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# INVESTIGATION OF PARTICULAR MATTERS ON THE LEAVES OF *PINUS NIGRA* ARN. SUBSP. *PALLASIANA* (LAMB.) HOLMBOE IN DENIZLI (TURKEY)

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**Abstract**

In urban environments, the main sources of emission of particular matters can be listed as follows: Emissions due to heavy traffic and industrial activities; emissions from power stations, local heating sources and road dust. Particular matters in variety of forms such as dust particles, globules, aggregates etc., accumulate on leaf surfaces of road side plants including higher plants. In this respect, investigation of leaf surfaces may indicate environmental pollution and other effects due to industrial and urban activities and thus, receive great deal of attention for environmental evaluations. *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe are among the most cultivated plants along the roadsides of the municipal area of Denizli city, TURKEY. In this study, the accumulation and the elemental analysis of particular matters on leaf surfaces of *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe were investigated using FESEM-EDS techniques during the years 2009 and 2010. Leaf samples were collected from roadsides of four different local areas of heavy traffic and densely urbanized, and from an area far away from heavy traffic. The chemical composition of particular matters on leaves showed distinct differences in between the two data. This finding indicates high level of increased urbanisation, growing industry and heavy traffic in some locations of Denizli city. As a result, leaves of higher plants may be used as bioindicators for the assessment of particular matters in urban areas.

## Introduction

It is a very well known and documented fact that rapid industrialisation and urbanisation results in heavy metal contamination in urban ecosystems. Roadsides of urban and industrial areas are mainly affected by particular matters released from plants, workshops, power generation, residential heating and motor vehicle emissions etc. Analysis of aerial metal pollutants in urban environments has been of growing interest with the goal to identify the extent and spread of the content of airborne particular matters.

In general plants are known to be in need of several major elements such as P, Ca, Mg for their normal growth process. However, excessive amounts of these elements in soil may have toxic effects to the plants (Agrios, 2005). These elements are deposited in different parts of plants such as on leaf surfaces as particular matters in dust forms. They are both originated from soil contamination and also from atmospheric pollution and contamination. Plants can be used as a useful bioindicators for the assessment of environmental pollution in soil and atmosphere of urban areas (Tomasevic *et al*., 2004; Madejón *et al.*, 2004, 2006; Gautam *et al.*, 2005; Mingorance & Rossini Oliva, 2006). Therefore, they have a great ecological importance and they have been attracting quite an attention of investigation. In this regard, several studies were carried out using plants such as mosses and lichens (Jalkanen *et al*., 2000; Riget *et al*., 2000; Szarek-Lukaszewska *et al*., 2002; Giordano *et al*., 2005) and higher plants (Aksoy & Ozturk, 1997; Hodson & Sangster, 1998; Aksoy & Sahin, 1999; Aksoy *et al*., 2000; Dongarra` *et al*., 2003; Celik *et al*., 2005; Aksoy & Demirezen, 2006; Yilmaz *et al*., 2006; Akguc *et al*., 2008) as bioaccumulators or bioindicators in environmental investigations.

The city of Denizli is in the southwest corner of the Aegean Region of TURKEY. It is a fast growing and industrialising city with about 500.000 inhabitants within the extent of the municipal area. The major industries in the city are textile, leather, machinery, manufacturing and metal working industries. In general, *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe are evergreen plants and are widely cultivated near the roadsides of the city, Denizli.

The purpose of this study is to compare the differences between the analysis of particular matters on the leaf surfaces of *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe collected in years 2009 and 2010 from the roadsides of different locations with varying degrees of high traffic and urbanisation density using Field Emission Scanning Electron Microscope-Energy Dispersive Spectroscopy (FESEM-EDS) techniques.

## Materials and Methods

The study area was selected as one of the most crowded districts of the city where the main route is for transportation vehicles, small and large size trucks and touristic tour busses, vans and mini vans etc., therefore it has a heavy traffic density. Some regions are in close proximity to industrial plants, factories and small/medium size workshops. The contaminated leaves were collected from four different regions along the roadsides of the study area (Table 1). For uncontaminated controls, leaves were collected from Honaz Mountain which is the highest mountain in the region located about 17 km southeast of Denizli city. All the samples were taken on trees at a height of at least 1 m above ground level in each region.

As has been widely demonstrated, SEM is one of the strongest techniques for a direct proof for the presence of atmospheric particles on leaf surfaces (Tomasevic *et al*., 2004). In this study, the analyses were carried out by FESEM method, observing the presence and shape of the particles, followed by EDS analysis to determine elemental composition of the particles on leaf surfaces. Portions of unwashed leaves were dried in an oven at 65 $^{o}C$ for 48 h. They were mounted on stubs and coated with gold micro-layers before FESEM-EDS observations. The micrographs of leaf samples and their corresponding EDS spectrums were taken. The weight percentages of the elements on the samples obtained from EDS analysis were given in Table 2.

**Table 1. Description of locations of leaves sampled along the roadsides.**

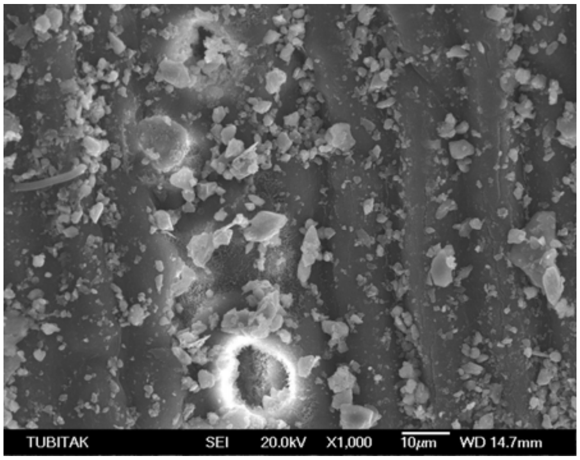
| Site no. | Site name | Distance from the city center and remarks |
|---|---|---|
| 1 | Campus | About 3 km |
| 2 | Kinikli | About 2 km |
| 3 | Ucgen | In center, have heavy traffic |
| 4 | Kayalar | About 4 km west of the highway entering city, have heavy traffic and close to the industrial area |

**Table 2. FESEM-EDS analytical data (average wt. %) on particles constituting on leaves of *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe collected in 2009 and 2010 along the roadsides in the study area with controls.**

| Elements | Elemental percentage (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Kayalar | | Ucgen | | Kinikli | | Campus | | Control |
| | 2009 | 2010 | 2009 | 2010 | 2009 | 2010 | 2009 | 2010 | |
| C | 52.59 | 20.11 | 58.29 | 28.29 | 52.31 | 33.48 | 54.42 | 31.89 | 76.80 |
| O | 34.41 | 32.71 | 28.87 | 10.55 | 43.33 | 31.98 | 35.90 | 31.83 | 22.13 |
| Na | 0 | 1.54 | 0 | 0.24 | 0 | 0 | 0 | 0 | 0 |
| Mg | 0.36 | 0.44 | 0.44 | 1.19 | 0.64 | 1.21 | 0.45 | 0.62 | 0 |
| Al | 0.66 | 1.55 | 0.55 | 2.00 | 0.85 | 2.07 | 1.24 | 1.40 | 0.15 |
| Si | 1.52 | 4.80 | 1.36 | 4.10 | 3.62 | 3.56 | 3.04 | 2.30 | 0.28 |
| P | 0 | 6.30 | 0 | 0 | 0 | 0.34 | 0 | 0 | 0 |
| S | 0.28 | 1.58 | 0 | 0.93 | 0.35 | 1.92 | 0 | 1.32 | 0 |
| Cl | 0 | 0 | 0 | 0 | 0 | 0.24 | 0.24 | 0 | 0 |
| K | 0.33 | 0 | 0.59 | 2.89 | 0.97 | 1.02 | 1.15 | 0 | 0.06 |
| Ca | 7.90 | 3.94 | 2.06 | 2.53 | 8.89 | 4.98 | 2.51 | 0.63 | 0.16 |
| Ti | 0 | 19.49 | 0.34 | 30.66 | 0 | 0.40 | 0 | 13.43 | 0 |
| Fe | 0.80 | 29.85 | 10.42 | 46.22 | 1.49 | 15.46 | 1.82 | 29.04 | 0 |
| Cu | 0.86 | 0 | 1.03 | 0 | 0.71 | 12.80 | 0.49 | 1.64 | 0.48 |
| Zn | 0.75 | 0 | 1.14 | 0 | 1.96 | 0.79 | 0 | 1.62 | 0 |
| Sb | 0 | 0 | 0 | 0 | 0 | 1.40 | 0 | 0 | 0 |
| Ba | 0 | 0 | 0 | 4.49 | 0 | 11.78 | 0 | 5.23 | 0 |
| AgL | 0 | 0.91 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La | 0 | 10.17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ce | 0 | 21.32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nd | 0 | 7.11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| W | 0 | 0 | 0 | 0 | 0 | 1.76 | 0 | 0 | 0 |
| Pb | 0.20 | 0 | 0.35 | 0 | 0.33 | 0 | 0 | 0 | 0 |

## Results and Discussion

The morphology and chemical characterization of the particles deposited on unwashed leaf surfaces were examined using FESEM-EDS analysis. The morphological characters of the particular matters obtained from the scanning electron microscopy from the contaminated leaf surfaces collected in years 2009 and 2010 showed that the particles were in irregular forms and in different sizes (Fig. 1a & b). They were found both as single particles and in clusters. On the other hand, uncontaminated leaf surfaces were found to be quite clear (Fig. 2). Typical examples of scanning electron microscopy micrographs and corresponding EDS spectrums of contaminated plant samples and control are shown in Fig. 3 and Fig. 4, respectively. The EDS spectrum of the particles exhibited some differences according the terms of 2009 or 2010 they were collected. The C content decreased in the year 2010, O content also showed a decrease in Ucgen and Kinikli regions in 2010, but it was similar in other regions for both years. For Mg, a slight increase was detected in Ucgen and Kinikli collected in the year 2010. Al and S exhibited moderately increase in all regions in 2010. The remarkable chances occurred in Kayalar and Ucgen for Si and Ti in 2010. They were found at high levels for Si in Kayalar and Ucgen; for Ti in Kayalar, Ucgen and Campus. Fe content is much higher than on the samples of all locations in 2010. Cu was found at higher level only in Kinikli in 2010. K and Zn percentages showed increase and decrease between the two years. Particularly, Ca decreased in Kayalar, Kinikli and Campus in 2010. The Cl content was detected on the samples of Kinikli in 2010 as the same ratio of the samples collected in Campus in 2009. The rare earth elements, Lanthanum (La), Cerium (Ce) and Neodymium (Nd) were present only in the samples of Kayalar in 2010. Likely, W and Sb in Kinikli, Na in Kayalar and Ucgen, P in Kayalar and Kinikli, Ag in Kayalar, Ba in Ucgen, Kinikli and Campus were detected in the same year. Pb was found on the samples of Kayalar, Ucgen and Kinikli in 2009. On the other hand, most of the elements detected on the contaminated leaves were absent on the controls (Table 2). In addition, Fig. 5 illustrates the comparative percentages of the elements on the leaves collected from study regions in 2009 and 2010. From this figure it can be suggested that some of the elements analyzed exhibited decrease and increase in percentation, some are completely absent in some regions of the study area.



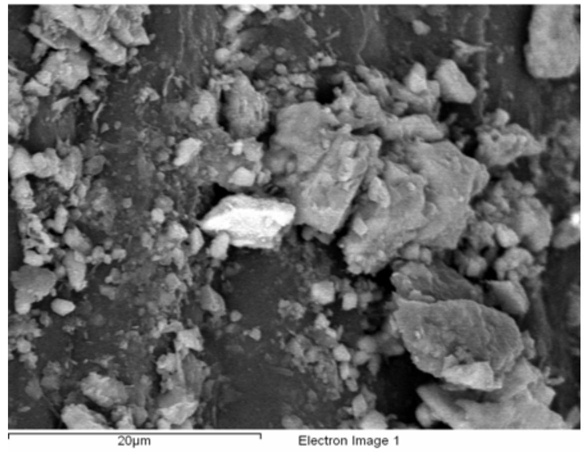




Fig. 1. Intense deposited particles on the leaf surface of *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe collected from Kayalar in 2009 (a) and 2010 (b).

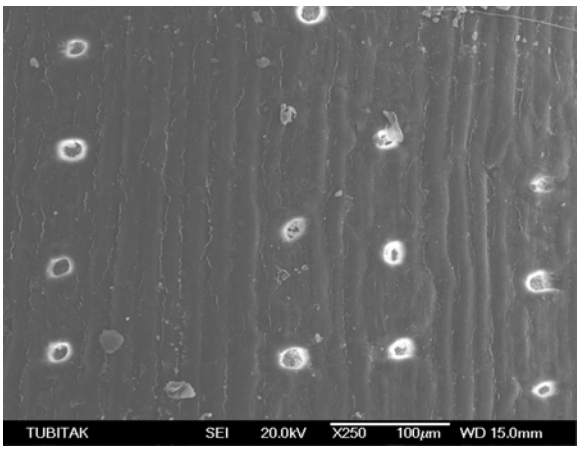



Fig. 2. Uncontaminated leaf surfaces collected from Honaz mountain showing extremely clear surface.

The data collected during both years of 2009 and 2010 reveals that many different sized and shaped particles in the form of dusts and aggregates are accumulated on leaf surfaces collected from the road sides of urbanised area. In addition, it is important to note that there is a distinct difference in the elemental composition of the particles in between the two data of 2009 and 2010.

Silicon is the second most abundant element in the soil. It plays an important role in growth, development and environmental stresses for plants (Epstein, 1994). As it has been previously mentioned, the co-localization of Al with Si in the epidermis of *Picea glauca* needles (Hodson & Sangster, 1998) may be related to the sequestration of heavy metal in plants. Likewise, the results may also indicate that Si accumulation may reflect a close relation with toxic element due to the protection mechanisms against deterioration by heavy metals of plants in highly urbanised area. The higher value of Fe on the samples collected in year 2010 may be due to automobile exhaustion which is considered as a typical cause in such heavy traffic. Other elements found in urban areas including Zn, Cu, Mg can be mostly anthropogenic. They are most probably originated from heavy traffic, renovation, and weathering and corrosion of building materials (Miguel *et al*., 1997). Zn, Cu, Mg were detected on the leaves collected from the locations having urbanisation and heavy traffic. The presence of Cu detected on leaves at high levels in Kinikli region may be the results of natural sources, however it may also have originated from vehicular pollution as discussed by Miguel *et al.*, (1997). Rare earth elements including 14 lanthanides are known to be the most abundant in the Earth's crust. It is also a known fact that these elements are utilized as part of some systems in industrial applications such as in automotive (e.g., as catalyst in three-way catalytic convertors and filtering devices of automobiles, trucks and buses), chemical, petroleum. In addition, fertilizers containing rare earth elements are widely used in agriculture in China (Tyler, 2004). Some minor amounts of rare earth elements such as La, Ce and Nd were also detected on the leaf surfaces collected from Kayalar region. The region has also industrial plants, factories, many small workshops and consequently very intense load of traffic circulation. The inhibition effect of La and Ce on primary root elongation, reduced the dry weight of roots and shoots and the content of mineral elements (Ca, Mg, K, Cu, Zn) in plant at the concentration of 0.5-25 mg/l to the individually and in combination in the culture medium was shown (Hu, 2002). Likely, Diatloff *et al*., (2008) reported that La or Ce decreased the growth of corn and mungbean at concentration above 0.2μM, but they can have positive effect at concentrations below 0.2μM. In this study, La and Ce may be soilborn originated. On the other hand, there is a strong possibility that in some Ce containing substance is added into diesel fuels for example as cerium oxide. Therefore, it can be originated from the exhaust gases from motor vehicles and accumulated on leaf surfaces of road side plants. Nd is known as the second most abundant rare-earth elements. It is found in ore minerals such as monazite and bastnasite. Also, it can be found in the environment mainly by petroleum-producing industries and in waste materials of household equipment. In rape, Nd treatment can relieve Ca-starvation symptom and increase the activities of protecting enzyme system, lower the extent of membrane lipid peroxidation, and inhibit membrane peroxidation process (Wei & Zhou, 1999). Nd detected on the samples of Kayalar in 2010 may indicate that it may be originated from soil, and also may play a biological role for *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe. Ti is found in soil. It is also known as an urban element in road dust (Miguel *et al*., 1997). The presence of Ti on the samples of Ucgen region may result from natural and/or urban sources in that area. Phosphorus (P) is known as an essential element for plant growth. However, internal precipitation of P by Al inhibits plant growth, since Al toxicity may be the cause of precipitation of P internally, thus inactivating P (Wright, 1943). In general, Al content increased in contaminated samples of the study area in year, 2010. Meanwhile, P was present on samples of Kayalar region in the same year. This data may indicate the relationship between P and Al on account of environmental toxication of plants. The phytotoxic effects of Ba on soybean plants by inhibiting photosynthesis were reported (Suwa *et al*., 2008). In 2010, the samples collected from Ucgen, Kinikli and Campus regions showed Ba accumulation as particular matters on leaf surfaces. Most probably, this pattern may influence the plant metabolism. Tungsten (W) is an another rare element which naturally occurs in the earth's crust and also comes from industrial areas. W accumulation is attracting great deal of attention of researchers in order to investigate its possible beneficial or toxic effects for organisms. Its toxicity has been reported in some studies (Jiang *et al*., 2007; Johnson *et al*., 2009; Adamakis *et al*., 2010). Strigul *et al*., (2005) have also suggested that metallic W in soils has some adverse environmental effects at or above the threshold level of 1% mass basis (i.e., 10 000 mg $kg^{-1}$). They also reported that plants and worms can be used in phytoremediation based technologies for the cleanup of W contaminated sites in their ability to take up W from soil in significant concentration. W is detected on the leaf samples as an accumulated particular matter in Kinikli region indicates the possibility of soil contamination originated from mineralisation or industrialisation. Due to such findings, it can be suggested that *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) can be used as bioindicators and/or bioaccumulators for W polluted soils.

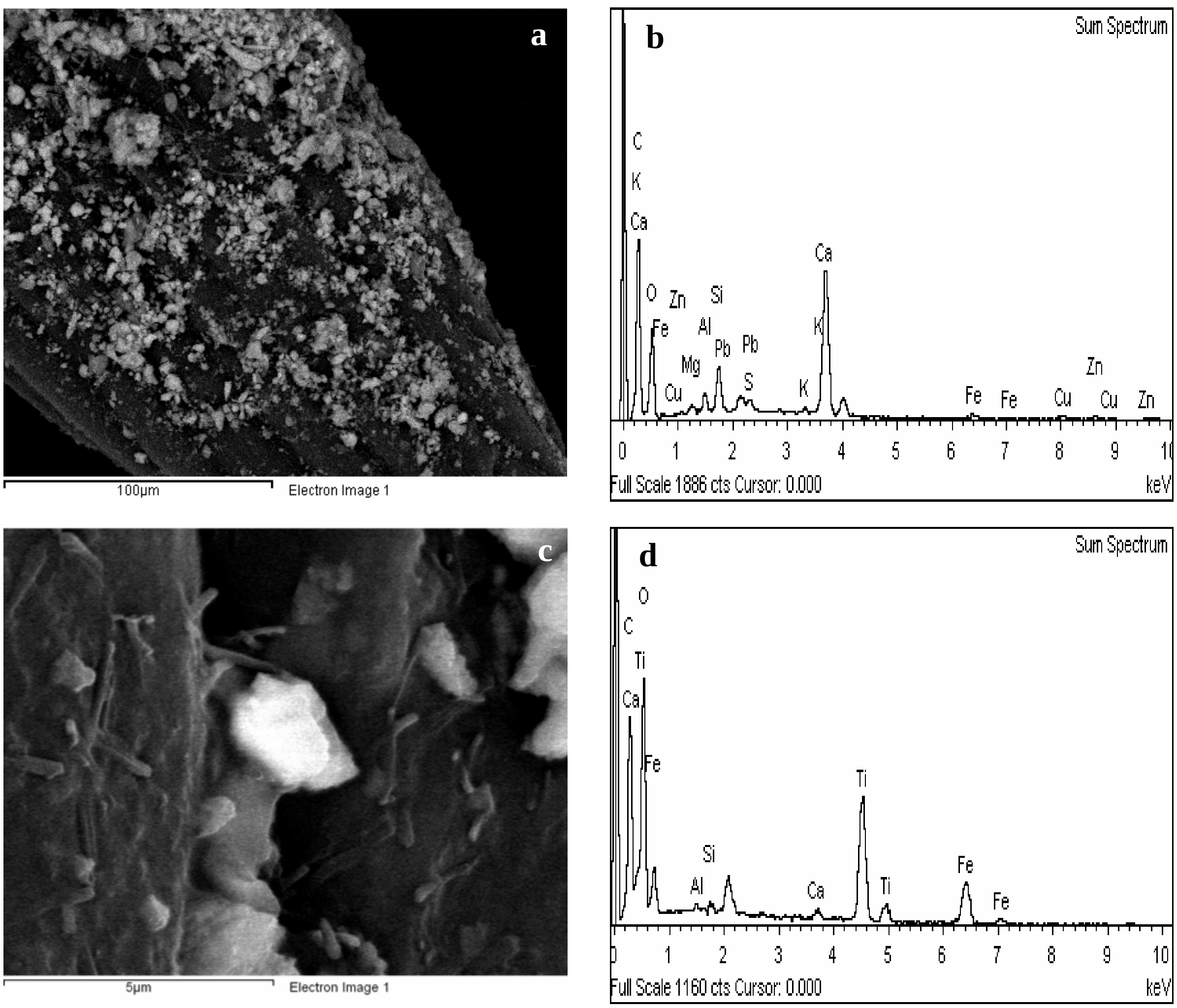



Fig. 3. Scanning electron micrographs and EDS spectrums of particulate matters on the samples of *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe collected from highly contaminated location, Kayalar (a and b for 2009; c and d for 2010).

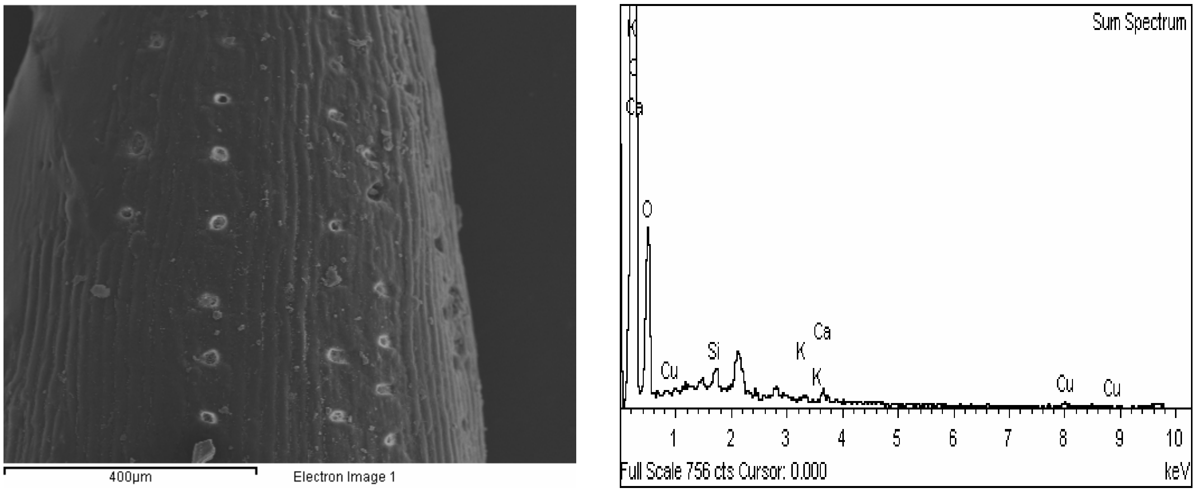



Fig. 4. Scanning electron micrograph and corresponding EDS spectrum of leaf sample collected from Honaz Mountain as control.

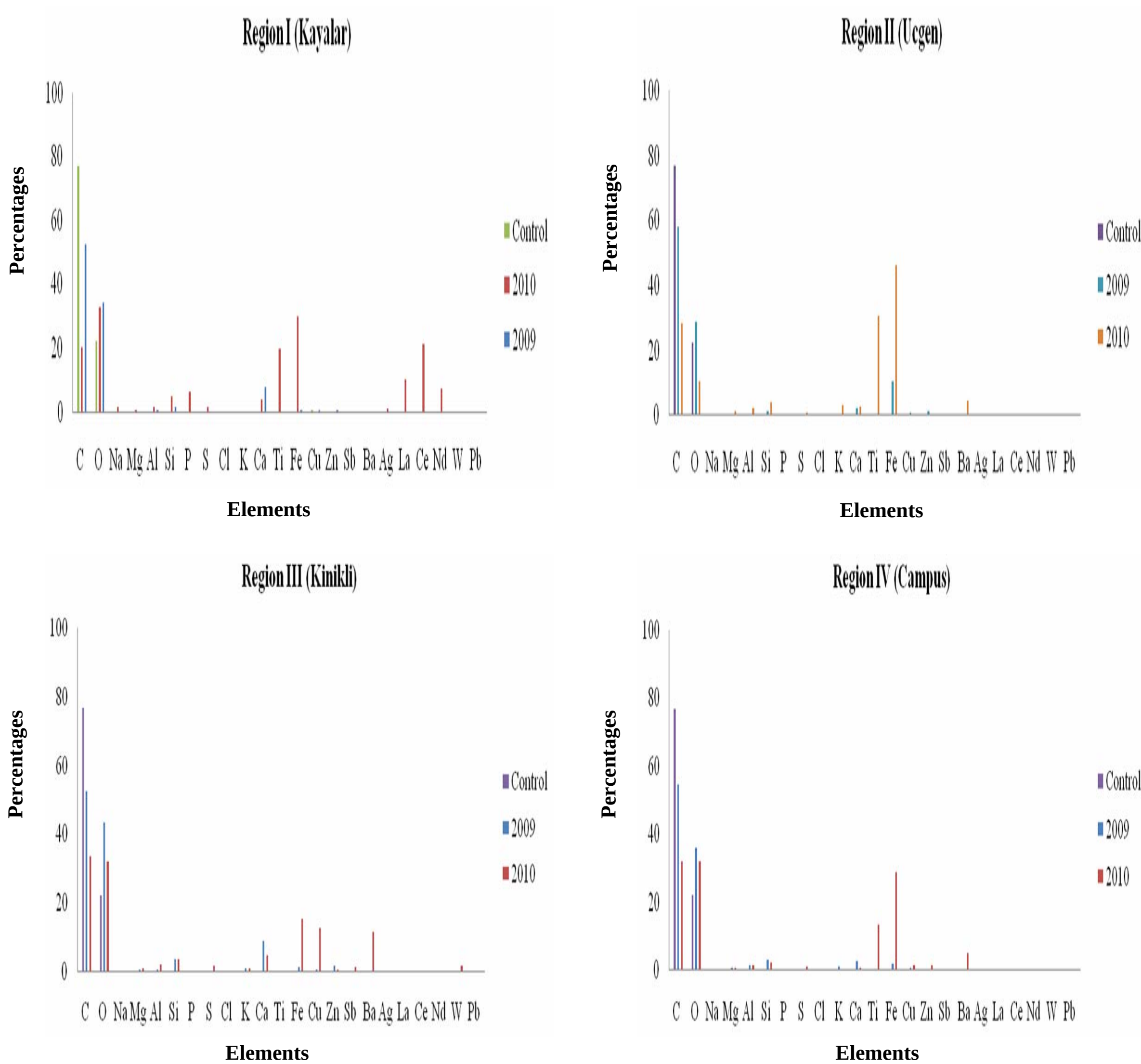



Fig. 5. Comparison of the element percentages on the leaves collected from selected regions in 2009 and 2010 compared with control samples.

## Conclusion

As a result, the determination of elemental contents of particular matters accumulated on leaf surfaces is important indication for the assessment of environmental pollution. In this study, some particular matters accumulated on the leaves of *Pinus nigra* Arn. Subsp. *pallasiana* (Lamb.) Holmboe in various regions of Denizli were detected and analysed using FESEM-EDS techniques in years, 2009 and 2010. It can be suggested that the distribution and elemental compositions of airborne and soilborne particular matters on leaf surfaces of *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe vary in locations depending on traffic volumes, urbanisation and industrial activities in years, 2009 to 2010. In conclusion, it can be suggested that roadside plants could be used as important indicators for the assessment of particular matters in the urban environment.

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