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Genetic insights into the mineral profiling of twenty-five accessions across three *Achillea* species

Mostafa Farajpour[1*], Mohsen Ebrahimi[2*], Mohammad Sadat-Hosseini[3] and Amin Baghizadeh[4]

Abstract

Background *Achillea* L., a medicinal plant belonging to the Asteraceae family, has a global distribution and has been employed for its therapeutic properties since ancient times. Different species within the *Achillea* genus are frequently employed to tackle various health concerns. While there has been considerable research conducted on the bioactive compounds found in these plants and their corresponding biological activities, there remains a notable gap in our understanding of their mineral composition. Therefore, the present study aimed to systematically evaluate the mineral profile within and across three *Achillea* species, namely *A. vermicularis*, *A. wilhelmsii*, and *A. tenuifolia*. The study focused on the analysis of eight minerals, namely Fe, Cd, K, Ca, Pb, Mg, Cu, and P, over a span of two crop years.

Results Based on the results, the mineral composition varied significantly among the species and accessions. In the study, the K (3.9–4.48%) content in all three examined species was found to be abundant. Comparatively, the Fe content in the *A. wilhelmsii* (952 mg/kg) and *A. vermicularis* (1047 mg/kg) species was more than double that of the *A. tenuifolia* (390 mg/kg) species. The Cu content, on the other hand, demonstrated similar levels across all three species. The highest concentrations of Mg (0.59%), Ca (1.79%), and P (0.62%) were observed in *A. wilhelmsii*, *A. tenuifolia*, and *A. vermicularis* species, respectively. Notably, *A. tenuifolia* species exhibited the lowest level of Pb, while the *A. vermicularis* species had the lowest level of Cd. In the studied plants, the minerals were found to have the following concentration order: K > Ca > P > Mg > Fe > Cu > Pb > Cd. The cluster analysis categorized the 25 accessions into three distinct groups, revealing no similarity among accessions within each species. The PCA plot, utilizing the first two PC, validated approximately 46% of the total variance. This confirmation solidified the outcomes derived from the cluster analysis.

Conclusions In conclusion, this study provides valuable insights into the mineral composition of *Achillea* species, highlighting the variability among species and accessions. The findings contribute to our understanding of the nutritional and therapeutic potential of these plants and can guide future research on their cultivation and utilization in various applications.

Keywords *A. wilhelmsii*, *A. tenuifolia*, *A. vermicularis*, Iron, Macroelements, Microelements

*Correspondence:
Mostafa Farajpour
m.farajpour@areeo.ac.ir
Mohsen Ebrahimi
mebrahimi@ut.ac.ir
Full list of author information is available at the end of the article

Background

The *Achillea* genus, renowned for its medicinal properties, belongs to the Asteraceae family and encompasses over 100 wild-growing species [1]. This genus includes well-known medicinal plants such as Yarrow (*Achillea* spp.), which has been used for millennia and requires meticulous consideration of raw material quality during collection and processing [2]. Chemical profiling of specialized metabolites in Achillea species is crucial for chemophenetic investigations. The historical use of *Achillea* plants in folk medicine spans millennia, with evidence even found in a 65,000-year-old Homo neanderthalensis tomb in Iraq [3]. Notably, species within this genus have been widely reported for their medicinal properties [2, 4, 5]. The pharmacological efficiency of Yarrow is attributed to key compounds such as; caffeoylquinic acids, flavonoids, and sesquiterpene lactones, contributing to multifunctional biological activity [6, 7]. The essential oils and extracts of this genus have been analyzed, revealing a wide array of phytochemicals contributing to its therapeutic properties [8].

Considering the extensive utilization of medicinal plants, numerous investigations have been carried out to analyze the mineral content of key plants employed for medicinal uses across diverse nations [9–12].

Sixteen essential elements are vital for the growth of plants and animals, with nine categorized as macronutrients and the remaining as micronutrients based on their concentration in plant tissues [13]. The human body lacks the ability to synthesize these essential elements and must obtain them through food. While carbon, hydrogen, and oxygen come from air and water, plants absorb other essential nutrients from the soil. The availability and utilization of nutrients by plants impact human health by ensuring food and nutritional security. Essential nutrients fulfill diverse roles in the human body, from structural components to physiological functions [14]. Imbalances in nutrient intake can disrupt crucial bodily functions, leading to severe health problems [15]. Conversely, an abundance of certain elements can adversely impact the accessibility of other elements, resulting in detrimental effects on human health. Mineral deficiencies continue to be a significant challenge in numerous underdeveloped countries.

The encouragement of integrating edible plants into human nutrition is prevalent, leading to a surge in the intake of wild, edible plants. Consequently, it becomes imperative to evaluate and oversee the mineral content across all consumed plants. This evaluation serves the purpose of directing consumers towards plant varieties rich in essential dietary elements to address deficiencies. Simultaneously, it aids in averting the overconsumption of elements that could potentially pose health risks.

The minerals analyzed in this study - iron, cadmium, potassium, calcium, lead, magnesium, copper, and phosphorus - were selected based on their importance for critical human physiological functions and nutritional needs. Iron is essential for red blood cell production and oxygen transport throughout the body. Low iron levels can lead to anemia with symptoms like fatigue and reduced cognition. Cadmium and lead are toxic heavy metals that can accumulate in the human body and cause serious organ damage over time. Their levels were analyzed to allow for an assessment of potential health risks. Calcium, potassium and phosphorus are major macro minerals necessary for maintaining strong bones and teeth. They also play regulatory roles in nervous system, muscle and cardiovascular functioning. Magnesium and copper participate in over 300 and 100 enzymatic reactions respectively, linked to energy production, immune response and neurological health. Deficiencies in these trace elements can increase risks for various diseases. By examining the full mineral profile provided by crops, insights about their nutritional quality and ability to meet dietary mineral intake recommendations can be gained. The selection of these specific minerals therefore focused on their established importance for critical physiological processes and overall human health.

Medicinal plants, exemplified by *Achillea* species, enjoy widespread utilization in Iran, underscoring their cultural and therapeutic significance. The essential oils derived from these plants exhibit a notable phytochemical diversity, rendering them valuable for medicinal applications [1, 16, 17]. This prompts speculation regarding their potential as reservoirs for essential minerals. Consequently, the objective of the present study is to systematically evaluate the mineral profile within and across three distinct *Achillea* species, namely *A. vermicularis*, *A. wilhelmsii*, and *A. tenuifolia*. By conducting a comprehensive mineral analysis, this research aims to contribute valuable insights into the potential mineral content of *Achillea* species, further enhancing our understanding of their multifaceted medicinal properties.

Materials and methods

Plant materials

In this study, the seeds were collected from 25 accessions of three *Achillea* species (*A. vermicularis* with 10 accessions, *A. wilhelmsii* with 8 accessions, and *A. tenuifolia* with 7 accessions), distributed in various regions in Iran (Table 1). The collection of plant samples was carried out under the permission granted by the Agricultural Research, Education, and Extension Organization of Iran. Voucher samples were stored at the Herbarium of the Research Institute of Forests and Rangelands in Tehran, Iran. The plants were identified by Dr. Mozaffarian from the Department of Botany at the Research Institute of

Table 1 Geographical location of 25 Iranian *Achillea* Sp accessions

Achillea sp.	Code	Voucher numbers	Province	City
A. wilhelmsii	W1	8451	Isfahan	Daran
	W2	15,796	Lorestan	Kuhdasht
	W3	17,628	Qom	Dastjerd
	W4	19,489	Kurdistan	Baneh
	W5	33,976	Yazd	Tabas
	W6	34,431	Hormozgan	Bandar-Abbas
	W7	35,561	Mazandaran	Polur
	W8	39,346	Qazvin	Tarom Sofla
A. vermicularis	V1	9687	Kurdistan	Sanandaj
	V2	9872	Kurdistan	Baneh
	V3	10,342	Yazd	Khatam
	V4	19,471	West Azerbaijan	Mahabad
	V5	19,488	West Azerbaijan	Mirabad
	V6	22,593	Kurdistan	Saqqez
	V7	23,155	Zanjan	Zanjan
	V8	26,032	Kurdistan	Divandarreh
	V9	35,179	West Azerbaijan	Khoy
	V10	35,181	West Azerbaijan	Salmas
A. tenuifolia	T1	14,234	West Azerbaijan	Salmas
	T2	14,300	Kurdistan	Divandarreh
	T3	25,948	Kurdistan	Dehgolan
	T4	25,977	Kurdistan	Saqqez
	T5	35,180	West Azerbaijan	Mahabad
	T6	39,374	Qazvin	Takestan
	T7	34,662	Kurdistan	Sanandaj

Forests and Rangelands (TARI) in Tehran, Iran, according to Flora Iranica [18]. Initially, the seeds were grown in a greenhouse, and once the plants reached approximately 10 cm in height, they were transferred to the field. The seedlings were cultivated using a randomized complete block design at the research farm of the College of Abouraihan, University of Tehran. Each accession was planted in 1 m^2 plots with sandy-loam soil. To assess the mineral content of the plants, they were harvested during the initial flowering stage.

In the region of Pakdasht, the climate exhibits distinct seasonal characteristics. Summers are characterized by sweltering heat, arid conditions, and clear skies, while winters are marked by very cold temperatures, dry air, and predominantly clear weather. Over the course of the year, the temperature typically varies from 1 to 38 °C, with rare instances of temperatures dropping below −3 °C or rising above 41 °C. Also, in the present study, all methods were performed in accordance with the relevant guidelines and regulations.

Mineral content

To analyze the mineral content, the samples underwent a series of steps. Firstly, they were dried in an oven at a temperature of 70 °C. After that, 1 gram of each sample was subjected to a furnace set at 550 °C for 6 h. The resulting ash was finely ground and dissolved in 100 mL milliliters of HCl (10%). The concentrations of Cd (cadmium), Cu (copper), Pd (palladium), and Fe (iron) in the plant samples were determined using an Atomic Absorption Spectrophotometer, specifically the Perkin Elmer Aanalyst 400 model, USA. The experimental conditions employed were in accordance with the methodology outlined by Munson et al. [19]. The determination of P (phosphorus) followed the procedure described by Cottenie et al. [20]. This involved using a spectrophotometer (Perkin Elmer Aanalyst 400, USA), specifically a Spekol instrument, and measuring the absorbance at a wavelength of 430 nm. The experimental conditions mirrored those specified by Attar et al. [21]. The concentration of K (potassium) was determined via flame photometry, a technique that utilizes the measurement of emitted light from excited potassium atoms. Lastly, the levels of magnesium and calcium were determined using the EDTA-Titration method as outlined by Piper [22].

Statistical analysis

The study was conducted over a span of two years in field conditions. To ensure the consistency of variance between the two years, an F test was performed and confirmed. As a result, a combined analysis was conducted for the minerals under investigation. The Statistical Analysis Software (SAS Institute, Cary, NC) version 9.1 for Windows was utilized for this combined analysis. For the classification of the accessions based on the arithmetic mean method (UPGMA), a heat map clustering technique was employed. The cluster and heat map correlation analysis were then presented as a colored heat map using MetaboAnalyst. To perform the Principal Component Analysis (PCA), the factoextra package in R [23] was utilized.

Results and discussion

Mineral contents

Mineral contents were analyzed in three species of *Achillea* sp. i.e. *A. wilhelmsii*, *A. vermicularis*, and *A. tenuifolia*. The study spanned two crop years and examined eight minerals Fe, Cd, K, Ca, Pb, Mg, Cu, and P.

For *A. wilhelmsii*, only Cu showed significance in both years ($P < 0.01$), indicating its sensitivity to environmental changes compared to other elements (Table 2). K exhibited the highest concentration among the studied elements. Accession W1, from Daran in Isfahan province, had the highest levels of Pb, Fe, and Cu in both years. Conversely, W4 had the lowest amount of Fe in both

Table 2 Mean values ± sem of eight elements content of 25 accessions of three *Achillea* Sp

	First year							
	Fe (mg/kg)	**Pb (mg/kg)**	**Cd (mg/kg)**	**Cu (mg/kg)**	**Mg (%)**	**Ca (%)**	**K (%)**	***P* (%)**
W1	1189 ± 12	14.8 ± 1.8	0.8 ± 0.2	15.8 ± 1.3	0.61 ± 0.1	0.66 ± 0.3	4.21 ± 0.3	0.45 ± 0.1
W2	786 ± 92	8.6 ± 0.2	1.2 ± 0.3	15.8 ± 0.1	0.45 ± 0.1	1.43 ± 0.1	4.56 ± 0.3	0.32 ± 0.1
W3	1020 ± 100	8.9 ± 1.1	0.7 ± 0.1	12.9 ± 1.2	0.67 ± 0.0	1.23 ± 0.0	3.89 ± 0.8	0.29 ± 0.0
W4	654 ± 14	7.6 ± 0	1 ± 0.2	11.6 ± 1.6	0.56 ± 0.1	0.97 ± 0.0	4.1 ± 0.2	0.44 ± 0.0
W5	987 ± 97	8.7 ± 0.3	1.2 ± 0.2	13.2 ± 1.5	0.74 ± 0.1	1.11 ± 0.2	4.32 ± 0.4	0.27 ± 0.1
W6	897 ± 99	11.3 ± 1.4	0.9 ± 0.3	12.1 ± 0.3	0.57 ± 0.0	0.87 ± 0.0	3.88 ± 0.8	0.32 ± 0.1
W7	689 ± 97	8.9 ± 0.9	0.4 ± 0.1	11.5 ± 0.3	0.39 ± 0.1	1.34 ± 0.2	3.54 ± 0.1	0.39 ± 0.1
W8	832 ± 46	9.3 ± 1.9	1.2 ± 0.3	10.7 ± 1	0.77 ± 0.1	0.87 ± 0.1	3.88 ± 0.5	0.38 ± 0.0
	Second year							
W1	1451 ± 16	13.8 ± 1.4	0.6 ± 0.1	17.4 ± 0.9	0.33 ± 0.1	0.9 ± 0.0	4.63 ± 0.7	0.37 ± 0.0
W2	1084 ± 59	6 ± 0.5	0.8 ± 0.2	15 ± 1.2	0.49 ± 0.0	1.71 ± 0.2	5.24 ± 0.3	0.38 ± 0.0
W3	1080 ± 93	7.3 ± 0.4	0.7 ± 0.1	16.3 ± 0.8	1.21 ± 0.1	1.91 ± 0.0	5.83 ± 0.7	0.49 ± 0.0
W4	548 ± 127	9.2 ± 1.1	0.8 ± 0.1	15.2 ± 0.9	0.38 ± 0.0	2.17 ± 0.5	4.94 ± 0.2	0.54 ± 0.1
W5	1143 ± 56	7.3 ± 0.5	0.8 ± 0.2	17.4 ± 0.6	0.68 ± 0.1	1.25 ± 0.2	4.76 ± 0.1	0.51 ± 0.0
W6	1141 ± 93	11.1 ± 0.2	0.7 ± 0	17.1 ± 0.9	0.37 ± 0.1	0.69 ± 0.2	1.52 ± 0.2	0.2 ± 0.1
W7	749 ± 66	4.7 ± 0.1	0.6 ± 0.2	15.7 ± 0.9	0.55 ± 0.1	1.8 ± 0.1	5.46 ± 0.4	0.53 ± 0.1
W8	982 ± 84	8.3 ± 0.2	1 ± 0.2	13.3 ± 0.4	0.65 ± 0.1	1.49 ± 0.3	4.28 ± 0.4	0.5 ± 0.1
Mean	952 ± 58.6	9.1 ± 0.65	0.84 ± 0.06	14.44 ± 0.56	0.59 ± 0.05	1.28 ± 0.11	4.32 ± 0.24	0.4 ± 0.03
t-test	n.s	n.s	n.s	**	n.s	n.s	n.s	n.s
T1	429 ± 28	5.6 ± 0.2	1 ± 0.1	15.8 ± 0.4	0.43 ± 0.0	1.78 ± 0.3	3.6 ± 0.5	0.56 ± 0.0
T2	301 ± 12	4.3 ± 0.3	0.6 ± 0.1	10.7 ± 0.9	0.63 ± 0.0	1 ± 0.2	4.2 ± 0.3	0.46 ± 0.0
T3	389 ± 31	4.5 ± 0.8	0.8 ± 0.1	15.4 ± 1.6	0.39 ± 0.0	1.38 ± 0.1	3.6 ± 0.3	0.48 ± 0.0
T4	345 ± 29	5.7 ± 0.8	0.9 ± 0.2	14.3 ± 0.9	0.17 ± 0.0	1.87 ± 0.2	2.98 ± 0.2	0.37 ± 0.0
T5	311 ± 33	5.2 ± 0.4	0.9 ± 0.3	11.3 ± 0.7	0.5 ± 0.0	2.89 ± 0.1	2.78 ± 0.1	0.41 ± 0.0
T6	587 ± 89	6.7 ± 0.6	1.5 ± 0.3	15.5 ± 1.6	0.19 ± 0.0	1.23 ± 0.1	3.43 ± 0.2	0.47 ± 0.0
T7	235 ± 52	6.5 ± 0.3	1.3 ± 0.6	11 ± 1.2	0.59 ± 0.2	1.1 ± 0.1	4.65 ± 0.1	0.4 ± 0.0
	Second year							
T1	433 ± 10	5.2 ± 0.2	0.6 ± 0.1	20.8 ± 1.5	0.51 ± 0.1	2.92 ± 0.3	4 ± 0.4	0.66 ± 0.0
T2	359 ± 6	3.5 ± 0.3	0.4 ± 0.1	13.9 ± 0.7	0.79 ± 0.1	1.36 ± 0.1	3.6 ± 0.1	0.54 ± 0.0
T3	377 ± 27	4.7 ± 0.6	0.8 ± 0.2	18.2 ± 0.6	0.55 ± 0.0	1.76 ± 0.1	3.84 ± 0.1	0.6 ± 0.0
T4	389 ± 21	4.5 ± 0.2	0.7 ± 0	13.5 ± 0.8	0.31 ± 0.0	2.05 ± 0.2	3.78 ± 0.1	0.43 ± 0.0
T5	357 ± 3	4.4 ± 0.6	1.1 ± 0.1	14.7 ± 1.5	0.44 ± 0.1	3.39 ± 0.1	4.14 ± 0.3	0.55 ± 0.0
T6	681 ± 2	6.1 ± 0.5	1.1 ± 0	14.5 ± 2.1	0.29 ± 0.0	1.13 ± 0.0	4.53 ± 0.0	0.45 ± 0.0
T7	279 ± 2	5.9 ± 0.3	0.7 ± 0.1	14.4 ± 1.3	0.83 ± 0.1	1.26 ± 0.1	5.51 ± 0.0	0.58 ± 0.1
Mean	1047 ± 118	6 ± 0.37	0.83 ± 0.06	15.4 ± 0.32	0.28 ± 0.04	1.33 ± 0.06	4.48 ± 0.12	0.62 ± 0.1
t-test	n.s	n.s	n.s	n.s	n.s	n.s	n.s	*
V1	589 ± 9	4.3 ± 0.4	0.67 ± 0.1	14.4 ± 0.3	0.44 ± 0.0	1.47 ± 0.0	4.78 ± 0.5	0.4 ± 0.0
V2	698 ± 2	5.1 ± 0.2	0.9 ± 0.1	15.3 ± 1	0.19 ± 0.0	1.1 ± 0.1	4.32 ± 0.3	0.38 ± 0.0
V3	2159 ± 58	7.3 ± 0.4	1.3 ± 0.2	17.2 ± 1.1	0.38 ± 0.0	1.33 ± 0.1	5 ± 0.3	0.33 ± 0.0
V4	1398 ± 94	6.3 ± 0	1.2 ± 0.3	17.1 ± 0.1	0.21 ± 0.1	1.2 ± 0.1	4.69 ± 0.0	0.52 ± 0.0
V5	676 ± 122	3.8 ± 0.2	1.2 ± 0.4	14.5 ± 0.3	0.15 ± 0.01	1.12 ± 0.0	3.9 ± 0.4	1.79 ± 0.2
V6	970 ± 10	8.4 ± 0.3	1.3 ± 0.4	14.6 ± 0.4	0.44 ± 0.0	1.47 ± 0.2	4.1 ± 0.3	0.43 ± 0.0
V7	936 ± 40	6.3 ± 0.6	0.9 ± 0.3	13.8 ± 0.3	0.1 ± 0.0	1.26 ± 0.1	3.74 ± 0.5	0.49 ± 0.1
V8	735 ± 47	4.4 ± 0.1	0.9 ± 0.2	14.5 ± 0.3	0.12 ± 0.0	1.33 ± 0.1	3.71 ± 0.0	0.5 ± 0.0
V9	1179 ± 170	8.5 ± 0.6	1 ± 0.3	16.9 ± 0.6	0.41 ± 0.0	1.1 ± 0.1	3.93 ± 0.6	0.41 ± 0.0
V10	932 ± 158	8.5 ± 0.4	0.5 ± 0.2	12.6 ± 0.7	0.1 ± 0.0	1.3 ± 0.2	4.94 ± 0.4	0.44 ± 0.0
	Second year							
V1	625 ± 50	3.3 ± 0.2	0.53 ± 0.0	15.4 ± 1	0.5 ± 0.0	1.67 ± 0.1	5.38 ± 0.1	0.66 ± 0.0
V2	778 ± 11	4.5 ± 0.3	0.9 ± 0.1	16.3 ± 0.5	0.29 ± 0.0	1.26 ± 0.1	4.44 ± 0.3	0.5 ± 0.0
V3	2641 ± 57	6.3 ± 0.1	0.9 ± 0.1	18.6 ± 1.2	0.56 ± 0.1	1.81 ± 0.1	4.8 ± 0.5	0.37 ± 0.1
V4	1426 ± 59	5.9 ± 0.7	0.6 ± 0	14.1 ± 0.7	0.27 ± 0.0	1.16 ± 0.1	5.47 ± 0.3	0.56 ± 0.1

Table 2 (continued)

	First year							
	Fe (mg/kg)	**Pb (mg/kg)**	**Cd (mg/kg)**	**Cu (mg/kg)**	**Mg (%)**	**Ca (%)**	**K (%)**	***P* (%)**
V5	730 ± 148	4.6 ± 0.4	0.6 ± 0	15.5 ± 0.3	0.09 ± 0.0	1.62 ± 0.2	3.74 ± 0.5	2.11 ± 0.2
V6	900 ± 34	6.8 ± 0.6	0.7 ± 0.1	16.6 ± 0.9	0.5 ± 0.0	1.67 ± 0.2	4.3 ± 0.4	0.45 ± 0.0
V7	802 ± 37	5.7 ± 0.1	0.7 ± 0.1	16.2 ± 0.1	0.14 ± 0.1	1.48 ± 0.2	4.58 ± 0.1	0.51 ± 0.0
V8	591 ± 48	5 ± 0.3	0.5 ± 0	13.9 ± 2.4	0.12 ± 0.0	1.41 ± 0.1	4.09 ± 0.2	0.52 ± 0.1
V9	1281 ± 71	8.3 ± 0.2	0.6 ± 0.1	15.7 ± 2.3	0.53 ± 0.1	0.46 ± 0.4	4.43 ± 0.2	0.43 ± 0.0
V10	904 ± 53	7.3 ± 0.8	0.7 ± 0.1	14.8 ± 0.7	0.14 ± 0.1	1.44 ± 0.0	5.22 ± 0.2	0.5 ± 0.1
Mean	390 ± 86	5.2 ± 0.25	0.89 ± 0.08	14.57 ± 0.72	0.47 ± 0.05	1.79 ± 0.02	3.9 ± 0.19	0.5 ± 0.02
t-test	n.s	n.s	**	n.s	n.s	n.s	n.s	n.s

years. Pb and Cd were identified as toxic elements, with the Daran accession showing the highest lead toxicity and accession W8 exhibiting the highest cadmium toxicity. Two accessions exceeded the permissible limit of Pb set by the World Health Organization (10 mg/kg) in both years. In terms of Mg, accession W8 had the highest levels in the first year, while accession W3 had the highest levels in the second year. All accessions had a calcium-to-phosphorus index higher than 1, indicating good food quality.

In *A. vermicularis*, the Cd element showed significance ($P < 0.01$) in both years, suggesting its sensitivity to environmental changes. Cu was the significant element for *A. wilhelmsii*. K had the highest concentration among the elements studied. Accession V3, from Khatam city in Yazd province, exhibited the highest average values for Fe, Cu, K, and Ca in the first year, and for Fe, Cu, Mg, Ca, and Cd in the second year. Accession V1 had the lowest amount of Fe in the first year, and V8 had the lowest amount in the second year. The Khoy accession (V9) showed the highest Pb toxicity, and the Khatam accession exhibited the highest Cd toxicity. All accessions remained below the permissible limit of Pb. Accession V1 had the highest percentages of Mg and calcium Ca in the first year, while V3 had the highest percentages in the second year. The Mirabad accession (V5) exhibited the highest percentages for the P element in both years. The calcium-to-phosphorus index for all accessions, except Mirabad, was higher than one, indicating good food quality.

In *A. tenuifolia*, the year had a significant effect only on the phosphorus element ($P < 0.05$). K had the highest concentration among the elements studied. Accession T6, from Takestan city in Qazvin province, had the highest average values for Fe, Pb, and Cd in both years, while the Sanandaj accession had the lowest amount of iron. Accession Divandareh (T2) had the lowest toxicity for both Pb and Cd in both years. All accessions remained below the permissible limit of Pb. Accession Salmas had the highest Cu content in both years. Accession T2 had the highest percentages of Mg in the first year, while T7 had the highest percentages in the second year.

In the conducted study, the K content in all three examined species, as presented in Table 2, was found to be abundant. This mineral plays a crucial role in regulating cellular acidity and alkalinity [24]. Comparatively, the Fe content in the *A. wilhelmsii* and *A. vermicularis* species was more than double that of the *A. tenuifolia* species.

Iron is a crucial element for various metabolic processes in living organisms, such as oxygen transport, DNA synthesis, and electron transport [25]. However, its levels need to be carefully controlled as excessive amounts can cause tissue damage due to the formation of free radicals. Disorders of iron metabolism are prevalent in humans and can lead to a range of conditions, including anemia, iron overload, and potentially neurodegenerative diseases [26]. Notably, *A. wilhelmsii* species exhibited the highest levels of Pb, while the *A. tenuifolia* had the highest levels of Cd. It is worth mentioning that the Pb levels in all three species remained below the permissible limit. However, certain accessions of specific species surpassed the permissible limit for Pb content.

Cd and Pb are highly toxic metals that have no known function in the human body and are not essential for living organisms [27]. Exposure to these metals, both in the environment and through occupations, poses a growing public health concern [27, 28].

The Cu content, on the other hand, demonstrated similar levels across all three species. The highest concentrations of Mg, Ca, and P were observed in *A. wilhelmsii*, *A. tenuifolia*, and *A. vermicularis* species, respectively. Mg is the second most abundant cation found within cells and has a positive impact on muscle function [29]. Calcium (Ca) is essential for various functions, including maintaining a healthy skeletal system, cell organelle structure, and regulating fluid balance within and outside cells [30]. Insufficient calcium levels can lead to osteoporosis, a prevalent issue in underdeveloped countries [31]. P is necessary for the human body and participates in multiple biological processes such as ATP and GTP production, the formation of teeth and bones, and acts as an energy source and signaling molecule in tissues [32].

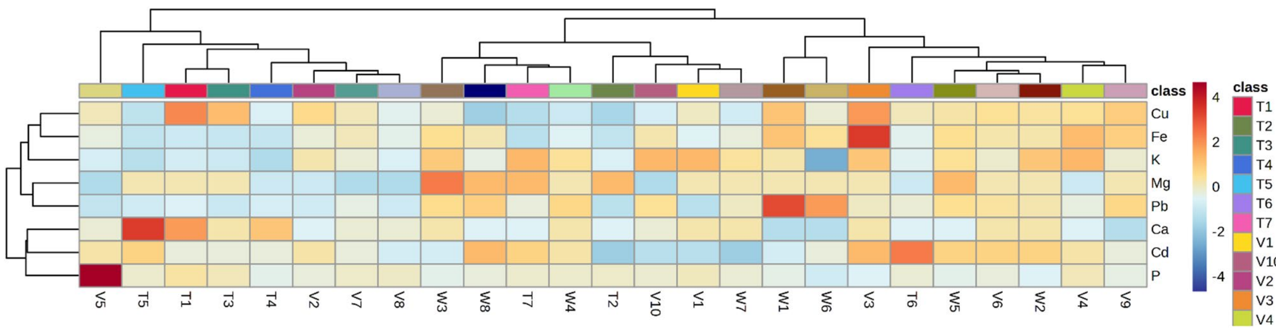

Fig. 1 Heatmap clustering of the 25 accessions of three studied *Achillea* sp. based on the eight measured minerals. The color scales represent the values were normalized by Z-score ((value-mean value)/standard error) for each character

Table 3 Mean values ± sem of eight elemental contents within three distinct groups identified through cluster analysis

Clustering	Fe (mg/kg)	Pb (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Mg (%)	Ca (%)	K (%)	*P* (%)
Group 1	561 ± 71	4.95 ± 0.5	0.84 ± 0.1	15.25 ± 1.7	0.28 ± 0.2	1.79 ± 0.7	3.83 ± 0.3	0.68 ± 0.5
Group 2	673.63 ± 83	6.7 ± 2	0.74 ± 0.2	13.4 ± 1	0.58 ± 0.2	1.4 ± 0.2	4.64 ± 0.5	0.47 ± 0.0
Group 3	1230 ± 115	8.4 ± 7.3	0.8 ± 0.9	16.3 ± 15.6	0.47 ± 0.4	0.78 ± 1.2	4.18 ± 4.3	0.42 ± 0.4

However, excessive intake of phosphorus and bioactive phosphates can have pathogenic consequences.

In the studied plants, the minerals were found to have the following concentration order: K > Ca > P > Mg > Fe > Cu > Pb > Cd. A similar concentration order was also observed in the mineral matter contents of *A. collina* becker ex rchb, a plant species from the flora of Bulgaria [33]. Hasimi et al. [34] reported that *A. filipendulina* was rich in P content.

Multivariate analysis

Cluster analysis was conducted on the measurement data of eight minerals in leaves from 25 *Achillea* sp. accessions across three species. The analysis categorized the accessions into three distinct groups, revealing no similarity among accessions within each species. Each group comprised accessions from different species (Fig. 1).

The first group, consisting of 8 accessions (V5, T5, T1, T3, T4, V2, V7, V8), exhibited the highest concentrations of Fe, Pb, and Cu, while displaying the lowest values for Ca and P (Table 3). This divergent trait portfolio suggests unique genetic factors influencing their mineral composition. These accessions show promise as either nutrient-enriched sources or for introducing targeted traits into new varieties. The second group, also comprising 8 accessions (W3, W8, T7, W4, T2, V10, V1, W7), demonstrated elevated levels of Cd, Ca, and P, alongside lower values for Fe, Pb, Mg, and K. Their elevated calcium and phosphorus combined with reduced toxic metals like cadmium positions them as varieties with potential health benefits. However, breeding efforts may aim to further optimize their nutritional balance.

The final group of nine accessions had characteristically high levels of magnesium and potassium, both important nutrients, alongside reduced cadmium and copper levels, minimizing toxicity risks. Their mineral profiles indicate suitability for medicinal applications.

Table 4 PCA based on the eight minerals of 25 *Achillea* Sp. Accessions

Label	Minerals	Principal components		
		PC1	PC2	PC3
1	Fe	0.81	0.38	0.05
2	Pb	0.76	-0.21	-0.10
3	K	0.42	-0.02	-0.10
4	Mg	0.29	-0.70	0.31
5	Cu	0.38	0.68	0.19
6	P	-0.46	0.52	-0.33
7	Cd	0.17	0.31	0.69
8	Ca	-0.59	0.01	0.61
-	Eigenvalue	2.24	1.5	1.1
-	% of variance	28.02	18.80	13.83
-	Cumulative%	28.02	46.82	60.65

Collectively, the division into groups highlights the wide natural variation present. With further genetic characterization, specific accessions from each cluster could be selected as parental lines to develop populations tailored to different uses. The knowledge generated on precise mineral signatures offers strategic guidance for future cultivation and breeding of these important *Achillea* species.

Principal Component Analysis (PCA) was applied to the eight measured minerals in *Achillea* sp. leaves. The first three principal components (PCs) explained 60.65% of the total variance (Table 4). PC1, contributing to 28.02% of the variance, exhibited a significant positive correlation with Fe, Pb, and K. PC2, explaining approximately 19% of the variance, was positively correlated with

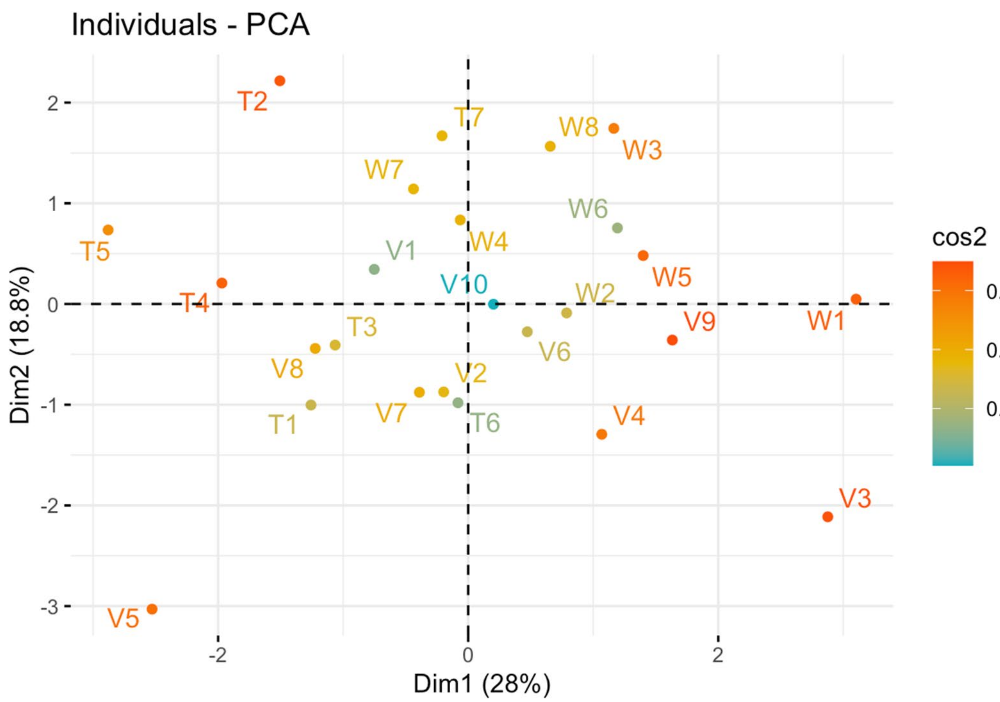

Fig. 2 PCA plot based on the two first PC of the 25 accessions of three studied *Achillea* sp

Cu and P and negatively correlated with Mg. PC3, positively correlated with Cd and Ca, accounted for 13.83% of the total variance. The PCA plot of the first two principal components confirmed the absence of similarity among accessions within each species, reaffirming that each group consisted of accessions from different species (Fig. 2).

The results showed significant variability in the mineral composition across the different plant accessions evaluated. As all plants were cultivated in the same location, environmental conditions and soil types could not account for this observed variation. However, genetic factors and the interaction between genetics and the local environmental conditions may help explain the variability. Each accession represents a different genetic makeup that can influence mineral uptake and distribution within the plant. Genetic properties like mineral transporter efficiency, nutrient use efficiency, and tolerance to stresses can impact elemental accumulation. The principal component analysis identified the first factor (PC1) as accounting for much of the variability, suggesting it may relate to underlying genetic or genotype-environment interaction effects. Plants with different genetic backgrounds growing in the same soil conditions can still differentially absorb and allocate minerals based on their inherent genetic properties. Furthermore, the interaction between an accession's genetics and environmental cues like temperature, precipitation or day length could also drive physiological responses affecting mineral content.

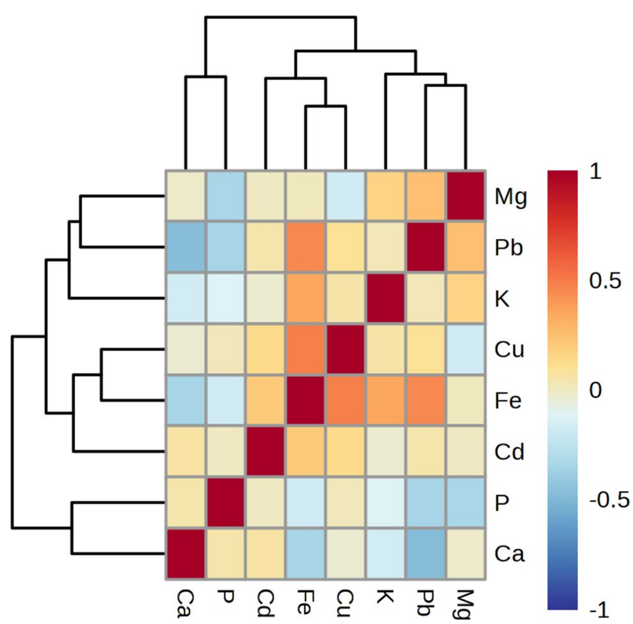

Fig. 3 Heat map correlation among the eight measured minerals of 25 accessions of three studied *Achillea* sp

The correlation analysis among accessions of three *Achillea* species revealed significant relationships between mineral concentrations. Ca exhibited a negative correlation with Pb and P, suggesting an inverse relationship between Ca and these elements (Fig. 3). Similarly, Pb showed a negative correlation with Ca and P. Fe demonstrated a negative correlation with Ca, indicating an inverse association between Fe and Ca. Cu and

Fe displayed a positive correlation, suggesting a direct relationship between these elements. K had a negative correlation with Fe, indicating an inverse association. Pb exhibited a positive correlation with Fe, suggesting a direct relationship. These correlation patterns provide insights into the intricate relationships among mineral elements in *Achillea* sp. accessions. The negative correlations between Ca and certain elements indicate potential antagonistic interactions, while positive correlations suggest cooperative relationships. Understanding the connections between minerals is crucial as they reveal insights into the sources of these elements [35]. Positive correlations indicate that the element pairs share a common source, while negative correlations confirm that their sources are independent [12]. Strong positive and negative correlations enable the prediction of one element based on another by employing simple regression models.

The observed variability in mineral composition across *Achillea* accessions has important implications for our understanding of their therapeutic properties and nutritional value. Accessions with naturally higher levels of bioactive minerals like iron, magnesium and calcium would likely confer greater health benefits [36]. Accessions genetically optimized for specific mineral traits could potentially be developed into cultivars tailored for different medicinal or dietary applications. Furthermore, these findings provide insights to guide selective breeding efforts aimed at enriching the nutritional and pharmaceutical potential of *Achillea*. Characterizing mineral diversity enables the selection and cultivation of elite accessions or hybrids with customized mineral profiles ideal for producing herbal medicines and high nutrient medicinal herbs. This knowledge can also support the standardization and commercialization of *Achillea* extracts and supplements.

Conclusion

The comprehensive analysis of mineral content in three *Achillea* species—*A. wilhelmsii*, *A. vermicularis*, and *A. tenuifolia*—across two growing seasons has provided valuable insights. In total, eight minerals were studied across 25 accessions of these *Achillea* species. The analysis revealed abundant K in all species, with Fe content more than doubling in *A. wilhelmsii* and *A. vermicularis* compared to *A. tenuifolia*. Cu levels were similar across all three species. Additionally, the highest concentrations of Mg, Ca, and P were found in *A. wilhelmsii*, *A. tenuifolia*, and *A. vermicularis*, respectively. Multivariate analyses, including cluster and principal component analysis, highlighted distinct mineral patterns and the absence of similarity among samples within each species. Correlation analysis unveiled complex relationships, providing insights into potential antagonistic and cooperative interactions among mineral elements in *Achillea* species. In conclusion, this research significantly enhances our understanding of mineral variations in *Achillea* species, providing valuable information for both scientific knowledge and potential applications in human health.

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MF and ME conceived and designed the research. MF Performed the statistical analysis and wrote the manuscript. MF and MSH conducted experiments. ME and AM elaborated on the results and discussion, while doing a critical reading of the manuscript.

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The ethical approval or individual consent was not applicable.

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Author details
[1]Crop and Horticultural Science Research Department, Mazandaran Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Sari, Iran
[2]Department of Agronomy and Plant Breeding, College of Abourihan, University of Tehran, Tehran, Iran
[3]Department of Horticultural Science, Faculty of Agriculture, University of Jiroft, Jiroft, Iran
[4]Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran

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