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Heavy metals and proximate analysis of Sihar (*Rhazya stricta* Decne) collected from different sites of Warcha salt mine, Salt Range, Pakistan

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Abstract

Rhazya stricta is an important medicinal plant species distributed all over the Warcha salt mine in Pakistan. Analysis of proximate composition and metals is a commendable job to assess the suitability of the exploitation of medicinal plants on a large scale by ethnic communities. The proximate analyses (moisture, fiber, ash, crude fats, proteins and carbohydrates) of all the samples collected from five different sites (Table 1) were carried out. Moreover, different inorganic elements in the samples were also determined. The maximum and the minimum moisture content in *Rhazya stricta* was observed at Site 4 (31.21%) and Site 2 (29.14%), respectively. The maximum and the minimum ash concentrations were recorded in the populations collected from Site 2 (7.48%) and Site 5 (6.25%), respectively. The maximum fat content was found in the Site 3 (3.91%) population, whereas the minimum contents of crude fiber were observed in the population from Site 2 (12.2%). The maximum concentration of crude protein was observed in the plants from Site 5 (9.93%), whereas the minimum contents of carbohydrates were observed in the plants from Site 2 (48.4%). Essential and nonessential elements like potassium (K), magnesium (Mg), iron (Fe), nickel (Ni), chromium (Cr), cadmium (Cd), cobalt (Co), copper (Cu), manganese (Mn), and zinc (Zn) were analyzed in the roots, stem and leaves of *Rhazya stricta*. The maximum K content (6836.886 mg/kg) was found in the roots of plants collected from Site 5 and the minimum in the leaves (5528.795 mg/kg) collected from Site 3. The highest concentration of magnesium was found in the roots of plants from Site 3 (2414.46 mg/kg), whereas the lowest concentration of iron was recorded in the leaves of Site 4 (31.45 mg/kg) plants. The maximum and the minimum concentrations of cobalt were detected in the roots of plants from Site 1 (0.320 mg/kg) and in the leaves of plants from Site 4 (0.135 mg/kg), respectively. The minimum concentration of leaf cadmium was recorded in plant population from Site 4, whereas in the same population the maximum level of root nickel was recorded. The highest concentration of copper was observed in the stem of plant population from Site 2 (0.297 mg/kg). The concentrations of most of the elements appraised in the present study are well below the overall permissible limits of these elements in medicinal plants determined by WHO, so this plant from the Warcha mine can be utilized without harm by herbal practitioners and pharmaceutical industry.

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Introduction

Medicinal plants play a commendable role in treating various types of diseases (Singh, 2015). A World Health Organization (WHO) survey reported that about 80% of the world's population depends on locally available plants for medication. Developing countries including Pakistan have inadequate medical setups, particularly in backward areas. Therefore, people use their indigenous species to treat major diseases such as diabetes, malaria, diarrhea, skin and respiratory disorders, and fungal and bacterial infections (Wazir et al*.,* 2007; Pirzada et al*.,* 2009; Dogan et al., 2011). Besides their role in medication, these medicinal plants are used as a food or as a main ingredient in cosmetics for maintenance of skin in intact form (Hosseinzadeh et al*.,* 2015). Although metals like Co, Ni, Cu, Mn, Zn and Fe are considered essential nutrients, because they regulate numerous processes of the living body, they become harmful and toxic when their concentration exceeds the recommended standards (Ugulu et al., 2019; Khan et al*.,* 2020; Wajid et al., 2020; Chen et al., 2021).

Metals can build up in the body, resulting in oxidative stress and clinical symptoms (Khan et al., 2018). Thus, painful gullet, upper body pain, headache, coughing, faintness, and lung disorders are observed with acute exposure to Cd (Casey, 1991), whereas long standing arsenic introduction can source cancer and inhibit protein synthesis (Singh et al*.,* 2007). Chronic lead poisoning (also called saturnosis) can damage the kidneys and reproductive system, while impairing a child's intellectual abilities (Needleman, 2004). In addition, Cr can also result in longing, sneezing, liquid nose, and ulcers, if inhaled in excess concentrations. In addition, high systemic concentrations of As in humans are distinguished by multifaceted medical syndromes as well as neurological disorders (Baruthio, 1992).

Natural processes of the ecosystem cause bioaccumulation of many toxic chemical compounds in the surroundings, and cause a harm to the environment (Ugulu and Erkol, 2013); these processes might be mining, industrial and agricultural, which cause various health issues to forages and livestock (Ugulu*,* 2015; Khan et al., 2019a, b). The food chain has these toxic metals as a component. However, metal toxicity risk is reduced by the production of safe animal feed, which could be beneficial for animal and human health (Nadeem et al*.,* 2019; Ugulu et al., 2022).

Heavy metal contaminations exist in the environment due to their bioaccumulation in the food chain, so various biological functions are disturbed by heavy metal toxicity (Makridis et al., 2012; Ugulu et al., 2016). Forages and animals accumulate heavy metals in their bodies that are risky for humans, because these trace metals are transported through the food chain (Hongyu et al., 2005; Munir et al., 2019). From one trophic level to a higher trophic level, biomagnifications and transport of heavy metals take place by more bioaccumulation of toxins occurring in the food of animals (Sahin et al., 2016; Siddique et al., 2019). The soil and forage heavy metal bioaccumulation causes gastrointestinal cancer in animals (Ugulu et al., 2009; Zhuang et al*.,* 2009; Dogan and Ugulu, 2013; Tariq et al., 2021).

Rhazya stricta Decne belongs to Apocynaceae family, and is mainly scattered in the Indian Sub-continent and the Middle East (Akhgari et al., 2015). This plant contains more than 100 alkaloids including terpenoid indole alkaloids (TIAs), which are further classified into 17 sub-groups (Vardhan and Shukla, 2017). Therefore, different parts of this plant are used in folk medicine to treat several diseases. Mostly, decoction of the plant parts is used to treat anti-pyretic, cancer, diabetes mellitus, helminthiasis, inflammatory conditions, rheumatism, sore throat, stomach diseases and skin diseases (Akhgari et al*.,* 2015). The purpose of the present research work was to appraise heavy metal concentrations in *Rhazya stricta* Decne (roots, stem and leaves) collected from five different sites of the Warcha mine area in the Salt Range (Pakistan). As *Rhazya stricta* is widely used by native people, so, it is a need of the time to analyze the proximate and metal components in this plant. This present research will assist medical practitioners, health-care professionals, planners, and especially the public to use this plant wisely.

Materials and Methods

Plants collection

Warcha Salt mine is an active salt mine located in Warcha village, Khushab District of Punjab, Pakistan, with reserves of over 1 billion tonnes of 98% pure, clear and crystalline sodium chloride salt. The Warcha mine became operational in 1872, and it still produces over 200,000 tonnes of rock salt per year, with the capacity to double its production (PMDC, 2021).

Fifteen replicates of plant samples were collected from five different sites (**Table 1**). Each part of the plant (stem, root, leaves) was separated from the main plant. All parts of the selected plant were rinsed with fresh running water and then with deionized water to get rid of contaminated agents. Furthermore, plant samples were dried in shade at room temperature (22–25 ^{o}C) and subsequently in an oven. The dried plant parts were crushed and their powdered forms were kept in polyethylene sampling bags for further processing.

Table 1: Locations of sites from where different populations of *R. stricta* were collected.

Sites	Coordinates	Elevation (m)
Site 1	32º17ʾ27 N-72º 21ʾ3 E	222.4
Site 2	32º17’31 N 72º 16’25 E	180.0
Site 3	32º51 N 77º 54.45 E	225.4
Site 4	32º59 N 79º 87 E	235.2
Site 5	32º37 N 73º31ʾ37 E	188.0

Proximate analysis

The proximate analyses (moisture, fiber, ash, crude fats, crude proteins and carbohydrates) of all the samples were determined. The moisture and ash were determined using the weight difference method. Fiber content was estimated from the loss in weight of the crucible and its content on ignition. Carbohydrates were determined when the sums of the percentages of moisture, ash, crude protein, ether extract and crude fiber were subtracted from 100. The nitrogen value, which is an integral component of proteins, was determined by the micro-Kjeldahl method, involving digestions, distillation and finally titration of the samples. The nitrogen value was converted to protein by multiplying a factor of 6.25. All the proximate values were reported in percentage (AOAC, 1990).

Digestion and mineral analysis of plant samples

Plant samples were digested by the wet digestion method. A proportion (2.0 g) of dried plant sample was digested in 20 ml of H_2SO_4. This solution was kept for one night for digestion at room temperature. The samples were heated until fumes came out. H_2O_2 was added again and again until the samples became colorless. Filtration was done with the Whatman filter paper. Then the samples were cooled. Distilled water was added to raise the volume of the solution to 50 ml. The processed samples were stored at room temperature in airtight plastic bottles for further analysis. All digested samples were subjected to an atomic absorption spectrometer for mineral analysis (Perkin Elmer AA Analyst 700).

Translocation Factor

Translocation Factor (TF) estimates the heavy metal transfer in the stem and leaves of a plant through the soil system (USEPA, 2000).

$$TF = C_{stem}/C_{root}$$
$$TF = C_{leaves}/C_{stem}$$

Statistical analysis

One-way ANOVA was applied for statistical analysis of the data using the Statistical Package for Social Sciences (SPSS 16) (Ugulu et al., 2008; Yorek et al., 2010a, b).

Results

Proximate Analysis

Tissue moisture (%)

The maximum moisture content was observed in plants from Site 4 (31.21%), whereas the minimum contents of moisture were observed in those from Site 2 (29.14%). The decreasing order of moisture content in plant populations was S4>S5>S1>S3>S2 (**Figure 1**).

Ash content (%)

The maximum ash content was observed in plants from Site 2 (7.48%), whereas the minimum ash contents were observed in those of Site 5 (6.25%). The decreasing order of ash content was S2>S4>S1>S3>S5 (**Figure 1**).

Fat content (%)

The maximum amount of fat was observed at Site 3 (3.91%), whereas the minimum was observed in those from Site 1 (3.62%). The decreasing order of fat content was S3>S2>S5>S1>S4 (**Figure 1**).

Crude fiber (%)

The maximum amount of fiber was observed in population from Site 5 (13.02%), whereas the minimum was observed in the Site 2 population (12.2%). The decreasing order of crude fiber was S5>S4>S3>S1>S2 (**Figure 1**).

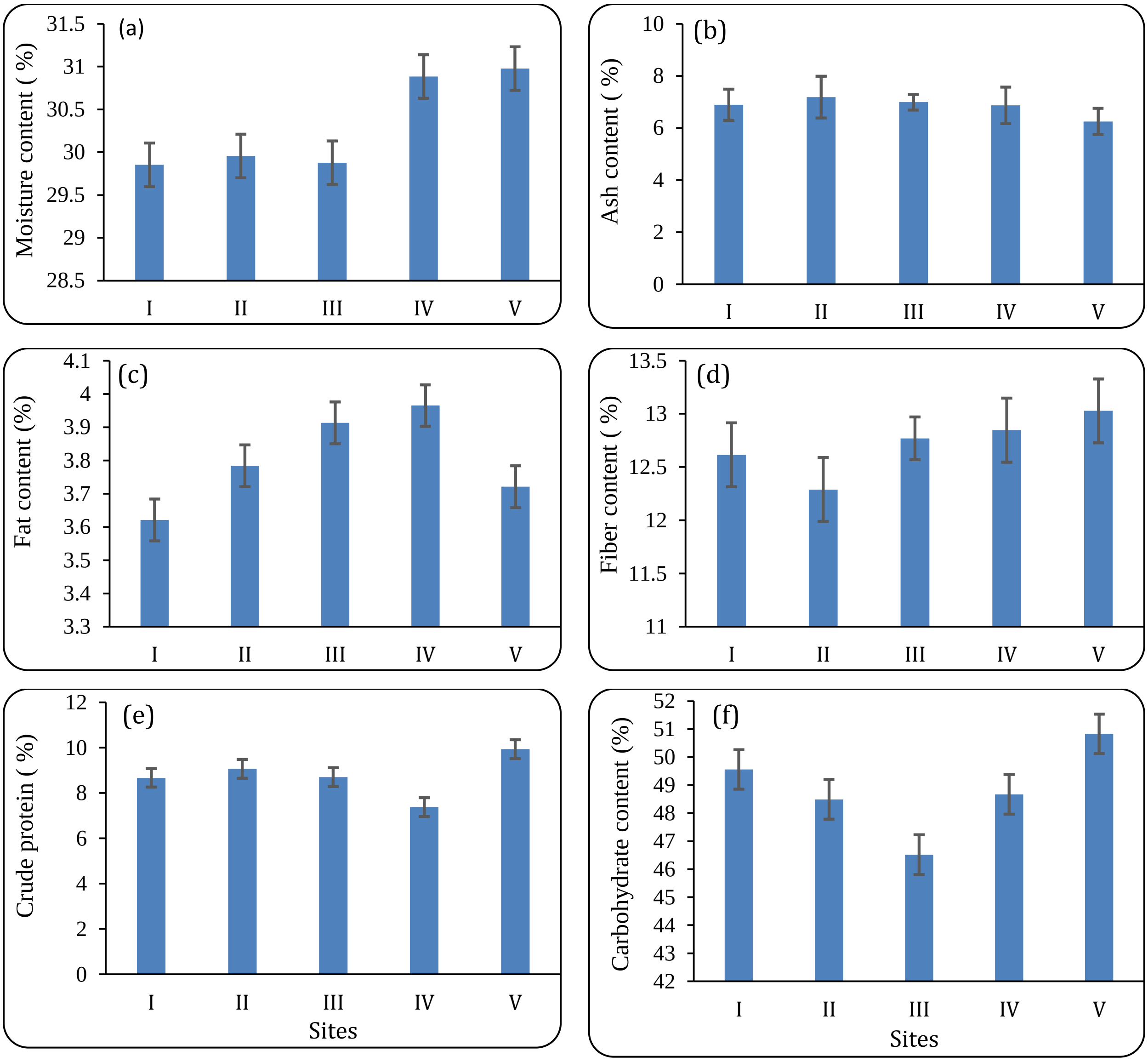

Figure 1: Moisture (a), ash (b), fat (c), crude fiber (d), crude protein (e) and carbohydrate (f) contents in *Rhazya stricta* (% + SE).

Crude protein (%)

The maximum amount of protein was observed in plants from Site 5 (9.93%), whereas the minimum was recorded in the Site 4 population (7.37%). The decreasing order of crude protein content was S5>S3>S1>S2>S4 (**Figure 1**).

Carbohydrates (%)

The maximum concentration of carbohydrates was observed in plants from Site 5 (50.82 %), while the minimum in the Site 2 plants (48.4%). The decreasing order of carbohydrates was S5>S4>S1>S3>S2 (**Figure 1**).

Concentrations of ions in leaves, roots and stem

Potassium

The maximum root potassium content was observed in the plants collected from Site 5 (6836.886 mg/kg), whereas the minimum was observed in the plants taken from Site 4 (6211.266 mg/kg). The decreasing order of root potassium concentration was S5>S3>S2>S1>S4. The maximum concentration of stem potassium was observed in the plants growing at Site 3 (6017.585 mg/kg), while the minimum was observed in those from Site 1 (5786.361 mg/kg). The decreasing order of stem potassium concentration was S3>S2>S5>S4>S1. The maximum concentration of leaf potassium was observed in the plants of Site 4 (6272.154 mg/kg), while the minimum was observed in those from Site 3 (5528.795 mg/kg). The decreasing order of leaf K was S4>S5>S2>S1>S3 (**Figure 2**).

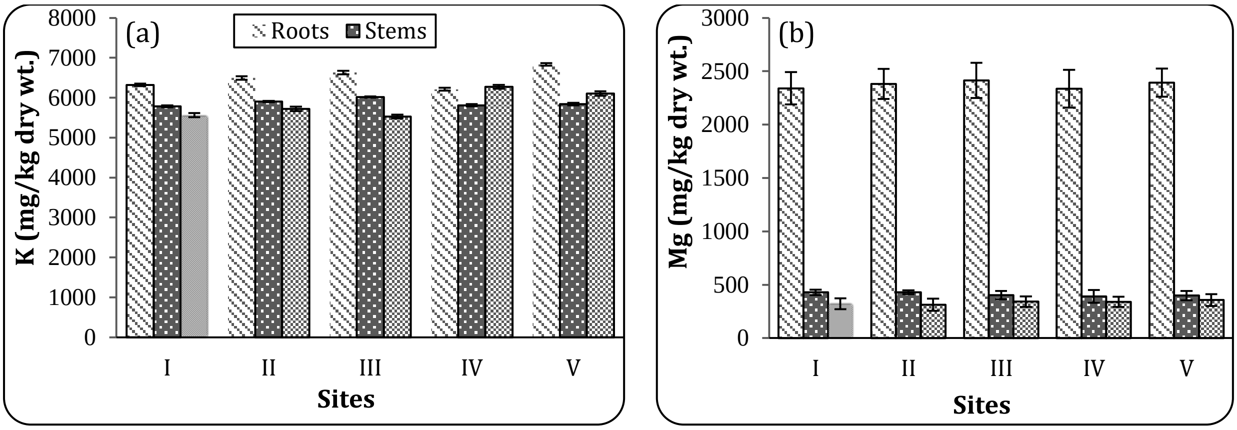

Figure 2: K and Mg concentrations in stem, leaf and roots of plants collected from different sites of Warcha Mine (mg/kg dry wt.).

Magnesium

The maximum and the minimum concentrations of root magnesium were observed in the plants from Site 3 (2414.460 mg/kg) and Site 4 (2335.101 mg/kg), respectively. The decreasing order of root magnesium concentration was S3>S5>S2>S1>S4. The maximum and the minimum concentrations of stem magnesium were observed in the plants collected from Site 2 (431.157 mg/kg) and Site 4 (391.315 mg/kg), respectively. The decreasing order of stem magnesium concentration was S2>S1>S5>S3>S4. The maximum and minimum concentrations of leaf magnesium were recorded in the plants from Site 5 (357.208 mg/kg) and Site 2 (312.348 mg/kg), respectively. The decreasing order of leaf magnesium concentration was S5>S3>S4>S1>S2 (**Figure 2**).

Iron

The maximum and the minimum concentrations of root iron were observed in the plants from Site 4 (31.336 mg/kg) and those from Site 5 (27.145 mg/kg), respectively. The decreasing order of root iron concentration was S4>S2>S1>S3>S5. The maximum and the minimum concentrations of stem iron were observed in the plants of Site 3 (31.475 mg/kg) and Site 2 (25.461 mg/kg), respectively. The decreasing order of root iron concentration was S3>S4>S5>S1>S2.The maximum and the minimum concentrations of leaf iron were recorded in the plants from Site 2 (34.525 mg/kg) and Site 5 (25.281 mg/kg), respectively. The decreasing order of leaf iron concentration was S2>S3>S1>S4>S5 (**Figure 3**).

Nickel

The maximum and the minimum levels of nickel were observed in the roots of plants from Site 4 (0.127 mg/kg) and those from Site 2 (0.069 mg/kg), respectively. The decreasing order of root nickel concentration was S4>S1>S5>S3>S2. The maximum and the minimum stem Ni levels were recorded in the Site 4 population (0.103 mg/kg) and that from Site 2 (0.09 mg/kg), respectively. The decreasing order of stem nickel concentration was S4>S1>S3>S5>S2. The maximum and the minimum concentrations of leaf nickel were observed in the plant population collected from Site 5 (0.103 mg/kg) and that from Site 4 (0.006 mg/kg). The decreasing order of leaf nickel concentration was S5>S3>S1>S2>S4 (**Figure 3**).

Cadmium

The maximum and the minimum concentrations of root cadmium were observed in the plant population from Site 4 (0.097 mg/kg) and that from Site 3 (0.057 mg/kg), respectively. The decreasing order of root cadmium concentration was S4>S5>S2>S1>S3. The maximum and the minimum levels of stem cadmium were found in the plants from Site 2 (0.095 mg/kg) and those from Site 1 (0.073 mg/kg), respectively. The decreasing order of stem magnesium concentration was S2>S5>S4>S3>S1. The maximum and the minimum concentrations of leaf cadmium were observed in the plants growing at Site 2 (0.107 mg/kg) and those at Site 4 (0.057 mg/kg). The decreasing order of leaf cadmium concentration was S2>S3>S1>S5>S4 (**Figure 3**).

Copper

The maximum and the minimum levels of root copper were noticed in the plant population from Site 4 (0.244 mg/kg) and that from Site 3 (0.118 mg/kg), respectively. The decreasing order of root copper concentration was S4>S2>S1>S5>S3. The maximum and the minimum levels of stem copper were recorded in the plants from Site 1 (0.280 mg/kg) and those from Site 2 (0.191 mg/kg), respectively. The decreasing order of stem copper concentration was S1>S3>S5>S4>S2. The maximum concentration of leaf copper was observed in the plants from Site 3 (0.160 mg/kg), whereas the minimum was observed in those from Site 2 (0.095 mg/kg). The decreasing order of leaf copper concentration was S3>S1>S5>S4>S2 (**Figure 3**).

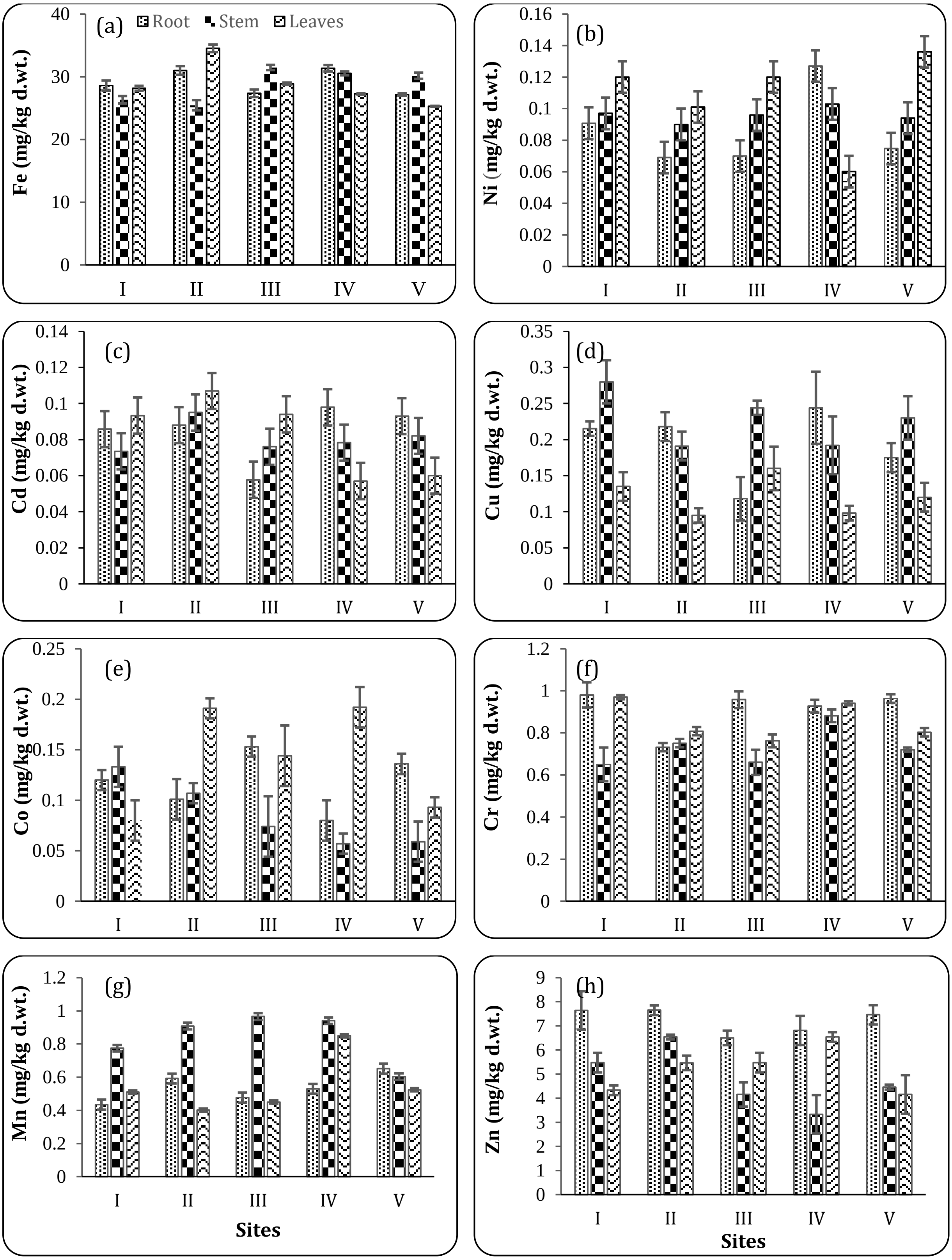

Figure 3: Fe (a), Ni (b), Cd (c), Cu (d), Co (e), Cr (f), Mn (g) and Zn (h) concentrations in leaf, roots and stem of plants collected from different sites of Warcha Mine (mg/kg dry wt.).

Cobalt

The maximum and the minimum levels of root cobalt were found in the plants from Site 3 (0.153 mg/kg) and those from Site 4 (0.080 mg/kg), respectively. The decreasing order of root cobalt concentration was recorded as S3>S5>S1>S2>S4. The maximum and the minimum concentrations of stem cobalt were observed in the plants from Site 1 (0.133 mg/kg) and those from Site 4 (0.057 mg/kg), respectively. The decreasing order of stem cobalt concentration was S1>S2>S3>S5>S4. The maximum and the minimum concentrations of leaf cobalt were observed in the plant population from Site 4 (0.192 mg/kg) and that from Site 1 (0.080 mg/kg), respectively. The decreasing order of leaf cobalt concentration was S4>S2>S3>S5>S1 (**Figure 3**).

Chromium

The maximum and the minimum concentrations of chromium were found in the roots of the Site 1 (0.980 mg/kg) plant population and that of Site 2 (0.732 mg/kg), respectively. The decreasing order of root chromium concentration was S1>S5>S3>S4>S2. The maximum and the minimum levels of Cr were recorded in the stems of the Site 4 (0.881 mg/kg) plant population, and that of Site 1 (0.650 mg/kg), respectively. The decreasing order of stem chromium concentration was S4>S2>S5>S3>S1. The maximum leaf Cr concentration was observed in the plants from Site 1 (0.970 mg/kg), whereas the minimum was in those of Site 3 (0.762 mg/kg). The decreasing order of leaf chromium concentration was S1>S4>S2>S5>S3 (**Figure 3**).

Manganese

The maximum and the minimum concentrations of root manganese were observed in the plants from Site 5 (0.592 mg/kg), and those from Site 1 (0.434 mg/kg), respectively. The decreasing order of root manganese concentration was S5>S2>S4>S3>S1. The maximum and the minimum levels of stem manganese were observed in the plant population from Site 3 (0.966 mg/kg) and Site 5 (0.603 mg/kg), respectively. The decreasing order of stem manganese concentration was S3>S4>S2>S1>S5. The maximum and the minimum levels leaf manganese were recorded in the plants from Site 4 (0.850 mg/kg) and Site 2 (0.401 mg/kg), respectively. The decreasing order of leaf manganese concentration was S4>S5>S1>S3>S2 (**Figure 3**).

Zinc

The maximum and the minimum concentrations of root Zn were observed in the plant population from Site 2 (7.650 mg/kg) and Site 3 (6.499 mg/kg), respectively. The decreasing order of root zinc concentration was S2>S1>S5>S4>S3. The maximum and the minimum stem Zn concentrations were recorded in the populations from Site 2 (6.539 mg/kg) and that from Site 4 (3.333 mg/kg), respectively. The decreasing order of stem zinc concentration was S2>S1>S5>S3>S4. The maximum leaf Zn concentration was observed in the Site 4 population (6.539 mg/kg), whereas the minimum in that from Site 5 (4.155 mg/kg). The decreasing order of leaf zinc concentration was S4>S3>S2>S1>S5 (**Figure 3**).

Translocation factor

Iron

Iron showed a high TF value for root to stem in plants from Site 3, while Fe TF for stem to leaves was considerably high in the plants from Site 3. The order of TF for root to stem was S3>S5>S>S1>S2, whereas that for stem to leaves was S3>S4>S1>S2>S5 (**Table 2**).

Magnesium

Magnesium showed a high TF value for root to stem in the plants from Site 1, while a high TF value of this element for stem to leaves was found in the plants from Site 5. The order of Mg TF for root to stem was S1>S2>S3>S5>S4, whereas that for stem to leaves was S5>S4 >S3>S1>S2 (**Table 2**).

Cadmium

High TF value for Cd for root to stem was recorded in the plants from Site 2, while that for stem to leaves was high in the plants from Site 2. The order of Cd TF for root to stem was S2>S3>S4>S5>S1, whereas that for stem to leaves was S2>S5 >S1>S3>S4 (**Table 2**).

Potassium

High K TF value for root to stem was recorded in the plants collected from Site 4, while for stem to leaves high K TF value was observed in the plants from Site 4. The order of TF for root to stem was S4>S1>S2>S3>S5, whereas that for the stem to leaves was S4>S35>S2>S1>S3 (**Table 2**).

Table 2: Translocation factor values of different elements.

Metal	Sites	Stem/Root	Leaf/Stem	Metal	Sites	Stem/Root	Leaf/Stem
	1	0.68	0.56		1	0.183	0.754
	2	0.67	0.43		2	0.181	0.724
Fe	3	0.91	0.78	Mg	3	0.167	0.849
	4	0.74	0.76		4	0.167	0.869
	5	0.89	0.34		5	0.166	0.896
	1	0.54	0.10		1	0.92	0.961
	2	0.89	0.54		2	0.91	0.97
Cd	3	0.32	0.76	K	3	0.91	0.92
	4	0.12	0.65		4	0.94	1.08
	5	0.65	0.54		5	0.85	1.04
	1	0.32	0.21		1	0.43	0.32
	2	0.55	0.98		2	0.54	0.36
Mn	3	0.21	0.43	Ni	3	0.53	0.12
	4	0.54	0.32		4	0.21	0.25
	5	0.53	0.54		5	0.89	0.65
	1	0.54	0.16		1	0.98	0.31
	2	0.32	0.96		2	0.25	0.98
Cr	3	0.98	0.32	Co	3	0.97	0.32
	4	0.31	0.41		4	0.54	0.43
	5	0.36	0.98		5	0.21	0.12
	1	1.43	1.52		1	1.40	0.30
	2	1.54	1.98		2	1.90	0.90
Zn	3	1.21	0.54	Cu	3	0.44	1.34
	4	1.98	0.31		4	0.43	1.65
	5	1.32	0.63		5	1.43	1.87

Manganese

High Mn TF for root to stem was recorded in the plants from Site 2, and that for stem to leaves was in the Site 2 population. The order of Mn TF for root to stem was S2>S4>S5>S1>S3, while that for stem to leaves was S2>S5>S3>S4>S1 (**Table 2**).

Nickel

Significantly high Ni TF for root to stem was found in the plant population from Site 5, while that for stem to leaves was in that from Site 5. The order of Ni TF for root to stem was S5>S2>S3>S1>S3, whereas that for the stem to leaves was S5>S2>S1>S4>S3 (**Table 2**).

Chromium

High Cr TF value for root to stem was observed in the plants from Site 3, and that for stem to leaves in the plants from Site 5. The order of Cr TF for root to stem was S3>S1>S5>S2>S4, whereas that for stem to leaves was S5>S2>S4>S3>S1 (**Table 2**).

Cobalt

Significantly high Co TF for root to stem was recorded in the plant population from Site 1, while that for stem to leaves was in the population from Site 2. The order of Co TF for root to stem was S1>S3>S4>S2>S5, whereas that for stem to leaves was S2>S4>S3>S1>S5 (**Table 2**).

Zinc

Considerably high Zn TF for root to stem was noticed in the plants from Site 4, while that for stem to leaves was high in the plants from Site 2. The order of Zn TF for root to stem was S4>S2>S1>S5>S3, while that for stem to leaves was S2>S1>S5>S3>S4 (**Table 2**).

Copper

High Cu TF value for root to stem was observed in the plants from Site 2, while that for stem to leaves was high in the plants from Site 5. The order of Cu TF for root to stem was S2>S5>S1>S3>S4, whereas that for stem to leaves was S5>S4>S3>S2>S1 (**Table 2**).

Discussion

Due to considerable importance of medicinal plants, local people harvest them to cure diseases without knowing their heavy metal composition. Similarly, herbal companies obtain raw herbs enriched with heavy metals from commercial suppliers to formulate medicines leading to heavy metal toxicity in humans and other organisms. Therefore, WHO (2005) recommended that heavy metal status must be analyzed in medicinal plants and in their raw material used to manufacture herbal medicines.

Proximate and nutrient analysis determines the nutritional significance of medicinal plants. As various medicinal plant species are also used in food, assessment of their nutritional significance can help analyze the significance of these plant species (Ugulu et al., 2009). The results of the proximate analysis showed considerable alterations in key biochemical and other contents in the plant under study. Ash, fat, crude fiber, protein and carbohydrates are the fundamental nutrients required for the sustainability of life. The maximum moisture content was observed in the plants from Site 4 (31.21%), whereas the minimum in those from Site 2 (29.14 %). The possible reason for more moisture content may have been due to the reason that plants generally growing in rocky and desert zones store up high quantity of moisture to prevent desiccation (Shad et al*.,* 2002). Similar findings have been earlier reported by Hussain et al. (2010) in plants collected from a dry-hit region.

The maximum fat content was found in plants growing at Site 3 (3.91%), whereas the minimum in those from Site 1 (3.62%). The values of fat in the present investigation were lower than those reported by Ghani et al. (2016) in *Solanum nigrum* plants collected from a drought-prone region. Lower values of fat in this population may have been due to the lofty and tough mountains of the hilly areas where minerals and water availability to plants are low (Shad et al*.,* 2002).

The maximum concentration of fiber was observed in plants brought from Site 5 (13.02%), whereas the minimum was observed in those from Site 2 (12.2%). The presence of a considerable amount of fiber is an essential requirement of the plant to survive in the rocky mountains deficient in water availability and desert areas for which the plant needs more fiber for strength so as to survive under harsh environmental conditions (Shad et al*.,* 2002).

The maximum protein content was observed in the plants from Site 5 (9.93 %), whereas the minimum in the plant population from Site 4 (7.37%). The variation in protein content observed in the five populations of *R. stricta* is expected in view of the differential adaptability of the populations to their specific habitats. Likewise, it is believed that genetically diverse cultivars of a crop may vary in protein content as earlier observed in different cultivars of *Rosa* species (Elinge et al., 2012). The differential pattern of carbohydrate accumulation in the five populations of *R. stricta* could also be explained due to their differential adaptation to their specific habitats. Our current study on the nutritional evaluation of *Rhazya stricta* has demonstrated that this plant is a good supplier of various nutrients (ash, proteins, fats, carbohydrates, fiber and minerals), and thus it is considered as a potential medicinal species to treat various diseases.

The permissible limit of iron in the medicinal plant is 20 mg/kg as recommended by WHO (2005). The concentration of iron in all five *R. stricta* populations was significantly lower than the permissible limit of 20 mg/kg as documented by WHO (2005). Nickel, in small quantities, is mandatory for the formation of red blood cells, since it regulates the lipid content in tissues (Ugulu, 2015). However, its slightly higher concentration may cause several diseases and disorders in humans (Khan et al., 2019c; Ugulu et al., 2019). The permissible limit of nickel in medicinal plants has been earmarked as 1.5 mg/kg (WHO, 2005). Again, the concentrations of Ni in the five grass populations were much lower than the permissible limit documented by WHO (2005).

The permissible limits of Cd, Cu, Co, Cr, and Mn in medicinal plants determined by WHO (2005) are reported as (Cd, 0.3 mg/kg; Cu, 10 mg/kg; Co, 0.04 mg/kg; Cr, 1.5 mg/kg; Mn, 200 mg/kg). In the present results, the concentrations of all five metals in the five populations were recorded to be much lower than the permissible limits of these metals reported by WHO (2005).

It can be concluded from the analysis of heavy metals in *Rhazya stricta* that all the metals were within the permissible limits recommended by the World Health Organization, and not a single metal exceeds its limit.

Metal movement from soil to root, and from root to shoot is generally reflected by the translocation factor (TF), which indicates the metal availability from the soil system to plant parts. Based on the metal translocation pattern, plants are identified as accumulators or excluders (Ugulu et al., 2021a, b). *Rhazya stricta* showed TF (translocation factor) values lower than one except for zinc, copper and magnesium. These findings show that *R. stricta* may be used as a phytostabiliser in metal-polluted areas.

The chemical analysis shows varying concentrations of heavy metals and proximate composition in different parts of *R. stricta* plant. Proximate analysis showed that there were significant amounts of ash, moisture, fat, crude fiber, crude protein and carbohydrates in different populations of *Rhazya stricta*.

Translocation factor also varied in different parts of the plant, i.e., from root to stem and from stem to leaves. However, the levels of most of the metals determined in the present investigation are well below the overall permissible limits of these elements for medicinal plants determined by WHO (2005), so this plant can be safely utilized by herbal practitioners and pharmaceutical industry.

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