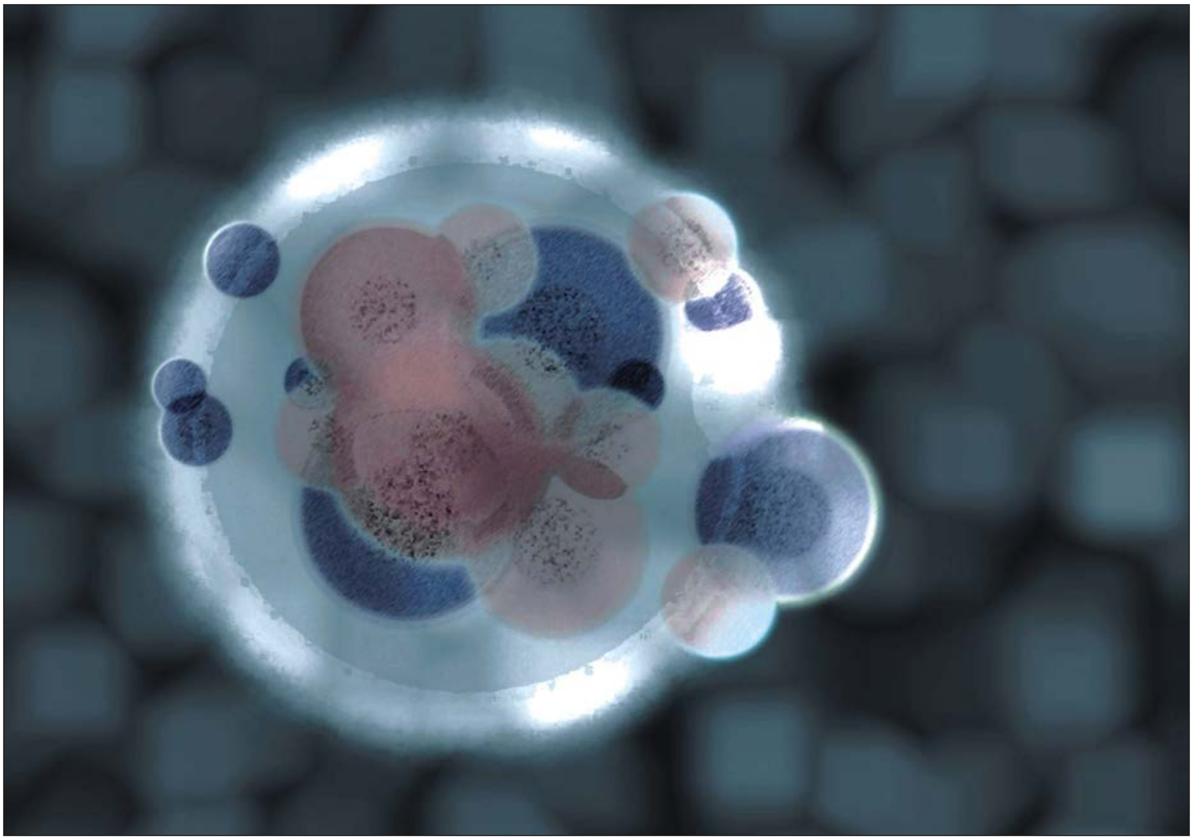

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Biomonitoring and Annual Variability of Heavy Metal Concentration Changes Using Moss (*Hypnum cupressiforme* L. ex. Hedw.) in Canakkale Province

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Abstract: Moss transplants of the species *Hypnum cupressiforme* L. ex Hedw. were used as active biomonitoring organisms. The moss samples were exposed at a Canakkale province site for 12 months for subsequent analysis of metal concentration (Cr, Cd, Pb, Fe, Ni, Mn). This field experiment was carried out to investigate the accumulation of the metals over a period of 3, 6, 9 and 12 months at 10 sample places. Results of heavy metal deposition indicated that metal concentration in the moss samples generally increased with the length of exposure and was higher at the roadside site. Heavy metal deposition was found in Canakkale province heavily polluted by Fe, Ni, Mn, Pb and Cr between the dates 12.06. 2002-17.03.2003. Consequently, oil and coal pollution is observed in Canakkale province. This pollution caused a very heavy metal deposition on moss especially on the lower grounds of the province in comparison to the higher ground. Apart from Cd, all different types of metal deposition are observed annually.

Key words: Biomonitoring, atmospheric deposition, heavy metals, *hypnum cupressiforme*

INTRODUCTION

Pollution by heavy metals over large areas and long periods of time may cause chronic damage to living organisms and must be carefully controlled. One way to determine the extent of environmental contamination is by measuring the levels of contaminants in plants. The use of mosses as biomonitors is a convenient method to determine levels of atmospheric deposition, as terrestrial mosses obtain most of their supply of mineral elements from precipitation and dry deposition of airborne particles. Mosses have therefore received increasing attention as a suitable tool for monitoring regional patterns of elemental deposition from the atmosphere in large scale studies in various countries, in areas close to industrial installations as well as in areas not expected to be contaminated[1,2]. Beside the fact that the "moss-method" is a useful tool for deposition measurement in the vicinity of stationary sources[3].

Methods based on the use of mosses for surveying heavy metal deposition were developed in Sweden at the end of the 1960s[4]. Biomonitoring using moss is a well-developed technique employed in many parts of the world to assess the concentrations of trace elements in the atmosphere and their potential sources. The suitability of the moss *Hypnum cupressiforme* L. ex Hedw. known as a one of the best biomonitor of atmospheric trace element pollution in several biomonitoring studies[2,5].

Primitive plants such as mosses have either poorly-developed or non-existent root system and therefore absorb most of their nutrients directly from the atmosphere, via either wet or dry deposition. Thus, mosses are also a best bioacumulator for heavy metal biomonitoring studies[6-8]. The lack of a thick cuticle promotes the migration of heavy metals and other elements to free cation exchange sites located on the walls of the cells in mosses[4]. Mosses require small amounts of heavy metals, e.g. Zn and Cu, for their metabolic functioning. Air pollution has a considerable effect on the element concentration in mosses. The absorption efficiency of mosses for heavy metals is affected by the proportion of free cation exchange sites, the form in which the heavy metals are present in deposition, the proportions of dry and wet deposition, ion exchange reactions and leaching. Furthermore, heavy metals and other cations also compete for the same cation exchange sites[4,9].

Mosses have been used large-scale, heavy metal deposition surveys mainly in Europe. During the past few decades the heavy metal emissions in Europe have decreased, but those in Asia have increased. As a result, heavy metal research has focused on those heavy metals that are readily transported over long distances, such as Hg, Cd and Pb[4].

Last few decades several biomonitoring studies were used mosses for heavy metal deposition in Turkey[8,10].
The first aim of this research was found first results for heavy metals deposition in Canakkale with active biomonitoring by moss, (*Hypnum cupressiforme* L. ex. Hedw.) and to obtain the pollution maps for six heavy

metals (Cr, Cd, Pb, Fe, Ni, Mn) using results of heavy metal deposition.

MATERIALS AND METHODS

Urbanization border of Canakkale used as the study area[11] Fig. 1. The sample places, where the experimental moss were located, were selected as the busy roads open to traffic, the military regions and government buildings considering the security of the samples. Two criteria were

Table 1: Sample places in the study area

No.	Sample places
1	Piri Reis Sitesi (On the coast)
2	18 March Hill (Military region, open area)
3	City Dump (500 m distance from coast-open area)
4	Yeni Kordon Sitesi (On the coast)
5	Yerel Gundem 21 (Nearby busy roads-onto the building roof)
6	Old SSK Hospital (Nearby busy roads-onto the hospital roof)
7	On Sekiz Mart University, Anafartalar Campus (Nearby busy roads-onto the roof of buildings)
8	Meyvecilik Uretme Istasyonu (Nearby busy roads-in the garden, to the open area)
9	Meteorology Park (On the coast-in the garden, to the open area)
10	Egitimciler Sitesi (Urban area)

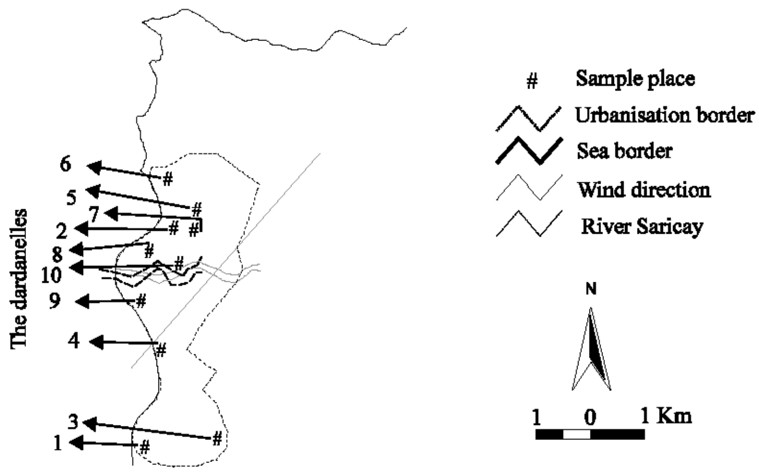

Fig. 1: Study area and sample places

Table 2: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 1 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	5.20±0.08	0.21±0.01	21.96±0.04	832.13±50.01	301.82±0.21	306.03±11.02
17.09.2002	2.40±0.20	0.30±0.06	17.93±2.8	449.76±370.48	274.74±44.86	268.52±6.85
17.12.2002	4.83±1.65	0.33±0.18	23.10±6.58	873.45±586.21	303.80±1.54	270.65±11.21
17.03.2003	4.16±0.01	0.41±0.01	21.54±0.01	849.48±25.71	174.23±0.01	345.77±5.18

Table 3: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 2 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	4.70±2.12	0.42±0.06	20.64±6.35	431.39±280.73	230.40±29.50	180.65±110.35
17.09.2002	3.49±0.20	0.48±0.17	26.40±5.54	695.20±368.29	224.03±28.91	355.55±175.50
17.12.2002	5.94±0.85	0.38±0.11	28.28±2.72	1133.19±272.68	181.86±35.39	422.68±19.23
17.03.2003	8.60±6.27	0.26±0.01	22.64±6.29	1226.23±225.12	171.84±2.07	438.54±289.68

Table 4: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 3 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	3.68±0.35	0.15±0.10	15.18±1.74	462.08±181.23	167.28±22.11	133.38±14.23
17.09.2002	4.81±0.71	0.40±0.11	27.91±3.09	720.68±52.12	203.71±19.77	349.97±31.07
17.12.2002	5.27±0.17	0.29±0.06	25.52±4.71	901.26±31.48	158.35±5.26	333.97±11.89
17.03.2003	8.77±1.12	0.30±0.07	19.40±2.20	980.81±70.12	152.88±8.17	333.87±17.45

Table 5: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 4 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	3.62±0.02	0.18±0.08	16.45±2.48	454.47±152.81	174.49±24.93	168.97±7.88
17.09.2002	1.99±0.01	0.28±0.14	14.92±6.11	240.25±166.33	165.44±46.16	223.40±24.89
17.12.2002	3.15±1.07	0.20±0.09	8.78±1.44	401.05±315.15	141.57±11.67	255.57±51.44
17.03.2003	10.31±6.80	0.18±0.05	28.10±6.38	1519.16±261.19	211.12±8.65	621.54±10.49

Table 6: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 5 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	3.21±0.01	0.17±0.05	10.31±1.62	179.79±112.18	109.27±17.62	112.32±24.41
17.09.2002	2.74±0.03	0.26±0.01	18.92±0.01	492.19±18.85	225.32±5.32	245.10±15.40
17.12.2002	5.88±0.01	0.24±0.01	25.83±0.01	1156.51±12.74	229.73±0.41	322.43±8.03
17.03.2003	12.41±2.99	0.18±0.04	28.69±1.13	1454.78±91.57	220.44±12.68	459.52±129.13

Table 7: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 6 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	3.83±0.64	0.19±0.07	14.38±3.80	232.84±141.08	219.34±14.93	191.52±65.06
17.09.2002	4.57±1.01	0.33±0.05	22.41±4.36	586.40±118.53	195.38±44.32	368.66±39.58
17.12.2002	8.75±2.20	0.27±0.02	22.30±8.49	997.44±496.26	202.56±10.96	300.72±162.11
17.03.2003	27.17±12.37	0.15±0.04	27.40±12.87	2097.58±521.12	325.02±20.04	577.64±185.21

Table 8: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 7 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	8.96±0.02	0.19±0.04	11.18±5.38	1198.15±69.82	195.23±22.01	307.00±139.24
17.09.2002	5.14±1.17	0.27±0.01	32.96±2.41	1377.89±523.94	294.77±40.31	478.80±47.32
17.12.2002	8.33±1.06	0.26±0.07	25.28±1.92	1163.37±124.11	122.98±65.03	422.57±54.24
17.03.2003	18.91±2.14	0.17±0.04	41.30±5.90	2427.64±560.21	188.02±25.70	711.41±163.12

Table 9: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 8 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	4.30±1.10	0.23±0.01	19.83±0.05	402.06±0.02	193.84±0.02	226.31±151.30
17.09.2002	4.27±0.12	0.25±0.09	29.44±16.94	508.06±47.93	208.46±97.07	185.32±310.31
17.12.2002	6.66±0.02	0.29±0.01	33.31±0.14	1206.58±35.92	186.08±0.82	397.94±22.01
17.03.2003	6.54±4.29	0.31±0.20	47.83±8.44	1297.28±71.12	209.65±30.59	346.76±258.38

Table 10: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 9 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	3.57±0.56	0.21±0.08	17.73±5.54	446.87±166.98	181.70±30.19	204.55±95.89
17.09.2002	4.60±0.81	0.20±0.10	25.63±6.09	850.15±117.98	179.74±58.38	242.41±18.11
17.12.2002	4.99±3.25	0.26±0.03	22.47±9.68	1316.94±364.30	172.72±17.46	387.53±153.81
17.03.2003	4.99±1.44	0.14±0.09	21.63±9.23	1568.36±199.40	162.11±13.28	506.33±56.01

Table 11: Heavy metal contents of moss samples, *Hypnum cupressiforme* in sample place 10 (ppm)

Sampling date	Cr	Cd	Pb	Fe	Ni	Mn
12.06.2002	2.91±0.01	0.16±0.08	16.54±4.78	508.67±160.84	178.66±8.95	214.51±28.11
17.09.2002	1.50±0.01	0.20±0.05	13.81±4.06	353.18±202.37	244.01±37.64	240.01±14.66
17.12.2002	2.33±2.30	0.23±0.01	13.81±9.33	386.46±6.46	204.51±12.35	196.94±11.51
17.03.2003	1.65±0.01	0.22±0.01	16.21±5.33	531.85±17.21	163.50±3.02	230.48±3.69

paid attention while selecting the 10 sample places; the distance to the city center and the density of settlement. Sample places are shown in Table 1. Arc VIEW 3.2 software programme is used for mapping heavy metal deposition in Canakkale province.

Plant material is *Hypnum cupressiforme* L. ex Hedw., a pleurocarpous moss species. The moss samples on the ground were obtained from forest region of Bayramic-Ayazma (Canakkale-Ida Mountain) in February 2002. The samples of *Hypnum cupressiforme* L. ex. Hedw. collected described on their native regions. Mosses were supplied with their local soil which was placed on plastic plots. Then the plots were transported and placed on sampling places. Atmospheric heavy metal deposition of *Hypnum cupressiforme* L. ex Hedw. monitored between the dates 12.03.2002 and 17.03.2003. Samples were obtained from sample places for heavy metal analyses four times at 12.06.2002 (Spring), 17.09.2002 (Summer), 17.12.2002 (Autumn), 17.03.2003 (Winter), respectively.

The moss samples were oven dried for 24 h at 100°C and then crushed and ground with an agate mortar and pestle. Subsequently, 1.5 g of each sample were subjected to a mixture of concentrated HNO_3 and H_2O_2 (5:2 v/v) in Pyrex beakers and heated at 120°C on a hot plate until the organic materials were oxidized. Each solution was evaporated to almost total dryness[12]. The residue was dissolved in 10 mL HCl and diluted to 50 mL with bidistilled water. Duplicate solution of each sample was prepared. Cr, Cd, Pb, Fe, Ni, Mn measurements were made by aspirating the sample solutions to Atomic Absorption Spectrophotometer (AAS).

In order to evaluate the precision of the measurements, the standard deviations of the analyses were calculated by dissolving four replicate samples and then measuring them (Table 2). Statistical data obtained from ANOVA and Tukey tests in Windows SPSS 11.0 software. The mean difference is significant at $p<0.05$ level.

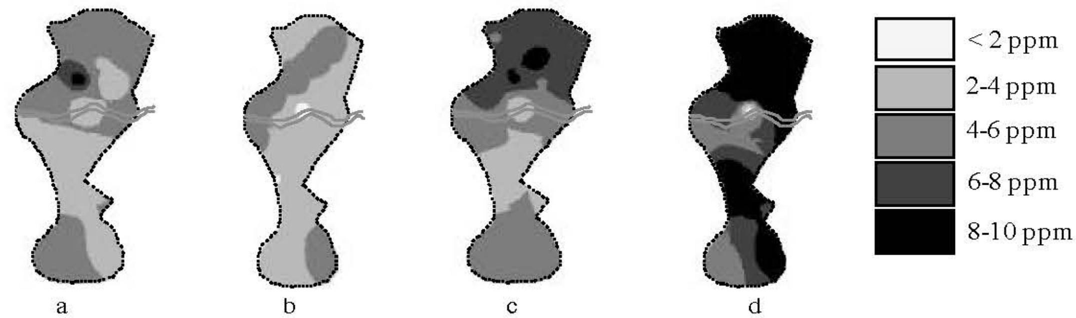

Fig. 2: Spatial and temporal variation of Cr in ppm (a: Spring, b: Summer, c: Autmn, d: Winter)

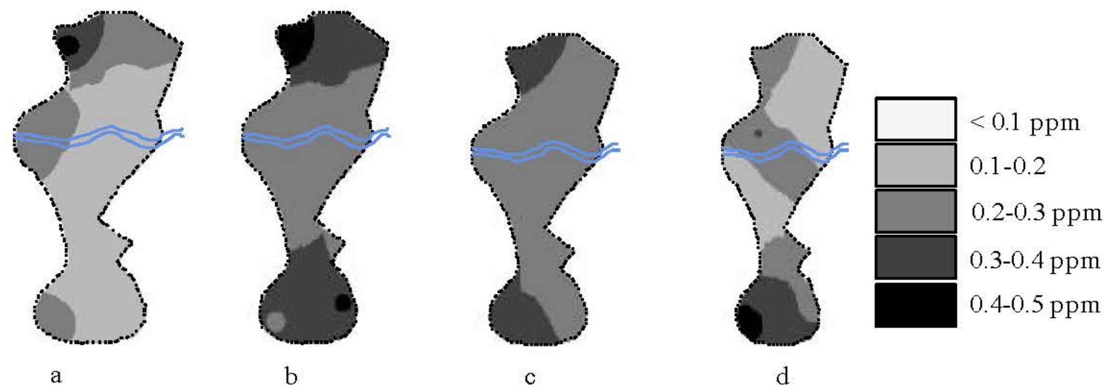

Fig. 3: Spatial and temporal variation of Cd in ppm (a: Spring, b: Summer, c: Autmn, d: Winter)

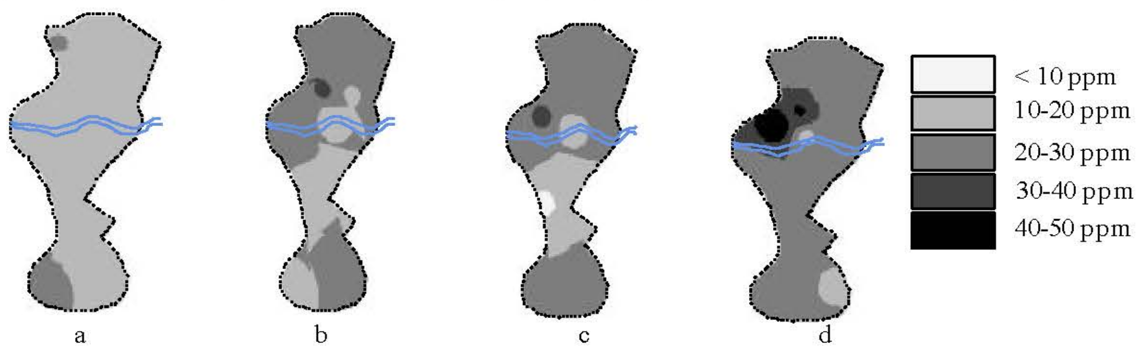

Fig. 4: Spatial and temporal variation of Pb in ppm (a: Spring, b: Summer, c: Autmn, d: Winter)

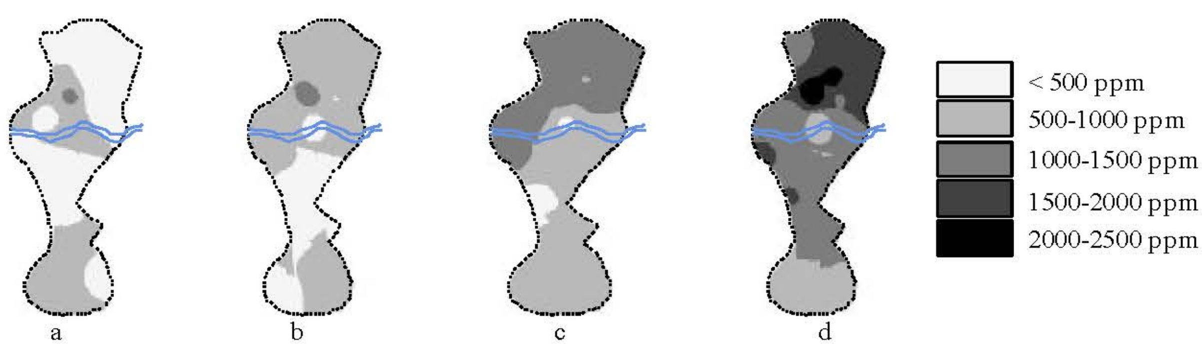

Fig. 5: Spatial and temporal variation of Fe in ppm (a: Spring, b: Summer, c: Autmn, d: Winter)

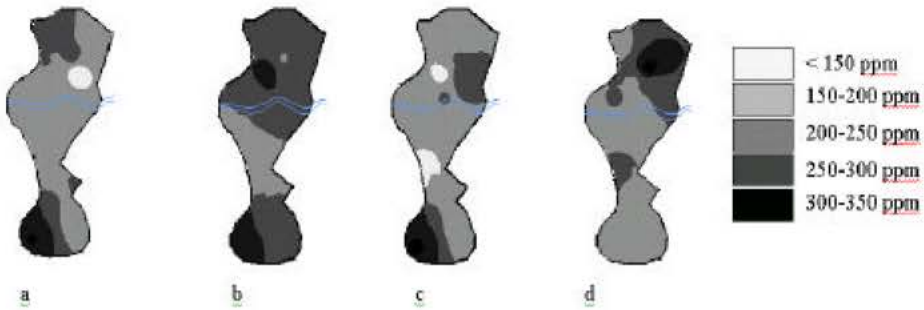

Fig. 6: Spatial and temporal variation of Ni in ppm (a: Spring, b: Summer, c: Autumn, d: Winter)

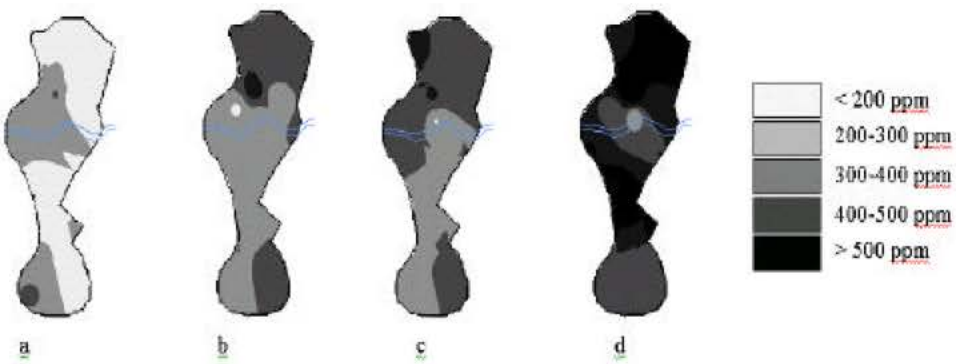

Fig. 7: Spatial and temporal variation of Mn in ppm (a: Spring, b: Summer, c: Autumn, d: Winter)

RESULTS AND DISCUSSION

The largest Cr value is observed at sample place 6 as 27.17(Table 7) ppm whereas the lowest is observed at sample place 10 as 1.50 ppm among all the stations (Table 11). The largest Cd value is obtained at sample place 2 as 0.48(Table 3) the lowest is at sample place 9 as 0.14 ppm (Table 10). In terms of Pb values sample place 8 has the largest as 47.83 ppm (Table 9) and the lowest is sample place 4 as 8.78 ppm (Table 5). Fe is observed the most at sample place 7 stations 2427.64 ppm (Table 8) and the least at the sample place 5 as 179.79 ppm (Table 6). For the Ni values the largest is monitored at the sample place 6 as 325.02 ppm (Table 7) and the lowest is at the sample place 5 as 109.27 ppm (Table 6). Sample place 7 gives the maximum Mn value as 711.41 ppm (Table 8) and sample place 5 gives the minimum as 112.32 ppm (Table 6).

When the results are compared, the sample place 5 is observed to have the minimum values for Fe, Ni and Mn deposition. On the other hand sample place 7 has the maximum values for Fe and Mn in comparison to the other sample places. This may be the result of the military zone covered by vegetation, which is next to the sample place 5; whereas sample place 7 has a dense settlement close by, especially having central heating systems burning coal.

When all the sample places are considered, the burning of coal or oil for heating and corrosion of the vehicle tires may be affective on the maximum deposition of Ni and Cr at the sample place 6 between the dates of 12.06.2002 and 17.03.2003. Pb deposition, which mostly due to exhaust emission has the maximum values on the samples observed on 17.03.2003 at the sample places 4, 5, 6, 7 and 8. The most important factor for this deposition is the busy roads close to the sample places.

There obtained mean difference ($p < 0.05$) on the Cr deposition between sample places 7 and 10, Cd deposition between sample places 2 and 3, Fe deposition between sample places 6 and 7, Ni deposition between sample places 1 and 6 at the statistical data collected on 12.06.2002.

According to the data gained 17.09.2002, Cr deposition between sample places 8 and 5 and Fe deposition between sample places 7 and 4 has a mean difference.

There obtained also mean difference ($p < 0.05$) on the Cr deposition between sample places 7 and 10, Pb deposition between sample places 8, 4 and 10, Ni deposition between sample places 1 and 4 at the statistical data collected on 17.12.2002.

Finally, statistical data of 17.03.2003 showed that Cr deposition between sample places 6, 1 and 10, Ni deposition between sample places 6, 1, 2 and 10 have mean differences ($p < 0.05$).

Most studies have reported significant correlation between Pb and Cd concentrations in mosses and in wet deposition[13,14]. On the contrary, Cd results did not support the data in Canakkale province. There found no great atmospheric Cd deposition results with Pb in *Hypnum cupressiforme* L. ex Hedw. However, Cd and Cr have particularly harmful effects and toxicity for organisms. Cd is mainly spread to the environment by the use of phosphate fertilizers and through emissions from metal industry. The Cd content of mosses less than 0,3 ppm in unpolluted area in Europe[15]. The main source of Cr is also iron-steel mills. The baseline of Cr in unpolluted regions is lower than 1 ppm[15]. Results have shown that Cd values found were slightly polluted only at sample place 2. However, maximum Cr values obtained in Canakkale province addressed strong pollution between the dates 12.06. 2002-17.03.2003.

Soil dust has been shown to have a significant effect on the Fe concentrations, as well as to some extent Ni, Cr and Cd concentrations[13,14,16]. In Canakkale province, the effect of soil contamination on metal concentrations is likely to be relatively small because, apart from a number of sample plots in study area, all of the moss samples were transported and placed from forested area (Bayramic-Ayazma) to research points, because sample places have been covered with vegetation. The main emission source of Fe is oil and coal burning and the steel industry[15]. In this addition, increasing Fe values indicated that similar anthropogenic factors in study area. Vargha *et al*.[15] showed that the content of Fe in mosses from unpolluted areas is generally lower than 500 ppm. Maximum Fe values found in Canakkale province also pointed out strong pollution by this element between the dates 12.06. 2002-17.03.2003. Fe results are similar to Poikolainen *et al*.[4] On the other hand, wind effect is very important in atmospheric deposition of heavy metals and wind transect in Canakkale province (Fig 1). Comparison of the Fe values between all sample places stated that, there has been a heavy Fe pollution in the study area.

The elemental composition of mosses within same species varies to some extent naturally in different areas as a result of variations climate[17]. Many studies reported that both natural and anthropogenic sources play a part in supplying Fe, Cr and Ni to the atmosphere, however, found that atmospheric deposition of Fe, Ni and Cr was mainly due to local sources rather than long-range transport[9,4]. On this occasion, the results of the study at Canakkale province are parallel to reference studies.

During the winter period (17.12.2002-17.03.2003), heavy metal deposition dramatically increased in *Hypnum cupressiforme* L. ex Hedw., especially sample place 5, 6 and 7, except Cd. It may be caused by the reason of this area being placed in lower ground of the area in urban. This geological situation is a possible factor for higher heavy metal deposition in these sample places than the others. Wind effect is very important for removing and scavenging of pollution emissions. The results indicated that, wind is not effective in this area (Fig. 2-7).

There are differences in the accumulation of individual heavy metals in mosses and the heavy metal concentrations in mosses are also affected by factors other than atmospheric pollution. The effect of other factors may be considerable especially in lower ground of the area in urban. Consequently, oil and coal pollution is observed in Canakkale province. This pollution caused a very heavy metal deposition on moss especially on the lower grounds of the province in comparison to the higher ground. Apart from Cd, all different types of metal deposition are observed annually.

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