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Original Article

Health Risk Assessment of Heavy Metals in the Medicinal Plants *Ziziphora clinopodioides* and *Echinophora platyloba* in West Islamabad and Sanandaj Regions

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Abstract

Background & Aims: Medicinal plants are part of traditional medicine, which has many uses in the treatment of diseases and human health. This research was conducted to evaluate heavy metals in two species of *Ziziphora clinopodioides* and *Echinophora platyloba*.

Materials and Methods: In each of the cities of Islamabad and Sanandaj, five sampling stations were determined, and from each station, 5 samples of *Kakuti* plant and 5 samples of Khosharizeh plant were collected from 3 different places of non-agricultural lands in the summer season of 2021. The mean data of heavy metals were compared with each other using a one-way analysis of variance and Duncan's test.

Results: The average of manganese (Mn) in *Z. clinopodioides* and *E. platyloba* (5112.95 and 4258.67 mg/kg) was significantly higher than other heavy metals ($P<0.05$). In addition, the average of arsenic in *Z. clinopodioides* and *E. platyloba* (0.002 mg/kg and 0.001 mg/kg) had significantly the lowest values among the studied heavy metals ($P<0.05$). The results of the health risk assessment showed that the highest risk index of heavy metals in *Z. clinopodioides* and *E. platyloba* in the age group of children was related to Mn (12.15 and 12.74, respectively). On the other hand, the lowest value of the risk index of heavy metals for the consumption of *Z. clinopodioides* and *E. platyloba* for children and adults was obtained for iron (0.48 and 0.32, respectively). The highest carcinogenic risk index of the *Kakuti* plant was observed in two age groups of children and adults regarding cadmium (0.099 and 0.098, respectively).

Conclusion: The analysis of the health risk assessment of heavy metals in the samples of *Z. clinopodioides* and *E. platyloba* in two regions of Islamabad and Sanandaj revealed that the consumption of these two plants is dangerous for the health of people of different age groups.

Keywords: Medicinal plants, *Kakuti*, Khosharizeh, Heavy metals, Risk assessment, Health

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1. Introduction

Medicinal plants have found widespread applications in the treatment, prevention, and management of diseases. Due to the numerous benefits of herbal medicines, the majority of the world's population depends on them in any way for various health benefits [1]. According to the World Health Organization (WHO), it is estimated that 65–80% of the world's population relies on traditional medicine [2], and the use of herbs to treat diseases, including cancer, is accepted in many countries around the world [3].

Medicinal plants also play an important role in the healthcare industry, cosmetics, traditional medicines, and food [4]. The issue of food security and safety of medicinal plants is of great interest considering that their harvesting takes place from pastures, plains, and mountains [1,2].

Heavy metal contamination of plant materials is a potential health hazard, especially for populations that are already vulnerable [3]. Heavy metals of non-human origin are always present in parent rocks and soils decomposed by weathering and erosion [5], but concentrations of heavy metals in certain ecosystems have also increased dramatically due to human activities [6]. Heavy metals are often formed as cations that interact strongly with the soil network and can be mobilized as a result of changing environmental conditions [7]. Due to their ability to tolerate potential toxic ions in the environment, plants can accumulate trace elements, especially heavy metals, in and on their tissues [8]. The uptake of heavy metals by plants can increase the potential for certain toxic elements to enter the food chain, so understanding how these elements progress through food webs and the effects of such elements on organisms is significant [9].

Several studies have been conducted on the heavy metal contamination of medicinal plants in different countries [10,11]. Despite this, considering the severe consequences that it may have on health and the environment, risk factor assessment, and environmental risk assessment have been conducted by researchers [12,13]. It has been shown that the contamination of heavy metals in herbal

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remedies is an area that requires immediate attention and whose potential risk to human health has already been confirmed [14]. It was reported that lead (Pb) was present in all 10 studied medical plant species, except for *Ocimum gratissimum*. Cadmium (Cd) was also detected in some medicinal plant species, while mercury (Hg) and arsenic (As) in all plant species were below detection. Therefore, plant species that grow in different environments absorb and accumulate different levels of heavy metals [1]. In another study in South Africa, levels of As, chromium (Cr), Cd, and Pb were reported to be higher than permissible levels in medicinal plants, and the risk index was higher than 1, indicating that the combined effects of heavy metal contaminants found in a particular plant product were associated with health [3]. In Indonesia, heavy metals such as Hg, Pb, As, and Cd *in Chromolaena odorata* were studied, and Cd concentrations were higher than the permissible limit [15]. In a study on 20 medicinal plant samples in Ghana, it was reported that some concentrations of Cr, Cd, As, manganese (Mn), and Pb analyzed in every 20 medicinal plant samples exceeded the WHO limit, while concentrations of Hg and nickel (Ni) for all samples were lower than the WHO limit [16]. The concentration of heavy metals in 33 species of medicinal plants studied in Turkey in industrial and mineral areas was slightly higher than the limits set by the WHO. As a result, to avoid the accumulation of these heavy metals, collecting aromatic herbs must be done from rural areas, near clean rivers, or mountainous areas far from highways, mines, and industrial areas [17]. *Ziziphora clinopodioides* is one of the plants belonging to the *Lamiaceae* family, which is found in many parts of Iran. For this plant, several therapeutic properties have been reported, including strengthening and relieving stomach pain, antifever, anti-inflammatory, and relaxing properties. In different powdered and dried areas, this plant is used as a dairy seasoning for frying foods and other items [18].

Khosharizeh, with its scientific name, *Echinophora platyloba*, is a pasture and edible plant that is an automobile in Iran. *Khosharizeh* is a matte green plant with a yellowish, sturdy, prickly, and single stem, and it is very branched, with interlaced branches. This plant is traditionally used as a food condiment and for yogurt and cheese aromatization. In addition to the treatment of gastrointestinal diseases and the control of abdominal cramps, this plant has antimicrobial, antispasmodic, and antifungal effects [19]. Heavy metals are important for human health, and the western regions of the country are the hubs of medicinal plants in Iran and have high consumption of these plants. Therefore, it seems that the concentrations of heavy metals in the case study on two *Ziziphora* and *Khriz* from the Islamabad-e-Gharb area located in the hills and foothills of Tajar Karam Panahabad village (the nearest removable area of these plants to the Industrial Zone in Islamabad West) and the Sanandaj Mountains are essential. Considering the high consumption of these two high-profile plants and their multiplicity in recent years due to the coronavirus disease and its harvesting from unknown places (i.e., mountains and foothills), the issue of food security and the consequences of heavy metals for the overuse of these drugs and its implications for society is highly necessary and researchable. For this reason, the closest place to the industrial estate in Islamabad Gharbhas chosen a high harvest rate of its mountains. There has been research in this field, but, to the best of our knowledge, there is no research on heavy metals in these two plants and on a case-by-case basis. Accordingly, this study sought to evaluate the levels of pollution and health risks caused by heavy metals to humans, specifically in the medicinal plants of *Kakuti* and *Khosharizeh*.

2. Materials and Methods

This descriptive cross-sectional study was conducted in the summer of 2021 in Islamabad and Sanandaj. Five sampling stations were identified in each region. In Islamabad and Sanandaj, 5 samples of *Kakuti* and 5 samples of *Khosharizeh* plants were collected from 3 different points of non-agricultural lands (Figure 1). A total of 100 samples of *Kakuti and Khosharizeh* were prepared, and complete plant samples were placed in a paper bag for transportation and transferred to the laboratory. The mountains are located around the village of Tajar Karam Panahabad from the functions of West Islamabad at a latitude of 46 degrees and 31 minutes, a width of 34 degrees and 6 minutes, a height of 135 meters above sea level, 65 km south of Kermanshah, and on the way to Kermanshah Khosravi. The climate of this city is moderate Mediterranean, and the annual rainfall averages 478 mm. Sanandaj is one of the cities of Iranian Kurdistan and the 21st largest city in Iran. Sanandaj is the capital of Kurdistan province in western Iran, which is located at an altitude of 1450–1538 meters above sea level, and in the mountainous region of Zagros and has a cold and semi-arid climate. The city is limited to Abidar Mountain to the west, the famous Sheikh Mountain to the north and Sirajuddin Mountain in the south, and is spread over an area of 3688.6 hectares.

In this study, inductivity-coupled plasma-optical emission spectrometry (ICP-OES) was used to measure the concentration of heavy metals.

Digested samples were injected into the ICP-OES model Varian 710-ES device manufactured by Varian Company in the United States, and the detection limit of μg/kg (ppb) was calibrated to determine the concentration of Hg, As, Cd, Pb, copper (Cu), zinc (Zn), Ni, iron (Fe), aluminum (Al), and Mn in each sample as mg/kg. For the chemical digestion of the leaves of *Kakuti and Khosharizeh*, 0.5 g of each leaf sample was digested in 9 mL of concentrated

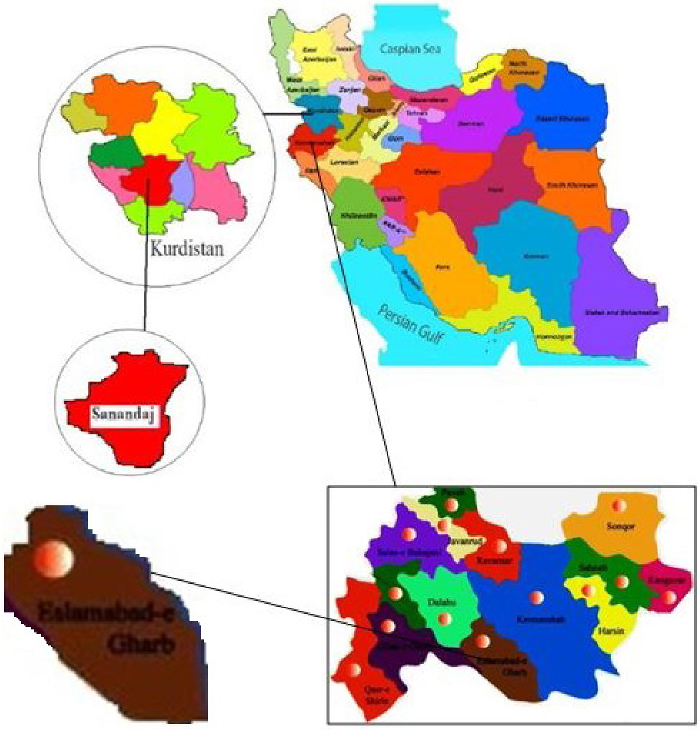

Figure 1. Geographical Location for Sampling *Ziziphora* and Other Medicinal Plants

nitric acid (to increase the solubility of heavy metals) for 15 minutes using an appropriate microwave system. Samples and acids were placed in suitable inert polymer microwave containers. The containers were sealed and heated in the microwave system. Temperature specifications are specified to allow specific reactions and include reaching 180 ± 5 ˚C in less than 5 minutes and remaining at 180 ± 5 ˚C for 9 minutes to complete specific reactions. After cooling, the contents of the sedimentation vessel and filter of 100 mL were passed and centrifuged, and the dilute volume of the liquid sample was prepared for injection into the machine [20]. The risk of consuming plant products contaminated with heavy metals for human health was assessed based on daily intake of metal, hazard quotient, and hazard index. Daily intake (estimated daily intake, EDI) was calculated to estimate the average daily intake of body metals, according to the body weight of a consumer. The amount of daily consumption was determined by Relation 1 as follows [21]:

Relationship 1: $EDI = C_{metal} \times IR / BW$

The risk index (HQ) is used to assess the non-carcinogenic risk to humans from prolonged exposure to heavy metals from vegetables, herbs, and fruits. If HQ is less than 1, it implies that no potential health effect is expected from exposure, while if HQ is higher than 1, it indicates that there are potential health risks due to exposure. HQ is calculated as a fraction of the dose determined to the reference dose as shown in Relation 2 [21]:

In this regard, the concentration of heavy metals in plant samples was measured as mg/kg, the IR mean intake of medicinal plants was determined 20 g/d for adults and 10 g/d for children, and the body weight (BW) was considered 70 kg for adults and 15 kg for children in this study [22]:

Relationship 2: $HQ = EDI / R_{FD}$

Where EDI is the average daily use of herbal medicines (mg/kg/d), and RfD denotes the reference dose of metal (mg/kg/d). RfD is an approximation of the tolerable daily exposure that a person is expected to have throughout life without any significant risk of harmful effects. RfD for Pb, Cu, Cd, Cr, As, Hg, Cu, Zn, Fe, Mn, and Ni was 0.004, 0.04, 0.001, 0.003, 0.0003, 0.0005, 0.04, 0.3, 0.7, 0.014, and 0.02 mg/kg/d, respectively [23].

The risk index (HI) helps assess the overall risk of carcinogenicity for human health through more than one heavy metal. Exposure to more than one pollutant leads to additive effects, as explained in Relation 3 [21]:

Relationship 3: $HI = \Sigma\, HQ_{metals}$

The data were analyzed using SPSS software, version 22. The means of treatments were compared using one-way analysis of variance and Duncan's test to determine the presence or absence of significant differences at the level of 5% ($P = 0.05$). An independent t-test was used to compare the concentration of heavy metals in the studied plants, Islamabad and Sanandaj. Excel software was utilized to draw charts and tables.

3. Results

The accumulation pattern of heavy metals in *Kakuti* in the Islamabad region was obtained as Mn < Al < Fe < Zn < Cu < Pb < Ni < Hg < Cd < As. In addition, in the samples obtained from the Islamabad region, the average of Mn metal was 4725.13 mg/kg, significantly higher than other heavy metals ($P < 0.05$). Further, the mean As content of 0.001 mg/kg significantly had the lowest values among the studied heavy metals ($P < 0.05$). The accumulation pattern of heavy metals in *Kakuti* in the Sanandaj region was obtained as Mn < Al < Fe < Zn < Cu < Ni < Cd < Hg = Cd < As.

Furthermore, as in the Islamabad region, in the *Kakuti* samples obtained from the Sanandaj region, the average Mn metal was 4437.31 mg/kg, significantly higher than other heavy metals ($P < 0.05$).

Moreover, the mean As content of 0.01 mg/kg significantly had the lowest values among the studied heavy metals ($P < 0.05$). Cd, Ni, Hg, and Zn ($P < 0.001$), as well as Mn and Pb ($P < 0.002$), were significantly different in the two regions, and according to the reported averages, their values in the Sanandaj region were significantly lower (Table 1).

The accumulation pattern of heavy metals in plant *Khosharizeh* in both Sanandaj and Islamabad regions was obtained as Mn < Al < Fe < Zn < Cu < Ni < Pb < Hg < Cd < As. Additionally, in the samples obtained from the Islamabad region, the average Mn metal was 4932.85 mg/kg, significantly higher than other heavy metals ($P < 0.05$).

Likewise, the mean As metal content of 0.005 mg/kg had the lowest value among the studied heavy metals ($P < 0.05$).

The average Mn metal in Khosharizah samples of the Sanandaj region was 4778.29 mg/kg, significantly higher than other heavy metals ($P < 0.05$).

Similarly, the mean As metal content of 0.007 mg/kg was significantly the lowest among heavy metals ($P < 0.05$). Cd, Ni, Hg, Cu, and Zn ($P < 0.001$) as well as Mn and Pb ($P < 0.002$) were significantly different in the two regions, and according to the reported averages, their values in Sanandaj were significantly lower (Table 1).

The results of the comparison of heavy metal concentrations in *Khosharizeh* and *Kakuti* plants were obtained as Mn < Al < Fe < Zn < Cu < Ni < Hg < Pb < Cd < As. In the studied samples, in *Khosharizeh*, the average Mn metal was 5112.95 mg/kg higher than other heavy metals ($P < 0.05$). In addition, the mean As metal content of 0.002 mg/kg had the lowest value among the studied heavy metals ($P < 0.05$). Further, in the samples of *Kakuti*, the average Mn metal was 4258.67 mg/kg higher than other heavy metals ($P < 0.05$). The mean As metal content (0.001 mg/kg) had the lowest value among the studied heavy metals ($P < 0.05$).

The results revealed that the amount of metals Ni, Mn, Fe,

Table 1. Statistical Parameters of Heavy Metals in *Ziziphora clinopodioides* and *Echinophora platyloba* From Islam Abad and Sanandaj

Location	Metals	*Ziziphora clinopodioides*		*Echinophora platyloba*	
		Mean ± SD (mg kg^{-1})	*P*	Mean ± SD (mg kg^{-1})	*P*
Islam Abad	Al	3334.35 ± 577.20	0.152	3551.23 ± 509.40	0.241
Sanandaj		3227.06 ± 679.40		3193.58 ± 508.67	
Islam Abad	As	0.001 ± 0	0.046	0.007 ± 0.003	0.003
Sanandaj		0.01 ± 0		0.005 ± 0.002	
Islam Abad	Cd	0.04 ± 0.02	0.001	0.011 ± 0.015	0.001
Sanandaj		0.02 ± 0.02		0.049 ± 0.028	
Islam Abad	Cu	23.61 ± 6.98	0.112	15.31 ± 6.51	0.002
Sanandaj		21.01 ± 4.20		25.38 ± 9.57	
Islam Abad	Fe	74.29 ± 14.94	0.014	72.50 ± 9.27	0.064
Sanandaj		58.94 ± 13.96		74.33 ± 11.05	
Islam Abad	Hg	0.06 ± 0.02	0.001	0.028 ± 0.019	0.001
Sanandaj		0.02 ± 0.01		0.058 ± 0.018	
Islam Abad	Mn	4725.13 ± 464.22	0.002	4778.29 ± 525.75	0.004
Sanandaj		4437.31 ± 222.60		4932.85 ± 494.31	
Islam Abad	Ni	0.29 ± 0.14	0.001	0.229 ± 0.17	0.001
Sanandaj		0.18 ± 0.14		0.289 ± 0.06	
Islam Abad	Pb	0.30 ± 0.13	0.002	0.073 ± 0.08	0.001
Sanandaj		0.09 ± 0.11		0.202 ± 0.09	
Islam Abad	Zn	35.39 ± 13.53	0.001	39.96 ± 20.87	0.001
Sanandaj		35.29 ± 7.38		41.23 ± 13.78	

Note. SD: Standard deviation.

Cu, and Zn ($P < 0.001$) in the two regions was significantly different, and according to the reported averages, their values in the Sanandaj area were significantly lower. One sample t-test was used to compare the concentrations of heavy metals such as Hg, Cu, As, Fe, Pb, Cd, Mn, Zn, Ni, and Al in two medicinal plants, *Khosharizeh* and *Kakuti*, according to the Iranian national standard and the WHO. The results demonstrated that the concentrations of heavy metals, except for Cd, in both plants were significantly different compared to the national standard limit of Iran and Ni in both plants compared to the global health standard ($P < 0.001$). In the two studied areas, these values were higher than national and global standards (Table 2).

The results of the health risk assessment of heavy metal concentration in *Kakuti* showed that the highest risk index of heavy metals in the age group of children was related to Mn (12.15). On the other hand, the lowest value of the heavy metals risk index for consumption of *Kakuti* in children and adults was 0.48 (Table 3).

The highest carcinogenic risk index in two age groups of children and adults in the Islamabad region was 0.099 and 0.098, respectively (Table 4).

Based on the results of the health risk assessment of heavy metal concentration in the *Khosharizeh* plant, the

Table 2. Comparison of the Concentrations of Heavy Metals in *Ziziphora clinopodioides* and *Echinophora platyloba* With the National Standard of Iran and the World Health Organization

Metals	Plant	Mean ± SD (mg kg^{-1})	*P*	NSI (mg kg^{-1})	*P*	WHO (mg kg^{-1})	*P*
Hg	Zc	0.049 ± 0.006	0.402	0.1	< 0.001	-	-
	Ep	0.051 ± 0.009			< 0.001		-
Cu	Zc	22.53 ± 3.59	0.005	1.5	< 0.001	1.5	< 0.001
	Ep	29.59 ± 9.39			< 0.001		< 0.001
As	Zc	0.001 ± 0.0009	0.272	1	< 0.001	1	< 0.001
	Ep	0.002 ± 0.0005			< 0.001		< 0.001
Fe	Zc	70.28 ± 4.82	0.008	1	< 0.001	1	< 0.001
	Ep	69.73 ± 2.07			< 0.001		< 0.001
Pb	Zc	0.197 ± 0.13	0.873	0.1	< 0.001	0.1	< 0.001
	Ep	0.089 ± 0.16			< 0.001		< 0.001
Cd	Zc	0.002 ± 0.0001	0.496	0.1	0.017	0.003	< 0.001
	Ep	0.021 ± 0.002			0.004		< 0.001
Mn	Zc	4258.67 ± 423.60	0.001	0.5	< 0.001	0.5	< 0.001
	Ep	5112.95 ± 204.52			< 0.001		< 0.001
Zn	Zc	47.31 ± 15.74	0.002	15	< 0.001	15	< 0.001
	Ep	42.42 ± 11.21			< 0.001		< 0.001
Ni	Zc	0.140 ± 0.001	0.001	0.07	< 0.001	1.5	0.142
	Ep	0.34 ± 0.086			< 0.001		0.005
Al	Zc	3885.58 ± 148.17	0.104	-	-	1.5	-
	Ep	3008.77 ± 352.48			-		-

Note. Zc: *Ziziphora clinopodioides*; Ep: *Echinophora platyloba*; NSI: National Standard of Iran; WHO: World Health Organization; SD: Standard deviation.

Table 3. Health Risk Assessment of Heavy Metals in *Ziziphora clinopodioides* From Islam Abad and Sanandaj for Adult Consumption

Metals	Islam Abad				Sanandaj			
	EDI (mg/kg/d)	HQ	CLI (mg/kg/d)	HI	EDI (mg/kg/d)	HQ	CLI (mg/kg/d)	HI
As	0.102	1.23	0.114	-	0.082	1.08	0.114	-
Cd	0.007	6.82	0.052	0.099	0.006	6.14	0.052	0.087
Cu	0.024	0.52	0.872	-	0.016	0.45	0.601	-
Fe	0.452	0.48	0.809	-	0.304	0.46	0.721	-
Hg	0.236	0.41	0.264	-	0.212	0.08	0.218	-
Mn	0.174	10.11	0.029	-	0.123	7.39	0.027	-
Ni	0.065	1.11	0.176	0.074	0.047	0.97	0.11	0.037
Pb	0.049	6.18	0.051	0.036	0.037	6.09	0.045	0.033
Zn	0.092	0.98	0.965	-	0.074	0.43	0.749	-

Note. EDI: Estimated daily intake; HQ: Hazard quotient; CLI: Consumption limit; HI: Hazard index.

highest risk index of heavy metals in the age group of children was 12.74 in the Islamabad region. The lowest value of the heavy metals risk index in *Khosharizeh* for adults with Fe in the Sanandaj area was 0.32 (Table 5).

The highest carcinogenic risk index in the two age groups of children and adults in Islamabad was obtained for Cd (0.109 and 0.111, respectively, Table 6).

4. Discussion

Heavy metals are biodegradable. Considering that the accumulation of heavy metals in human tissues and their adverse effects cause disease and carcinogenesis, the maximum control of heavy metals in medicinal plants is one of the issues that should be considered to maintain the health of consumers and achieve food safety [3,6].

Table 4. Health Risk Assessment of Heavy Metals in *Ziziphora clinopodioides* From Islam Abad and Sanandaj for Children Consumption

Metals	Islam Abad				Sanandaj			
	EDI (mg/kg/d)	HQ	CLI (mg/kg/d)	HI	EDI (mg/kg/d)	HQ	CLI (mg/kg/d)	HI
As	0.072	1.42	0.114	-	0.042	1.19	0.114	-
Cd	0.016	7.32	0.052	0.098	0.003	6.38	0.052	0.095
Cu	0.020	0.49	0.970	-	0.018	0.43	0.733	-
Fe	0.412	0.48	0.852	-	0.384	0.46	0.718	-
Hg	0.098	0.54	0.624	-	0.098	0.68	0.251	-
Mn	0.174	1.15	0.030	-	0.124	9.09	0.046	-
Ni	0.024	1.61	0.094	0.038	0.018	1.03	0.281	0.027
Pb	0.041	8.70	0.049	0.036	0.037	7.09	0.042	0.029
Zn	0.095	0.49	1.645	-	0.071	0.23	0.980	-

Note. EDI: Estimated daily intake; HQ: Hazard quotient; CLI: Consumption limit; HI: Hazard index.

Table 5. Health Risk Assessment of Heavy Metals in *Echinophora platyloba* From Islam Abad and Sanandaj for Adult Consumption

Metals	Islam Abad				Sanandaj			
	EDI (mg/kg/d)	HQ	CLI (mg/kg/d)	HI	EDI (mg/kg/d)	HQ	CLI (mg/kg/d)	HI
As	0.102	0.86	0.138	-	0.069	0.99	0.109	-
Cd	0.008	7.22	0.058	0.109	0.007	6.64	0.055	0.047
Cu	0.038	0.72	1.41	-	0.028	0.55	0.973	-
Fe	0.349	0.34	0.714	-	0.278	0.32	0.682	-
Hg	0.416	1.96	0.299	-	0.401	1.21	0.121	-
Mn	0.304	12.15	0.038	-	0.213	9.47	0.029	-
Ni	0.076	2.83	0.197	0.084	0.062	1.47	0.16	0.059
Pb	0.042	6.45	0.053	0.041	0.034	6.12	0.049	0.038
Zn	0.061	0.67	0.817	-	0.059	0.54	0.007	-

Note. EDI: Estimated daily intake; HQ: Hazard quotient; CLI: Consumption limit; HI: Hazard index.

Table 6. Health Risk Assessment of Heavy Metals in *Echinophora platyloba* From Islam Abad and Sanandaj for Children Consumption

Metals	Islam Abad				Sanandaj			
	EDI (mg/kg/d)	HQ	CLI (mg/kg/d)	HI	EDI (mg/kg/d)	HQ	CLI (mg/kg/d)	HI
As	0.062	0.73	0.084	-	0.042	1.01	0.103	-
Cd	0.008	7.84	0.060	0.111	0.007	7.04	0.049	0.050
Cu	0.042	0.76	1.525	-	0.034	0.59	0.980	-
Fe	0.381	0.39	0.791	-	0.201	0.48	0.734	-
Hg	0.386	1.84	0.318	-	0.474	1.37	0.177	-
Mn	0.362	12.74	0.046	-	0.178	9.11	0.036	-
Ni	0.070	2.96	0.141	0.089	0.085	1.62	0.180	0.064
Pb	0.039	6	0.059	0.053	0.034	5.86	0.057	0.047
Zn	0.066	0.601	0.901	-	0.062	0.59	0.832	-

Note. EDI: Estimated daily intake; HQ: Hazard quotient; CLI: Consumption limit; HI: Hazard index.

In this study, the concentration of Mn in the medicinal plants *Kakuti* and *Khosharizeh* vulgaris in the two regions of Islamabad West and Sanandaj was significantly higher than other heavy metals ($P<0.05$).

Further, the mean As metal content of two medicinal plants had the lowest values among heavy metals ($P<0.05$). Mn is an essential and important element for plant growth and development, maintains a metabolic role in different parts of plant cells [24], and can perform two different functions as an enzymatic cofactor as well as a metal with catalytic activity in biological activities [25]. In addition, Mn plays a role in diverse plant processes such as chloroplast development, purine and urea catabolism, phospholipid biosynthesis, DNA repair, and histidine biosynthesis [26, 27]. Therefore, the high concentration of Mn in both medicinal plants *Kakuti* and *Khosharizeh* sativa is probably due to the mentioned cases and the application of Mn in plant physiology. Different amounts of heavy metals, including Mn, have been reported in a study of eight medicinal plant species in Sanandaj [28], which is consistent with the results of this study. Furthermore, *Ziziphora clinopodioides*, *Centaurea virgata*, and *Cirsium congestum* have determined the amounts of heavy metals and Mn [29, 30], which confirms the results of this study on *Kakuti* and *Khosharizeh*. The researchers reported a concentration of 0.0001–0.002 mg/kg in medicinal plants [31], which is in line with the results of this study. As is a toxic element that has no physiological or biological role in living organisms [32], and probably the reason for its low content is related to the medicinal plants of *Kakuti* and *Khosharizeh*. It can probably be mentioned that the alkaline nature of the soil, the amount of organic matter, and the soil texture are responsible for the transfer of heavy metals from soil to plants [33]. Cd, Ni, Hg, Cu, and Zn ($P<0.001$) as well as Mn and Pb ($P<0.002$) in *Kakuti* were significantly different in the two regions, and according to reported averages, their values were significantly lower in the Sanandaj region. Moreover, in the medicinal plant of *Khosharizeh*, the amount of metals Ni, Mn, Fe, Cu, and Zn ($P<0.001$) in the two regions was significantly different, and according to the reported averages, their values were significantly lower in the Sanandaj region. All elements are naturally present in the environment with different concentrations, among which are toxic and dangerous elements. In fact, natural environments are contaminated with toxic elements on a global scale, with varying amounts and concentrations [19]. Element contamination can be of natural and abnormal origin, and industrial and agricultural activities can also cause heavy metals to enter the environment, where polluting metals pollute soil and climate and ultimately harm the environment [15]. Therefore, due to the sampling of medicinal plants *Kakuti* and *Khosharizeh* in the vicinity of Islamabad industrial town, it is likely that the concentration of heavy metals in samples in this region is higher than in Sanandaj. Habibollahi et al, in a study on the medicinal plants of Mentha spicata, *Mentha spicata*, *Glycyrrhiza glabra*, *Cichorium intybus*, *Foeniculum vulgare*, and *Echium amoenum* in Sistan and Baluchestan province, reported that the most important reason for the low level of heavy metals in the studied medicinal plants was the natural growth of these plants in remote areas of urban and industrial areas that are not affected by environmental pollutants [22]. This confirms the results of heavy metal concentration in medicinal plants of *Kakuti* and *Khosharizeh* communis in the Sanandaj area compared to Islamabad Gharb. Many factors, such as the abundance of heavy metals in the soil, excessive application of fertilizers and chemical toxins, as well as the presence of industrial and mineral wastes in nature and soil, may lead to the accumulation of heavy metals in plants [5,7]. The analysis of health risk assessment of heavy metals in *Kakuti* and *Khosharizeh* samples in two regions of Islamabad and Sanandaj showed that Hg, Pb, Ni, and Mn metals were higher than 1 in the age groups of children and adults, but the risk index for Zn, Fe, Cd, Cu, As, and Al was lower than 1. The carcinogenic risk indexes of toxic metals Cd, Pb, Ni, and As in *Kakuti* and *Khosharizeh* samples in Islamabad and Sanandaj regions were higher than 10^4. In one study, the amount of target hazard quotient of metals such as As, Hg, Pb, Cd, and Cu, as well as the hazard index values of the studied metals in the medicinal plants of mint (*Mentha spicata*), licorice (*Glycyrrhiza glabra*), chicory (*Cichorium intybus*), fennel (*Foeniculum vulgare*), and borage (*Echium amoenum*), in the Sistan and Baluchestan region, have been reported to be lower than 1 [22]. In another Brazilian study, for six medicinal plant samples, all recorded hazard index values (Cd, Pb, cobalt, Cu, Fe, Cr, barium, and As) were reported for adults (30 years old) and children (6 years old) with an abundance of exposure of 90 days per year less than 1, demonstrating a non-carcinogenic adverse effect caused by this pathway. However, the long-term health risk is high, and the non-carcinogenic adverse effect is not negligible. In fact, the presence of elements such as Pb, Cd, Cr, and cobalt can harm human health [34]. The presence of potassium, magnesium, sodium, phosphorus, Al, Fe, Zn, Mn, Cu, Ni, and selenium in dry samples and herbal tea has been reported in three species of medicinal plants, namely, *Bauhinia forficata*, *Eleusine Indica*, and *Orthosiphon stamineus*.

The studied medicinal plants had high concentrations of potassium and phosphorus and contained more magnesium, sodium, Al, Fe, Mn, Ni, Zn, and Cu than the limits set by the WHO. All hazard index values in medicinal plants are in the safe range for human consumption [35]. Likewise, in Ghana, EDI and hazard quotient of Hg, As, Pb, Cd, Cr, Ni, and Mn in 20 medicinal plant samples were less than the recommended tolerable intake and less than 1 for the reported , and the amount of carcinogenic risk was within the acceptable range for human consumption, indicating the absence of potential long-term health risks for consumers [16]. In another study, 33 Eastern Mediterranean medicinal plants in Turkey were measured the recommended dietary allowance for trace elements and the estimated target hazard quotient and hazard index for heavy metals. The concentration of heavy metals in some of the studied plants distributed in industrial and mineral areas was slightly higher than the limit set by the WHO [17]. Very low concentrations of some metals can be toxic and can cause serious health problems if medicinal plants containing metals accumulated in their leaves [35].

5. Conclusion

In this study, the concentration of Mn in the medicinal plants of *Kakuti* and *Khosharizeh* in two regions of Islamabad West and Sanandaj was higher than other heavy metals. Mn is an essential and important element for plant growth and development. Therefore, the high concentration of Mn in both medicinal plants, *Kakuti* and *Khosharizeh*, is probably due to the application of Mn in plant physiology. The accumulation patterns of heavy metals in *Kakuti* plants in the Islamabad and Sanandaj areas were Mn < Al < Fe < Zn < Cu < Pb < Ni < Hg < Cd < As and Mn < Al < Fe < Zn < Cu < Ni < Pb < Hg = Cd < As, respectively. The comparison of heavy metal concentrations in both *Ziziphus* and *Capsicum* L. was Mn < Al < Fe < Zn < Cu < Ni < Hg < Pb < Cd < As. Analysis of the health risks of heavy metals in *Kakuti* and *Khosharizeh* samples in Islamabad and Sanandaj regions showed that the consumption of these two herbs is dangerous for the health of different age groups. It is recommended that a comprehensive map of soil contamination is prepared for medicinal plant-growing areas. Moreover, comprehensive and complete studies should be carried out on medicinal plant species that are resistant to heavy metals and on accumulating plant species from soils and these plant species should be introduced to the EPA. Considering that the accumulation of heavy metals in these two species of medicinal plants have not been studied in Iran, it is necessary to investigate this topic further and compare the heavy metals of these two plants in different vegetative regions throughout Iran.

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Competing Interests

There is no conflict of interests between the authors.

Ethical Approval

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