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Determination of Some Major and Trace Elements in Cladodes of Barbary fig (*Opuntia ficus-indica* Mill.) by X-ray Fluorescence Spectrometry

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Abstract

Barbary fig (*Opuntia ficus-indica*) has attracted great attention in extensive rural agriculture for its potential agronomic, medicinal, and environmental benefits. However, there is only incomplete information about its chemical profile. Energy-dispersive X-ray fluorescence (EDXRF) spectrometry was applied to determine the concentrations of 11 major and trace elements (Br, Cr, Cu, Fe, K, Mn, P, Rb, Sr, Yb, and Zn) in cladodes of *O. ficus-indica* and the adjacent soil. For analytical accuracy, the standard reference materials CRM–IAEA 336 (Lichen) and CRM–NIST 1646a (Estuarine Sediment) were used. The relationships between the chemical elements were established by Pearson's correlation coefficient (*r*) and principal component analysis (PCA). The results show that K, P, Fe, and Mn were the dominant essential elements in *O. ficus-indica* cladodes; however, Br, Cr, Cu, Rb, Yb, and Zn were present at low concentrations. The cladodes showed high enrichment with K, Sr, and Br (BEF > 1), but the values of this coefficient were below 1 for the remaining elements. The PCA showed that in the *O. ficus-indica* cladodes, the higher concentrations of Br, K, and Sr were correlated; conversely, the highest contents of Cr, Cu, Fe, Mn, P, Rb, Yb, and Zn were retained in the soil. The present findings enabled us to determine that *O. ficus-indica* has a high ability to accumulate K, P, Fe, and Mn in its cladodes. Therefore, the data obtained from the analysis of this cactus will be useful for nutritional and medicinal purposes.

Keywords Cactus · Trace elements · BEF · Plant nutrition · Cladodes · EDXRF technique

Introduction

The prickly pear or Barbary fig (*Opuntia ficus-indica*) is an important fruit species in the arid and semi-arid zones of North Africa, where it plays a main role in rural agriculture [10, 20]. In these areas, *O. ficus-indica* is comestible as a fruit for human consumption and as forage for sheep and goats, and is cultivated as an ornamental species. It is used in hedges, as a wind break, and against soil erosion and desertification [14, 21]. Its fruits and oil seeds are used in cosmetics manufacture and for medicinal purposes. *Opuntia ficus-indica* is a cactus native of and well adapted to the Mediterranean climate, where it is most often grown in shallow, nutrient-poor soils on rocky substrates and is subjected to long periods of drought [15].

Cactus cladodes are rich in dietary fiber (> 43%), carotenoids, polyphenols, vitamin C (ascorbic acid), and some essential elements [30]. The seeds have a high content of linoleic acid (an unsaturated fatty acid) [26]. The natural colors of the fruits are related to the presence of betacyanins and betaxanthins, which have antioxidant properties [29]. The anti-ulcerogenic effect of *O. ficus-indica* is attributed to the presence in the cladodes of mucilage and pectin (polymers) that inhibit the production of ethanol-induced ulcers and gastritis [6, 16]

Essential trace elements such as Fe, Cu, Mn, and Zn are indispensable for plant growth at very low concentrations; however, other elements such as Rb, Sr, and V are known to stimulate plant development, but their functions are not yet known. These elements are involved in the activation of some enzymes such as dehydrogenases and oxidases, as well as in carbohydrates metabolism and chlorophyll and nucleic acid synthesis [12]. However, substantial concentrations of essential trace elements in the rhizospheric biotope lead to plant toxicity [11].

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Analytical techniques employing energetic beams, such as ICPMS, ICPOES, and ICPAES, are widely used for trace element determination in plant material. However, these procedures require acid digestion and pretreatment of the samples prior to measurement, which make them slower and produce residues. In contrast, other analytical tools like EDXRF and PIXE are suitable alternative techniques which have many advantages such as sample preparation simplicity, low cost, and quicker elements quantification [5, 9, 13, 17].

Energy-dispersive X-ray fluorescence (EDXRF) is a non-destructive analytical tool for measurement of macro- and micro-(trace) elements in a wide range of sample types, including pharmaceutical, agricultural, geological, and environmental materials. By using short bursts of high-precision exciting radiation, this technique permits multi-elemental analysis without chemical reagents [18, 23].

In spite of its economic and ecological relevance, there are still not enough data regarding the mineral composition of *O. ficus-indica* and the interrelationships between the elements present in cladodes and in the soil in which this cactus is grown. In this sense, the purpose of the present study is to determine the main major and trace elements in cladodes of *O. ficus-indica* and its ability to translocate trace metals from soil to cladodes.

Material and Methods

Species Description and Sample Collection

Opuntia ficus-indica (Cactaceae family) is a drought-resistant plant with crassulacean acid metabolism (CAM) [25] (Fig. 1). The green cladodes or "nopalitos" (35–45 cm long and 20–30 cm wide) have a spatulate form and are strongly succulent with abundant small spines. These cladodes can store substantial amounts of water in their parenchymatic tissues. Barbary fig has a superficial root system (max. depth of 30 cm) which extends horizontally to absorb the small amount of moisture provided by light rains in arid climates [33]. The red, purple, or yellow flowers (2–3 cm in length) are hermaphrodites, with 4 attached carpels enclosed in a floral cup and an inferior receptacle (ovary). The fruits (figs or prickly pears) are enclosed in a thick skin, covered with small prickles, and contain up to 270 seeds [28].

Fresh cladodes of *O. ficus-indica* and their associated soil were collected from the *Zaâfrane* location of Djelfa province, Algeria (34° 49′ 52″ N latitude, 2° 52′ 38″ E longitude, 852 m elevation). According to the approach described by Nedjimi [22], three parallel transects of 100 m were traced across the plantation of *O. ficus-indica* with a 100 m interval between the transects. In each transect, at a regular interval (10 m), ten cacti were randomly harvested (5 mature cladodes/cactus) and they were mixed to make up a composite sample.

The soils in the sampling site are typically calcareous in nature (pH = 7.6–8 and EC = 50 dS m^{-1}) with a silt-sand texture and low organic matter content (2–3%). Using a stainless steel sampling auger, three replicate composite soil samples (100 g) were randomly collected at 20–30 cm depth (rooting zone) at the same sites at which the cactus samples were taken. Each of the soil samples consisted of a mixture of 3 subsamples.

Sample Preparation

The plant material was washed with double-distilled water in order to remove dust, desiccated in an oven at 65 °C for 72 h, and subsequently powdered to fine particles (size fraction of 200 μm) using an agate mortar and pestle. The soil samples were oven-dried (65 °C/72 h) and passed through a 2-mm stainless steel mesh sieve. For each sample (plant and soil), 120 mg of powdered material was compressed using a 150-tonne hydraulic press apparatus and made into pellets of 13 mm diameter and 1–2 mm thickness. Triplicates of each sample were made. Two biological reference materials,

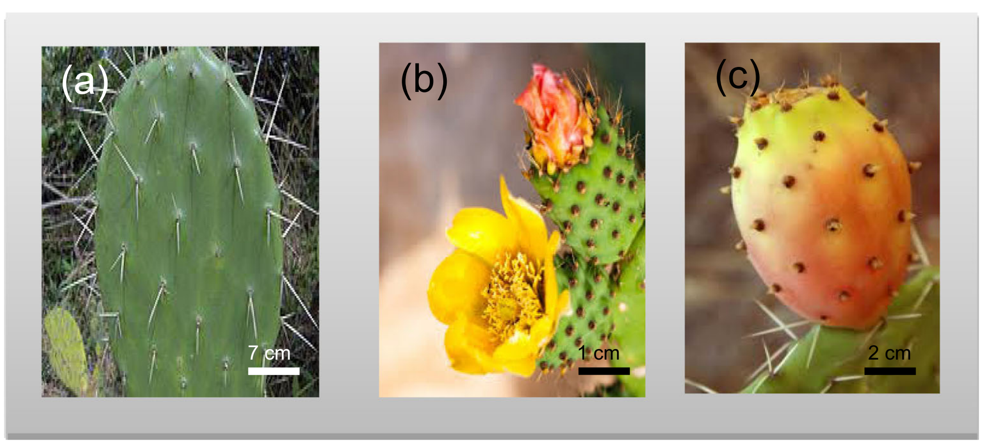

Fig. 1 *Opuntia ficus-indica* Mill. **a** Cladode. **b** Flower. **c** Fig

namely CRM–IAEA 336 (Lichen) and CRM–NIST 1646a (Estuarine sediment), were employed as controls for analytical accuracy.

EDXRF Spectrometry Analysis

A PANalytical X-ray fluorescence spectrometer (Epsilon 3 XL) was applied to measure the trace element concentrations in both cladode and soil samples. The setup consists of an Ag anode X-ray tube operated at 50 kV and 3 mA maximum voltage and a beam current of 1 nA. The X-rays emitted were recorded by an Si(Li) detector SDD with a resolution of 135 keV for the line Mn-Kα (5.9 keV) and an active surface of 5 mm^2 provided with a thin beryllium window of 8 mm thickness. The concentrations of the analytes were determined using several removable primary filters consisting of Ti, Ag, Al–50, Al–200, and Cu–300. The spectra were collected with an acquisition time of 1800 s using Epsilon 3 software.

Measurement and Statistical Analysis

The element concentrations (μg g^{-1}) were measured using the following equation [8]:

$$C_x = C_s\left[\frac{i_x.m_x}{i_s.m_s}\right]$$

where C_x and C_s denote the element concentration in the cladode sample and CRM, respectively, i_x and i_s denote the net intensity of the element concentration in the cladode sample and CRM, respectively, and m_x and m_s are the weight of the sample and CRM, respectively.

U_{score} is an important statistical parameter often used to monitor the accuracy of the analytical technique. This parameter was measured using the following equation [32]:

$$|U_{score}| = \frac{(x_{lab} - x_{cert})}{\sqrt{\sigma^2{}_{lab} + \sigma^2{}_{cert}}}$$

where x_{lab}, σ_{lab}, x_{cert}, and σ_{cert} denote the laboratory value of the element in the CRM, the uncertainty value of x_{lab}, the certified value, and the uncertainty value of x_{cert}, respectively. The XRF performance is assessed as satisfactory if $U_{score} \le 1$ and unsatisfactory for $U_{score} > 1$.

The bioaccumulation element factor (BEF) was calculated, to evaluate the transfer of trace elements from soil to *Opuntia* cladodes, using the following equation [7, 22]:

$$BEF = C_{plant}/C_{soil}$$

where C_{plant} and C_{soil} denote the content of the element in the cladode and soil sample, respectively.

Pearson's correlation coefficient (r) at $P < 0.05$ was used to identify the correlations among the various element concentrations. Principal component analysis (PCA) was carried out to assess the relationships among the chemical elements in the cladodes and soil. The data were statistically analyzed using the STATISTICA 12.0 software package

Results and Discussion

Accuracy Estimation

The EDXRF methodology was validated using two standard reference materials. The certified and quantified values of IAEA 336 lichen and NIST 1646a estuarine sediment were similar (good reliability and precision). For all chemical elements, the U_{score} values of the two CRMs were ≤ 1 (Table 1), indicating the reliability of the analytical technique.

Elemental Analysis and Bioaccumulation Factor

Eleven trace element concentrations—namely Br, Cr, Cu, Fe, K, Mn, P, Rb, Sr, Yb, and Zn—were determined using EDXRF analysis. The average concentrations are shown in Table 2. *Opuntia ficus-indica* cladodes were relatively rich in five major and trace elements, namely K, P, Sr, Fe, and Mn, for which the mean values were 32886.94 μg g^{-1} (K), 251.31 μg g^{-1} (P), 108.38 μg g^{-1} (Sr), 44.24 μg g^{-1} (Fe), and 17.33 μg g^{-1} (Mn). The elements Rb, Zn, and Br were present in minor amounts (8.68, 7.60, and 5.95 μg g^{-1}, respectively), with Cu, Cr, and Yb present in trace amounts, the mean values being 1.71 μg g^{-1} (Cu), 1.08 μg g^{-1} (Cr), and 0.05 μg g^{-1} (Yb).

The concentrations of chemical elements in the soil adjacent to the plants descended in the following order: P (45000 μg g^{-1}) > K (6630 μg g^{-1}) > Fe (6530 μg g^{-1}) > Mn (142.47 μg g^{-1}) > Sr (62.56 μg g^{-1}) > Zn (17.3 μg g^{-1}) > Rb (15.8 μg g^{-1}) > Cr (15.7 μg g^{-1}) > Cu (7.95 μg g^{-1}) > Br (5.1 μg g^{-1}) > Yb (0.76 μg g^{-1}).

The *O. ficus-indica* cladodes had a substantial amount of K (32886.94 μg g^{-1}). A similar K value (28985 μg g^{-1}) was determined in young neem (*Azadirachta indica*) leaves, collected in the Thiruvallur district in Tamil Nadu, southern India, by proton-induced X-ray emission (PIXE) [5]. In the present investigation, the concentration of P in *O. ficus-indica* cladodes was 251.31 μg g^{-1}. This result is in agreement with de Aragão Tannus et al. [3], who determined trace elements in *Maytenus ilifolia* from Brazil using ICPOES. The Sr concentration (108.38 μg g^{-1}) obtained in the present study is higher than the values determined in *Tinospora cordifolia* (20.5 μg g^{-1}) and *Moringa oleifera* (59.3 μg g^{-1}) by PIXE [9]. The Mn and Fe contents of the *Opuntia* samples are lower than those found by Kulal et al. [13]. These latter authors determined trace elements concentrations in some medicinal plants (*Justica adhatoda*, *Mesua ferea*, and *Ocimum sanctum*) using the EDXRF technique.

Table 1 Quality assessment of the XRF results based on comparison of quantified and certified values of CRM–IAEA 336 (Lichen) and CRM–NIST 1646a (Estuarine sediment). Values represent mean ± standard error of mean ($n = 3$)

Elements	CRM–IAEA 336 (Lichen)			CRM–NIST 1646a (Estuarine sediment)		
	Certified value	Measured value	$\|U_{score}\|$	Certified value	Measured value	$\|U_{score}\|$
Br	12.90 ± 1.53	12.71 ± 1.77	0.09	nr	–	–
Cr	1.06 ± 0.75	1.15 ± 0.04	0.12	40.90 ± 1.90	38.50 ± 1.66	0.95
Cu	3.60 ± 0.55	3.18 ± 0.13	0.74	10.01 ± 0.34	9.86 ± 0.14	0.41
Fe	430 ± 23.17	423 ± 12.52	0.27	20080 ± 390	19889.58 ± 192.37	0.44
K	1840 ± 56.44	1856 ± 42.89	0.23	8640 ± 160	8616.13 ± 888.55	0.03
Mn	63 ± 2.33	61.98 ± 1.74	0.35	234.50 ± 2.80	238.80 ± 14.90	0.28
P	610 ± 21.3	622 ± 18.77	0.42	270 ± 10	284 ± 78	0.18
Rb	1.76 ± 0.69	1.58 ± 0.54	0.21	(38)	36.90 ± 2.52	–
Sr	9.30 ± 0.63	9.11 ± 0.80	0.20	(68)	61.20 ± 3.21	–
Yb	0.037 ± 0.01	0.032 ± 0.03	0.16	nr	–	–
Zn	30.4 ± 1.47	30.18 ± 1.83	0.09	48.9 ± 1.6	47.95 ± 3.42	0.25

Value in parenthesis is non-certified; *nr* not reported

In plant nutrition, K is the most important major element, being involved in stomatal control and acting as an activator of numerous enzymes [1]. It is required for cell division and meristematic differentiation [19]. Phosphorus is an important constituent of the plasma membrane and plays an important role in both energy metabolism (ATP and ADP) and nucleotides and phospholipids synthesis [31]. Manganese is required in oxido-reduction reactions. It is a constituent of certain enzymes, such as pyruvate carboxylase (PC), pyruvate oxidase (POX), and pyruvate dehydrogenase (PDH), and plays an important role in photosynthesis [11]. Iron (Fe) is involved in photosynthesis (the electron transfer system) and in nucleic acids synthesis. It acts as an activator of many enzymes—such as catalase (CAT), peroxidase (POD), and cytochrome oxidase (CYT_b, CYT_c, and CYT_f) [19]. Strontium (Sr) is not an essential trace element for higher plants, but is easily taken up by the root system due to its high similarity to Ca [11].

The BEF results (Table 2) show that K was greatly enriched in *Opuntia* cladodes (BEF = 4.96), Br and Sr were also enriched, to a lower extent (BEF = 1.17 and 1.73, respectively), and the other elements, having BEF < 1 (Cr, Cu, Fe, Mn, P, Rb, Yb, and Zn), are not expected to bioaccumulate to any significant level in the cladodes.

Table 2 Mean values of chemical elements ($\mu g\ g^{-1}$) and bioaccumulation element factor (BEF) determined in *O. ficus-indica* cladodes and their adjacent soil. Values represent mean ± standard error of mean ($n = 3$)

Elements	Cladodes	Soil	BEF
Br	5.95 ± 0.51 (a)	5.10 ± 0.60 (a)	1.17 ± 0.15
Cr	1.08 ± 0.43 (b)	15.70 ± 0.90 (a)	0.07 ± 0.02
Cu	1.71 ± 0.46 (b)	7.95 ± 2.09 (a)	0.21 ± 0.12
Fe	44.24 ± 13.18 (b)	6530 ± 70 (a)	0.01 ± 0.01
K	32886.94 ± 31.38 (a)	6630 ± 90 (b)	4.96 ± 0.35
Mn	17.33 ± 9.80 (b)	142.47 ± 15.89 (a)	0.12 ± 0.06
P	251.31 ± 9.19 (b)	45000 ± 322 (a)	0.01 ± 0.03
Rb	8.68 ± 1.06 (b)	15.80 ± 2.60 (a)	0.55 ± 0.19
Sr	108.38 ± 4.93 (b)	62.56 ± 8.52 (b)	1.73 ± 0.41
Yb	0.05 ± 0.03 (b)	0.76 ± 0.05 (a)	0.07 ± 0.01
Zn	7.60 ± 1.86 (b)	17.30 ± 0.70 (a)	0.44 ± 0.15

Values with different letters are significantly different ($P < 0.05$, Tukey's test)

Table 3 Results of principal component analysis (PCA) of chemical elements

Elements	Principal components			
	PC1	PC2	PC3	PC4
Br	0.13	− 0.99	− 0.04	0.05
Cr	− 0.99	− 0.01	− 0.02	− 0.02
Cu	− 0.95	0.23	− 0.22	0.04
Fe	− 0.99	0.02	− 0.00	− 0.01
K	0.99	0.09	0.05	0.09
Mn	− 0.99	− 0.03	0.01	− 0.02
P	− 0.99	0.06	− 0.03	0.01
Rb	− 0.97	− 0.21	0.01	0.06
Sr	0.92	− 0.36	− 0.11	− 0.09
Yb	− 0.97	− 0.21	0.15	− 0.03
Zn	− 0.99	− 0.15	0.00	− 0.02
Eigenvalues	9.58	1.29	0.09	0.03
Cumulative eigenvalues	9.58	10.87	10.96	10.99
Cumulative %	87.12	98.83	99.64	99.88
% Variance	87.12	11.70	0.82	0.23

Significant correlations at $P < 0.05$

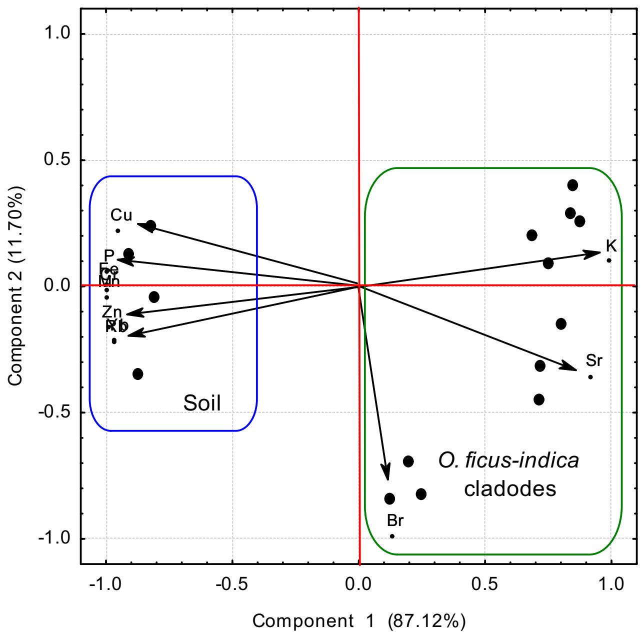

Fig. 2 Principal component analysis (PCA) ordination diagram of *O. ficus-indica* cladodes and their soil correlated to chemical element contents

Principal component analysis (PCA) was performed to assess the interdependences among the 11 elements accumulated in the *Opuntia* cladodes and adjacent soil. The component loadings show that the first principal component (PC1) explained 87.12% of the total variance (Table 3) and was positively associated with the describing variables K (0.99) and Sr (0.92). In contrast, PC1 was negatively correlated with Cr, Cu, Fe, Mn, P, Rb, Yb, and Zn, for which the loading values were in the range 0.95–0.99. The PC2 accounted for 11.70% of the total variance and was negatively correlated with Br, with a loading value of 0.99 (Table 3).

The projection of the data sets on the PC1 and PC2 plane (Fig. 2) indicates that *O. ficus-indica* cladodes were correlated with higher concentrations of Br, K, and Sr, while the highest contents of Cr, Cu, Fe, Mn, P, Rb, Yb, and Zn were retained in the soil (negatively correlated with PC1).

Pearson's Correlation Coefficients (*r*)

Pearson's correlation coefficients (*r*) calculated for the relationships among the different chemical elements are displayed

Table 4 Pearson correlation coefficient (*r*) of chemical elements in *O. ficus-indica* cladodes and their adjacent soil

Cladodes	Br	Cr	Cu	Fe	K	Mn	P	Rb	Sr	Yb	Zn
Br	1.00										
Cr	0.70**	1.00									
Cu	1.00***	0.70**	1.00								
Fe	0.97***	0.85***	0.97***	1.00							
K	− 0.85***	− 0.97***	− 0.85***	− 0.95***	1.00						
Mn	0.87***	0.96***	0.87***	0.96***	− 1.00***	1.00					
P	− 0.98***	− 0.84***	− 0.98***	− 1.00***	0.94***	− 0.96***	1.00				
Rb	0.99***	0.78**	0.99***	0.99***	− 0.90***	0.92***	− 1.00***	1.00			
Sr	0.85***	0.97**	0.85***	0.95***	− 1.00***	1.00***	− 0.94***	0.91***	1.00		
Yb	− 0.92***	− 0.36 ns	− 0.92***	− 0.79**	0.56*	− 0.60*	0.81***	− 0.86***	− 0.57*	1.00	
Zn	0.98***	0.83***	0.98***	1.00***	− 0.94***	0.95***	− 1.00***	1.00***	0.94***	− 0.82***	1.00
Adjacent soil	**Br**	**Cr**	**Cu**	**Fe**	**K**	**Mn**	**P**	**Rb**	**Sr**	**Yb**	**Zn**
Br	1.00										
Cr	0.95***	1.00									
Cu	− 1.00***	− 0.92***	1.00								
Fe	0.95***	1.00***	− 0.92***	1.00							
K	− 0.89***	− 0.70***	0.93***	− 0.70**	1.00						
Mn	1.00***	0.97***	− 0.99***	0.97***	− 0.86***	1.00					
P	− 0.11 ns	0.20 ns	0.20 ns	0.20 ns	0.56*	− 0.06 ns	1.00				
Rb	0.90***	0.99***	− 0.86***	0.99***	− 0.60*	0.92***	0.33 ns	1.00			
Sr	0.97***	0.85***	− 0.99***	0.85***	− 0.97***	0.96***	− 0.34 ns	0.77**	1.00		
Yb	0.99***	0.98***	− 0.98***	0.98***	− 0.82***	1.00***	0.01 ns	0.95***	0.94***	1.00	
Zn	1.00***	0.97***	-0.99***	0.97***	-0.85***	1.00***	-0.04 ns	0.93***	0.95***	1.00***	1.00

Significant correlations at $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; *ns* non-significant

Table 5 Comparison of selected chemical elements (μg g^{-1}) reported in cladodes of *Opuntia species* from different regions of the world

Species	*O. ficus-indica*	*O. dillenii*	*O. polyacantha*	*O. macrorhiza*
Country	Algeria	Spain	USA	Tunisia
	(This study)	[4]	[27]	[2]
Cr	1.06 ± 0.75 (a)	0.144 ± 0.036 (b)	-	-
Cu	3.6 ± 0.55 (b)	0.334 ± 0.054 (c)	25 (a)	3.3 ± 0.50 (b)
Fe	430 ± 23.17 (a)	1.53 ± 0.31 (d)	200 (b)	60 ± 10 (c)
K	1840 ± 56.44 (a)	908 ± 251 (b)	1300 (b)	43 ± 1.0 (c)
Mn	63 ± 2.33 (b)	5.09 ± 3.80 (c)	583 (a)	42 ± 5.0 (b)
P	610 ± 21.3 (a)	-	110 (b)	-
Zn	30.4 ± 1.47 (b)	1.29 ± 0.49 (d)	138 (a)	8.0 ± 1.0 (c)

Means across rows followed by different letters indicate significant difference at $P < 0.05$ according to Tukey´s multiple range test

in Table 4. For the *Opuntia* cladodes, all the chemical elements were significantly correlated ($P < 0.05$), except for Cr, which had no correlation with Yb. The same pattern was observed for the adjacent soil, with significant relationships among almost all the chemical elements ($P < 0.05$). Apart from the pair K-P ($r = 0.56$, $P < 0.05$), P was not associated with any of the chemical elements.

Comparison with Other *Opuntia* spp.

The comparison of our data with those reported for other *Opuntia* spp. from different regions of the world is shown in Table 5. The *O. ficus-indica* cladodes in this work generally had concentrations of K and Fe double those of *O. dillenii* and *O. polyacantha*, respectively, while their Zn content was very low compared to *O. polyacantha*. The Cu content of *O. ficus-indica* was comparable with that of *O. macrorhiza* but lower than that of *O. polyacantha*, while its P content was around six times higher than that of *O. polyacantha*. However, the Algerian species showed higher accumulation of Mn than *O. macrorhiza* and *O. dillenii*. These differences in elemental contents among *Opuntia* spp. could be due to differences in genotypic characters and environmental factors [5, 24].

Conclusion

This work has evaluated the concentrations of some major and trace elements (Br, Cr, Cu, Fe, K, Mn, P, Rb, Sr, Yb, and Zn) in cladodes of *O. ficus-indica* and their adjacent soil collected from Algerian arid zones. The uptake of these elements from soil and their transfer to cladodes was assessed by calculation of the bioaccumulation element factor (BEF). This showed that (1) *O. ficus-indica* cladodes were relatively rich in some essential elements such as K, P, Fe, and Mn; (2) the contents in the cladodes declined in the order: K > P > Sr > Fe > Rb > Zn > Br > Cu > Cr > Yb; and (3) this species had a particularly high transfer factor for K, Br, and Sr.

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Data Availability All data generated or analyzed during this study are included in this published article.

Compliance with Ethical Standards

Conflict of Interest The author declares that he has no conflict of interest.

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