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RESEARCH ARTICLE

The use of olive tree (*Olea europaea* L.) leaves as a bioindicator for environmental pollution in the Province of Aydın, Turkey

Dilek Turan · Cemre Kocahakimoglu · Pınar Kavcar ·
Handan Gaygısız · Levent Atatanir · Cafer Turgut ·
Sait C. Sofuoglu

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Abstract

Introduction In this study, olive tree leaves, collected from 50 sampling sites throughout the Province of Aydın, Turkey, were used to estimate level of pollution by measuring Al, As, B, Ba, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, and Zn concentrations and calculating pollution factor (PF) values.

Materials and methods After sample preparation, collected leaves were microwave digested, and extracts were analyzed by an inductively coupled plasma-mass spectrometer.

Results and discussion The maximum PF values were ≥ 10 for a number of elements ranging from 11–13 (Al, As, Cr, Fe, Mn, Ni) to >100 for Cu, Li, and Na. Urban–rural and roadside–nonroadside concentration comparisons showed that some of the elements (As, Cu, and Pb) were at significantly higher levels on urban and/or roadside sampling sites.

Summary and conclusion Correlations and factor analysis showed that there may be common sources for some elements, which included several soil types and anthropogenic activities. Based on the results of the statistical source apportionment, possible sources were narrowed down with help of the constructed elemental concentration maps. In conclusion, utilization of olive tree leaves for biomonitoring and assessment of environmental pollution was shown to be possible in the Mediterranean region where they are indigenous and cultivated.

Keywords Elemental concentrations · Olive leaves · Biomonitoring · Source apportionment

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D. Turan · C. Kocahakimoglu · P. Kavcar · S. C. Sofuoglu (✉)
Department of Chemical Engineering,
İzmir Institute of Technology,
Güllbahçe, Urla, 35430 İzmir, Turkey
e-mail: cemilsofuoglu@iyte.edu.tr
e-mail: saiticemil@iit.edu

H. Gaygısız · S. C. Sofuoglu
Environmental Research Center, İzmir Institute of Technology,
Güllbahçe, Urla, 35430 İzmir, Turkey

L. Atatanir · C. Turgut
Faculty of Agriculture, Adnan Menderes University,
Aydın, Turkey

Present Address:
C. Kocahakimoglu
Department of Chemical and Biological Engineering,
Koc University,
Sariyer, 34450 İstanbul, Turkey

1 Introduction

Since plants provide not only a cheap but also an easy way to monitor the trace elemental concentrations in the environment, their different parts were used as bioindicators of trace elemental contamination. The use of leaves as bioindicators of environmental pollution has been studied, more and more, to assess their suitability (Maisto et al. 2004; Tomasevic et al. 2005; Rossini Oliva and Mingorance 2006), to assess effect of a specific pollution source (Madejon et al. 2006; Aghabarati et al. 2008), to differentiate between background (unpolluted) and polluted sites (Maisto et al. 2004; Rossini Oliva and Mingorance 2006), and to monitor or assess the level of pollution in an area (Çelik et al. 2005; Rossini Oliva and Mingorance 2006; Al-Khlaifat and Al-Khashman 2007; Gratani et al. 2008).

Leaves of evergreen species are considered to be better traps because of higher accumulation on a longer period of time. Thus, olive trees are preferred species especially in

regions where they are indigenous, such as in the Mediterranean countries. Aghabarati et al. (2008) used olive tree leaves and fruit to compare contamination at a site irrigated with municipal effluent and at a site irrigated with well water. Madejon et al. (2006) employed wild olive leaves to biomonitor concentration of trace elements in an area affected by a spill from an open-pit pyrite mine.

Wild and cultivated olive trees are abundant in parts of Turkey with the Mediterranean climate. However, they were not employed in biomonitoring efforts (Çelik et al. (2005) used *Robinia pseudo-acacia* L. leaves; Tuncel et al. (2004) used *Xanthoria parietina*; Dogan et al. (2007) used the bark of *Pinus brutia*) except for a study by Tuna et al. (2005) that investigated the effect of three thermal power plants by the help of olive leaves for the determination of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn pollution. The average concentrations were 0.3, 2.2, 2.0, 6.9, 242, 42, 3.9, 8.3, and 22 mgkg⁻¹, respectively, which were significantly higher than the unaffected control sites.

Bioindication index (BI, Schulz et al. 1999) or in other words pollution factor (PF, Elik et al. 2000; Pöykiö et al. 2005) was described as the ratio of the maximal content to the minimal content in an area or the ratio of the determined contamination (concentration in a polluted area) to the base level contamination (concentration in an unpolluted, background, site), respectively. It can be used as an overall indicator of anthropogenic inputs over a study area, and as a rough measure of the relative degree of pollution to assess contamination in an area. The latter definition (PF = determined concentration/background concentration by Elik et al. (2000)) was adopted in this study; so that relative degree of pollution at each sampling site can be assessed for each measured element.

This study aimed to investigate the applicability of olive tree leaves for biomonitoring and assessment of environmental pollution in a region where they are indigenous and cultivated; by characterizing spatial elemental pollution distribution in the Province of Aydın, Turkey, using olive leaves. Elemental concentration maps and PF were used as assessment tools. Source apportionment was conducted using bivariate correlation and factor analysis.

2 Materials and methods

2.1 Sampling

In this study, olive tree leaves (*Olea europaea* L.) were collected from 50 sampling sites in the Province of Aydın from September to November in 2008. The sampling sites were chosen randomly to represent the Province of Aydın, for mapping purposes, using topographic (1/25,000) and land use (1/100,000) maps, and Landsat ETM+ images

from years 2006 and 2007. However, it was made sure that no other agricultural activity has been conducted at the site or the neighboring fields. The study area and the sampling sites are illustrated in Fig. 1. The leaves were collected from five to ten trees at each sampling site. The leaves were collected from both the upper and the lower parts of trees. After all were thoroughly mixed, 300 g were taken from the mix. The samples were kept in the freezer held at -20°C until sample preparation for digestion.

2.2 Digestion

For all analyses, cleaning, and sampling procedures, trace organic and chemical free MilliQ water (Millipore Elix 5) and high-purity solvents were used. All glassware and plastic bottles were washed once with tap water and three times with MilliQ water and were kept in 20% nitric acid bath (Merck, 65%) for at least 3 h.

The samples were equilibrated to room temperature before being placed in an oven for drying. The leaves were dried in a laboratory oven at 70°C for 6 h before grinding. The samples were manually ground in plastic bags in order to avoid contamination. After grinding, the samples were acid digested by the use of a microwave acid digestion procedure. A portion of the sample was weighted to 0.25 g by the aid of a plastic spatula directly into the microwave containers. After that, 7 ml of ultrapure HNO₃ (Merck, 69%) and 1 ml H₂O₂ (Merck, 35%) mixture was added. Then, the digestion program (room temperature to 180°C in 10 min, then 15 min at 180°C, power: 1,200 W, maximum allowable pressure 400 psi) was applied in the microwave (CEM Mars Xtraction ESP-1500). After digestion, the sample was completed to 50 ml with ultrapure water.

2.3 Analytical methods

The analytical instrument used in this work was inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce series). A five-level external calibration was performed for each element prior to the analysis of samples. The calibration curves were deemed acceptable when $R^2 > 0.99$. The operating conditions of the ICP-MS were as follows. Integration time 0.1 s, sampling period 0.31 s, acquisition time 22.76 s, RF power 1,550 W, RF matching 1.78 V, carrier gas 0.9 L/min, makeup gas 0.18 L/min, nebulizer pump 0.12 rps, He gas flow 4 mL/min, H₂ gas flow 4 mL/min. Readings were taken as replicates.

2.3.1 Quality assurance/quality control

In order to examine the laboratory capability, effectiveness of microwave digestion and the accuracy of ICP-MS

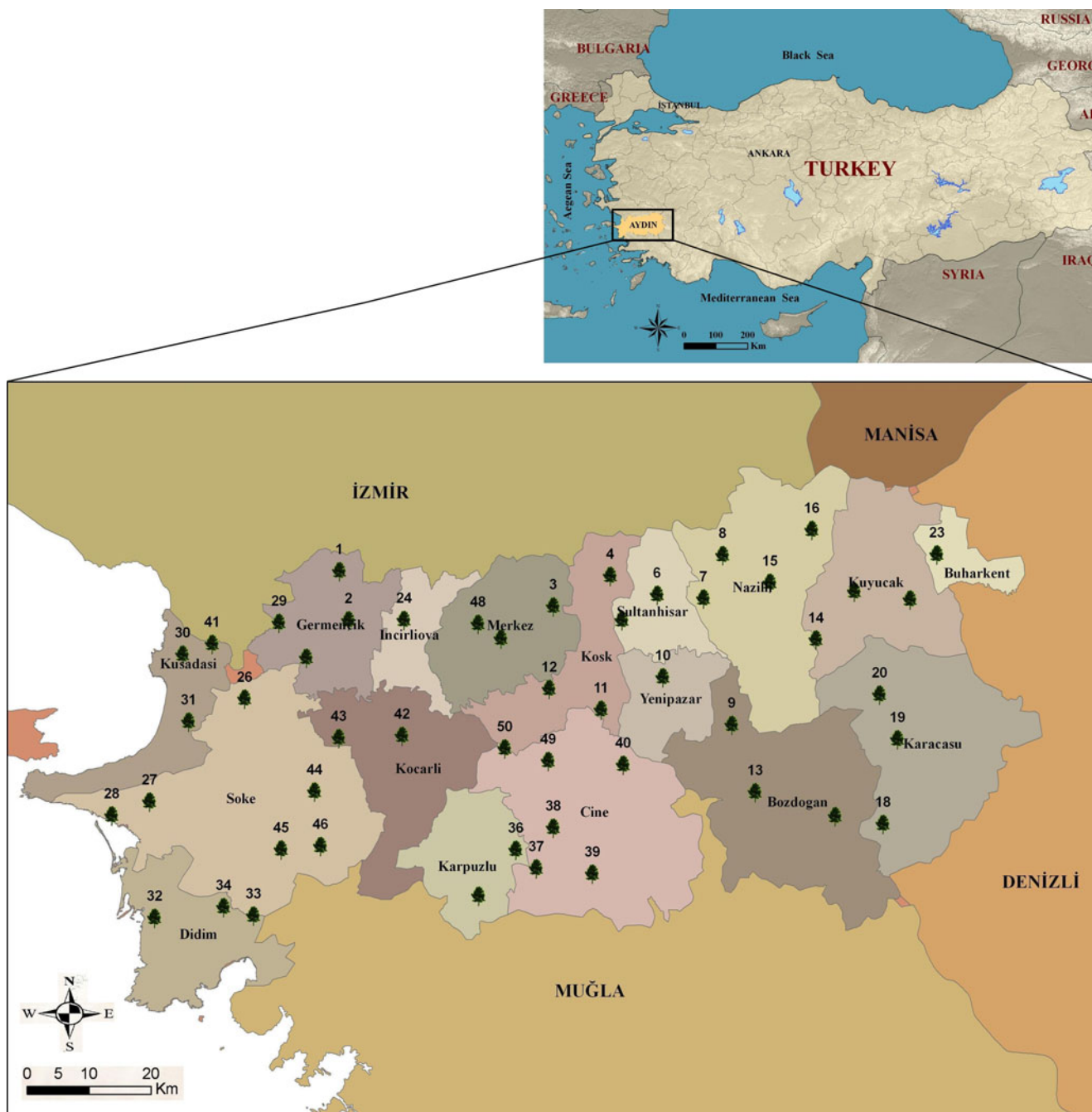


Fig. 1 The study area and the sampling sites

analysis, a certified reference material, blank samples, and replicate samples were used. Five blanks were treated as samples and analyzed. Limit of Detection (LOD) value for each analyte was calculated as the mean of the five blanks plus three standard deviations (see Table 1). Three samples were weighed from a certified reference material (BCR-062, Community Bureau of Reference), digested and analyzed, which contained nine elements. Percent recoveries are presented in Table 1 as the mean of the three values. The percent difference between the duplicates (two samples

were weighed from the original sample, and digested and analyzed as separate samples, $n=3$) were acceptable (<20%) except for Cd and Pb (see Table 1). Since Cd was generally below detection limit (BDL), it had not been included in data analysis. Because of the large percent difference (67%) in Pb concentrations between the duplicates, these should be considered with caution. The percent difference between the replicates (a sample was divided into two parts after digestion) was <10% except only for Na with 11% (see Table 1).

Table 1 Limit of detection, recoveries, and replicate differences

Metal	LOD ($\mu\text{g/l}$)	Recovery (%)	Duplicate difference (%)	Replicate difference (%)
Al	350	87	1.0	9.6
As	0.4	99	8.4	<1
B	13	63	3.5	2.7
Ba	16	–	8.4	6.1
Ca	20,700	–	4.2	7.1
Cd	0.4	–	278	2.9
Co	1.4	126	24	4.6
Cr	9.5	113	3.0	6.6
Cu	27	107	4.2	5.1
Fe	742	–	6.7	5.2
K	825	–	5.7	4.6
Li	5.3	–	11	5.8
Mg	620	–	3.6	5.8
Mn	12	113	2.9	11
Na	2,660	–	15	5.5
Ni	14	58	18	<1
Pb	9.5	122	67	6.8
Sr	19	–	7.5	9.6
Zn	1,040	–	21	<1

2.4 Mapping

Sampling sites have been examined by using Remote Sensing methods. Database has been created by using Geographic Information Systems (GIS). Remote sensing and GIS are very helpful in process of problem solving and decision making in terms of monitoring and controlling data. Well recognized 50 sampling sites were selected and measured with GPS. Coordinates, altitudes, concentrations were entered as attributes in ArcGIS 9.3. Continuous surfaces that show elemental concentrations at unknown locations were constructed by using the interpolation function in the spatial analysis module, which uses Inverse Distance Weighting interpolation technique. The scale of the constructed concentration maps was 1/700,000. The concentrations were classified into varying colors using the reclassify function.

2.5 Statistical methods

Statistical analyses were performed using SPSS (Release 12.0). *T* test, Mann–Whitney, and Kolmogorov–Smirnov tests were used to test the differences between data groups. Since the number of sampling sites in each group was low ($n=8$), the nonparametric tests were applied in addition to the *t* test. *T* test was used for difference in the mean values of two groups assuming unequal variances, whereas Mann–Whitney and Kolmogorov–Smirnov tests were used to test for difference between the medians of two groups. In this study, *p* values <0.05 were considered to point a significant difference between the compared groups. Factor analysis

was applied to infer on the source contribution to pollution, which was conducted using varimax rotated principal component analysis. Eigen values >1 and loadings >0.45 were regarded significant for interpretation of factors.

3 Results and discussion

3.1 Concentration and pollution factor levels

The descriptive statistics calculated for the concentrations of trace elements found in olive tree leaves are presented in Table 2 for the Province of Aydın. Concentrations below the limits of detection (LOD) are denoted with BDL (below detection limit) in the table. A PF_{\max} value was calculated for each element, which is the ratio of the maximum concentration in the area to the minimum concentration, analogous to BI (Schulz et al. 1999) and PF (Pöykiö et al. 2005). The calculated factor values are given along with the values from the literature for each trace metal in Table 2. PF values of >10 was taken as significantly inflated, as it was pointed out by Cinar and Elik (2002) that in cases when PF values differ by orders of magnitude for samples collected in this same general area, they can be treated as a reasonably valid measure of the anthropogenic pollution. This criterion (>10) is commonly used in evaluation of soil contamination by enrichment factor (Yay et al. 2008). The PF_{\max} values were >10 for Al, As, Cr, Cu, Fe, Mn, Ni, Pb, and Sr among mainly anthropogenic elements and elements that have both crustal and anthropogenic sources.

Table 2 Descriptive statistics for olive leaf trace element concentrations and PF values

Concentration (µg/kg)						PF _{max}			
	<i>n</i> =50	Average	SD ^a	Median	Min	Max	This Study	Schulz et al. (1999)	Pöykiö et al. (2005)
Al		7,925	6,170	6,290	3,060	37,300	12	1,500	–
As		8.0	5.5	6.2	2.6	33	13	386	–
B		566	248	523	228	1,680	7	30	–
Ba		723	623	505	73	2,689	37	–	–
Ca		385,400	161,000	335,000	186,200	874,100	5	15	–
Co		5	2.7	4.4	2.2	19	9	–	–
Cr		22	13	20	BDL	91	11	20	12.4
Cu		265	571	141	33	3,850	116	36	–
Fe		7,675	5,000	6,685	2,770	35,800	13	94	5.3
K		326,900	123,650	299,000	97,000	581,500	6	–	–
Li		25	60	11	BDL	400	608	–	–
Mg		81,000	39,300	68,150	31,600	177,500	6	–	–
Mn		1,400	725	1,167	353	4,491	13	–	–
Na		6,600	3,515	8,075	BDL	11,800	574	–	–
Ni		102	78	88	18	383	22	820	3
Pb		27	18	22	BDL	87	64	36	–
Sr		2,675	2,650	1,900	175	16,750	95	23	–

SD standard deviation, *Min* minimum, *Max* maximum, *PF* pollution factor

In comparison to the values reported from Germany (Schulz et al. 1999) measured at a known contaminated and an uncontaminated site, the index values of Al, As, B, Ca, Fe, and Ni in Germany were much higher than those of in Aydın, i.e., the contamination in Aydın in terms of these six elements were not as high as the contamination in Germany. It should be noted that Schulz et al. (1999) used pine tree barks as biomonitoring agents. Except for Cu, Pb, and Sr, all measured concentrations were lower than those reported by Schulz et al. (1999). In turn, PF values for these three elements were higher than those of Schulz et al. (1999) hinting at contamination. In addition, the index values reported by Pöykiö et al. (2005) calculated using scots pine (*Pinus sylvestris* L.) barks as bioindicators, were either similar to (Cr) or lower than (Fe, Ni) those determined in this study. Although comparison of concentrations reported for different biomonitoring agents is not appropriate because of the differences in accumulations rates, the use of PF lets one to make the comparisons since those are normalized concentrations. In addition, there were no reports of PF levels based on olive leaf trace element concentrations. On the other hand, even different parts of the same agent may give different results. For example, Rossini Oliva and Mingorance (2006) studied airborne heavy metal pollution using aboveground plant (*Pinus pinea* L. and *Nerium oleander* L.) parts and found that Al, Ba, Cr, Cu, Fe, Mn, and Pb concentrations in bark and leaves were higher than those in wood, who concluded that

plant bark and leaves accumulate pollutants straight from the atmosphere and they are deposited mainly on the surface, whereas wood accumulates elements mainly from the soil; and the ratio between bark and leaves to wood reflects the effect of direct atmospheric contamination.

The results of this study showed that, in general, there was a large variation in trace elemental concentrations in olive tree leaves in the province which may be caused by anthropogenic influence and/or different soil types with variable elemental profiles. The coefficient of variation (CV) were relatively low for abundant crustal elements (0.38, 0.42, and 0.49 for K, Ca, and Mg respectively), while it ranged from 0.54 (Co) to 2.2 (Cu) for mainly anthropogenic elements. Elements that may be of both crustal and anthropogenic origins had similar CV values to mainly anthropogenic elements. Elemental soil profiles dominated by crustal elements (Al, Ca, Fe, Mg, K) and sea-salt-Na may be considered as typical for the Mediterranean region (Vaccaro et al. 2007; Yay et al. 2008). It may be postulated that although not as significant as in Germany (Schulz et al. 1999) there was a contamination of Co, Cr, Cu, Li, Na, Ni, Pb, and Sr in Aydın when CV and PF_{max} values were considered in conjunction with the literature reported index values.

3.2 Sources

Firstly, the sampling sites were grouped as urban–rural and roadside–nonroadside in order to elaborate on the elements

emitted in the urban environment and the inter-city roadways as contamination sources. Descriptive statistics of the concentrations for each group were calculated and presented in Table 3 for urban–rural, and Table 4 for roadside–nonroad side comparisons for the anthropogenic elements. The tables also include the mean±standard deviation of PF values for each group. The difference in mean elemental concentrations was tested using *t* test assuming unequal variances.

Although all of the mean urban concentrations were higher than the rural ones, the only statistically significant differences were for Cu and Pb. However, since duplicate differences were rather large for Pb (see “Quality assurance/quality control (QA/QC)” in “Materials and methods” section) we may state that the differences in the mean urban and rural sampling sites were significant for Cu only. All of the urban median concentrations were higher than rural except for Sr. Statistical testing was repeated with Mann–Whitney test for the difference in medians. Both Cu and Pb were identified as having significant differences ($p \leq 0.05$); however, it was confirmed by Kolmogorov–Smirnov test for Pb ($p=0.09$) but not for Cu ($p=0.27$). Although all roadside mean concentrations (except for Mn) were greater than nonroadside mean values, all of the differences were not significant except for As.

Na may be considered as a sea-salt element. In order to observe the change in average Na concentrations with

Table 3 Comparison of urban and rural sampling site concentrations and PF values

Concentration (µg/g)						
	Mean	Median	SD	Max	Min	PF
Urban (n=8)						
As	8.6	8.1	4.9	17	2.7	3.1±1.8
Cr	23	22	4.7	32	19	2.7±0.5
Cu	179*	163	65	319	121	5.4±2.0
Mn	1,385	1,279	381	1,980	836	3.9±1.0
Ni	86	78	47	186	39	4.8±2.6
Pb	42*	37	23	87	18	5.7±3.1
Sr	2,316	1,873	1,377	5,473	1,390	13.2±7.8
Rural (n=8)						
As	6.4	5.7	2.1	10.9	4.4	2.5±0.8
Cr	20	19	6.7	29	BDL	2.0±1.1
Cu	112*	115	52	180	33	3.4±1.6
Mn	1,380	1,226	542	2,321	813	3.9±1.5
Ni	54	56	26	99	18	3.0±1.5
Pb	23*	15	14	42	BDL	3.1±1.9
Sr	2,024	1,911	963	3,916	710	11.5±5.5

SD standard deviation, Min minimum, Max maximum, PF pollution factor (mean±standard deviation)

* $p < 0.05$

Table 4 Comparison of roadside–nonroadside sampling site concentrations and PF values

Concentration (µg/g)						
	Mean	Median	SD	Max	Min	PF
Roadside (n=8)						
As	10*	10	4.5	17	3.8	3.9±1.7
Cr	24	23	4.5	32	19	2.8±0.5
Cu	165	158	78	319	76	5.0±2.4
Mn	1,242	1,150	389	1,980	806	3.5±1.1
Ni	95	87	53	210	39	5.3±3.0
Pb	33	25	17	62	18	4.5±2.2
Sr	2,460	2,062	1,316	5,473	1,390	14±7.5
Nonroadside (n=8)						
As	6.4*	5.7	2.2	11	4.2	2.5±0.8
Cr	19	19	6.7	29	BDL	2.0±1.1
Cu	109	115	48	172	33	3.3±1.5
Mn	1,503	1,248	623	2,321	813	4.3±1.8
Ni	73	56	75	254	18	4.1±4.2
Pb	22	15	15	42	BDL	2.9±2.0
Sr	1,898	1,911	1,086	3,916	579	11±6.1

SD standard deviation, Min minimum, Max maximum, PF pollution factor (mean±standard deviation)

* $p < 0.05$

respect to distance from the Aegean Sea, three areas were drawn in such a way that one represents the closest region to the sea, three represents the furthest region from the sea. Distributions of Na concentrations are presented in Fig. 2. As the distance from the sea increases, Na concentrations were decreasing. The difference in the mean and median values of region-1 and region-2, and region-1 and region-3 were all significant ($p \leq 0.10$), which actually may confirm that sea-salt Na (Yatkin and Bayram 2008a) influence the land up to a distance.

Secondly, probable sources were identified and apportioned using two methods of statistical analysis: correlation matrix and factor analysis. Correlation matrix is used to infer on the elements with common sources. Table 5 shows the Pearson correlation matrix for elements that were

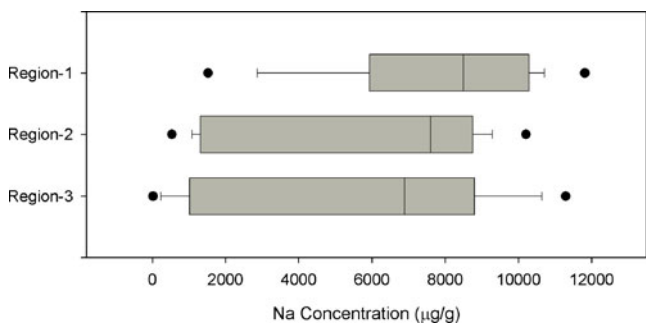
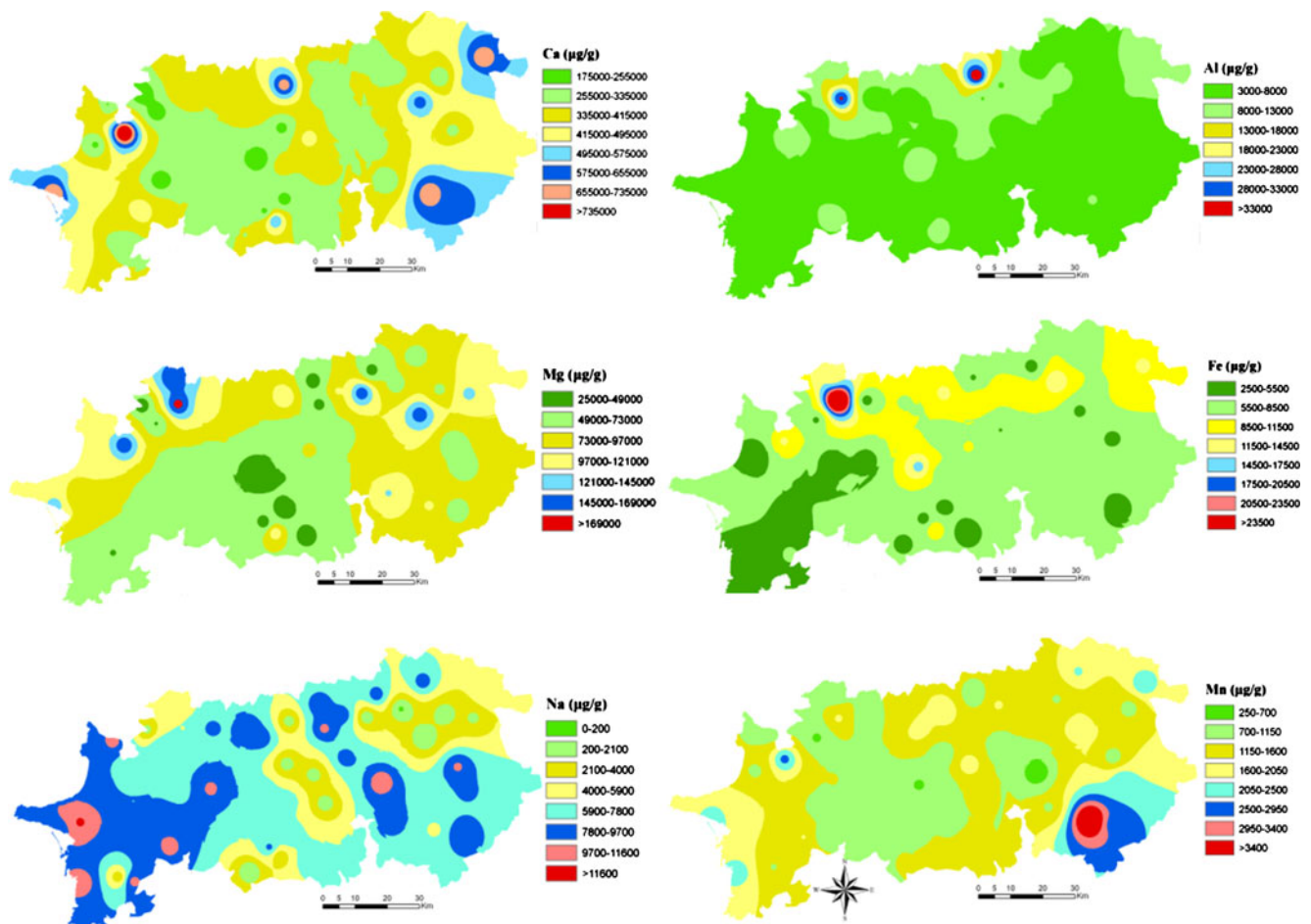


Fig. 2 The box-plots of Na concentrations versus proximity to the sea

Table 6 Scores of varimax rotated factor analysis

	Factor-1	Factor-2	Factor-3	Factor-4	Factor-5
Al	0.94				
As	0.87				
B			0.55	0.47	
Ba				0.55	
Ca		0.77			
Co	0.88				
Cr	0.95				
Cu		0.74			
Fe	0.95				
K				0.85	
Li	0.87				
Mg			0.71		
Mn		0.82			
Na				-0.72	
Ni					0.67
Pb					0.77
Sr			0.84		
Variation (%)	38	17	9	8	6
Cumulative Variation (%)	38	55	64	72	78

**Fig. 3** Concentration maps of crustal elements (Ca, Mg, and Na) and elements of both crustal and anthropogenic origin (Al, Fe, and Mn)

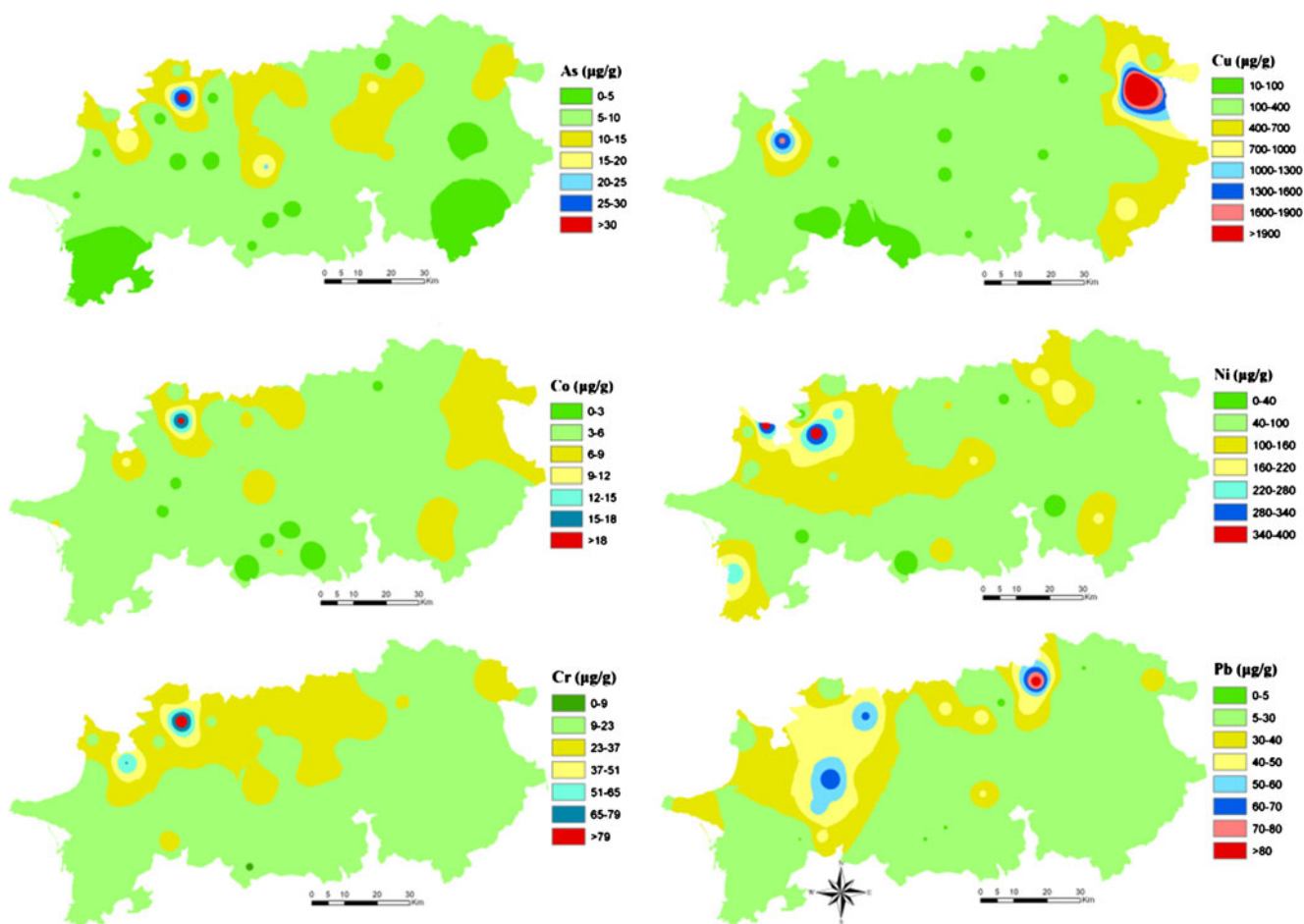


Fig. 4 Concentration maps of anthropogenic elements: As, Co, Cr, Cu, Ni, and Pb

mal power plant, mining sites and processing plants, tobacco-processing plants, and city and town centers spread around the province. Agricultural activities are abundant in the province ranging from fruits and vegetables to tobacco in which various pesticides with different formulations are used. The concentration maps can be effectively employed to identify the pollution sources at the hot spots. Fuel–oil, coal, wood, and olive oil cake burning in residential areas and industrial plants, traffic emissions, mining and mineral processing, tobacco processing, fertilizing, pesticides, municipal and industrial wastewater, and solid wastes are possible sources of the studied elements (Fogg and Rahn 1984; Pacyna and Pacyna 2001; Yoshinaga et al. 2001; Howe et al. 2004; Wang et al. 2006; Cetin et al. 2007; Yatkin and Bayram 2008b; Farooqi et al. 2009; Giraud et al. 2009; Houhou et al. 2009; Zhang et al. 2009) in the province. Na is generally a sea-salt element that is typical for the Mediterranean region. Figure 3 shows that the highest Na concentrations were found in regions close to the shoreline. In addition to marine salts, Na may also be present in emissions of coal and olive oil cake burning, and traffic (Cetin et al. 2007; Yatkin and Bayram 2008b). Inland

high concentration spots in Aydın may indicate those types of sources for Na.

4 Summary and conclusion

The Province of Aydın was determined to be contaminated with several elements when CV and PF values were considered. Sodium concentrations showed that sea-salt particles were a source of contamination. Comparison of urban–rural, and roadside–nonroadside concentrations and PF values showed higher concentrations in urban and roadside sampling sites, however the differences were generally not significant. Several possible sources were identified using statistical techniques which included different types of soil and anthropogenic activities. The obtained information were further clarified by examining spatial variation on the constructed elemental concentration maps pointing to organized industrial parks, mining activities, and tobacco processing as major sources, in addition to the more common sources such as coal burning and traffic emissions. In conclusion, olive leaves were

found to be suitable bioindicators of pollution in the Mediterranean where they are indigenous and cultivated.

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