

Research Article

Heavy metal accumulation by Mosses as affected by roadside pollutants

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Abstract: Mosses sampling were collected from 10 sites within the Erbil governorate in October 2015 during dry condition. The samples were air dried and crushed to fine particulates using rotary, then after the heavy metals concentration were determined by Genius XRF spectrophotometer (Skyray Instruments, USA). Location Map of selected mosses distribution were made. The results revealed that the mean values of Pb was 52.25mg/kg, Hg was 0.01mg/kg, Mn was 162.8mg/kg, Fe was 1.655%, Cu was 8.27mg/kg, Zn was 162mg/kg, Ti was 851.1mg/kg, Co was 6.28mg/kg, Ni was 57.57mg/kg, As was 6.84mg/kg, Se was 0.19mg/kg and Cr was 9.73mg/kg. The statistical results indicated that there were significant difference ($P < 0.05$) between sites and mosses species. The data obtained in this study was compared with that of WHO standards, the results revealed that the concentration of studied heavy metals were less than of WHO standards.

Keyword: Dry condition, Erbil, Iraq, Heavy metal, Mosses.

1. Introduction

Mosses are green plants, without true roots, stems, leaves and lack cuticle and vascular system (Aziz, 2012), picking up nutrients and other chemical substances, mainly from wet and dry deposition by surface area and diffusion from substrate; thus mosses adsorb and absorb pollutants and nutrients directly from the atmosphere (LeBlanc *et al.*, 1974; Govindaparyi *et al.*, 2010). The accumulation of air pollutants depends on the high surface-volume ratio of mosses tissue (Santelmann and Gorham, 1988; Ekpo *et al.*, 2012). The monitoring of heavy metal and nitrogen concentrations in naturally growing mosses allows determination of spatial patterns and temporal trends of heavy metal and nitrogen deposition in different manners (Harmens *et al.*, 2008; 2011 and 2013). Since terrestrial epipellic and epilithic mosses growing on the ground have a high capacity to retain many trace elements. Therefore, they are suitable for assessment and biomonitors of atmospheric deposition of heavy and trace elements (Abdullah *et al.*, 2014). In this respect, mosses were used for 45 years from 1968 as an indicator for air quality assessment and monitoring the deposition of air born heavy metals (Steinnes *et al.*, 1997 and Ekpo *et al.*, 2012). A single or a few or more than 30 elements studied simultaneously as biomonitoring (Harmens *et al.*, 2008). In Norway, the moss technique has been used regularly for the last

20 years in deposition surveys on a national scale (Steinnes *et al.*, 1997). Since these chemical substances are transported across international boundaries far from their sources and they persist in the environment, bioaccumulate occur through the food chain, and cause a risk to human health and the environment. International action has been taken in the form of two international agreements to protect human health and the environment (Bykowszczenko *et al.*, 2006; Harmens *et al.*, 2011; Harmens *et al.*, 2013).

In Nigeria, the concentration of six heavy metals were determined in five mosses species and used as bioindicator (Ojo *et al.*, 2012). In European country since 1990, the moss survey has been repeated at five-yearly intervals; in 2005 the lowest concentrations of metals in mosses were generally found in Scandinavia, the Baltic States and northern parts of the UK. The concentrations were generally found in Belgium and south-eastern Europe. However, the results of European studies from 1990 to 2005 indicated that the concentration of metals in mosses has declined, especially that of arsenic, cadmium, iron, lead and vanadium by 52-72%, followed by copper, nickel and zinc by 20-30%, while the reduction of mercury concentration was not significant (12%) and of chromium (2%), Harmens *et al.*, 2008, 2011, 2013 and in some cities in Nigeria (Ekpo *et al.*, 2012).

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Since there are no studies using mosses in this field of science in Iraq and Kurdistan region. The aim of this study is to estimate environmental air pollution caused by heavy metal deposition on the main road in Erbil Governorate.

2. Materials and Methods

2.1 Site description

Erbil is the capital of Iraqi Kurdistan region. It's located in the north part of Iraq; Erbil borders of Turkey to the north and Iran to the east. Geographically Erbil is elevated by about 411m above sea level. Erbil Governorate covers an area of 164840 km² in the north of Iraq (Fig. 1). Erbil Governorate longitude is 42° 15'E to 46° 30'E and latitude is 34° 25'N to 37° 50'N. In order to conduct the present study, 10 locations were selected from the north of Erbil governorate and inside Erbil city as described in (Table 1). The studied locations were covered the major area as possible based on the traffic activity as most of them located on the main roads of Erbil city and Hamilton main road toward Soran district. The area of this study located between 36° 19' 5177N, 44° 009414E and 36° 37.562N, 43° 34.422E (Hama *et al.*, 2014).

2.2 Geology, soil, and climate

Geologically, Erbil situated within recent sediment which belongs to Paleocene age that represents old river

sedimentation, which came from Bakhtiari formation (WFP, 2002). The stone of these sediments differs and compose of lenticels and stereographs with stone, sand, silt and alluvial, the thicknesses of the sediments vary largely within Erbil governorate (Muhammad, 2003).

The soil of Kurdistan Region of Iraq is calcareous because it is originated from limestone and dolomite of different formation mostly. Erbil soil is brown which occurs under the effect of climatic conditions. The soil has a brown surface layer of about 25-30cm depth gradient into a brownish-gray to whitish horizon of lime accumulation (Buringh, 1960; Brady, 1974). Soil formation of Erbil province ranges from mountainous to foothill soil; mountainous soil formation exists in high folded zone and characterized by dark brown to black color on the upper surface, while it's pink and yellowish color in subsurface and consists of lime and about 4-8% organic matter (Guest, 1966). In foothills, the soils are sandy with clay and suitable for agriculture, in the undulating terrain the soil is generally gravelly, sandy and clay or loam of grayish color and no gravels. Generally, the topsoil is calcareous and may contain 1-2% of organic matter in foothills the soil are sandy with clay and suitable for agriculture, while in the undulating terrain the soil is generally gravelly, sandy with clay and of a brownish color. In the plain, the soils are deep and consist of silty and sandy clay or loam of grayish color, and no gravels (Buringh, 1960).

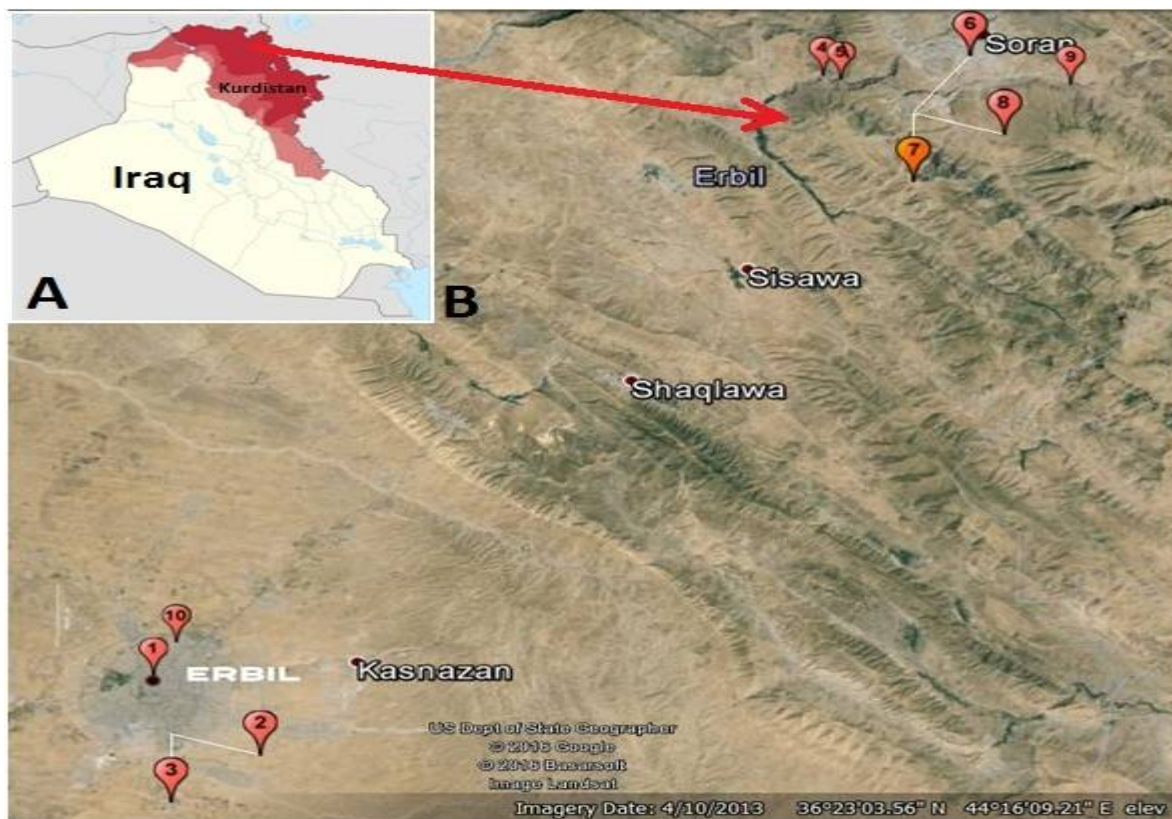


Fig. 1a. Map of Iraq, Kurdistan; Fig. (1b). Map of studied area shows the sampling location.

Table 1. Description of the studied location.

#	Name of location	Latitude / longitude	Description
1	Center of Erbil, Aras streets	36° 195177N/ 44° 009414E	It is located inside Erbil city beside Aras street which is the road between Tairawa and Ibn-Al Mstawfi quarter. Hundreds of vehicles per day are passing.
2	College of Science	36° 152526N/ 44° 019854E	It is located opposite of cafeteria of College, has very low traffic activity.
3	College of Science	36° 152833N/ 44° 021064E	This is located beside Hall of Environment in College of Science, has very low traffic.
4	Gali Ali Beg beside waterfall	36° 631183N/ 44° 445962E	70 Kilometers away from Erbil city, near Gali Ali Beg road. Its main road between Erbil and Soran district, with high traffic load; thousands of vehicles are passed by this area on daily basis; hot and dry in summer, moist, cloudy, rainy in winter up to 1000mm/year.
5	Gali Ali beg main road to Soran district	36° 627812N/ 44° 452166E	Several Kilometers away from Erbil city and few Kilometers far from Gali Ali Beg waterfall. Its main road between Erbil and Soran district, it is a high traffic road; Hundreds of vehicles are passed by this area on daily basis; hot and dry condition in summer, moist, cloudy, rainy in winter up to 1000mm/year.
6	Bekhal-Rawanduz, Malla nabi well	36° 36.143N/ 044° 30.013E	A few Kilometers away from Rawanduz; characterized by hot and dry condition in summer, moist, cloudy, and rainy in winter.
7	Bekhal-Rawanduz, near Malla nabi well	36° 36.143N/ 044° 30.15.85E	A few Kilometers away from Rawanduz characterized by hot and dry in summer, moist, cloudy, and rainy in winter.
8	Bekhal-Rawanduz, near Malla nabi well	36° 36.12.53N/ 044° 30.14.42E	A few Kilometers away from Rawanduz, characterized by hot, dry in summer and moist, cloudy, rainy in winter.
9	Jondian	36° 37.562N/ 044° 34.422E	A few Kilometers away from Soran district, this is the main road to Haji Omran the border point between Iraq and Iran. It is a high traffic road; Hundreds of vehicles are passed by this area on daily. It has the same climate.
10	Gulan Street, Erbil City	36° 219213N/ 44° 024302E	It is a high traffic road; hundreds of vehicles are passed by this area on daily basis.

*Funaria Hygrometrica Hedw.**Grimmia pulvinata Hedw.**Tortula ruralis Hedw.**Cirriphyllum piliferum Hedw.*(a) *Brachythecium rivulare Hedw.*(b) *Brachythecium rivulare Hedw.***Plate 1. The indicator mosses species used in this study.**

The climate of Erbil area is most closely approaches the Iran-Turanian type, and it's similar to that of the other parts of Kurdistan region and the other northern parts of Iraq, which is semi-arid and characterized by hot dry summer and moderately rainy cold winter. The higher altitude parts of the area have colder winters and receive more precipitation than the area of lower elevations (WFP, 2002). Usually, precipitation occurs during the months from September until April, rainfall, and humidity play a great role on the climate, all together with temperature. The climate is characterized by the assurance of four seasons, cold winter and mild growth periods of spring, hot dry summer and autumn pointed out that period from June to the end of January is rainless, whereas the wettest months are between March and April (Hama *et al.*, 2014).

3. Mosses

In this study, the used mosses plants were represented in the Table below.

Sites	Identified Sample
1	<i>Funaria Hygrometrica</i> Hedw.
2	<i>Funaria Hygrometrica</i> Hedw.
3	<i>Funaria Hygrometrica</i> Hedw.
4	<i>Tortula ruralis</i> (Hedw.) G. Gaertn., B. Mey. and Scherb.
5	<i>Brachythecium rivulare</i> Hedw.
6	<i>Grimmia Pulvinata</i> Hedw.
7	<i>Tortula ruralis</i> (Hedw.) G. Gaertn., B. Mey. and Scherb.
8	<i>Cirriphyllum piliferum</i> Hedw.
9	<i>Funaria Hygrometrica</i> Hedw.
10	<i>Tortula ruralis</i> (Hedw.) G. Gaertn., B. Mey. and Scherb.

3.1 Analyze of plant materials

All plant and soil samples were air dried first for 10 days at room temperature. In order to obtain concentrations on a dry matter basis, the samples were further dried in the dryer for 24 h at 50°C, then samples were segregated, cleaned and powdered. The dried plant material was ground in a laboratory mill into fine powder (average particle diameter 100µm). To avoid contamination, the mill was thoroughly cleaned and dried after each grinding.

The powdered dried of plant tissues (leaves) were analysed by XRF spectrophotometer or Genius XRF, Skyray Instrument Inc (Steinnes, 1980; Røyset *et al.*, 1995; Ene *et al.*, 2010). The data was statistically analyzed using analysis variance (2 way ANOVA) and when the result where significant was applied L.S.D. The L.S.D value as mentioned by Abdulla *et al.*, (2014) was calculated by:

$$L.S.D = t. 0.05 \sqrt{\frac{2m^2}{N}} \quad \text{or/and} \quad L.S.D = \sqrt{\frac{2S^2}{N}}$$

4. Results

4.1 Temperature

During of the studied period, the highest temperature value (37.05°C) was recorded at Makhmour location during July 2015 while lowest temperature value (6.2°C) was recorded at Choman district during the month of March 2015 (Table 3).

Table 3. Monthly mean air temperature (°C) values for the of study location within Erbil governorate during wet condition Mar, Apr and May and dry condition Jul, Aug and Sept 2015.

Location	Wet condition			Dry condition		
	Mar.	Apr.	May	Jul.	Aug.	Sep.
Erbil center	10.21	15.7	20.11	36.2	35.0	27.8
Choman district	6.2	14.6	20.8	31	28	25.9
Soran district	7.4	17.4	22.7	31.3	31.1	27.4
Rawanduz district	9.6	16	22	30.3	31.1	27
Harir district	6.5	16.3	24.5	31.2	33.8	29.9
Shaqlawah district	6.5	15.7	19.6	28.1	28	23.3
Salahaddin sub-district	11.2	21.95	27.1	35.9	35.15	30.8
Makhmour district	12.3	25.05	28.3	37.05	36.7	32
Mean	8.73	17.83	23.13	32.63	32.35	28.01

Source: Meteorological Center of Erbil, LSD (P<0.01) = 7.44

4.2 Humidity

The relative humidity of studied location during the studied period was presented in Table 4. Results revealed that during wet condition the humidity percentage was more than during dry condition, in wet condition it was ranged from 30-74.2, while during dry condition ranged from 18.5-55.6

Table 4. Monthly relative humidity (%) and rainfall value of the locations within Erbil Governorate during wet condition March, April, May and dry condition July, August and, September 2015.

Area	Humidity %			Rainfall (mm/years)					
	Wet			Dry			wet		
	Mar	Apr	May	Jul	Aug	Sep	Mar	Apr	May
Choman district	74.2	64.8	59.6	53.3	31.7	51.3	126.4	52.6	17.3
Soran district	71.7	64.4	62	52.2	53.3	55.6	108.3	51.2	17.1
Rawanduz district	73.1	66.2	63	53	52	51.3	123	52.3	17.7
Harir district	61.1	53	32.5	19	18.9	18.5	165	32.3	6.7
Shaqlawah district	64.7	54	43.5	21	18.9	18.5	167	34.5	12
Salahaddin sub district	62	50	42	22	29	29	72.1	14.5	26.8
Makhmour district	53	40	30	25	25	28	26.5	8	3.3
Mean	65.7	56.1	47.5	35.1	32.7	36.0	112.6	35.1	14.4

Source: Meteorological Center of Erbil, LSD of humidity (P<0.01) = 31.2, LSD of rainfall (P<0.01) = 47.79

Table 5. The mean concentration (mg/kg⁻¹) of heavy metal in mosses in the studied samples in dry condition.

Sites	Pb	Hg	Mn	Fe%	Cu	Zn	Ti	Co	Ni	As	Se	Cr
1	100	0.0	213	2.25	300	*	1560	7.9	51	16	0.2	40
2	58	0.0	220	2.24	69	659	1954	7.7	59	12	0.2	-
3	93	0.0	290	2.64	114	*	*	10	82	6.8	0.1	32
4	35	0.0	100	1	25	140	403	4.5	125	3.1	0.1	6.6
5	29	0.0	63	0.66	26	389	380	3.3	22.7	4.1	0.4	-
6	46	0.0	202	2.18	25	100	1209	7.9	49	7	0.3	-
7	44	0.0	151	1.55	26	82	685	5.8	45	5.6	0.1	-
8	33	0.0	56	0.71	40	59	471	3.4	44	2.8	0.1	-
9	41.5	0.1	116	0.96	32	113	369	4.2	30	4	0.1	-
10	43	0.0	217	2.36	25.7	78	1480	8.1	68	7	0.3	18.7
Total	522.5	0.1	1628	16.55	682.7	1620	8511	62.8	575.7	68.4	1.9	97.3
mean	52.25	0.001	162.8	1.655	68.27	162	851.1	6.28	57.57	6.84	0.19	9.73
S.D	24.69	0.031	77.52	0.76	86.30	212.53	611.27	2.34	29.22	4.18	0.11	14.72

* Over range of the instrument reading,

- Not detected.

Table 6. The mean Concentration (mg/ kg⁻¹) of heavy metals of two moss species samples in dry condition collected in October at study sites.

Heavy Metal	Mosses spp.							Total	Mean	S.D	LSD
	Funaria				Tortula						
	Sites				Sites						
	1	2	3	9	4	7	10				
Pb	100	58	93	41.5	35	44	43	414.5	59.21	26.46	14.14
Hg	0	0	0	0.1	0	0	0	0.1	0.014	0.03	0.020
Mn	213	220	290	116	100	151	217	1307	186.71	67.30	35.97
Fe (%)	2.5	2.24	2.64	0.96	1	1.55	2.36	13.25	1.89	0.71	0.38
Cu	300	69	114	32	25	26	25.7	591.7	84.52	100.60	53.77
Zn	*	659	*	113	140	82	78	1072	153.14	249.81	133.52
Ti	1560	1954	*	369	403	685	1480	6451	921.57	674.39	360.47
Co	7.9	7.7	10	4.2	4.5	5.8	8.1	48.2	6.88	2.11	1.132
Ni	51	59	82	30	125	45	68	460	65.71	30.96	16.54
As	16	12	6.8	4	3.1	5.6	7	54.5	7.78	4.61	2.46
Se	0.2	0.2	0.1	0.1	0.1	0.1	0.3	1.1	0.15	0.07	0.042
Cr	40	0	32	0	6.6	0	18.7	97.3	13.9	16.65	8.90

* Over range of the instrument reading;

- Not detected.

4.3 Heavy metals in mosses

Heavy metal concentration in studied mosses plants and location represented in Tables 5 and 6. The results were as follows:

Pb: The highest concentration was 100mg/kg⁻¹ recorded in *Funaria* at Aras Street at Erbil center location, while the minimum concentration was 35mg/kg⁻¹ recorded in *Tortula* at Gali Ali Bag road, with the mean value of 59.21.

Hg: The highest concentration was 0.1mg/kg⁻¹ recorded in *Funaria* at Jondian, Soran district location, while the minimum concentration was 0.0mg/kg⁻¹ recorded in all location except Jondian location, the mean value was 0.014.

Mn: The highest concentration was 290mg/kg⁻¹ recorded in *Funaria* at College of Science, Erbil city, while the minimum concentration was 100 mg/kg⁻¹ recorded in *Tortula* under Gali Ali Beg waterfall, the mean value was 186.71.

Fe (%): The highest concentration was 2.64% recorded in *Funaria* at College of Science, while the minimum concentration was 0.96% recorded in *Funaria* at

Jondian, Soran district location, the mean value was 1.89.

Cu: The highest concentration was 300mg/kg⁻¹ recorded in *Funaria* at Aras Street at Center of Erbil, while the minimum concentration was 25mg/kg⁻¹ recorded in *Tortula* under Gali Ali Beg waterfall, the mean value was 84.52.

Zn: The highest concentration was over range recorded in *Funaria* at Aras Street and at College of Science, while the minimum concentration was 78mg/kg⁻¹ recorded in *Tortula* at Gulan Street-Erbil City, the mean value was 153.14.

Ti: The highest concentration was over range recorded in *Funaria* at College of Science, while the minimum concentration was 369mg/kg⁻¹ recorded in *Funaria* at Jondian, Soran district location, the mean value was 921.57.

Co: The highest concentration was 10mg/kg⁻¹ recorded in *Funaria* at College of Science, while the minimum concentration was 4.2mg/kg⁻¹ recorded in *Funaria* at Jondian, Soran district location, the mean value was 6.88.

Ni: The highest concentration was 125mg/kg⁻¹ recorded in *Tortula* under Gali Ali Beg waterfall, while the minimum concentration was 30mg/kg⁻¹ recorded in *Funaria* at Jondian, Soran district location, the mean value was 65.71.

As: The highest concentration was 16mg/kg⁻¹ recorded in *Funaria* at Aras Street at Erbil city location, while the minimum concentration was 3.1mg/kg recorded in *Tortula* under Gali Ali Beg waterfall, the mean value was 7.78.

Se: The highest concentration was 0.3mg/kg⁻¹ recorded in *Tortula* at Gulan Street-Erbil City, while the minimum concentration was 0.1mg/ kg⁻¹ recorded in *Funaria* at College of Science, Jondian-Soran district location and in *Tortula* under Gali Ali Beg waterfall and near Malla Nabi Water well Bekhal-Rawanduz road, the mean value was 0.15.

Cr: The highest concentration was 40mg/kg⁻¹ recorded in *Funaria* at Aras Street at Erbil city, while the minimum concentration was 6.6mg/kg⁻¹ recorded in *Tortula* under Gali Ali Beg waterfall, the mean value was 13.9.

4.4 Heavy Metals in Soil

The concentration of heavy metal, in the soil of some location was shown in (Table 7). The result reviewed that in Erbil city center and the main road, was as follows for Pb ranged from 0.12 to 0.91, Cd from 0.80 to 3.02, for Cr from 77.19 to 195.85, for Cu from 18.01 to 33.18, for Zn from 126.60 to 156.16 and for Mn 299.44 to 586.04 (ppm). The similar results was obtained by (Kaminski and Landsberger, 2000; Ojo *et al.*, 2012 and Qadir, 2011) who studied the heavy metal concentration in lichens within Erbil governorate. No relationship found between heavy metal concentration in mosses and soil of some sites; this is similar to the result of (Abdullah *et al.*, 2014).

Table 7. Heavy metal concentration (ppm) in mosses and in the soil of some main road of mosses sampling within Erbil city.

Heavy Metal	Main Road				Total	Mean	S.D	L.S.D
	Erbil	Rawanduz	Gali Ali Beg	Shaqlaw				
Pb	0.71	0.12	0.15	0.91	1.89	0.4725	0.398361	0.281684
Cd	3.02	1.01	0.9	0.8	5.73	1.4325	1.061803	0
Cr	166.19	195.85	146.94	77.19	586.17	146.5425	50.42215	35.65385
Cu	35.18	22.82	34.86	18.01	110.87	27.7175	8.658816	6.122707
Fe	1345.11	15013.04	1648.32	1023.76	19030.23	4757.558	6841.742	4837.843
Zn	245.78	144.77	156.16	426.6	973.31	243.3275	130.2646	92.11096
Mn	328.74	412.88	586.04	299.44	1627.1	406.775	128.8196	91.08922

*In natural subsoil Pb = 0.05-5.0 ppm;

*In shoot of herbaceous roadside plants = 12.0-35.0 ppm;

*In roadside tree plant 0.0, 5.0 – 23.25 ppm.

5. Discussion

As in the present study of which more attention paid to *Funaria Hygrometrica*, *Tortula ruralis*, *Brachythecium rivulare*, *Cirriphyllum piliferum*, *Grimmia Pulvinata*, investigations carried out in Poland concerned with the *Hypnum cupressiforme* var. *filiforme* and *Orthodicranum montanum* mosses, they were useful as good bioindicators for environmental pollution by heavy metals. These taxons were chosen because of their abundance on the examined area as well in the industrial zone and in the clean or not polluted area. In the majority of samples, low contents of molybdenum and high contents of zinc were found. In the area of the steel factory near the main road from Erbil to Gwer sub-district. Other workers stated that the influence of industrial and communication factors play a great role in higher contents of metals such as Cd, Zn, Mo, Ni and Pb in samples of mosses (LeBlanc & Rao, 1974; Baranowska and Srogi, 2000).

Concerning polluted and unpolluted area along the main road and Erbil center the results agree with that of Baranowska and Srogi, (2000). They found that the mean heavy metal concentrations in mosses collected in 1995 across Poland were: Zn – 43.0µg/g d.m., Fe –

362.0µg/g d.m., Ni – 1.4µg/g d.m., Cd – 0.44µg/g d.m. and Cr – 1.50µg/g d.m. In 1998, comparison of mosses collected in Silesia-Kraków Industrial Region and control area in northeastern Poland showed that the concentrations of metals in *Pleurozium schreberi* from industrial regions are several folds higher than in the mosses from sparsely industrialized control area (respectively: for Zn – 150µg/g d.m. and 37.4µg/g d.m.; for Fe – 1226µg/g d.m. and 298µg/g d.m.; for Ni – 2.5µg/g d.m. and 1.4µg/g d.m.; for Cd – 2.2µg/g d.m. and 0.1µg/g d.m.; for Cr – 6.2µg/g d.m. and 0.8µg/g d.m. (Frontasyeva *et al.*, 1994; Aslan *et al.*, 2011; Ekpo *et al.*, 2012).

In the present study, this result in agreement with the results of Santelmann and Gorham (1988) who worked with heavy metals in (mg/gm) in *Sphagnum* mosses with particular references to Cd: 0.18–0.43, Cr: 0.36–1.23, Cu: 2.77–3.89, Fe: 318– 1080, Mn: 59–127, Pb: 15.7–21.8 and Zn: 21.9–30.1 and agree with results of Rühling and Tyler (1968) and of Bykowszczenko *et al.*, (2006) that working with a heavy metal concentration in moss *Pleurozium schreberi* in Poland. He revealed that the Cr in 2002 was 38.233, 0.25 and 0.8 (mg/gm d.m) and in a linear relationship with the results of Ekpo *et al.*, (2012) in Nigeria, while the

concentration Pb: 3.5-11.212, Cr: 0.03-2.23, Cu: 5.07-10.93, Cd: 0.21-0.56, Mn: 3.51-10.91, Fe: 289.6-604.96, and Zn was 9.98-31.81. However, there is no relationship found between heavy metal concentration in mosses and soil of some sites, this is similar to the result of (Abdullah *et al.*, 2014).

The data obtained in this study was compared with that of WHO revealed that the concentration of studied were less than of WHO recommendation (Steinnes, 1980; Steinnes *et al.*, 1997; Harmens *et al.*, 2013, 2011, 2008).

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