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BIOMONITORING OF HEAVY METALS ACCUMULATION IN WILD PLANTS GROWING AT SOON VALLEY, KHUSHAB, PAKISTAN

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Abstract

Ruminants' health is badly affected by the hazardous effects of heavy metals. Due to the scarcity of water, feed production in many regions is very low, so mostly ruminant animals are fed with wild plants of the region. The aim of this study was to evaluate the heavy metals concentration in native forage plants of Soon Valley, Khushab, Pakistan. Twenty forage plants, which are mostly consumed by the ruminants, were selected. The concentrations of heavy metals, cobalt (Co), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) were determined by using Atomic Absorption Spectrophotometer (AAS). Results from heavy metals analysis revealed that the highest Co, Cu, Zn, Fe, and Mn contents were observed in *Digitaria sanguinalis* (0.30 mg/kg), *Hordeum leporinum* (15.64 mg/kg), *Hordeum leporinum* (36.46 mg/kg), *Achnatherum hymenoides* (26.04 mg/kg) and *Hordeum leporinum* (28.26 mg/kg), respectively. Statistically, the results of the analyses of variance (ANOVA) for Cu, Co, Fe, and Zn showed highly significant ($p<0.05$) difference in all plant samples. The results of this research showed that heavy metal accumulation values in the studied plants were in the normal natural concentration range.

Key words: Biomonitoring, Trace metals, Wild plants, Pollution, Pakistan

Introduction

Spreading of xenobiotics into the ecosystems has resulted in environmental pollution and degradation (Yorek *et al.,* 2016; Khan *et al.*, 2018a; Ugulu, 2020). It has been observed that heavy metals in combination with a variety of other toxic compounds enter into the environment through the pesticide use, industries, power generators, municipal waste, smoke from automobile and refuse to burn in agriculture (Dogan *et al.*, 2010; Durkan *et al.*, 2011; Dogan *et al.*, 2014a, b). These metals can accumulate in plant tissues from air or water (Bayor *et al.*, 2009). Both chronic and acute responses are seen in animals consuming the plants that have taken up metals (Sahin *et al.*, 2016; Ugulu *et al.*, 2019a, b; Khan *et al.*, 2019a, b). Heavy metals are the main reason for various health problems. Their accumulation in the body results in the cessation of brain and liver cells and finally leads to the complete damaging of the organs. These metals also result in the reduced functioning of the kidney. Against scenarios like this, monthly observations are required to monitor the level of contaminants (Abou-arab & Aboudonia, 2001).

On the other hand, heavy metals play a crucial role in plant growth, but their higher concentration exerts a toxic effect on plants (Ugulu, 2015a; Khan *et al.*, 2020a, b). Many important functions are played by manganese (Mn), iron (Fe) and zinc (Zn) in the biological mechanism of organisms. Copper (Cu) being trace mineral is required in a low quantity in biological entities. The difference in Cu concentration in different plant parts depends upon ecological factors like nitrogen supply and chemical characteristics of soil, age, and type of plant species (Ugulu &Baslar, 2010; Khan *et al.*, 2018c). Cobalt (Co) is the main building block of vitamin B_{12} which plays an important role in metabolism. Anemia occurs in ruminants due to the Co deficiency and Fe and Cu are also interrelated with vitamin B_{12} in hematopoietic cells/ processes. Harsh emaciation and loss of appetite are also associated with the shortage of Co (Sahin *et al.,* 2016; Khan *et al.*, 2019c; Nadeem *et al.*, 2019).

Accidental or intentional spilling of wastewater into agricultural resources can introduce heavy metals to the food chain (Ugulu *et al.*, 2009; Khan *et al.*, 2020c). All trophic levels are affected by movement of metals through the food chain (Wajid *et al.,* 2020). In this direction, the present study was performed to evaluate the heavy metal contents of wild forages grown in Soon Valley of Pakistan.

Materials and Methods

Study area: The present research was performed in Soon Valley, Khushab, Pakistan. The central town of the valley is Noshehra and the valley is located in the North West side of the Khushab (Fig. 1). Soon Valley is also known by another name, the Central Salt Range. The valley is encircled from the east by Kalar Kahar, north by Potohar plateau, west by Indus River at some distance and south by Punjab Plains. Valley is located geographically between coordinates 71. 50' 33" to 72. 30' 07" East and 32. 26' 11" to 32. 41' 18" North.

Sample collection: Twenty plants which were most preferably grazed by the ruminants were collected from the valley. Those plants were collected which were not smashed by any material. Complete plants with all organs were collected. Different parts of plants were obtained by using knives and diggers. A first aid box, long shirt, long trousers, hat, and boot were present in order to avoid any undesirable happenings during plant collection. Small brown paper bags were used for the storage of plants. Plants were identified at sites and also after their collection. In order to remove impurities and dust particles, the plant samples were washed with distilled water. The plants were air-dried and then put into the oven at 70°C temperature for 5 to 7 days. These dried plants than used for further analysis.

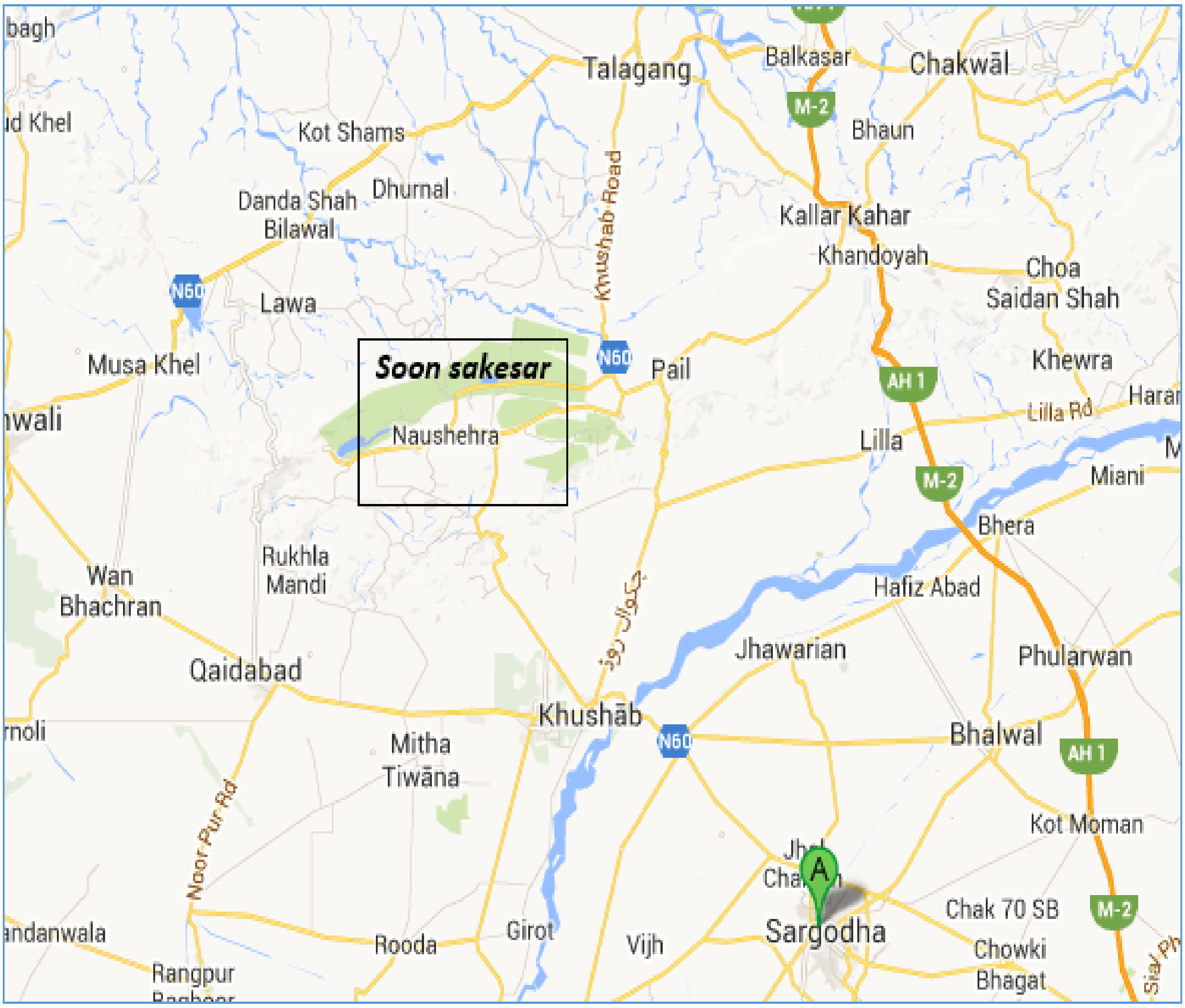

Fig. 1. Study area.

Plant sample identification: The plants were identified in the department of Biological Sciences, Sargodha University. The plants scientific and common name along with their families is given below (Table 1).

Plant sample preparation: Plant digestion was done by taking one gram dried plant sample into the digestion flask and addition of H_2SO_4 (2 mL) and H_2O_2 (4 mL) and putting this flask for 30 minutes into the digestion chamber. When the fumes stopped to evaporate from the flask, then the flask was removed from the digestion chamber. 2 mL of H_2O_2 was further added to achieve complete digestion and the flask was again placed in the digestion chamber for heating. The process was repeated till the achievement of transparent solution. Following this, the sample was taken out from digestion chamber, distilled water was added to dilute the sample in twice the amount of sample for making the final volume of 50 mL after filtration by Whatmann filter paper. The soilution was finally preserved in labeled plastic bottles and stored till further analysis (Ugulu *et al.*, 2016).

Heavy metal analysis: After the digestion of the all plant samples these were analyzed in order to check the mineral status of these plants with the help of Atomic Absorption Spectrophotometer Perkin-Elmer AAS-5000. Quantity of a single element in a specific sample can be determined by using the method of Atomic absorption spectroscopy.

The present analysis was performed using AAS (Atomic Absorption Spectrophotometer) using the method given by Lindsay and Norvell (1978) in order to evaluate the quantity of minerals accumulated in medicinal plants which were mainly used by the ruminants and people of the area. The following metals were under study: manganese (Mn), cobalt (Co), iron (Fe), copper (Cu) and zinc (Zn), and. The detection limits of these metals are presented in Table 2.

Statistical analysis: For statistical analysis, the SPSS software version 17 was used. The one-way analysis of variance (ANOVA) was performed to compare the heavy metal accumulation between different plant species. The significance of means was at the probability levels of 0.001, 0.01 and 0.05 as suggested by Steel & Torrie (1980).

Table 1. Plants with scientific, family and common name.

No.	Plant species	Family	Common name
1.	*Abutilon theophrasti*	Malvaceae	Indian mellow
2.	*Achnatherum hymenoides*	Poaceae	Indian rice grass
3.	*Aconitum columbianum*	Ranunculaceae	Columbian monkshood
4.	*Adenocaulon bicolor*	Asteraceae	America trail plant
5.	*Agoseris glauca*	Asteraceae	Pale agoseris
6.	*Artemisia rothrockii*	Asteraceae	Timberline sagebrush
7.	*Brassica rapa*	Brassicaceae	Canola
8.	*Capsella bursa-pastoris*	Brassicaceae	Shepherd's purse
9.	*Ceanothus velutinus*	Rhamnaceae	Red root
10.	*Collomia linearis*	Polemoniaceae	Tiny trumpt
11.	*Conyza Canadensis*	Asteraceae	Horse weed
12.	*Cynodon dactylon*	Poaceae	Dhoob
13.	*Digitaria sanguinalis*	Poaceae	Hairy crabgrass
14.	*Dodonaea viscose*	Sapindaceae	Hop bush
15.	*Erigeron divergens*	Asteraceae	Spreading fleabane
16.	*Galium aparine*	Rubiaceae	Goose grass
17.	*Hordeum leporinum*	Poaceae	Barley grass
18.	*Lepidium latifolium L*	Brassicaceae	Pepper grass
19.	*Monarda punctata*	Lamiaceae	Horse mint
20.	*Saccharum spontaneum*	Poaceae	Kanas grass

Table 2. Detection limits of atomic absorption spectrophotometer.

No	Metal	Detection limits
1.	Cu	1.5 (Flame AA)
2.	Co	9 (Flame AA)
3.	Fe	5 (Flame AA)
4.	Zn	1.5 (Flame AA)
5.	Mn	1.5 (Flame AA)

Results and Discussion

In this study, 20 different plant species gathered from Soon Valley used as biomonitors with the purpose of determining the accumulation levels of heavy metals. The concentration of the Fe, Zn, Co, Mn, and Cu (mg/kg) in various samples of plants taken from Soon Valley are given in Table 3.

Copper is still generally utilized for electrical hardware and has likewise an extensive variety of different applications in horticulture (supplements, pesticides, and fungicides), wood conservation, and therapeutic applications (Calfee & Little, 2017). According to the findings of the analyses, the concentration of Cu ranged from 10.60 mg/kg to 15.64 mg/kg in all types of plant samples (Table 3). The minimum recorded concentration was observed in *Brassica rapa* and the maximum content was found in *Hordeum leporinum*. In *Artemisia vulgaris* mean Cu concentration was found lower as compared to the findings Khan *et al*., (2008). Lasisi *et al*., (2005) also detected the higher Cu concentration in Alafia bateri oliver (herbal plant) in comparison to present study.

The Co is generally released into the environment from anthropogenic sources such as mining, discharge from industries and atmospheric deposition (Cornelis, 2005). The heavy metal analyses showed that the Co contents in various plant species ranged from 0.12 mg/kg to 0.30 mg/kg. The maximum concentration of Co was noticed in *Digitaria sanguinalis* and the lowest was investigated in *Ceanothus velutinus* (Table 3). Kulhari *et al*., (2013) reported the lower level of Co in the stem of *Ficus religiosa* as compared to Co contents in the present research. Anon., (1980) also suggested the lower Co concentration. A number of vital roles played by Co in many biological processes and it is the main component of the vitamin B_{12}, but due to its interaction with P and Cu, it is considered as poisonous heavy metal (Zhang *et al*., 2009; Ugulu *et al*., 2019c).

The essential emission sources of Fe are the steel industry, coal-burning, and intensive traffic (Ugulu, 2015b). In the present investigation, the Fe contents varied from 17.35 mg/kg to 26.04 mg/kg in all types of plant samples. The maximum value of Fe noticed in *Achnatherum hymenoides* while the minimum was investigated in *Adenocaulon bicolor* (Table 3). The concentration of Fe in the current research was much higher than the findings of Prohp *et al*., (2006). Owolabi (2012) suggested the lower Fe concentration in Cowpea variety (Kannanado). Fe is an essential element needed by the body and protein chelated Fe plays an important role in the biological mechanism of living organisms. However, the higher Fe concentration in infants leads to death by poisoning. Gastrointestinal discomfort is established in individuals if the ingestion of Fe increases more than 20 mg/kg while temperate intoxication results if Fe in the elemental form increases more than 40 mg/kg and severe toxicity results if ingestion exceeds more than 60 mg/kg which may also be lethal (Spanierman, 2011).

Table 3. Mean values of heavy metal contents in wild plants growing at Soon Valley (mg/kg).

Plants	Co	Cu	Zn	Fe	Mn
Abutilon theophrasti	0.28	12.10	31.83	22.93	27.60
Achnatherum hymenoides	0.15	11.58	32.03	26.04	28.13
Aconitum columbianum	0.18	10.82	32.06	23.71	27.73
Adenocaulon bicolor	0.25	12.62	33.72	17.35	25.95
Agoseris glauca	0.27	12.32	35.20	23.21	24.60
Artemisia rothrockii	0.17	11.43	31.02	20.86	25.98
Brassica rapa	0.18	10.60	30.07	22.43	24.74
Capsella bursa-pastoris	0.22	15.34	32.09	18.57	24.48
Ceanothus velutinus	0.12	11.53	32.39	19.88	27.07
Collomia linearis	0.26	14.69	35.01	19.17	26.91
Conyza canadensis	0.17	12.78	33.67	20.34	26.72
Cynodon dactylon	0.23	13.19	34.82	19.89	28.09
Digitaria sanguinalis	0.30	12.44	31.40	24.44	23.58
Dodonaea viscose	0.21	12.19	30.94	22.45	22.61
Erigeron divergens	0.15	13.60	33.27	19.88	25.26
Galium aparine	0.28	12.19	32.60	23.51	27.69
Hordeum leporinum	0.27	15.64	36.46	21.54	28.26
Lepidium latifolium	0.27	13.56	34.86	21.30	24.15
Monarda punctata	0.21	14.55	32.70	25.07	25.05
Saccharum spontaneum	0.16	14.40	31.89	23.90	26.06
Min.:	0.15	10.60	30.07	19.17	22.61
Max.:	0.30	15.64	36.46	26.04	28.26
Mean:	0.2165	12.8785	32.9015	21.8235	26.033

Table 4. Analysis of variance for heavy metals of different plant species.

Source of variation	Degree of freedom df	Mean squares				
		Cu	Co	Fe	Mn	Zn
Plant Spp.	19	6.373***	.009***	16.122***	8.358ns	8.470***
Error	40	1.353	.001	3.579	5.558	2.722

***=Significant at 0.001 level, ns=Non-significant

The Mn level rises in the plant growth in especially shoots and roots (Kulkarni *et al.*, 2006). For this reason, the Mn concentration in plant is an important factor for plant growth. According to the results of the analyses, the Mn concentration ranged from 22.61 mg/kg to 28.26 mg/kg in all types of the plant collected from Soon Valley. The highest Mn levels were noticed in *Hordeum leporinum* and the lowest in *Dodonaea viscosa* (Table 3). Lasisi *et al*., (2005) reported the higher Mn concentration in the stem of *Berlinia confusa* in relation to the current research. Kulhari *et al*., (2013) estimated the lower Mn values than the current Mn content in the studied plants. Khan *et al*., (2008) suggested the higher concentration as compared to the present study. Mn concentration in all types of plant samples was within the maximum permissible range as suggested by (Anon., 1984). For most of the enzymes, Mn acts as a co-factor so it is an important trace element (Ugulu *et al.*, 2012). As compared to other metals it is less toxic, however higher concentration more than 5 mg/m^3 results in the neurological disorders (Kulhari *et al.*, 2013).

Zinc is utilized in various chemical productions such as plastic, pigments, lubricants, pesticides and corrosion protection on steel components (Unver *et al.*, 2015). In the present investigation, the value of Zn in various plant species differed from 30.07 mg/kg to 36.46 mg/kg. Results revealed that *Brassica rapa* contained the lowest Zn contents while the *Hordeum leporinum* contained the highest (Table 3). Khan *et al*., (2008) reported the much higher Zn value in *Stevia rebaudiana* plant species as compared to our value. Lasisi *et al*., (2005) also estimated the higher Zn concentration in the stem of *Daracaena fragran*. Zn becomes harmful for living organisms only in high concentration, however, it is an important component of many proteins (Kulhari *et al*., 2013; Ugulu *et al.*, 2020).

Statistically, analysis of variance (ANOVA) of data for Cu, Co, Fe, and Zn showed highly significant (p<0.05) difference in all plant samples (Table 4). However, according to the ANOVA results, Mn showed non-significant (p>0.05) deviation in all plant species. Considering that different plant species may have different abilities regarding heavy metal accumulation, it can be said that these results are the expected results. However, studies aiming to compare collected plants at the level of random species may be useful to define the accumulation abilities of any plant species (Ugulu, 2019).

For land plants the normal natural concentration of heavy metals range from Cu: 2-20 mg/kg, Co: 0.1-10 mg/kg, Fe: 20-700 mg/kg, Mn: 20-700 mg/kg, Zn: 20-400 mg/kg (Ahmad *et al.*, 2018). Comparing these findings with the results of the present study, it was determined that the accumulation of heavy metals was within the permissible range among all plant samples. For this reason, it is concluded that there is no heavy metal pollution in the study area.

Conclusion

The results of this research showed that heavy metal levels in the studied plants were in the normal natural concentration intervals. That's why the most important finding of the present study is that there is no heavy metal contamination in the study area. However, the results of this study may be useful to guide the identification of the sources of the accumulation of heavy metals. For instance, while Cu and Zn mainly originate from anthropogenic actions such as steelworks, cement industry, and mining operations. Fe originates from both anthropogenic and natural sources.

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