 to −20‰) were close to the values of XP collected in Germany (−25.3 to −17%) by Beck and Mayr (2012). The presence of similar carbon sources, for example, domestic heating, in sampling areas of both studies was effective in the closeness of the values. The δ13C values of XP from a rocky substrate (France) showed the more positive average and closer range (−19.1 to −19.0‰) than those grown on an organic substrate (other studies). The observed δ13C differences could be caused by the uptake of respiratory CO₂, light level, water availability and additional organic carbon uptake on different substrates (Beck and Mayr, 2012).

### 3.3. Source apportionment of elements by using positive matrix factorization and G-score maps

The PMF model solution with six sources was obtained with the justification of bootstrap and displacement tests. The six estimated sources are given in Table 1 and as a pie chart in Fig. S1. The identification of factors was made with the comparison of chemical profiles and pollution maps.

Factor 1 explained 2.56% of total concentration was characterized with the elements Sr (49.7%), Cd (43.9%), Zn (43.6%), Cu (39.1%), As (36.1%), Ba (30.1%), Co (25.9%), Pb (23.6%), Bi (22.1%), Ni (19.4%), Mn (17.1%), Hf (13.4%), Cr (12%), Na (10.8%), and Ti (10.4%). Zinc, Cu, As and Pb are the inorganic marker elements of non-ferrous metal industries. Besides, Ba and Sr came from the mechanical abrasion of brake and tyres (Calvo et al., 2013). Arsenic, Bi and Tl are indicator elements of coal combustion (Yenisoy-Karakas, 2000; Calvo et al., 2013; Karadeniz, 2017). This combination of traffic, industrial and coal combustion indicators is the sign of the urban atmosphere of Bolu. Hypsll Trajectory Frequency was drawn and winds from WSW direction were dominantly explained in Fig. S2. The city is covered by high altitude mountains and winds coming from the Marmara region, Black Sea region and Europe are entering from the WSW direction to the city center (Yenisoy-Karakas et al., 2012). Consequently, lichen metabolisms were directly affected not only Bolu city but also the industrial cities like Istanbul, İzmir. The G scores of Factor 1 were mapped and the G scores of samples in lower altitudes (the city center) were found to be higher than the others. Especially the city center and villages around the city center (third zone) are under the influence of urban atmosphere (Fig. 3).

It was reported that elements like K, Rb, Cs, and Mg were enriched in the lichens or plant materials (Yenisoy-Karakas, 2000; Frati et al., 2005). In our study, two factors were represented by these elements. Factor 2 was characterized by the nutrient elements K (77%), Mg (44%), Rb (26%), Zn (25%), Cu (19%). Factor 5 was also characterized by Rb (63%) and Cs (18%). Factor 2 and Factor 5 were accounted for 44.2% and 7.15%, respectively. Since the concentration of K element in the lichen metabolism was exchangeable depending on the heavy metal accumulation or stress (Brown and Brown, 1991; Adamo et al., 2003), lichens in the highly urbanized areas like third zone, including the city center, were affected by the heavy metal accumulation and had lower G scores in Factor 2. The first, second and fourth zones had higher G scores in Factor 2. These areas are generally less dense settlement areas or villages. The concentration maps of K and Rb elements showed similar trends in the pollution maps (Figs. S3 and S4) because of both being metabolism elements. The difference in the pollution maps came from the higher concentrations of K in the borderline areas (mostly the northern part of the area) where the villages are located. In the villages, the most common way of heating was the wood-burning in stoves and this explained the high concentrations of K in the lichens. The third zone (the city center) is the highly polluted area in the city. Therefore, the concentrations of K and Rb in the samples in some parts of that area were lower than the other areas due to the most probably the exchange of heavy metals with K and Rb.

Factor 3 was accounted for 23% of total elemental concentrations in the lichen data and involved the elements Al (59.4%), Y (48.4%), Lu (45.9%), Tb (43.9%), Tm (43.7%), Er (43.1%), Ho (43.1%), Sc (42.2%), Dy (41.9%), Tb (41.3%), Fe (39.9%), Mg (39.2%), Sm (38.8%), V (38.7%), Gd (38.6%), Tb (36.9%), Eu (36.1%), La (35.3%), Ge (35.1%), Nd (34.7%), Pr (34.6%), Ce (33.2%), Ti (31.3%), Na (28.3%), Mn (25.5%), Cr (24.6%), Ni (24.3%), Co (24.3%), Li (22.7%), and Pb (20.4%). Aluminum, Fe, Na, Ti, Mn, Sc, Sm, Ce, La, Cr, and V are the known earth elements (EC, 2004) and these elements were explained in the range of 59–24%. The other elements except for Pb and Co were also known as rare crustal elements (Yenisoy-Karakas, 2000). The elements of Pb and Co are mostly traffic and coal-related. The concentrations of each element near to agricultural areas, TEM and D-100 ways and sand quarries in Factor 3 were higher than the other areas in both seasons. In addition, the higher contributions for this factor were observed in sites relatively close to agricultural areas and sand quarries. As a result, the contribution of contaminated soil to lichens was observed in Factor 3.

Factor 4 was explained by the elements like Li (45.4%), Ba (35.7%), Na (35.6%), Ni (33.8%), Fe (32.3%), Cr (29.5%), Pb (28.0%), Mn (27.7%), Co (25.8%), Sr (22.0%), As (21.4%), Al (19.6%), Zn (19.3%), and Cu (18.7%) and some of the crustal elements (between 58.2% and 22.0%). This factor explained 17.5% of the total concentration. Aluminum, As, Ba, Co, Cr, Fe, Li, Mn, Na, Ni, Pb, Sr, Zn, and Cu are emitted by car brake linings and car brake dust (Thorpe and Harrison,
Vanadium, Ni, Mn, Fe, Cr, and As are also sources of oil burning (Calvo et al., 2013). In the G score map (Fig. 4), high scores were obtained especially close to highways. Besides, the concentration map of the Pb supported G score map (Fig. S5) since the lichens show long term accumulation. Therefore, it was seen that Pb had still been the tracer of vehicle emission in lichens although Pb in aerosol samples collected between 1st of March 2013 and 1st of March 2014 in Bolu was not found to be a tracer of the traffic emission (Karadeniz, 2017). The new tracer element for traffic, Ba, was higher near to TEM and D-100 highways and general roads due to mechanical abrasion of brakes (Calvo et al., 2013) (Fig. S6) and the trend of concentration distribution of Ba was similar to both G score map and the concentration map of Pb.

Factor 6 was characterized by the elements Tl (81%), Cs (38%), Bi (38%), Cd (20%), Cr (20%), As (16%), and Pb (17%), and so it was

### Table 1

<table>
<thead>
<tr>
<th>Factors</th>
<th>Elements</th>
<th>Sources</th>
<th>Explained percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor 1</td>
<td>Sr (49.7%), Cd (43.9%), Zn (43.6%), Cu (39.1%), As (36.1%), Ba (30.1%), Co (25.9%), U (24.5%), Pb (23.6%), Bi (22.1%)</td>
<td>Urban environment</td>
<td>2.56%</td>
</tr>
<tr>
<td>Factor 2</td>
<td>K (77%), Mg (44%), Rb (26%), Zn (25%)</td>
<td>Lichen nutrient elements</td>
<td>44.2%</td>
</tr>
<tr>
<td>Factor 3</td>
<td>Al (59.4%), Y (48.4%), Lu (45.9%), Yb (43.9%), Tm (43.7%), Er (43.1%), Ho (43.1%), Sc (42.2%), Dy (41.9%), Tb (41.3%), Fe (39.9%), Mg (39.2%), Sm (38.8%), V (38.7%), Gd (38.6%), Tb (36.9%), Eu (36.1%), La (35.3%), Ce (35.1%), Nd (34.7%), Pr (34.6%), Ce (33.2%), Ti (31.3%), Na (28.3%), Mn (25.5%), Cr (24.6%), Ni (24.3%), Co (24.3%), Li (22.7%), Pb (20.4%)</td>
<td>Contaminated soil</td>
<td>23.9%</td>
</tr>
<tr>
<td>Factor 4</td>
<td>Be (58.2%), Li (45.4%), Hf (43.8%), Ti (40.8%), Ge (36.7%), U (36.5%), Nd (35.9%), Pr (35.9%), Ba (35.7%), Na (35.6%), Tb (34.7%), Sm (34.1%), Y (34.0%), La (34.0%), Ni (33.8%), Eu (33.5%), Sc (33.2%), Gd (32.9%), Fe (32.3%), Tb (31.9%), Dy (31.5%), Ho (30.9%), Er (30.4%), Yb (30.4%), Tb (30.0%), Cr (29.9%), Lu (29.3%), Pb (20.0%), Mn (27.7%), Y (26.4%), Co (25.8%), Sr (22.0%), As (21.4%)</td>
<td>Traffic and mixed soil</td>
<td>17.5%</td>
</tr>
<tr>
<td>Factor 5</td>
<td>Rb (63%)</td>
<td>Lichen metabolism</td>
<td>7.15%</td>
</tr>
<tr>
<td>Factor 6</td>
<td>Tl (81%), Cs (38%), Bi (38%), Cd (20%), Cr (20%)</td>
<td>Coal combustion</td>
<td>4.64%</td>
</tr>
</tbody>
</table>

Fig. 3. G-score map for Factor 1.

Fig. 4. Map of G scores for Factor 4.
called as coal combustion factor. Coal was the dominant fuel in the city center until 2013. It has still been in the usage in small villages (for example in the second zone). Arsenic, Cd, Tl, Pb are in the list of most hazardous elements found in coal (Finkelman and Gross, 1999). The elements As and Tl are mostly found in the pyrite mineral phase of the coals (Finkelman and Gross, 1999). Bismuth, As, Tl, Cd, Pb, and Cr are mostly also found in fly ash of coal combustion (Querol et al., 1995). Especially, As is the tracer element for Turkish coal (Yenisoy-Karakas, 2000). As a result, the lichen samples collected in the second zone had higher G scores (Fig. 5). Because samples were influenced by coal combustion in the area. Bismuth (Fig. S7) and Tl (Fig. S8) concentration maps were very similar to G score map. The concentrations of As were greater especially in village areas in the third zone except for the areas with economically high living standards where natural gas was used for residential heating (Fig. S9). This factor was accounted for 4.64% of the total concentration.

3.4. Evaluation of isotope ratios with distribution maps

The usage of different types of fossil fuel for domestic heating leads to a significant difference among the δ13C values of the city center and rural areas (Fig. S10). Coal was one of the main fuel forms that were used in rural areas whereas the usage of natural gas was dominant in the city center. When burned, coal emits almost two times more carbon dioxide (CO₂) than natural gas (U.S. Energy Information Administration, 2019). CO₂ emissions from fossil fuel combustion caused depleted ¹³C (more negative δ13C values) in rural areas. Also, Saurer et al. (1995) and Kwak et al. (2009) stated that SO₄, NOx and ozone cause less negative δ¹³C value in higher plants due to stomatal closure and carboxylation enhancement. These inorganic pollutants, especially NO₃, are mostly found in the urban atmosphere. Whereas CO₂ emissions from coal-burning led to the depleted δ¹³C values in rural areas, usage of natural gas instead of coal and the presence of other inorganic pollutants contributed to more positive δ¹³C values in urban areas.

When δ¹⁵N values in Fig. S11 were evaluated, agricultural areas and urbanized regions could be clearly distinguished. The δ¹⁵N value was higher through the major roads (TEM, D-100, and tourism-related roads). The more positive δ¹⁵N values indicated the impact of N oxides that were the result of urban N emissions (Boltersdorf et al., 2014). Agriculture and poultry activities resulted in the emission of NH₃ whose abundance causes depletion in ¹⁵N (lower δ¹⁵N value) (Boltersdorf et al., 2014; Pinho et al., 2017). Hence, the most negative δ¹⁵N values were obtained in the regions where agriculture (the second and fifth zones) and poultry activities (the second zone) were performed.

3.5. Effect of the altitude gradient on stable isotopes in the lichen

The natural isotopic variations of stable isotopes in the XP were examined with an altitude gradient between 700 m and 1200 m above the sea level. The δ¹³C and δ¹⁵N values showed slight changes with the altitude gradient in both seasons (Fig. 6).

The sharp increase in δ¹³C values could be a result of the variance of photosynthetic activity with altitude. It was known that ozone which leads to a decrease in photosynthesis rate was found in the highest levels at high altitudes (Ruoss and Vonarburg, 1995; Cuna et al., 2007). Thus, higher ozone levels occurring at the higher altitude affected the rate of photosynthesis and indirectly led to less negative δ¹³C values in the summer season. However, δ¹³C values showed the opposite trend in the winter season. By supposing colder weather conditions occurring at higher altitudes, domestic heating activities increased in that season resulted in the rise of CO₂ concentrations. So, more negative δ¹³C values were obtained with increasing altitude in that season.

Concerning δ¹⁵N, more negative values were obtained with decreasing altitude in the summer season. Boltersdorf et al. (2014) indicated that more negative δ¹⁵N values can be a result of NH₃ emissions. Hence, lower δ¹⁵N values determined at lower altitudes can be explained with the widespread presence of NH₃ emission sources such as chicken process plants, agricultural activities and poultry farming at lower altitudes in the summer season. By contrast, agricultural activities that lead to more negative δ¹⁵N values were not common in the winter season. Instead of this, urban N emissions emitted from sources such as vehicular traffic and domestic heating had more influence on δ¹⁵N values in the winter season. These emission sources caused more positive δ¹⁵N values in the city center and surroundings which represent lower elevations (Boltersdorf et al., 2014).

3.6. Comparison of accumulation capacities of lichens and pine needles

In biomonitoring studies, the choice of biomonitor organisms has prime importance. Which species should be selected is based on the common biomonitor species in the study area. Additionally, an ideal biomonitor organism should have sufficient uptake efficiency for the target pollutants (Sloof, 1993). In our study, the most common lichen species was the XP in the area and it has high uptake efficiency. For the next study in which if this suitable common lichen species cannot be found, another form of biomonitor will be needed. The most suitable form of biomonitor organism can be pine needle. Since the pine
trees are dominant in the region. Lichens and pine needles are also among the most preferred bioindicators (Conti and Cecchetti, 2001; Paoli et al., 2014; Rudawski and Leski, 2005; Al-Alawi and Mandiwana, 2007; Kwak et al., 2009). It is aimed to determine whether the accumulation capacity of pine needle was comparable with lichens. The obtained data can be used to do an intercalibration (Sloof, 1993; Yenisoy-Karakas and Tuncel, 2004b) between two biomonitor species by calculating the ratio of median elemental concentrations of the lichen samples to that of pine needle samples. Fig. S12 shows the ratios in two seasons.

Ratios changed between 0.28 (Ca) and 11 (Be) in the summer season while the ratios were between 0.31 (Ca) and 9.7 (As) in the winter season. The high ratios (8–11) were obtained for Be, Lu, Th, Yb, As, Sn and Ti but high values were also found for Ho, Cd, Hf, Tm, Er, Dy and Al. These elements are the ones with the highest values for both graphs: in summer and winter. This indicated a significantly higher uptake rate of these elements by Xanthoria parietina as compared to pine needle. However, the ratio lower than 1 was calculated for the Ca, Sr, Mn, Mg, K and Zn elements. The higher uptake rate obtained for most of the elements allows a more precise definition of polluted zones and early reflection of air quality. Although XP had a higher accumulation capacity in the detection of most of the elements, pine needle was another good biomonitor organism to accumulate the elements in our study area.

Fig. 7 showed stable isotope signatures of lichen and pine needle. The isotope values of XP in summer ranging from −18 to 1.4‰ for δ15N and from −24 to −21‰ for δ13C. The δ15N and δ13C values were discriminated the lichen from pine needle. They were found in the range of −8 to 6‰ (δ15N) and −24 to −30‰ (δ13C) for pine needle in the summer season. The differences in the isotope values of different species were related to physiological and biochemical processes, that varied from one species to another (Galimov, 2000). In the winter season, XP ranged from −15 to −0.4 for δ15N and from −24 to −20‰ for δ13C whereas pine needle ranged from −22 to −29 for δ13C and from −9 to 3 for δ18N. Seasonal variation in δ13C and δ15N were also caused by species differences of biomonitor organisms. However, the isotopic signatures were broadly similar in both seasons with the lowest δ13C and the highest δ15N values occurring in pine needle.

4. Conclusion

Determination of 51 elements and stable isotopes (δ15N, δ13C) have been performed in lichen Xanthoria parietina and pine needle samples in high altitude plateau in Turkey. The Mann-Whitney (Wilcoxon) W test was used to compare the seasonal accumulation of elements in XP. The results of the test did not show a statistically significant difference between the seasons at the 95% confidence level (p = 0.05) in XP. The elements which are the markers of coal combustion such as As and Cd showed an increase in the winter season whereas the lichen metabolism elements such as K, Mg and Rb showed an increase in the summer season.

This is the first study of Turkey comparing lichens and pine needles as biomonitors in the determination of stable carbon and nitrogen isotope ratios in addition to elements. The results showed that significantly higher amounts of metals were accumulated by XP than by pine needles. The way of discrimination of pine needle sample from lichen sample could be possible by using stable isotopes since the lowest δ13C and the highest δ15N values were obtained in pine needle in both seasons. In addition, with the help of δ15N values, agricultural areas and urbanized regions could be clearly distinguished in the pollution map. The abundance of NH4 from the agriculture and poultry activities caused the depletion in 15N (lower δ15N value). Also, the depleted δ13C values obtained in rural areas are strong evidence of atmospheric CO2 concentration originating from coal burning.

PMF version 5.0 model was used for source apportionment using the elements determined in XP and the distribution maps of G scores were drawn to visualize the spatial distribution of factors in the sampling area. A solution with six factors was found to be the most realistic result. Lichen metabolism and nutrient elements, contaminated soil, coal combustion, traffic and mixed soil, and urban environment were the possible sources. Lichen metabolism was affected by heavy metal accumulations especially in the highly urbanized areas of the sampling region. These sources of heavy metals were coal combustion, traffic, and small scale industries. The element Pb was found to be still the tracer of vehicle emission due to the long term accumulation of particulates in lichens. The new tracer element for traffic, Ba, had a similar spatial distribution with Pb element in lichens. The higher contributions of soil elements like Al, Sc, Fe, Sm, V, La, Ce, Ti, Na, Mn, and Cr were observed in sites that are relatively close to agricultural areas and sand quarries. The concentration of As, the tracer element of Turkish coal, was greater in village areas where coal has still been used for residential heating. The elements Bi and Tl might have been other marker elements for coal combustion showing similar trends with As in both the concentration distribution maps and the PMF results.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106212.

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Fig. 7. δ13C versus δ15N values of lichen and pine needle.
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