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Evaluation of Trace Element and Heavy Metal Levels of Some Ethnobotanically Important Medicinal Plants Used as Remedies in Southern Turkey in Terms of Human Health Risk

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Abstract
Heavy metal accumulation in medicinal plants has increased dramatically in recent years due to agricultural and industrial activities leading to pollution of natural sources. This study is focused on the concentration of trace elements and heavy metals in aboveground parts of 33 medicinal plants from the Eastern Mediterranean of Turkey. Results showed that the Al concentrations varied between 4.368 and 1104.627, the B level varied between 47.850 and 271.479, Ca values ranged between 1971.213 and 22,642.895, Cd concentrations ranged between 0.011 and 0.651, Cr contents varied between 1.371 and 41.692, Cu values varied between 13.278 and 42.586, Fe concentrations varied between 20.705 and 1276.783, K levels ranged between 652.143 and 14,440.946, Mg concentrations varied from 336.871 to 1869.486, Mn contents varied between 46.383 and 849.492, Na concentrations varied between 167.144 and 3401.252, Ni values varied between 0.065 and 9.968, Pb levels ranged between 1.311 and 16.238, and Zn concentrations ranged between 67.250 and 281.954 mg kg^{-1}, respectively. Furthermore, Recommended Dietary Allowance (RDA) values for trace elements and estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) for heavy metals were calculated. The concentrations of heavy metals in some studied plants distributed in industrial and mining regions were found as slightly higher than the acceptable limits determined by WHO. Consequently, in order to prevent this heavy metal accumulation, when collecting medicinal aromatic plants, rural areas, close to clean rivers, or mountainous areas should be preferred, away from highway, mining, and industrial areas.

Keywords Herbal medicines · Mineral content · Health hazards · ICP-OES · Environmental pollution

Introduction

The earliest archeological evidence on the relationship between humans and plants dates back 1.2 million years. Recently, chemical analysis of human dental calculus belonging to ancient times has demonstrated that 70–80% of daily calorie was provided from plant products [1]. In recent years, the demand for medicinal plants has been increasing especially due to the chemical side effects of drugs although the use of plants for medicinal purposes in traditional treatment methods in history has lost its old value due to technological developments. It is known that 80% of the world's population benefits from medicinal plants against diseases and more than 80,000 plant species are used for medicinal purposes [2]. Like other plants, medicinal plants also need macroelements (Ca, Cl, N, Na, P, K, Mg, etc.) and trace elements (B, Fe, Cu, Mn, Zn, etc.) for their structure, growth, and metabolic activities in maintaining a proper life. Their uptake from the environment is crucial since these elements cannot be synthesized by the organism itself [3].

These trace elements have important roles in metabolic activities such as seed and fruit formation, pollen health, fertilization, protein synthesis, formation and transport of carbohydrates, transport of calcium, and formation of hormones, development of the cell tissues, the formation of roots and flowers, the structural and physiological stability of plant tissue, cell division, cell wall formation, cell expansion, and activation of enzymes in plant metabolism [4, 5]. Essential nutrient deficiency causes necrosis in young leaves, vegetative and reproductive growth, blossom-end rot of fruits, the significant losses in crop yield and quality, cell breakdown, reduction in

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plant fertility, loss of membrane integrity, and inhibition of the cell expansion in plant functions [6–8].

However, numerous anthropogenic activities such as industry, fossil fuel burning, mining, smelting, forest fires, traffic, municipal wastes, sewage disposal, chemical fertilizers, and pesticides have caused dramatically increased pollution in the period since the Industrial Revolution [2, 9, 10]. Consequently, many natural aquatic and terrestrial ecosystems have been contaminated by heavy metals like aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), iron (Fe), lead (Pb), nickel (Ni), silver (Ag), and zinc (Zn) elements [11, 12]. Numerous medicinal plants may accumulate heavy metals while growing in their natural habitats. Transition of heavy metals and trace elements from environments to plants is greatly affected by soil pH. At different pH levels, some heavy metals can be accumulated less, while others can reach extreme levels that cause toxicity [13]. Salinity is another factor affecting both uptake and accumulation of heavy metals and trace elements [14]. It is also dependent on the competition rates between the plant species growing in the same lands [15]. In addition, it is known that there is a decrease in the concentrations of trace elements in plants grown in densely populated and polluted environments compared to those grown in unpolluted ones [16]. Moreover, some plants are also capable of accumulating extraordinarily high amounts of heavy metals, well above the levels found in most other plant species, without showing any phytotoxic effects. Among these plants, which are called as hyperaccumulators, some could be used for medicinal purposes and can cause toxic effects when consumed [2, 17].

It was reported that heavy metal pollution can be deadly harmful to living species including humans. Instead of treating various diseases, toxic heavy metals can pass directly or indirectly to humans, causing cardiovascular and stomachic diseases; excretory, nervous, and respiratory system diseases; cancer; and even death [18–20]. In addition, relevant data show that pollution causes at least 9 million early deaths, and this number is many times higher than the deaths caused by the COVID-19 pandemic [21, 22]. Therefore, it is very important to analyze the accumulation of heavy metals in medicinal plants traditionally used by humans, to monitor pollution, and to understand the effect on plant and animal species in their natural habitats.

The aim of the present work was to determine the concentrations of the nutritional trace elements and heavy metals in medicinal plants traditionally used in the East Mediterranean Turkey in terms of human health risk. It is an attempt to increase the knowledge available and provide protection for natural and cultural ethnobotanical legacy.

Material and Methods

Plant Material Sampling and Study Area

Aboveground parts of a total of 33 medical plant samples were collected from mining, farming, industrial, and rural sites of the East Mediterranean of Turkey during their flowering time in 2020 and 2021 (Fig. 1). Plant taxa were identified using the "Flora of Turkey" [23–25]. Detailed information on their collected localities and scientific and vernacular names is also given together with their ethnobotanical uses and pharmacological activities for each plant taxa (20 perennial, and the others annual or biannual) belonging to 23 plant families and 30 genera (Table 1). Phytogeographical origins of the studied medicinal species were determined as Irano-Turanian (9 taxa), Mediterranean (9 taxa), Euro-Siberian elements (4 taxa), East Mediterranean, and cosmopolitan elements (1 taxon each), while the phytogeographical origins of the remaining taxa were unknown. *Erodium amanum* of the studied taxa is endemic to Turkey, while the others are commonly distributed. Life forms of the studied medicinal species were determined as Hemicryptophyte (14 taxa), Therophyte (8 taxa), Chamaephyte (6 taxa), Geophyte (4 taxa), and Phanerophyte (1 taxon) according to the Raunkiaer method [26].

The study area falls into the 35° 48′–37° 00′ N, 35° 46′–36° 41′ E in the east Mediterranean part of Turkey. The climate is clearly Mediterranean type with 18.1 °C annual temperature and 1.078 mm annual rainfall. The study area has about 2000 plant taxa, and a rich ethnobotanical heritage transferred from generation to generation since this region is known to be the cradle of many civilizations throughout history [2].

Determination of Trace Element Level and Statistical Analysis

Plant samples were isolated by transferring into sterile bags and dried in glass petri dishes at 80 °C for 48 h. Plant samples were grounded in porcelain mortar, powdered, and weighed. Samples weighed in the range of 0.200–0.250 g were transferred to Teflon Wessel. For plant samples, 8 ml of 65% HNO_3 (Merck) solution was added to Teflon Wessels. Prepared samples were digested by using a Berghof-MSW2 brand model microwave device. After the process, the samples were transferred to 50-ml sterile falcon tubes using ultrapure water by filtering with a blue band Whatman filter, then they were filled up to a total volume of 50 ml with ultrapure water. The quantities of Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn elements

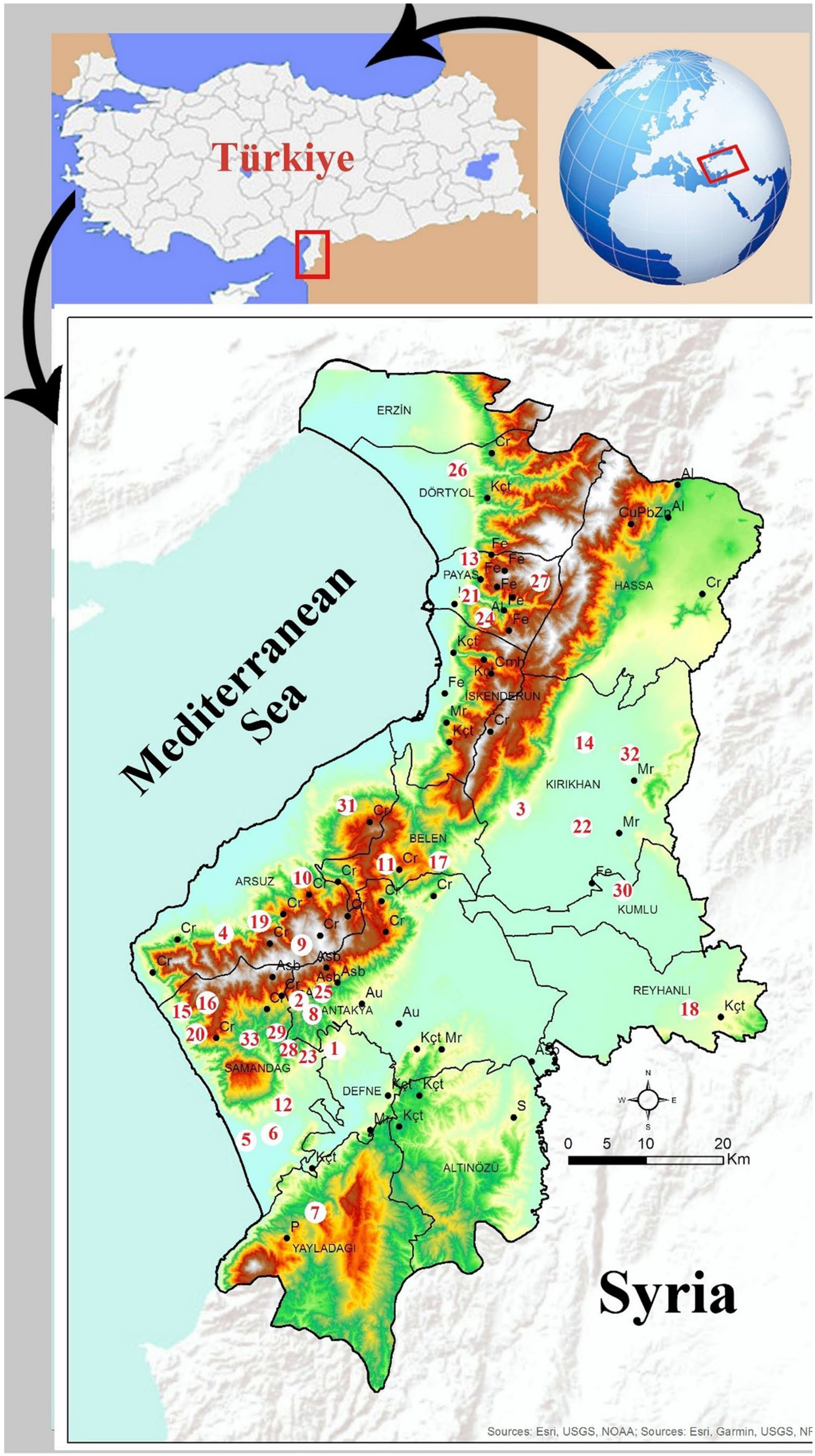


Fig. 1 Localities of the studied medicinal plants and mineral maps of the study area modified from MTA [27]

Table 1 List of wild medicinal plants investigated with their related information

No	Taxa	Element/growth and life form	Common/vernacular name	Ethnobotanical use, ailments treated, and pharmacological activity	Locality
1	*Adiantum capillus-veneris* L. Pteridaceae	IR/H/P	Common Maidenhair/Baldırı kara	To throat afflictions, coughs, bronchitis, and expel worms from the body. It is used as antitussive, emetic, abortive, weakly expectorant, laxative, antidandruff, detoxicant in alcoholism, astringent, demulcent, depurative, weakly emmenagogue, emollient, pectoral, febrifuge, galactogogue, refrigerant, stimulant, sudorific, and tonic [28–30]	Harbiye
2	*Alcea apterocarpa* (Fenzl) Boiss. Malvaceae	IR/H/P	Hollyhock/Gülfatma hatmi	Allergy, stomach ailments, pulmonary disorders like asthma, bronchitis, expectorant, cough, cold, menstrual irregularity, urinary system disorders, kidney stones, intestinal disorders, wounds, inflammation, skin diseases, and diuretic [31–36]	Kızıldağ
3	*Ammi visnaga* (L.) Lam. Apiaceae	M/H/B	Toothpick-plant/Diş Otu	Mild anginal symptoms, asthma, cough, spastic bronchitis, painful menstruation and as regulate menstruation, intestinal diseases, antispasmodic, urinary calculi, diuretic, bladdar and kidney stones, dental diseases, atherosclerosis, tonic, gingivitis, digestive, carminative, appetizer [37, 38]	Kırıkhan
4	*Anacamptis pyramidalis* (L.) Rich. Orchidaceae	M/G/P	Pyramidal orchid/Cam salebi, Peynir çiçeği	Diarrhea, cold, flu, body warmers, vasodilator, tonic, pleasure, psychedelics, medicinal tea. It has positive effects on fertility and pregnancy in women, drink as a salep, used as a thickening agent in ice cream production and adhesive for stitching shoe parts, also ornamental plant [39–41]	Arsuz
5	*Cakile maritima* Scop. Brassicaceae	ES/Th/A	Sea rocket/Deniz teresi, kumteresi	Helps prevent the occurrence of atherosclerosis, heart attack, stroke and other cardiovascular diseases, prevents blood clotting, antipyretic, antimicrobial, diuretic, appetizer, purgative, antiscorbutic, digestive, edema, jaundice, detoxfy the lungs [38, 42, 43]	Çevlik
6	*Centaurium erythraea* subsp. *erythraea* Rafn. Gentianaceae	ES/H/B	Common centaury/Tukul otu, kırmızı kantaron	Stomach pains, gastritis, ulcers, indigestion and loss of appetite [28, 44]	Çevlik
7	*Cephalaria syriaca* (L.) Schrad. Caprifoliaceae	IN/Th/A	Cephalaria/Pelemir	Antimicrobial, antioxidant, sedative, antidiabetic, analgesic,. seeds are mixed with bread flour for flavor in the baking industry, in leather business [45–47]	Yayladağı
8	*Cistus creticus* L. Cistaceae	ES/Ch/P	Pink rock-rose, hoary rock-rose/Karağan, laden	For constipation, diabetes, as cardiovascular, nutritional, digestive, stimulant, expectorant, antifungal, astringent [28, 48–50]	Kızıldağ
9	*Cynoglossum creticum* Mill. Boraginaceae	IR/H/B	Dog's tongue/Köpek dili	Emollient, hydrated burns, wart treatment, antitumoral and antispasmotic [51, 52]	Arsuz
10	*Daphne sericea* Vahl Thymelaeaceae	M/Ph/P	Daphne/Develik otu	Toothache, rheumatic pain, malaria and blood cancer, ulcer, skin diseases, menstruation, as diuretic, diaphoretic, used in broom construction. It is also used to plant in the gardens [53–55]	Arsuz
11	*Dianthus orientalis* Adams, Weber & Mohr Caryophyllaceae	IR/Ch/P	Georgian pink/Kızhanım, yarkaranfili	Toothache, mouth freshener, and anti-spasm [56, 57]	Belen
12	*Dianthus strictus* Banks & Sol. Caryophyllaceae	IR/Ch/P	Wild pink/Yer karanfili	Toothache, teeth inflammation, antimicrobial activity [58, 59]	Çevlik

Table 1 (continued)

No	Taxa	Element/growth and life form	Common/vernacular name	Ethnobotanical use, ailments treated, and pharmacological activity	Locality
13	*Drimia maritima* (L.) Stearn Liliaceae	M/G/P	Squill, maritime squill/Ada soğanı	Hemorrhoids, warts, skin problems; it is also diuretic in human and as repellent for mice and rat poison in granaries and silos within veterinary medicine [28, 29, 60]	İskenderun
14	*Echium italicum* L. Boraginaceae	M/Th/B	Talian viper's bugloss/Sığırdili	Wound, rheumatic pains, cholesterol, cold, sore throat, as nervous system relaxant, carminative, soporific, diuretic and expectorant, anti-inflammatory [28, 38, 56, 61, 62]	Gölbaşı
15	*Epilobium hirsutum* L. Onagraceae	IN/H/P	Great willowherb/Yakı Otu	Epilepsy, whooping cough, cold, cough, muscle spasm, constipation, sedative hemostatic, astringent [63, 64]	Samandağ
16	*Erica manipuliflora* Salisb. Ericaeae	M/Ch/P	Autumn heather/Funda	Kidney stones, weight loss, common cold, urethritis, constipation, arthritis, diuretic. It is also used as fuel and in making broom [55, 65–67],	Samandağ
17	**Erodium amanum* Boiss. & Kotschy Geraniaceae	IR/H/P	Filarees/Hatay iğneliği	Chronic and acute rheumatic disorders [68]	Belen
18	*Erodium cicutarium* subsp. *cicutarium* (L.) L Hér. Geraniaceae	IN/Th/A	Pine weed, storksbill/Dönbaba Otu, iğnelik	For constipation, uterine hemorrhage, laxative, snake/scorpion bites, wart, as stops bleeding, astringent, anti-inflammatory, hemostatic. It is used in traditional food "Kömeç" [54, 64, 67, 69, 70]	Reyhanlı
19	*Gladiolus kotschyanus* Boiss. Iridaceae	IR/G/P	Gladiolus/Karga soğanı	For cold, flu, as aphrodisiac and emetic [28, 70]	Arsuz
20	*Lavatera punctata* All. Malvaceae	M/Th/A	Annual tree mallow/Hatmi gülü, saracak	To treat bronchitis, cough, sedative to minor pain, inflammation, digestive system disorders, constipation, ulcer, hemorriod, urinary system infections, ashtenia, skin disease such as wounds, sores, insect stings, throat pains, used as diuretic gargle, in preparing soap, shampoo and cosmetic reams that nourish the skin and remove wrinkles [71]	Samandağ
21	*Michauxia campanuloides* L Hér. ex Aiton Campanulaceae	EM/H/B	Rough-leaved michauxia/Gercimek	Analgesic, wound healing, antioxidants, anti-inflammatory. It is also eaten fresh or cooked as food [36, 72]	Payas
22	*Plantago lanceolata* L. Plantaginaceae	IR/H/P	Narrowleaf plantain/Sinirli ot, Damar otu	Abscess, respiratory problems, cough, wounds, insect bites, pimples ulcer, gastric pain, hemorrhoids, embolism, gynecologic diseases, urinary inflammations, shortness of breath, suppurative, cardiovascular diseases, protect to lung health, also as antiparasitic, vulnerary, astringent, expectorant, slimming, and anti-inflammatory [29, 34, 49, 71, 73–75]	Kırıkhan
23	*Primula vulgaris* Huds. Primulaceae	ES/H/P	Primule/Çuha çiçeği	Cough, insomnia, rheumatism, tranquilizer, injury, inflammations, sedative, diuretic and expectorant, chest emollient and diaphoretic. Raw in salads with lemon/vinegar and olive oil as food. Ingredient of "risotto". Young leaves and flowers used to make liqueur in Italy [28, 76–78]	Karaçay
24	*Ruta chalepensis* L. Rutaceae	IR/Ch/P	Fringed rue/Sedef otu	Headache, rheumatism, stomach pain, arthritis, back ache, skin diseases, evil eye, eye and ear infections, gallstone, head lice as hair tonic, keep away from bad spirits. It is also used as digestive, sedative, as a sudorific, antispasmodic, vermifuge, aborative, and emmenagogue [45, 48, 79–82]	Payas

Table 1 (continued)

No	Taxa	Element/growth and life form	Common/vernacular name	Ethnobotanical use, ailments treated, and pharmacological activity	Locality
25	*Ruta suaveolens* DC. Rutaceae	IN/H/P	Yelow rue/Sarı Çanak	Antibacterial and antifungal [83, 84]	Kızıldağ
26	*Scabiosa columbaria* subsp. *columbaria* L. Caprifoliaceae	IN/H/P	Wild scabious/Uyuz Otu	Treat diphtheria, diarrhea, skin ailments such as scabies, wound bruises, sores and hyperpigmentation, sterility, heartburn, diuretic, antifungal, antibacterial and antiprotozoal. In addition, it is used to make baby powder and as side dish in food [28, 85–87]	Dörtyol
27	*Silene aegyptiaca* subsp. *aegyptiaca* (L.) L.f Caryophyllaceae	IN/Th/A	Campion/Yapışkan otu	Wound, eczema, sore throat, fever, and urinary tract [10, 59, 80]	Payas
28	*Solanum nigrum* L. Solanaceae	CO/G/A	Black nightshade/İt üzümü	Rheumatism, cough, dysentery, chronic bronchitis, tumors, cancer, odontalgia, mouth ulcers, stomach, intestinal distress, bloating, hemorrhoid, pains, constipation, anemia, liver diseases, inflammation, skin diseases, poisonous, also used as diuretic, analgesic, antispasmodics, vasodilator, sedative, narcotic, CNS depressant, analgesic, antioxidant, anti-inflammatory, gastroprotective, hepatoprotective, antipyretic, antitumoral, laxative, and aphrodisiac [29, 67, 80, 88–92]	Karaçay
29	*Thalictrum orientale* Boiss. Ranunculaceae	M/H/P	Meadow-rue/Çayırsedefi	To snake bite, jaundice, rheumatism, as tonic, aperient, diuretic, stomachic, bitter, and antiseptic [93]	Karaçay
30	*Tribulus terrestris* L. Zygophyllaceae	IN/Th/A	Puncturevine, Small caltrops/Deve çökerten, çoban çökerten	For treatment asthma, urinary tract disorders, cardiovascular system diseases, chronic malaria, diabet, for tumors and ulcers, especially for festering ulcers of the gums, kidney, gall and bladder stones, gonorrhea, headaches, skin diseases such as eczema, athlete's foot, wart treatment, eye inflammations, strong side pains, infertility. It is also used as diuretic, tonic, astringent, purgative, aphrodisiac, energizing remedy, and tonic in folk medicine [52, 66, 94–96]	Kumlu
31	*Vaccaria hispanica* (Mill.) Rauschert Caryophyllaceae	M/Th/A	Cow cockle, cow herb/İnek otu	To treat tumors, amenorrhea, breast infections, promote diuresis, malta fever, activate blood circulation, stimulate milk let-down. It is used as analgesic, ointments in skin diseases (eczema and psoriasis), relieve carbuncles, emmenagogue, as stimulant, and diaphoretic [29, 70, 91, 96]	Iskenderun
32	*Verbena officinalis* L. Verbenaceae	IN/H/P	Vervain, Hors whip/Mineçiçeği	Treat muscular-skeletal disorders, skin diseases, wounds, diarrhea, diabetes, stomach pain, bathe the body of children infected with scabies or lice, bone fractures, reduce pain and discoloration of bruises, gain weight, liver diseases, kidney stone, fatigue, insomnia, dysentery, menstrual flow and milk secretion, fever and menstrual cramps, sterility, and to prevent miscarriage in women. It is also used as a tonic, antipyretic, anthelmintic, antihaemorrhagic, antispasmodic, antiscorbutic, astringent, diuretic, diaphoretic, emmenagogue, febrifuge, galactagogue, stimulant, sedative, and purgative [48, 67, 97–100]	Kırıkhan

Table 1 (continued)

No	Taxa	Element/growth and life form	Common/vernacular name	Ethnobotanical use, ailments treated, and pharmacological activity	Locality
33	*Withania somnifera* (L.) DUNAL Solanaceae	IN/Ch/P	Winter cherry/Güvey Feneri	External use as analgesic on painful areas inflamed skin, boils, scabies, vitiated condition of vata, fever, painful swelling, constipation, eye inflammations, leucoderma, tissue building, arthritis, memory, stabilizes blood sugar, stress reduction, anxiety treatment, depression, nervous breakdown, insomnia, sedative. It has been as astringent, adaptogenic, diuretic, hepatoprotective, somniferous, thermogenic, stimulant, aphrodisiac, and tonic. It has anti-tumor, antibiotics, anxiolytic, and antidepressant activities [28, 91, 92, 101]	Samandağ

A annual, *B* biannual, *Ch* chamaephyte, *CO* cosmopolitan, *ES* Euro-Siberian, *EM* East Mediterranean, *G* geophyte, *H* hemicryptophyte, *IN* imperfectly known, *IR* Irano-Turanian, *M* Mediterranean, *P* perennial, *Ph* phanerophyte, *Th* therophyte*(endemic)

(mg kg^{-1} dry weight) were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES/PerkinElmer-Optima 7000DV) [2].

Quality Control and Quality Assurance

All chemicals used in this study were of analytical grade. Ultrapure water (Human-Zener Power I) was used as a solvent in dilution processes and in all experimental steps. Elemental values of medicinal plant samples were determined in triplicate (for linearity). All elemental concentrations were determined with a very low margin of error of ± 0.72–1.95% (RSD) using the prepared calibration standards (Table 2). The EPA 3051A Analytical Method for ICP-OES was applied using Mars Microwave to dissolve medicinal plant samples. Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, and Zn contents of the samples were determined using ICP-OES (PerkinElmer Optima 7000DV).

The advantages of ICP are long working hours, providing little or no organic molecules, a few number of ionization interferences, and the ability to sequentially analyze a large number of elements [102]. Calibration standards were prepared by diluting 1000 mg L^{-1} of ICP multi-element standard solution (Merck) in order to calculate the concentration values of each element examined for medicinal plant samples. Calibration curves using calibration standards prepared in 8 different concentrations were obtained for each element and found to be $R^2 > 0.999$ (Table 2). After the initial calibration, the calibration standards were reanalyzed after every ten samples during the experimental process, and their margins of error were checked to prove that the initial calibration parameters remained constant throughout the analysis process.

The accuracy and consistency of the elemental analyzes were also proved by repeated analysis of multi-element calibration solutions with known concentrations. LoD (limit of detection) and LoQ (limit of quantification) values were determined by analyzing blank solutions and calculated for each element (Table 2):

$$\mathrm{LoD} = \varnothing_{\mathrm{n}} \times \mathrm{SD}$$

$$\mathrm{LoQ} = \varnothing_{\mathrm{q}} \times \mathrm{SD}$$

In the given equations, the term SD denotes the standard deviation of ten replicates of the blank solution, while the values of $\varnothing_n$ and $\varnothing_q$ explain multiplying factors of 3 and 10, respectively [103, 104]. To determine the elemental compositions of the medicinal plant samples, the spectral lines were selected as listed in Table 2 according to the related literature [104–106].

Risk Assessment for Toxic Metals (Cd–Cr–Ni–Pb)

Exposures of Cd, Cr, Ni, and Pb leading up to the health risks to consumers through consumption of plant leaves were

Table 2 The analytical method parameters of ICP-OES

Elements	Spectral lines (nm)	Plasma torch position	LoD (mg kg^{-1})	LoQ (mg kg^{-1})	RSD (%)	R^2
Al	396.153	Axial	0.122	0.407	1.26	.999904
B	249.677	Axial	0.073	0.243	0.82	.999911
Ca	317.933	Radial	0.731	2.437	1.11	.999872
Cd	228.802	Axial	0.009	0.030	0.92	.999916
Cr	267.716	Axial	0.015	0.050	1.62	.999888
Cu	327.393	Axial	0.082	0.273	0.83	.999759
Fe	238.204	Axial	0.291	0.970	1.04	.999893
K	766.490	Radial	0.513	1.710	1.33	.999860
Mg	285.213	Radial	0.278	0.927	0.87	.999914
Mn	257.610	Axial	0.133	0.443	1.14	.999883
Na	589.592	Radial	0.188	0.627	1.95	.999729
Ni	231.604	Axial	0.008	0.027	0.72	.999831
Pb	220.353	Axial	0.022	0.073	0.95	.999879
Zn	213.857	Axial	0.136	0.453	1.09	.999909

LoD limit of detection, *LoQ* limit of quantification, *RSD* relative standard deviation, $R.^2$ determination coefficient

assessed according to the estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) [107].

$$\mathrm{EDIn} = (\mathrm{Cn} \times \mathrm{IR} \times \mathrm{TRn})/(\mathrm{BW} \times 1000);\ n = \mathrm{Cd} - \mathrm{Cr} - \mathrm{Ni} - \mathrm{Pb} \quad (1)$$

In this equation, Cn is the toxic metal concentration in the plant samples (mg kg^{-1}); *n* is the toxic metals (CdCr-Ni-Pb); IR is the uptake rate of the plant for an adult person (11.4 g/person/day); TRn stands for the transference rate of the toxic metal; and BW represents body weight (70 kg of adult person) [107, 108]. The TR values used in this study are 6.6% for Cd, 42% for Cr, 30% for Ni, and 19.8% for Pb [107, 109, 110].

$$\mathrm{THQn} = \mathrm{EDIn}/\mathrm{RfDn};\ n = \mathrm{Cd} - \mathrm{Cr} - \mathrm{Ni} - \mathrm{Pb} \quad (2)$$

THQ was used for the quantitative evaluation of the potential non-carcinogenic effects of each individual toxic metal [107]. A THQ value less than 1 indicates no significant risk of carcinogenicity to the exposed population. RfDn (mg/kg/day) for each metal (*n*) represents the reference dose determined by FAO/WHO. EDIn is the daily average exposure dose (mg/kg/day), and THQn is the target hazard quotient of the toxic metal. The combined risk of multiple toxic metals on general human health through the consumption of medicinal plants may arise from exposure to multiple pollutants. Therefore, the hazard index (HI) was used to estimate the total health hazards that are carcinogenic caused by exposure to multiple toxic metals.

$$\mathrm{HI} = \Sigma\mathrm{THQn} = \mathrm{THQCd} + \mathrm{THQCr} + \mathrm{THQNi} + \mathrm{THQPb} \quad (3)$$

In this equation, HI represents the total health risk due to exposure to toxic metals. An HI value less than 1 indicates that the negative effects of toxic metals on human health are less likely to occur. On the other hand, if the HI value is greater than 1, it is very likely that toxic metals cause adverse effects on human health.

Evaluation of Recommended Dietary Allowance Values

In order to reveal the Dietary Reference Intake potentials of *E. cicutarium*, *M. campanuloides*, and *P. vulgaris* used as daily food among the plants examined in this study, Recommended Dietary Allowance (RDA) values were calculated for Ca, Cu, Fe, K, Mg, Mn, Na, and Zn elements [10, 17].

$$\mathrm{RDA}(\%) = (\mathrm{MV}/10) \times (100/\mathrm{RDAst})$$

In this equation: RDA (%) is Recommended Dietary Allowance percentages in 100 g/dw;

MV is trace element values in the studied plant samples (mg/kg; ppm);

RDAst is international standards (mg per 100 g/dw).

Results and Discussion

The mean concentrations of micronutrients and toxic metals (mg/kg) in the most commonly used whole plants are shown in Table 3. Our experimental results showed that heavy metal and trace element in the plant samples vary between 4.368 (*E. hirsutum*) and 1104.627 (*E. amanum*) for Al, 47.850 (*P. lanceolata*) and 271.479 (*E. italicum*) for B, 1971.213 (*C. erythraea*) and 22,642.895 (*R. chalepensis*)

Table 3 Heavy metal and nutritional trace element levels of selected medicinal plants in the study area

No	Sample name	Al	B	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Zn	LOC
1	*A. capillus-veneris*	12.483 ± 0.272	101.561 ± 1.945	4545.385 ± 119.083	0.016 ± 0.001	2.830 ± 0.083	26.201 ± 0.211	130.194 ± 4.433	8635.624 ± 85.139	1000.238 ± 32.730	82.061 ± 1.515	503.810 ± 18.998	3.565 ± 0.040	5.009 ± 0.153	140.885 ± 3.774	Harbiye
2	*A. apterocarpa*	62.940 ± 0.658	223.196 ± 2.566	10,088.831 ± 186.943	0.191 ± 0.002	12.808 ± 0.113	32.688 ± 0.283	337.327* ± 6.484	6392.263 ± 185.039	1479.764 ± 20.115	196.346 ± 2.718	477.602 ± 14.299	9.968* ± 0.127	11.235* ± 0.403	190.263* ± 5.076	Kızıldağ
3	*A. visnaga*	10.704 ± 0.184	83.071 ± 1.420	4300.883 ± 66.607	0.324 ± 0.007	1.485 ± 0.053	22.673 ± 0.730	49.446 ± 0.715	6608.546 ± 90.567	1322.420 ± 19.887	128.183 ± 3.538	674.497 ± 0.577	5.663 ± 0.087	14.694* ± 0.361	157.072 ± 5.881	Kırıkhan
4	*A. pyramidalis*	127.444* ± 2.807	89.622 ± 2.351	5251.597 ± 56.297	0.030 ± 0.001	28.675* ± 0.503	42.586* ± 0.467	943.275* ± 33.376	7819.771 ± 171.934	1214.133 ± 28.629	157.653 ± 2.836	219.159 ± 2.452	6.548* ± 0.241	9.731 ± 0.299	136.538 ± 2.679	Arsuz
5	*C. maritima*	16.267 ± 0.578	65.792 ± 2.073	3464.545 ± 53.263	0.189 ± 0.004	2.652 ± 0.050	18.668 ± 0.320	22.448 ± 0.686	7754.438 ± 119.035	875.517 ± 19.202	46.383 ± 1.389	3401.252 ± 68.736	3.849 ± 0.072	6.947 ± 0.099	155.018 ± 2.882	Çevlik
6	*C. erythraea*	18.866 ± 0.620	69.064 ± 2.575	1971.213 ± 52.150	0.025 ± 0.001	1.675 ± 0.033	24.193 ± 0.755	73.541 ± 2.222	3921.095 ± 144.236	1217.983 ± 35.328	138.629 ± 2.001	335.597 ± 4.541	4.655 ± 0.168	14.110* ± 0.358	126.607 ± 3.821	Çevlik
7	*C. syriaca*	20.404 ± 0.691	143.475 ± 3.612	7798.552 ± 257.648	0.234 ± 0.004	2.579 ± 0.057	18.376 ± 0.384	153.038 ± 3.347	8051.242 ± 305.518	1191.553 ± 41.174	101.361 ± 1.373	332.125 ± 7.909	3.565 ± 0.120	9.084 ± 0.082	116.819 ± 2.386	Yayladağı
8	*C. creticus*	29.065 ± 0.872	123.374 ± 2.781	4589.942 ± 82.929	0.062 ± 0.001	2.138 ± 0.069	18.392 ± 0.241	209.195 ± 7.838	3339.331 ± 63.893	1281.769 ± 24.354	64.298 ± 1.631	447.876 ± 9.919	2.565 ± 0.050	4.788 ± 0.145	255.220* ± 5.386	Kızıldağ
9	*C. creticum*	5.173 ± 0.167	165.186 ± 6.201	7014.266 ± 116.617	0.052 ± 0.001	1.645 ± 0.027	20.379 ± 0.331	20.705 ± 0.517	6568.387 ± 217.977	635.121 ± 8.484	62.717 ± 0.785	554.941 ± 11.553	7.497* ± 0.083	1.572 ± 0.034	162.066* ± 2.392	Arsuz
10	*D. sericea*	27.647 ± 0.896	169.528 ± 4.378	6202.073 ± 207.262	0.651* ± 0.010	2.301 ± 0.036	15.062 ± 0.511	148.464 ± 4.844	2045.893 ± 26.212	491.950 ± 14.160	180.410 ± 4.211	355.669 ± 9.492	2.549 ± 0.062	13.238* ± 0.331	172.306* ± 3.624	Arsuz
11	*D. orientalis*	14.167 ± 0.242	70.898 ± 1.799	3407.970 ± 78.543	0.030 ± 0.000	4.572 ± 0.157	15.795 ± 0.216	115.138 ± 1.207	5704.594 ± 124.780	570.600 ± 19.409	90.301 ± 3.369	773.488 ± 25.322	3.565 ± 0.073	3.306 ± 0.037	163.093* ± 3.944	Belen
12	*D. strictus*	7.260 ± 0.153	55.135 ± 1.055	4548.075 ± 146.049	0.014 ± 0.000	3.320 ± 0.058	34.173* ± 0.351	74.849 ± 0.836	3427.818 ± 122.067	1072.707 ± 30.100	149.396 ± 3.483	675.079 ± 8.583	2.655 ± 0.074	3.194 ± 0.044	197.437* ± 3.878	Çevlik
13	*D. maritima*	27.911 ± 0.776	61.429 ± 1.587	3307.599 ± 29.687	0.290 ± 0.009	13.630 ± 0.281	32.494 ± 0.552	885.232* ± 30.050	652.143 ± 11.980	336.871 ± 11.892	223.776 ± 7.310	347.529 ± 8.360	0.065 ± 0.002	8.968 ± 0.285	281.954* ± 8.523	Payas
14	*E. italicum*	24.888 ± 0.731	271.479 ± 3.923	18,804.908 ± 161.010	0.175 ± 0.003	2.050 ± 0.024	31.040 ± 0.691	46.032 ± 1.138	11,138.114 ± 118.596	650.055 ± 17.187	116.261 ± 4.315	1358.839 ± 30.174	3.985 ± 0.079	3.824 ± 0.103	150.300 ± 1.519	Gölbaşı
15	*E. hirsutum*	4.368 ± 0.108	60.464 ± 0.757	2778.920 ± 47.055	0.056 ± 0.001	1.435 ± 0.047	13.278 ± 0.490	38.034 ± 0.337	4762.516 ± 69.720	650.131 ± 14.847	306.192 ± 7.136	286.786 ± 10.191	4.259 ± 0.103	8.466 ± 0.235	122.322 ± 1.302	Samandağ
16	*E. manipuliflora*	16.164 ± 0.466	105.908 ± 1.397	1976.876 ± 54.523	0.057 ± 0.002	3.600 ± 0.073	17.134 ± 0.416	116.735 ± 1.826	1212.857 ± 13.148	690.534 ± 22.956	125.018 ± 3.474	510.984 ± 14.066	2.649 ± 0.040	4.909 ± 0.176	114.752 ± 3.029	Samandağ
17	*E. amanum*	1104.627* ± 19.532	51.531 ± 0.470	9917.536 ± 172.296	0.277 ± 0.002	41.692* ± 0.794	22.868 ± 0.867	1276.783* ± 43.511	1179.682 ± 39.654	1869.486 ± 41.879	849.492* ± 12.493	318.655 ± 11.975	1.910 ± 0.023	13.127* ± 0.348	132.047 ± 3.306	Belen
18	*E. cicutarium*	98.542 ± 2.255	105.687 ± 3.378	7020.149 ± 109.243	0.030 ± 0.001	12.338 ± 0.145	25.650 ± 0.814	925.854* ± 26.176	7806.074 ± 232.395	1131.164 ± 12.268	450.336* ± 6.929	1461.447 ± 13.803	1.665 ± 0.051	4.070 ± 0.095	123.035 ± 3.097	Reyhanlı
19	*G. kotschyanus*	11.221 ± 0.147	93.326 ± 2.799	4245.664 ± 138.012	0.117 ± 0.004	11.230 ± 0.236	23.307 ± 0.320	378.653* ± 12.384	3580.621 ± 122.457	919.003 ± 26.492	91.507 ± 2.676	451.607 ± 5.911	7.377* ± 0.196	6.634 ± 0.199	168.072* ± 5.471	Arsuz
20	*L. punctata*	16.336 ± 0.485	105.284 ± 1.141	9067.320 ± 125.293	0.030 ± 0.000	5.347 ± 0.120	24.560 ± 0.725	52.045 ± 0.474	6642.243 ± 82.965	1466.225 ± 43.089	102.404 ± 3.825	974.768 ± 9.860	4.076 ± 0.154	1.951 ± 0.027	152.822 ± 1.445	Samandağ
21	*M. campanu-loides*	26.161 ± 0.611	98.635 ± 2.502	3237.05 ± 100.048	0.036 ± 0.001	6.655 ± 0.169	32.163 ± 0.459	132.692 ± 1.364	5946.37 ± 147.837	1036.238 ± 35.812	66.891 ± 1.956	568.111 ± 6.698	4.174 ± 0.124	12.637* ± 0.462	188.849* ± 6.624	Denizciler
22	*P. lanceolata*	14.130 ± 0.296	47.850 ± 1.254	9834.250 ± 224.431	0.060 ± 0.001	2.544 ± 0.021	14.511 ± 0.339	140.637 ± 3.182	12,604.985 ± 477.188	527.618 ± 5.528	48.071 ± 1.447	513.197 ± 9.582	0.565 ± 0.011	4.420 ± 0.111	97.077 ± 1.826	Kırıkhan
23	*P. vulgaris*	27.227 ± 0.855	84.538 ± 2.702	4453.111 ± 36.416	0.011 ± 0.000	1.371 ± 0.034	16.830 ± 0.418	124.241 ± 3.818	4954.564 ± 99.949	420.939 ± 15.983	114.076 ± 3.873	314.522 ± 11.373	6.488* ± 0.188	2.793 ± 0.073	82.727 ± 2.658	Karaçay
24	*R. chalepensis*	46.533 ± 1.571	62.519 ± 2.272	22,642.895 ± 638.181	0.052 ± 0.000	8.152 ± 0.209	26.985 ± 0.351	685.198* ± 15.899	10,526.846 ± 360.488	895.325 ± 15.552	199.895 ± 6.585	1555.327 ± 56.014	2.987 ± 0.083	6.335 ± 0.067	67.250 ± 0.900	Belen
25	*R. suaveolens*	8.336 ± 0.244	105.754 ± 2.643	6562.571 ± 130.178	0.109 ± 0.003	1.610 ± 0.050	21.948 ± 0.802	35.103 ± 1.333	7816.065 ± 291.150	529.192 ± 11.980	90.602 ± 3.186	298.547 ± 4.160	3.040 ± 0.105	1.311 ± 0.047	159.749 ± 1.815	Kızıldağ
26	*S. columbaria*	92.267 ± 0.930	84.407 ± 2.427	7257.958 ± 118.506	0.028 ± 0.000	6.841 ± 0.138	24.380 ± 0.867	603.153* ± 14.413	7111.105 ± 127.691	722.854 ± 21.832	166.052 ± 1.689	373.538 ± 5.147	6.484* ± 0.128	7.756 ± 0.235	113.974 ± 1.221	Dörtyol
27	*S. aegyptiaca*	158.763* ± 5.088	73.565 ± 2.294	15,236.985 ± 164.020	0.101 ± 0.003	23.301* ± 0.874	22.234 ± 0.558	948.763* ± 31.120	11,805.95 ± 325.441	1565.29 ± 49.867	251.28 ± 4.658	1795.63 ± 46.427	8.489* ± 0.275	7.213 ± 0.121	75.346 ± 1.791	Payas

Table 3 (continued)

No	Sample name	Al	B	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Pb	Zn	LOC
28	*S. nigrum*	21.492 ± 0.288	107.069 ± 0.874	7070.535 ± 183.639	0.165 ± 0.004	4.927 ± 0.175	36.410* ± 1.044	38.845 ± 0.863	12,011.674 ± 398.063	1063.577 ± 9.794	227.501 ± 3.095	167.144 ± 3.352	3.762 ± 0.031	16.238* ± 0.496	139.582 ± 2.650	Karaçay
29	*T. orientale*	15.805 ± 0.446	128.635 ± 3.120	5905.281 ± 212.872	0.037 ± 0.001	3.739 ± 0.104	18.716 ± 0.594	103.487 ± 1.407	7870.115 ± 260.150	872.606 ± 19.311	98.723 ± 3.444	176.999 ± 5.148	7.046* ± 0.173	12.369* ± 0.401	75.431 ± 1.508	Karaçay
30	*T. terrestris*	16.861 ± 0.556	170.148 ± 3.363	14,360.246 ± 543.215	0.107 ± 0.004	1.572 ± 0.042	30.967 ± 0.851	142.661 ± 2.102	9789.486 ± 273.570	937.987 ± 23.178	353.633 ± 7.173	316.240 ± 4.561	2.026 ± 0.043	4.841 ± 0.126	149.106 ± 2.925	Kumlu
31	*V. hispanica*	11.374 ± 0.273	133.583 ± 4.643	20,937.946 ± 638.130	0.258 ± 0.003	3.425 ± 0.130	20.577 ± 0.754	36.860 ± 0.563	8925.750 ± 251.287	1038.636 ± 31.663	316.980 ± 5.605	385.992 ± 6.783	5.945 ± 0.141	5.951 ± 0.175	179.555* ± 6.378	Arsuz
32	*V. officinalis*	13.218 ± 0.467	106.097 ± 2.168	6842.383 ± 125.946	0.019 ± 0.001	2.691 ± 0.102	26.592 ± 0.671	51.756 ± 1.842	1764.215 ± 22.566	1443.031 ± 43.925	79.609 ± 2.900	1643.364 ± 25.032	3.589 ± 0.121	2.444 ± 0.061	177.575* ± 4.429	Kırıkhan
33	*W. somnifera*	6.495 ± 0.135	126.923 ± 4.011	3550.011 ± 92.523	0.045 ± 0.001	2.816 ± 0.024	41.286* ± 0.816	25.565 ± 0.661	14,440.946 ± 299.944	1649.894 ± 49.146	69.487 ± 0.624	385.557 ± 12.465	8.655* ± 0.275	3.413 ± 0.060	189.916* ± 2.513	Samandağ

*Beyond permissible limit defined by WHO

for Ca, 0.011 (*P. vulgaris*) and 0.651 (*D. sericea*) for Cd, 1.371 (*P. vulgaris*) and 41.692 (*E. amanum*) for Cr, 13.278 (*E. hirsutum*) and 42.586 (*A. pyramidalis*) for Cu, 20.705 (*C. creticum*) and 1276.783 (*E. amanum*) for Fe, 652.143 (*D. maritima*) and 14,440.946 (*W. somnifera*) for K, 336.871 (*D. maritima*) and 1869.486 (*E. amanum*) for Mg, 46.383 (*C. maritima*) and 849.492 (*E. amanum*) for Mn, 167.144 (*S. nigrum*) and 3401.252 (*C. maritima*) for Na, 0.065 (*D. maritima*) and 9.968 (*A. apterocarpa*) for Ni, 1.311 (*R. suaveolens*) and 16.238 (*S. nigrum*) for Pb, 67.250 (*R. chalepensis*) and 281.954 (*D. maritima*) for Zn (mg/ kg), respectively (Table 3).

The results obtained from our study were also compared with the permissible limits set for medicinal plants by FAO/WHO and the American Herbal Products Association (AHPA). It was detected that the heavy metal concentrations determined in the study materials were mostly within the permissible limits [111–122].

Numerous countries such as Brazil, Canada, China, Germany, India, Malaysia, Republic of Korea, Poland, Singapore, Union of Europe, UK, Thailand, and Turkey have implemented their own programs to ensure safe plant-based production regarding the heavy metal content (Table 4).

When comparing our results according to the acceptable limits, it was shown that Al levels of *A. pyramidalis*, *E. amanum*, and *S. aegyptiaca* were higher than the permissible limits, and they were within normal limits in other taxa; the content of Cd was slightly higher than the permissible limits in *D. sericea*, while it was within the permissible limits in all others; the levels of Cr were higher than the acceptable limits in *A. pyramidalis*, *E. amanum*, and *S. aegyptiaca*, while they were within the acceptable limits in others; the value of Cu was slightly higher than the acceptable limits in *A. pyramidalis*, *D. strictus*, *S. nigrum*, and *W. somnifera*, while it was within the acceptable limits in others; the values of Fe were found as higher than the permissible limits in *A. apterocarpa*, *A. pyramidalis*, *E. amanum*, *E. cicutarium*, *G. kotschyanus*, *R. chalepensis*, *S. columbaria*, *S. aegyptiaca*, and *D. maritima*, while they were within the acceptable limits in others; the concentrations of Mn were slightly higher than the permissible limits in *E. amanum* and *E. cicutarium*, while they were normal in others; the concentrations of Ni were slightly higher than the permissible values in *A. apterocarpa*, *A. pyramidalis*, *C. creticum*, *G. kotschyanus*, *P. vulgaris*, *S. columbaria*, *S. aegyptiaca*, *T. orientale*, and *W. somnifera*, while they were within normal limits in others; the contents of Pb were slightly higher than the permissible limits in *A. apterocarpa*, *A. visnaga*, *C. erythraea*, *D. sericea*, *E. amanum*, *M. campanuloides*, *S. nigrum*, and *T. orientale*; and Zn concentrations were found as slightly higher than the permissible limits in *A. apterocarpa*, *C. creticus*, *C. creticum*, *D. sericea*, *D. orientalis*, *D. strictus*, *G. kotschyanus*, *M. campanuloides*, *D. maritima*, *V. hispanica*,

Table 4 Standards of permissible levels of heavy metals for herbal products from different countries or organizations

Standards		Cd	Cr mg kg^{-1}	Cu	Fe	Mn	Ni	Pb	Zn
Brazil	Herbal tea	0.4							
Canada	Raw herbal materials	0.3	2					10	
	Finished herbal products	0.06*	0.02*					0.02*	
Chinese Pharmacopoeia	Herbal materials	1						10	
Germany	Herbal products	0.2						5	
India	Herbal tea							10	
Japanese Pharmacopoeia	Herbal tea							20	
Malaysia	Finished herbal products							10	
Republic of Korea	Herbal materials							30	
Poland	Herbal materials	0.1						0.2	
Singapore	Finished herbal products			150				20	
Thailand	Herbal materials, finished herbal products	0.3						10	
Taiwan	Herbal products	0.5						10	
Turkish Food Codex	Fresh herbs	0.2						0.1	
Union of Europe	Herbal materials	0.2							
British Pharmacopoeia	Herbal drugs	1						5	
USP	Herbal drugs	0.3						10	
AHPA	Herbal products	4.1**						10**	
FAO/WHO	Medicinal plants	0.3	2	20–150		2	1.5	10	
	Edible plants	0.21	0.02	3	20		1.63	0.43	27.4
GSMPP	Herbal materials	0.3			20			5	

AHPA American Herbal Products Association, *GSMPP* Green Standards of Medicinal Plants and Preparations for Foreign Trade and Economy, WM/T 2–2004, *USP* United States Pharmacopeia 4th

*Milligrams per day

**Micrograms per day

V. officinalis, and *W. somnifera* taxa, while they were within normal limits in other taxa (Table 3).

Normally, plants need and tend to uptake some heavy metals (Cu, Fe, Mn, Ni, and Zn) in low concentrations as trace elements for plant biochemistry and physiology, but their higher concentrations have toxic effects for both plant metabolism and its consumer people [17]. Long-term heavy metal exposure can lead to (ROS) resulting in lipid peroxidation, enzyme inactivation and DNA damage, inhibited respiration and gas exchange, reduced photosynthesis, diminished water balance, and disturbed carbohydrate and nutrient uptake metabolisms resulting in visible symptoms such as root blackening, necrosis, chlorosis, wilting, stunted plant growth, senescence, general reductions in biomass production limited seed numbers, and even death in plant metabolism [18, 123–125].

When the damages of heavy metal toxicity on human health are evaluated, higher levels of Al in the human body can cause Alzheimer, dialysis dementia, neurotoxicity, dermatitis, lung and bone disease, kidney disease, microcytic anemia, and lung and bladder cancer [126, 127]. The toxicity of Cd has harmful multiple human functions including pulmonary emphysema, gastrointestinal disorder, lung cancer, renal dysfunction, kidney stones, high blood pressure, skeletal defects, and bronchitis [18, 128, 129]. Cu overload has been associated with anemia, gastrointestinal, cardiovascular, liver and kidney dysfunction, intestinal irritation, abdominal pain, cramps, and nausea [128, 130, 131]. An excessive level of Cr can lead to nervous system damage, lung cancer, liver and kidney problems, fatigue, and irritability [128, 130]. Fe toxicity can result in diarrhea, vomiting, gastrointestinal bleeding, metabolic acidosis, shock, hypotension, tachycardia, cardiovascular and central nervous system problems, and possibly death [130–132]. A high concentration of Ni may lead to nausea, headache, cough, epigastric, chest pain, gastrointestinal problems, and weakness [127]. Mn toxicity causes damage to cardiovascular and central nervous systems. Pb toxicity is excessively harmful due to causing infant encephalopathy, developmental delay, and mental retardation in children, congenital paralysis, nervous system damage, gastrointestinal damage, and liver and kidney diseases [128]. A high amount of Zn can lead to skin diseases, vomiting, chest tightness, nausea, excitement, coldness, unconsciousness, liver damage, and pulmonary edema [131].

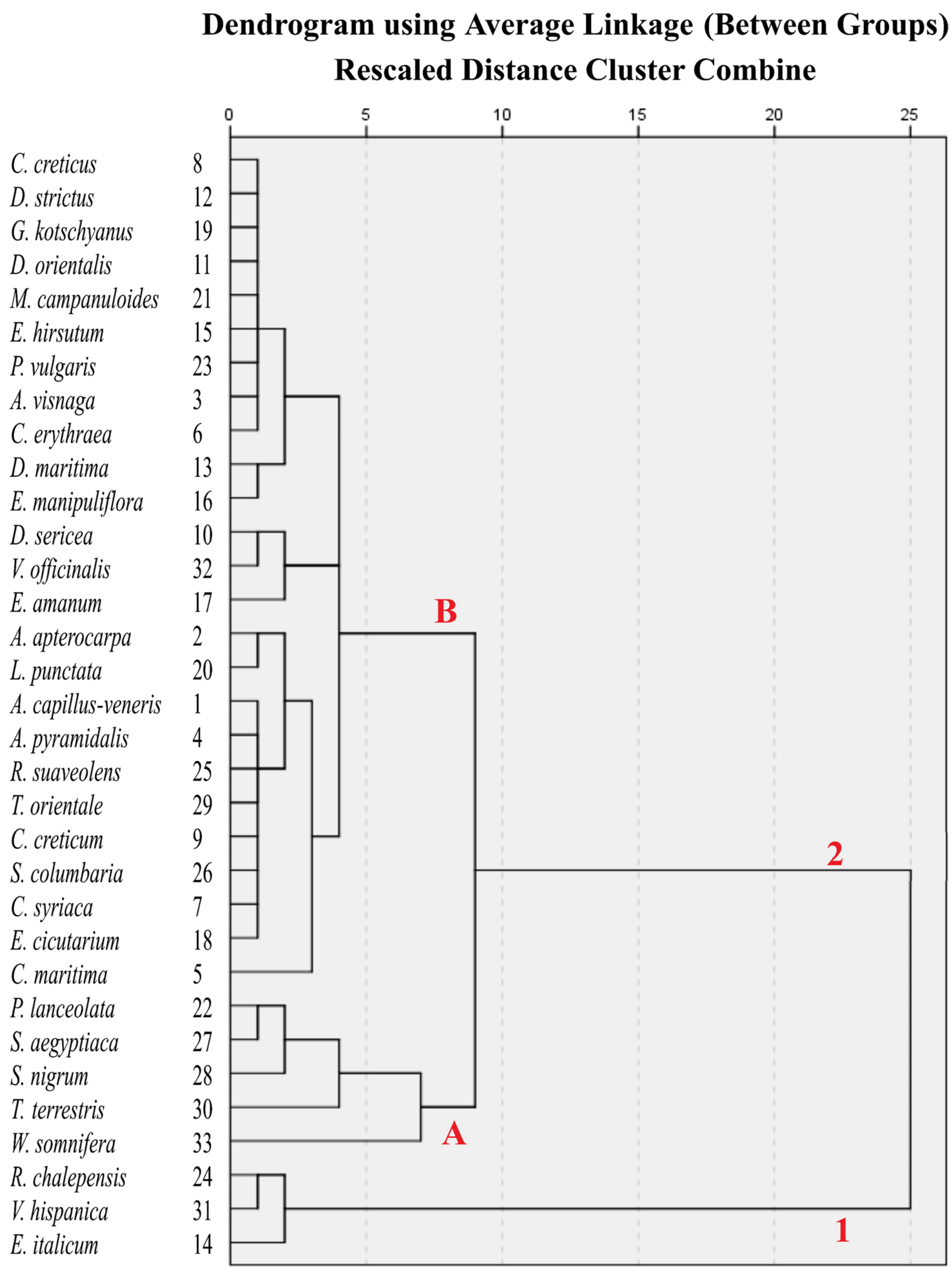
On the map of Hatay mines of MTA, it is seen that there is a Cr mine in the Kızıldağ, Arsuz, and Belen regions that have soils rich in Cr, and there are iron and steel, fertilizer, cement, and paint factories in the Payas-İskenderun regions [27]. According to our evaluation using the results of the total heavy metal profile, high Cr concentrations in *A. pyramidalis*, *E. amanum*, and *S. aegyptiaca* show that these samples were collected from soils rich in Cr (Samandağ, wArsuz, and Belen), while Al, Fe, Cu, Cr, Ni, Pb, and Zn accumulation of *M. campanuloides*, *S. aegyptiaca*, *D. maritime*, and *R. chalepensis* taxa indicates that these samples were collected from industrial areas and highways with heavy traffic (Iskenderun and Payas). In addition, contamination was observed in the *A. visnaga* and *S. columbaria* samples collected from agricultural soils (Kırıkhan and Dörtyol) as high levels of Ni were detected. Finally, lower levels of heavy metal contamination were observed in plant samples collected from relatively uncontaminated areas (Fig. 1; Table 3).

Our results revealed that the highest heavy metal accumulations were determined in *A. apterocarpa*, *A. visnaga*, *A. pyramidalis*, *G. kotschyanus*, *M. campanuloides*, *S. aegyptiaca*, *S. nigrum*, and *W. somnifera*. These species can be utilized in green phytoremediation technology for cleaning heavy metal–polluted agricultural soil and water resources. Similarly, previous studies were in agreement with our results, reporting that the species mentioned could be considered as natural hyperaccumulators [92, 133, 134].

Hitherto, numerous previous studies have been carried out on trace element and heavy metal accumulation in medicinal plants in many different parts of the world. In previous studies, concentrations of Cd and Cr in edible plants from rural areas of Eastern Iran were investigated and it was found that Cd and Cr values were 0.45 and 0.648 in *A. capillus-veneris* and 0.016 and 0.704 mg kg^{-1} in *P. lanceolata* [135]. The toxic heavy metal levels of *P. lanceolata* at the smelter area near Meza valley (Serbia) were measured, and they were found to be 0.5–16.0 for Cd, 59.4–338.7 mg kg^{-1} for Fe, 1.4–195.9 mg kg^{-1} for Pb, and 33.3–799.5 mg kg^{-1} for Zn, clearly higher than the permissible limits. Also, the content of various heavy metals in *P. lanceolata* growing in metal-contaminated and non-contaminated soil in southern parts of Poland has been measured for monitoring soil pollution [136]. The results of the study demonstrated a wide range between 1 and 13.8 for Cd, 175.7 and 1065.6 for Fe, 6.03 and 121.3 for Pb, and 101.3 and 420.1 mg kg^{-1} for Zn in the samples from non-metalliferous and metalliferous sites, which shows that plants in the polluted area have high heavy metal content as compared to those in non-polluted regions. Similarly, these elements were found within normal limits in the related taxa according to our results. In another study, accumulation of heavy metals (Cu, Ni, Pb, and Zn) in natural plants collected from the Sarcheshmeh copper mining site (Iran) was measured. Concentrations of Cu and Pb were found to be clearly higher than the permissible limit in *E. hirsutum* (1300–1581 and 20 mg kg^{-1}), *P. lanceolata* (128 and 11 mg kg^{-1}), and *S. nigrum* (119 and 12 mg kg^{-1}) expectedly, while the Ni and Zn values were within the normal limit in these taxa [137]. However, it was found that Cu, Ni, and Zn accumulations in *S. nigrum* collected from the nearby agricultural area (Karacay) were above the permissible limits, while other values were found to be within normal levels in *E. hirsutum* and *P. lanceolata*. Trace element (Ca, Mg, Na) and heavy metal (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) levels of the *S. nigrum* and *W. somnifera* samples in industrial areas in Islamabad (Pakistan) and also sold in Indian herbalist markets were investigated, and it was reported that the content of Cd (0.65 and 0.97 mg kg^{-1}), Cu (311.88 mg kg^{-1}), and Pb (37.8, 36.47, and 19.87 mg kg^{-1}) was clearly above the permissible limits set by the WHO, while the others were within normal limits [90, 92, 138]. In another study, *W. somnifera* samples were collected from the field of the PCSIR Lab. complex in Karachi (Pakistan) and it was determined that the concentrations of Cu (245.7 and 135.8 mg kg^{-1}), Fe (9417.7 and 3750.3 mg kg^{-1}), Mn (833.5 and 452.6 mg kg^{-1}), Ni (16.2 and 10.3 mg kg^{-1}), Pb (60.6 and 23.3 mg kg^{-1}), and Zn (422.2 and 375 mg kg^{-1}) in shoot and leaves were above the permissible limits [101]. Similarly, they were found that Cu and Pb in *S. nigrum* and Cu, Ni, and Zn in *W. somnifera* collected from the nearby agricultural area (Samandag-Karacay) were above the permissible limits, while the other metals were within normal limits. For *R. chalepensis*, the concentrations of Cd and Pb metals were measured from different locations in Ethiopia and Jordan, and the studies suggested that the mean values of Cd (0.58–0.71) and Pb (22.8) mg kg^{-1} were above normal limits in the samples from industrial and agricultural areas [139, 140]. However, it was found that only Fe accumulations in *R. chalepensis* collected from the nearby industrial area (Belen) were above the permissible limits, while the other metals were within the permissible limits in our study. The results of all obtained from previous studies and our results show that plant samples in the polluted area have high heavy metal content as compared to non-polluted regions.

Herein, the dendrogram constructed by using the results of average linkage cluster analysis revealed 2 main groups as groups 1 and 2 (Fig. 2). Group 1 containing *R. chalepensis*, *V. hispanica*, and *E. italicum* showed a general distribution in close regions (Payas-İskenderun) having similar habitat characteristics, and the members of this subgroup were probably affected by the similar environmental conditions. They are also Mediterranean element, therophyte, and annual taxa. Group 2 included A and B subgroups. Subgroup A comprised *P. lanceolata*, *S. aegyptiaca*, *S. nigrum*, *T.*

Fig. 2 Dendrogram constructed from studied medicinal plants based on the element composition of samples. It is generated with a hierarchical cluster analysis with average linkage (between species)



terrestris, and *W. somnifera* which are generally annual taxa and showed distribution generally in close ranges (Payas-Samandağ/Kırıkhan-Kumlu). The other 25 taxa were placed in subgroup B.

The human body needs at least 22 mineral elements in order to maintain its metabolic activities in a healthy way. Plants are the most important natural sources for these essential nutrients [141]. Therefore, the Recommended Dietary Allowance (RDA) values for the elements Ca, Cu, Fe, K, Mg, Mn, Na, and Zn were calculated in *E. cicutarium*, *M. campanuloides*, and *P. vulgaris* taxa used as daily food (Table 5). These RDA values are as follows: the RDA value of Ca was measured in *E. cicutarium* > *P. vulgaris* > *M. campanuloides*; the RDA values of Cu and Zn were found in *M. campanuloides* > *E. cicutarium* > *P. vulgaris*; the RDA values for Fe and Mn were found in *E. cicutarium* > *P. vulgaris* > *M. campanuloides*; the RDA values for K, Mg, and Na were found in *E. cicutarium* > *M. campanuloides* > *P. vulgaris*. As it is known, these plants have edible properties. This indicates their possible usability as dietary supplements.

When the functions and importance of trace elements studied on human health are evaluated, Ca affects many intracellular and extracellular processes, and is essential

Table 5 Average level of minerals in the medicinal plants and RDA values (mg 100 g.$^{-1}$) of selected minerals (*for adults, ** for premenopausal women)

Element	RDA	*E. cicutarium*		*M. campanuloides*		*P. vulgaris*	
		Amount	RDA (%)	Amount	RDA (%)	Amount	RDA (%)
Ca	1000 mg*	702.01	70.20	323.71	32.37	445.31	44.53
Cu	0.9 mg*	2.56	284.99	3.22	357.36	1.68	187.01
Fe	8 mg*	92.59	1157.32	11.77	147.07	12.42	155.30
	18 mg**		514.36		65.37		69.02
K	4700 mg*	780.61	16.61	594.64	12.65	495.46	10.54
Mg	420 mg*	113.12	26.93	103.62	24.67	42.09	10.02
	320 mg**		35.35		32.38		13.15
Mn	2.3 mg*	45.03	1957.98	6.69	290.83	11.41	495.98
	1.8 mg**		2501.87		371.62		633.75
Na	1500 mg*	146.14	9.74	56.81	3.79	31.45	2.10
Zn	11 mg*	12.30	111.85	18.88	171.68	8.27	75.21
	8 mg**		153.79		236.06		103.41

for the development, growth,, and maintenance of teeth and bone, and the stability of the cytoskeleton [142]. It also plays an important role in vitamin D absorption, regulation of the activity of intracellular enzymes, and neuronal transmission [143]. Ca deficiency can lead to congestive heart failure, cardiac arrhythmias, scurvy, sarcopenia, rickets, and osteoporosis in the human body [144, 145]. Dairy products contain high levels of calcium, and the RDA value for Ca is reported as 1000 mg day^{-1}. According to our results, the lowest and highest RDA values were found to be 323.71 (in *M. campanuloides*) and 702.01 (in *E. cicutarium*) for Ca. In other words, the daily consumption of 100 g of *M. campanuloides* meets ~ 32.37% of daily Ca need and this rate is ~ 70.20% for *E. cicutarium* (Table 5).

Cu plays a key role in metabolic activities such as bone mineralization, brain development, immune system, energy metabolism, maintenance of hematopoietic activity, growth, maturation of red and white blood cells, and Fe transport and metabolism [146]. Deficiency of Cu can lead to many health problems such as retarded growth and bone abnormalities, anemia, leukopenia, and gray hair [147]. Some foods such as grain products, seafood, meat and offal, fruits, and vegetables are rich in Cu element, and the RDA for Cu is reported as 0.9 mg per day [148]. The lowest and highest RDA values were found to be 1.68 (in *P. vulgaris*) and 3.22 (in *M. campanuloides*). Daily consumption of 100 g of *P. vulgaris* meets ~ 187.01% of daily Cu need, while *M. campanuloides* is ~ 357.36% in adults (Table 5).

Fe plays an essential role in biological processes, including DNA biosynthesis, transport of hemoglobin, the formation of oxygen transport proteins and enzymes in electron transfer and oxidation reductions, electron transfer in the redox cycling, and cellular energy generation [149–151]. Fe can be obtained from food sources such as eggs, pulses, cereals and black mulberry, legumes, vegetables, and fruits. The RDA value for Fe is reported as 8 mg day^{-1} for men and 18 mg day^{-1} for women [152]. However, Fe deficiency has been associated with many different diseases including chronic anemia in adults, weakness, fatigue, weakened immune function, reduced cognitive function in children, delays in childhood development, sepsis, miscarriage, maternal and perinatal death, and low birth weight during pregnancy [132, 151–154]. In our study, the lowest and highest RDA values were found to be 11.77 in *M. campanuloides* and 92.59 in *E. cicutarium*, respectively. In other words, the daily consumption of 100 g M. *campanuloides* meets ~ 147.07% of daily Fe need in men and ~ 65.37% in women, while this ratio is ~ 1157.32% and ~ 514.36% in *E. cicutarium*, respectively (Table 5).

K is critical for nerve, skeletal, and cardiac muscle activity [155]. Deficiency of K can lead to high cardiac arrhythmias, blood pressure, kidney disease, muscle cramps and weakness, bone demineralization, constipation, fatigue, nausea, and vomiting [145]. It can be obtained from vegetables, fruits, and foods, including tomato, potato, blueberries, beet greens, yogurt, and milk [156, 157]. The RDA for K has been reported as 4700 mg day^{-1} for men [158]. Our study revealed that the RDA values for K were between 495.46 in *P. vulgaris* and 780.61 in *E. cicutarium*, which shows that daily consumption of 100 g *P. vulgaris* provides ~ 10.54% and *E. cicutarium* ~ 16.61% of the required daily amounts of K, respectively (Table 5).

Mg is necessary for energy production, transport of electrolytes across cell membranes, permeability of cell membranes, preservation of the anatomical and functional integrity of various cellular organelles, muscle contraction, development of bone structure, neuron excitability, synthesis of DNA and RNA, and release of hormones and neurotransmitters [142, 159]. Mg deficiency causes atrial fibrillation, appetite, weakness, hypertension, atherosclerosis, cerebrovascular disease, congestive heart failure, migraines, asthma, Alzheimer's, nausea, hypocalcemia, fatigue and hypophosphatemia, premenstrual syndrome, dermatological allergy, rapid heartbeat, and mental disorders [159, 160]. The RDA for Mg is listed as 420 mg day^{-1} for men and 320 mg day^{-1} for women [158]. Our results show that the RDA values of Mg were found between 42.09 in *P. vulgaris* and 113.12 in *E. cicutarium*, which means that the daily consumption of 100 g *P. vulgaris* provides ~ 10.02% and 13.15% and *E. cicutarium* ~ 26.93% and 35.35% of the daily requirements of Mg for men and women, respectively (Table 5).

Mn is an essential nutrient for several vital biological processes including synthesis and activation of many enzymes, antioxidant defense, glucose and lipid metabolism, bone development, catalysis of hematopoiesis, immune response, and neural functions [161, 162]. Common dietary sources of Mn include cereals, rice, tea, and nuts. However, Mn deficiency results in problems such as birth defects, impaired growth and fertility, poorer bone formation, abnormal glycosylation patterns, carbohydrate and lipid metabolism, skeletal problems, and general psychomotor disability [162–164]. The RDA values for Mn are stated as 2.3 mg day^{-1} for men and 1.8 mg day^{-1} for women [152]. In our results, minimum and maximum RDA values for Mn were found to be 6.69 in *M. campanuloides* and 45.03 in *E. cicutarium*. As a result, daily consumption of 100 g of *M. campanuloides* provides ~ 290.83% and 371.62%, while *E. cicutarium* provides ~ 1957.98% and 2501.87% of the daily requirements of Mn for men and women, respectively (Table 5).

Na plays a critical role in healthy muscle, nerve activity, acid–base balance, and plasma volume [155]. Na deficiency can cause severe health problems like weakness, headache, lethargy, anorexia, nausea, vomiting, muscle cramps, confusion, seizures, coma, and altered consciousness [145]. Our analyses demonstrated that our studied medicinal plants are rich in trace element such as Ca, K, Mg, and Na. The RDA for Na is stated as 1500 mg day^{-1} for men [165]. The RDA values of Na were found between 31.45 in *P. vulgaris* and 146.14 in *E. cicutarium* in our results. Additionally, daily consumption of 100 g of *P. vulgaris* provides ~ 2.10% of daily required Na and ~ 9.74% of *E. cicutarium* (Table 5).

Zn is a critical element for several vital biological functions and processes, such as bone structure, protein synthesis, immune system functions, DNA synthesis, cell division, and development of pregnancy, for growth during childhood and adolescence. It is also involved in more than 300 metalloenzymes, as well as Zn matrix metalloproteinases. Common dietary sources of Zn include oysters, whole grains, crab, red meat, and beans [166–168].

Thus, Zn deficiency results in a number of detrimental effects, such as immune systems disorders, weight loss, infertility and hypogonadism in males, mental disorders, anorexia, depression, mental lethargy, decreased wound healing, skin lesions, rough skin, acrodermatitis, alopecia, diarrhea, loss of appetite, and retarded growth in children [166]. The RDA value of Zn is presented as 11 mg day^{-1} for men and 8 mg day^{-1} for women [169, 170]. According to our results, the lowest RDA values of Zn were found in *P. vulgaris* (8.27), while it was the highest in *M. campanuloides* (18.88). Moreover, a daily intake of 100 g of *P. vulgaris* provides ~ 75.21% and 103.41% of the daily requirement for men and women, while *M. campanuloides* provides ~ 171.68% and 236.06% of the daily requirement (Table 5). However, it is estimated that more than 60% of the world's 6 billion people are iron (Fe) deficient and more than 30% are zinc (Zn) deficient. In addition, high calcium deficiencies of Ca, Mg, and Cu are common in many developed and developing countries [141]. Therefore, our RDA results clearly demonstrate that the studied plant species have a high potential for essential elements.

As a result of our study, it was seen that the EDI, THQ, and HI values determined for studied medicinal plants were within the acceptable limits. Considering the determined THQ values and cumulative HI values for Cd, Cr, Ni, and Pb, the risk of adverse effects in adults was within the permissible limits after exposure to the four toxic heavy metals tested through the consumption of medicinal plants. The lowest and highest HI values were found to be 0.012 for *P. lanceolata* and 0.154 for *E. amanum*, respectively (Table 6). Furthermore, risk assessment of toxic metals in these plants is important not only for assessing their adverse effects on human health, but also for obtaining realistic estimates of their uptake through this traditional use.

Additionally, the Joint FAO/WHO Expert Committee on Food Additives [112] has allocated the maximum daily intake of toxic elements for a 70-kg adult person (TDI: tolerable daily intake). TDI values are considered to be within permissible limits for toxic heavy metals such as As, Br, Cd, Pb, and Sb, which have hazardous effects for human body and metabolism [10]. Our results suggested that the Cd and Pb values of all the medicinal plants investigated when compared with the TDI values were beyond the TDI limits (for Cd 0.014 and Pb 0.021 mg/day/70 kg adult person). These results demonstrate to us that the use of medicinal plants is a phenomenon that should be evaluated scientifically.

Conclusion

Long-term environmental damages caused by pollutants such as mining, agricultural activities, burning of fossil fuels, and wastewater, which have increased due to rapid population growth in recent years, affect many plant species living in terrestrial and aquatic ecosystems. In addition, highly toxic metals in soils can threaten wildlife and human health through the consumption of contaminated plants. In order to prevent this heavy metal accumulation, when collecting medicinal aromatic plants, rural areas, close to clean

Table 6 EDI (mg/kg/day), THQ of Cd, Cr, Ni, and Pb and HI values for adults* associated with the consumption of medicinal plants samples in the study

No	Species	Cd		Cr		Ni		Pb		HI
		EDI	THQ	EDI	THQ	EDI	THQ	EDI	THQ	
1	*A. capillus-veneris*	1.76E−05	5.86E−05	1.94E−02	9.68E−03	1.74E−02	1.16E−02	1.62E−02	1.62E−03	0.023
2	*A. apterocarpa*	2.06E−04	6.85E−04	8.76E−02	4.38E−02	4.87E−02	3.25E−02	3.62E−02	3.62E−03	0.081
3	*A. visnaga*	3.48E−04	1.16E−03	1.02E−02	5.08E−03	2.77E−02	1.84E−02	4.74E−02	4.74E−03	0.029
4	*A. pyramidalis*	3.27E−05	1.09E−04	1.96E−01	9.81E−02	3.20E−02	2.13E−02	3.14E−02	3.14E−03	0.123
5	*C. maritima*	2.03E−04	6.76E−04	1.81E−02	9.07E−03	1.88E−02	1.25E−02	2.24E−02	2.24E−03	0.025
6	*C. erythraea*	2.66E−05	8.87E−05	1.15E−02	5.73E−03	2.27E−02	1.52E−02	4.55E−02	4.55E−03	0.026
7	*C. syriaca*	2.52E−04	8.39E−04	1.76E−02	8.82E−03	1.74E−02	1.16E−02	2.93E−02	2.93E−03	0.024
8	*C. creticus*	6.65E−05	2.22E−04	1.46E−02	7.31E−03	1.25E−02	8.35E−03	1.54E−02	1.54E−03	0.017
9	*C. creticum*	5.56E−05	1.85E−04	1.13E−02	5.63E−03	3.66E−02	2.44E−02	5.07E−03	5.07E−04	0.031
10	*D. sericea*	7.00E−04	2.33E−03	1.57E−02	7.87E−03	1.25E−02	8.30E−03	4.27E−02	4.27E−03	0.023
11	*D. orientalis*	3.24E−05	1.08E−04	3.13E−02	1.56E−02	1.74E−02	1.16E−02	1.07E−02	1.07E−03	0.028
12	*D. strictus*	1.51E−05	5.04E−05	2.27E−02	1.14E−02	1.30E−02	8.65E−03	1.03E−02	1.03E−03	0.021
13	*D. maritima*	3.12E−04	1.04E−03	9.32E−02	4.66E−02	3.18E−04	2.12E−04	2.89E−02	2.89E−03	0.051
14	*E. italicum*	1.89E−04	6.29E−04	1.40E−02	7.01E−03	1.95E−02	1.30E−02	1.23E−02	1.23E−03	0.022
15	*E. hirsutum*	6.01E−05	2.00E−04	9.82E−03	4.91E−03	2.08E−02	1.39E−02	2.73E−02	2.73E−03	0.022
16	*E. manipuliflora*	6.12E−05	2.04E−04	2.46E−02	1.23E−02	1.29E−02	8.63E−03	1.58E−02	1.58E−03	0.023
17	*E. amanum*	2.97E−04	9.91E−04	2.85E−01	1.43E−01	9.33E−03	6.22E−03	4.23E−02	4.23E−03	0.154
18	*E. cicutarium*	3.18E−05	1.06E−04	8.44E−02	4.22E−02	8.14E−03	5.42E−03	1.31E−02	1.31E−03	0.049
19	*G. kotschyanus*	1.25E−04	4.18E−04	7.68E−02	3.84E−02	3.60E−02	2.40E−02	2.14E−02	2.14E−03	0.065
20	*L. punctata*	3.23E−05	1.08E−04	3.66E−02	1.83E−02	1.99E−02	1.33E−02	6.29E−03	6.29E−04	0.032
21	*M. campanuloides*	3.88E−05	1.29E−04	4.55E−02	2.28E−02	2.04E−02	1.36E−02	4.07E−02	4.07E−03	0.041
22	*P. lanceolata*	6.46E−05	2.15E−04	1.74E−02	8.70E−03	2.76E−03	1.84E−03	1.43E−02	1.43E−03	0.012
23	*P. vulgaris*	1.14E−05	3.80E−05	9.38E−03	4.69E−03	3.17E−02	2.11E−02	9.01E−03	9.01E−04	0.027
24	*R. chalepensis*	5.57E−05	1.86E−04	5.58E−02	2.79E−02	1.46E−02	9.73E−03	2.04E−02	2.04E−03	0.040
25	*R. suavedens*	1.17E−04	3.90E−04	1.10E−02	5.51E−03	1.49E−02	9.90E−03	4.23E−03	4.23E−04	0.016
26	*S. columbaria*	2.99E−05	9.98E−05	4.68E−02	2.34E−02	3.17E−02	2.11E−02	2.50E−02	2.50E−03	0.047
27	*S. aegyptiaca*	1.08E−04	3.60E−04	1.59E−01	7.97E−02	4.15E−02	2.76E−02	2.33E−02	2.33E−03	0.110
28	*S. nigrum*	1.78E−04	5.93E−04	3.37E−02	1.69E−02	1.84E−02	1.23E−02	5.24E−02	5.24E−03	0.035
29	*T. orientale*	4.02E−05	1.34E−04	2.56E−02	1.28E−02	3.44E−02	2.29E−02	3.99E−02	3.99E−03	0.040
30	*T. terrestris*	1.15E−04	3.85E−04	1.08E−02	5.38E−03	9.90E−03	6.60E−03	1.56E−02	1.56E−03	0.014
31	*V. pyramidata*	2.77E−04	9.23E−04	2.34E−02	1.17E−02	2.90E−02	1.94E−02	1.92E−02	1.92E−03	0.034
32	*V. officinalis*	2.01E−05	6.69E−05	1.84E−02	9.20E−03	1.75E−02	1.17E−02	7.88E−03	7.88E−04	0.022
33	*W. somnifera*	4.82E−05	1.61E−04	1.93E−02	9.63E−03	4.23E−02	2.82E−02	1.10E−02	1.10E−03	0.039

DI estimated daily intake, *THQ* target hazard quotient, *HI* hazard index

*70 kg of adult person

rivers, or mountainous areas should be preferred, away from highways and mining and industrial areas. In addition, this accumulation should be controlled by similar heavy metal analyses carried out periodically in widely used medicinal plants, and the results should be shared with local users and managers. It is clearly seen from our results that in most of the analyzed plant samples, the selected heavy metals are within the permissible limits set by international authorities or in some plants slightly above these limits. Briefly, it was observed that the heavy metal levels in the collected plants were generally at consumable levels, although they were slightly higher in the samples collected from the areas close to the industrial zones and mining and agricultural areas.

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Author Contribution F. Karahan: conceived and designed the present study, collected and analyzed the plant samples, and did the writing—review and editing.

Data Availability The author can confirm that all data generated or analyzed are included in the article.

Declarations

Ethics Approval This work is a systematic and meta-analysis review that did not need ethical approval and did not receive any technical or financial support from any institution and was carried out by the authors at their own personal expense.

Conflict of Interest The author declares no competing interests.

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