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Seasonal Assessment of some Potentially Toxic Elements with Possible Animal Health Risks in *Atriplex canescens* (Pursh) Nutt.

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Abstract

In the present investigation Br (Bromine), Cr (Chromium) and Pb (Lead) concentrations were assessed employing X-ray florescence spectrometry to evaluate seasonal variation of these elements in the xero-halophyte *Atriplex canescens* (Pursh) Nutt., a shrub with high pastoral value. The results showed that *A. canescens* and its surrounding soil have similar accumulation patterns for Br and Pb across seasons, but Cr concentrations in shrubs are higher in spring than other seasons. The seasonal mean contents of trace elements in *A. canescens* descend in the following order: Cr (8.33 µg g^{-1}) > Br (5.34 µg g^{-1}) > Pb (0.24 µg g^{-1}). The maximum element transfer factor (ETF) of Cr and Pb was recorded in summer and autumn. However, no significant difference between seasons was found for Br. Principle component analysis (PCA) showed that Br, Cr and Pb were associated negatively with soil during all seasons. In contrast, *A. canescens* was only associated to Cr contents in the spring. Results provide evidence also that the levels of Br, Cr and Pb were within the safety-limits recommended by the National Research Council (NRC) guidelines for animal nutrition.

Keywords Dietary intake · Feed safety · Livestock nutrition · Saltbush · Season · Toxic elements · XRF analysis

Introduction

Pasture forage can contribute significantly to nutrition and health of livestock, because forage provides the majority of macro and micro-nutrients indispensable for animal health. Heavy metals (HMs) can also be present in fodder plants in levels that could be potentially toxic to animals. Accumulation HMs in agricultural and pasture lands is associated in almost all cases to human activities, such as mining, smelting, power transmission, traffic, and chemical agricultural products (Mujeeb et al. 2020). Heavy metals, such as lead (Pb), cadmium (Cd), and mercury (Hg) can induce toxicity even when present at trace concentrations (Nedjimi 2021). The threat related to these toxic elements in pasture and rangelands can be intensified by their bioaccumulation in ruminants grazing therein (Miranda et al. 2009). These elements pose a potential risk not only to farm animals but also to human health, as milk and meat consumption constitute an important part of the human diet and can be an important vector of toxic elements (Pareja-Carrera et al. 2014).

The target plant material in this study was *Atriplex canescens* (Pursh) Nutt. (Amaranthaceae family), a halophytic shrub introduced from North America to north African arid rangelands (Nedjimi 2014). Nowadays, this saltbush constitutes one of the most important forage species, not just because of its nutritive value, but due to its adaptation to harsh conditions (drought and salinity) and effectiveness in land restoration (Nedjimi 2018, 2020). Although shrubs of *A. canescens* are a good source of essential elements, it is well known that they bioaccumulate certain toxic metals, which in some cases can exceed the permissible levels for animal nutrition, depending on the soil in which the plants grow (Topcuoğlu et al. 2019). There are many publications focused on the nutritional value of *A. canescens*, including cellulose, crude fiber, tannin and crude protein (Pinos-Rodríguez et al. 2007; Medjekal and Bousseboua 2016; Mellado et al. 2018); however, little attention has been paid to their trace element contents (Nedjimi 2020).

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Analysis of HM contents in soil is generally used to indicate the level of soil pollution. Nevertheless, vegetative analysis is considered a useful tool to determine HMs translocation from soil to edible parts of plants (Hasan et al. 2020). Monitoring the level of potentially toxic elements in the ration formulation for livestock, is one of the most essential tools to controlling feed safety, in order to establish tolerable uptake of HMs to avoid any potential animal health risk (Khan et al. 2018; Ali et al. 2022; Ge et al. 2022).

There are many analytical procedures like Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), X-ray Fluorescence Spectroscopy (XRF), and Proton Induced X-ray Emission (PIXE) employing energetic beams to quantify the contents of trace element in plant samples (Amet and Fitoussi 2020; Elayaperumal et al. 2021; Wang et al. 2021). Among these analytical techniques, XRF is a highly precise procedure for analyzing HMs in all geological and biological matrices. This technique has a high sensitivity, great accuracy and low detection limit without chemical digestion (Shaltout and Hassan 2021).

The present study provides, for the first time, a seasonal quantification of the levels of three toxic trace elements in *A. canescens*, the most halophytic species planted in the Algerian central steppe, as a source of fodder for livestock. The objectives of this work were: (1) to characterize seasonally three potentially toxic elements, Br, Cr and Pb in *A. canescens* and in the soil in which plants were growing (2) to determine the chemical element enrichment (ETF) in plants in relation to their soil, and finally (3) to assess possible health risks to livestock browsing this halophyte.

Materials and Methods

The field work was conducted in the *El khanfra* plantation in Zaafrane aera, Djelfa, Algeria (34°49′52″ N, 2°52′38″ E; 852 m a.s.l.). The pastoral plantation of *A. canescens* (9-y-old) was planted with 1 000 shrubs ha^{-1} (individual plant spacing of 2.5 m x 4 m) and grazed by livestock in two browsing seasons (autumn and spring). This zone received about 280 mm of rainfall in 2018, with lowest quantity (5 mm) in July and the highest (86 mm) in September. The monthly average minimum and maximum temperatures ranged between 4 °C in February and 34 °C in July. The sampling site is underlain by sedimentary calcareous rock with loam-sandy soils.

Three 150 m transects (blocks) were selected across the plantation of *A. canescens* with a distance of 100 m between different sampling transects. Ten *A. canescens* shrubs were randomly chosen from each transect. Leaves plus young (non-lignified) stems (10–15 cm in length) were collected seasonally [January-15 (winter), April-15 (spring), July-15 (summer) and October-15 (autumn)] from each transects, and each composite vegetal sample was derived from ten sub-samples. To ensure the homogeneity, each composite sample was replicated three times ($n=3$) and each replicate comprised a mixture of 10 subsample plants (i.e. 10 shrubs per transect).

Before chemical analysis, *A. canescens* samples were washed with deionized–water in to eliminate any surface particles and dust, and oven–dried at 65 °C for 72 h. The dry samples were powdered (to diameter < 2 μm) using an agate–mortar and pestle (Nedjimi 2020).

As with plant collection, soil samples were collected seasonally at the same site from where plant samples were clipped at a depth of 20–25 cm (the root-zone), using a pedologic auger with a diameter of 2.5 cm. Soil samples were mixed to make a composite soil sample for each transect, dried at 65 °C for 72 h and passed through a 2 mm sieve (Nedjimi 2018). For homogeneity, each composite sample was replicated three times ($n=3$) and each replicate included the mixture of 10 subsample soils.

A set PAN Analytical-Epsilon 3.xl spectrometer consisting of a Silicon Drifted Detector (SDD5) with a resolution 135 keV at Mn-Kα (5.9 keV) and Ag X-ray tube was used to estimate Br, Cr and Pb in soil and *A. canescens* samples. The X-ray tube was operated at a maximum voltage of 50 kV and electric current up to 300 mA. For all samples and standards, a period of 2100 s was used to collected spectra X. Epsilon 3 software was employed for data acquisition.

Precision of the analytical method was checked for *A. canescens* samples by analyzing certified reference material CRM–IAEA 336 (Lichen) from the International Atomic Energy Agency, Vienna, Austria, and for soil samples by analyzing CRM–ISE 868 (Sandy soil) reference material from Wageningen Analytical Laboratories, Netherlands.

The Br, Cr and Pb concentrations were measured using the following relation (Gama et al., 2017):

$$\rho_x\left(\mu g g^{-1}\right) = \rho_s\left[\frac{i_x.w_x}{i_s.w_s}\right]$$

Where ρ_x and ρ_s were the Br, Cr and Pb concentrations (μg g^{-1}) in the *A. canescens* sample and standard, i_x and i_s were the net intensities of Br, Cr and Pb concentrations in the *A. canescens* sample and standard, and w_x and w_s were the weight (mass) of the sample and standard respectively.

The |Z|-score was defined as:

$$|Z| - \text{score} = \mathrm{R}_{lab} - \mathrm{R}_{ref}/\sigma_{ref}\ (\text{Santos etal., 2020})$$

Table 1 Quality assessment of the XRF measurement based on comparison of measured and certified values of CRM–IAEA 336 (Lichen) and CRM–ISE 868 (Sandy soil). Values represent means ± standard error of means ($n = 3$)

Elements	CRM–IAEA 336 (Lichen)			CRM–ISE 868 (Sandy soil)		
	Certified value	Measured value	|Z|-score	Certified value	Measured value	|Z|-score
Br	12.9 ± 1.70	11.41 ± 1.37	0.88	7.02 ± 0.81	6.93 ± 0.74	0.11
Cr	1.06 ± 0.19	1.15 ± 0.04	0.47	8.46 ± 1.49	7.98 ± 1.73	0.32
Pb	4.90 ± 0.84	5.09 ± 0.71	0.23	44.0 ± 4.64	43.47 ± 6.71	0.11

Table 2 Seasonal variations of Br, Cr and Pb (μg g^{-1}) determined in leaves of *A. canescens* and the soil in which the plants were growing. Values represent means ± standard error of means ($n = 3$). Values with different letters indicate significant difference at $P < 0.05$ according to the *Tukey*′s multiple range test

Element	Br	Cr	Pb
	Atriplex canescens		
Winter	3.88 ± 0.46 (b)	4.04 ± 1.22 (c)	0.09 ± 0.01 (b)
Spring	4.40 ± 1.36 (b)	11.63 ± 5.43 (a)	0.09 ± 0.01 (b)
Summer	6.63 ± 0.25 (a)	8.07 ± 4.11 (ab)	0.40 ± 0.06 (a)
Autumn	6.44 ± 0.23 (a)	9.55 ± 0.53 (ab)	0.39 ± 0.03 (a)
Seasonal mean	5.34 ± 0.57	8.33 ± 2.82	0.24 ± 0.02
	Soil		
Winter	67.4 ± 6.30 (b)	23.13 ± 5.25 (b)	0.56 ± 0.09 (b)
Spring	73.81 ± 3.67 (b)	72.70 ± 14.63 (a)	0.59 ± 0.05 (b)
Summer	117.45 ± 20.17 (a)	62.10 ± 13.42 (a)	1.28 ± 0.37 (a)
Autumn	124.93 ± 18.16 (a)	22.49 ± 8.21 (b)	1.26 ± 0.21 (a)
Seasonal mean	95.90 ± 12.08	45.11 ± 17.88	0.93 ± 0.18

Where R_{lab}, R_{ref}, and σ_{ref} are the laboratory result, the reference value, and uncertainty with the reference value, respectively. The laboratory performance is evaluated as satisfactory if |Z|-score ≤ 2, questionable for 2 < |Z|-score < 3, and unsatisfactory for |Z|-score ≥ 3.

In order to evaluate the trace element bioaccumulation capacity of *A. canescens* shrubs, we calculated the element transfer factor (ETF) which is defined as a ratio of trace element concentration in plant tissue to the concentration in the soil below plants. This factor was calculated using the following formula (Arumugam et al. 2018):

$$\mathrm{ETF} = \mathrm{C}_{Atriplex}/\mathrm{C}_{Soil}$$

Where $C_{Atriplex}$ and C_{Soil} indicate the element concentrations in *A. canescens* and soil respectively.

Data were statistically analyzed by one-way ANOVA using the STATISTICA 12.0 software package. Principal component analysis (PCA) was executed to investigate the relationships between toxic metals in *A. canescens* and its related soil. Significant differences between element concentrations were analyzed using *Tukey*'s multiple range test at a significance level of $P < 0.05$. Pearson correlation coefficient (r) was used to explore possible interferences between the pairs of toxic elements as well as the seasons. All data were represented in terms of means ± standard error of means (SE).

Result and Discussion

The comparison of measured values of the certified reference materials to their certified values are shown in Table 1. For all elements, our experimental values are in good agreement with the certified values for both CRMs. The |Z|-score values for CRM–IAEA 336 and CRM–ISE 868 were below 1 which indicates the reliability of analytical method. The concentrations of Br, Cr and Pb in leaves of *A. canescens* and their soil are presented in Table 2. Each trace element had the same seasonal fluctuation in terms of increasing and/or decreasing concentrations. The seasonal mean concentrations of chemical elements in *A. canescens* descend in the following order: Cr (8.33 μg g^{-1}) > Br (5.34 μg g^{-1}) > Pb (0.24 μg g^{-1}). However, in soil below plants the order of these elements was: Br (95.90 μg g^{-1}) > Cr (45.11 μg g^{-1}) > Pb (0.93 μg g^{-1}).

A one-way ANOVA indicated a significant effect of the season on Br and Pb concentrations ($P < 0.01$) (Table 3). Elemental analysis revealed that *A. canescens* collected in autumn and summer had higher concentrations of Br and Pb. However, the lowest concentrations of these elements were recorded in spring and winter. Cr concentration was significantly ($P < 0.05$) higher during spring (11.63 μg g^{-1}) than in winter (4.04 μg g^{-1}) (Table 2).

For soil in which *A. canescens* was growing, Br and Pb concentrations remained similar during summer and autumn seasons. However, a significant ($P < 0.01$) decrease in Br and Pb was found during winter and spring (Table 2). A one-way ANOVA indicated that Cr concentration varied

Table 3 A one-way ANOVA results of the effects of seasons on element transfer factor (ETF), and Br, Cr and Pb concentrations in *A. canescens* leaves and its related soil

Independent variables	df	Sum of squares	Mean Squares	*F*-Ratio	*P*-Value
Atriplex canescens					
Br	3	17.64	5.88	10.87	**
Cr	3	92.51	30.83	2.55	*
Pb	3	0.28	0.09	81.23	***
Soil					
Br	3	7820.6	2606.9	13.19	***
Cr	3	6134.52	2044.84	3.72	*
Pb	3	1.44	0.48	9.68	**
ETF					
Br	3	0.0098	0.0033	0.22	ns
Cr	3	0.21	0.07	4.14	**
Pb	3	0.08	0.03	5.14	**

Significance levels are reported as not significant (ns), *** $P<0.001$, ** $P<0.01$ or * $P<0.05$

significantly ($P<0.05$) by season (Table 3). Predominantly, spring and summer concentrations were higher (72.70 and 62.10 µg g^{-1} respectively) than in autumn and winter (22.49 and 23.13 µg g^{-1} respectively) (Table 2).

It is evident from this investigation that Br, Cr and Pb contents in *A. canescens* vary between seasons with a clear reduction of these elements during winter. Comparable results were also founded in others pasture species (Tahir et al. 2017; Kovacik et al. 2017; Nedjimi 2018). Rainy seasons (winter and spring) seem to have affected the elemental contents during the study period; there was probably a significant flushing of elements by rainfall through the profound horizons. By way of contrast, there were high concentrations of these elements found during the whole dry seasons (summer and autumn), probably due to relatively low rainfall and high temperatures what entrained capillarity phenomenon (Nedjimi 2018).

Trace element migration in soils is influenced by many factors including pH, OM, CEC, soil colloids and climate (Kabata-Pendias 2011; Gjengedal et al. 2015). Among these factors soil pH was the principal parameter determining trace element bioavailability over the seasons. Our studied soil is typically calcareous with little OM and high carbonate contents (Halitim 1988). In calcareous soils (pH > 7), the negative charges of colloids dominate and may lead to reduce the trace element availability in soil solution (Jalali and Najafi 2018). During the dry seasons (summer and autumn), $CaCl_2$ and $MgCl_2$ are the major components in the Algerian arid rangelands due to high evaporation and low precipitation. The deposition of these components on the surface of the soil leads to decreases soil pH and consequently trace elements availability (Pédrot et al. 2009).

Lead (Pb) is considered one of the most toxic HMs and presents a major health risk for domestic animals. Chronic exposure of animal to Pb can causes anemia, birth defects and infertility (Zantopoulos et al. 1999; Pareja-Carrera et al. 2021). According to NRC (2007), a concentration less than 30 mg Pb kg^{-1} of diet DM was considered safe permissible limit for ruminants.

At low concentration (0.1 to 2.0 mg kg^{-1}), Cr acts as an essential trace element for normal glucose metabolism by activation of glucose tolerance factor (GTF) indispensible for insulin secretion (Haldar et al. 2009; Lashkari et al. 2018). No adverse effects were observed in ruminants fed 4.0 mg Cr kg^{-1} (NRC, 2007). However, when present in high concentrations it can causes several syndromes, including liver and kidney damage, lung cancer and pathologic transformation in DNA (Pillay et al. 2003). For animal health, fodder should contain less than 1 g Cr kg^{-1} of diet DM (NRC, 2007).

Bromine is not an essential trace metal for plants or animals and can be toxic even in small amounts, leading to dysfunctions of many biological activities in animals (Kabata-Pendias 2011; Nielsen 2017). To avoid toxicity, NRC (2007) suggests a maximum level of 200 mg Br kg^{-1} for ruminants.

A one-way ANOVA shows that season had a significant effect ($P<0.01$) on the element transfer factor (ETF) of Cr and Pb (Table 3). ETF for Cr was significantly ($P<0.01$) higher during autumn (ETF = 0.48) than in the other seasons. For Pb the maximum values of this factor were reached in autumn and summer (0.31 and 0.34 respectively). However, the ETF of Br did not vary significantly ($P>0.05$) across seasons (Fig. 1). For all elements, the transfer factor values are below the upper critical level (ETF < 1). This indicates a poor translocation rate of the majority of toxic elements to plants, and no obvious bioaccumulation of these elements occurred in *A. canescens*.

To determine any correlations that exist between the toxic trace elements and season, principal component analysis (PCA) was carried out. Results show that trace metals composition in *A. canescens* and its related soil revealed 2 principal components (PCs) that account of 96.86% of the total variance (Table 4). The first component (CP1) that accounted for 80% of the total variation was related negatively to Br, Cr and Pb by high loadings values (0.79–0.95) during all seasons (Table 4). However, the second component (CP2:16.86% of variance) was mainly associated negatively to Cr by loadings value of 0.60 (Table 4). Figure 2 displays the plot of CPA ordination. This projection shows that *A. canescens* samples were correlated mainly to the highest concentrations of Cr in spring. However, the soil had the highest contents of Br, Cr and Pb in the summer, autumn and spring seasons.

The relationship between the seasons and toxic trace metals was elucidated using a Pearson correlation analysis

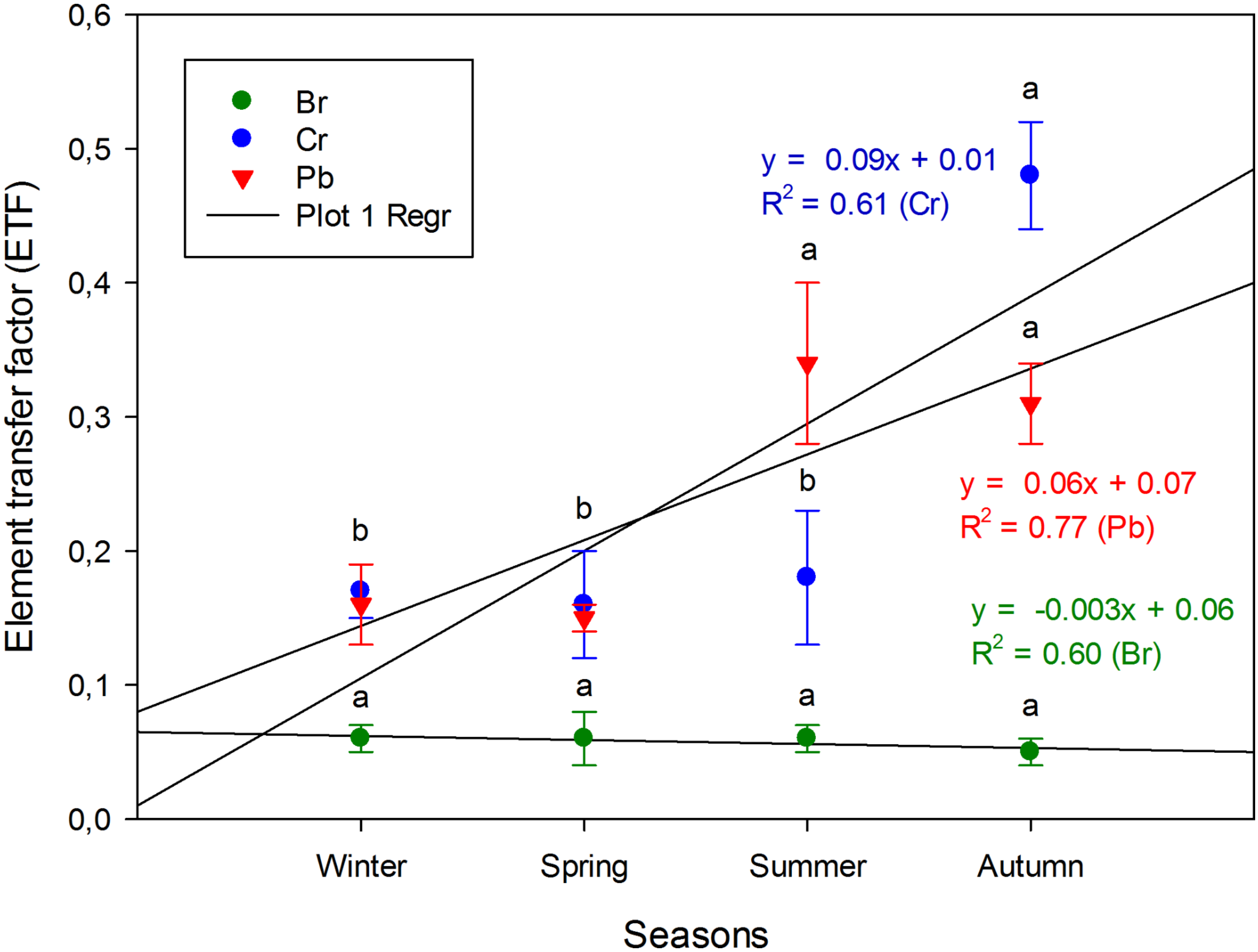

Fig. 1 Element transfer factor (ETF) of Br, Cr and Pb from soil to *A. canescens* shrubs. Values represent means ± standard error of means ($n = 3$). Lines give the regression curves. Different letters above values indicate significant difference at $P < 0.05$ according to the *Tukey´s* multiple range test

Table 4 Results of principal component analysis (PCA).

Elements	Principal components	
	PC1	PC2
Br	-0.95	0.22
Cr	-0.79	-0.60
Pb	-0.93	0.29
Eigenvalues	2.39	0.50
Cumulative eigenvalues	2.39	2.90
Cumulative %	80.00	96.86
% Variance	80.00	16.86

Significant correlations at $P < 0.05$

(r) (Table 5). For *A. canescens*, season was positively correlated with Pb content ($r = 0.87$) and negatively with Br ($r = -0.76$), whereas a negative correlation was found between Br and Pb ($r = -0.86$). No significant correlations were established between Cr and the other elements. However, in soil, a strong positive correlations between season–Pb ($r = 0.79$), season–Br (r = 0.86) and Br–Pb (r = 0.88) were found (Table 5).

The estimation of potential daily intakes (PDIs) of Br, Cr and Pb that a ruminant (body weight 45 kg) would ingest from *A. canescens* leaves was determined assuming an intake of 1 kg of DM day^{-1} $sheep^{-1}$. To estimate the possible risks posed to animal health, these values were compared with the maximum tolerable level (MTL) established by National Research Council (NRC, 2007). According to the NRC, concentration of Pb, Br and Cr in vegetables should not exceed 30, 200 and 1000 µg g^{-1} respectively. Results given in Table 6 show clearly that all element concentrations in *A. canescens* were below the MTL set by NRC guidelines without any animal health risk.

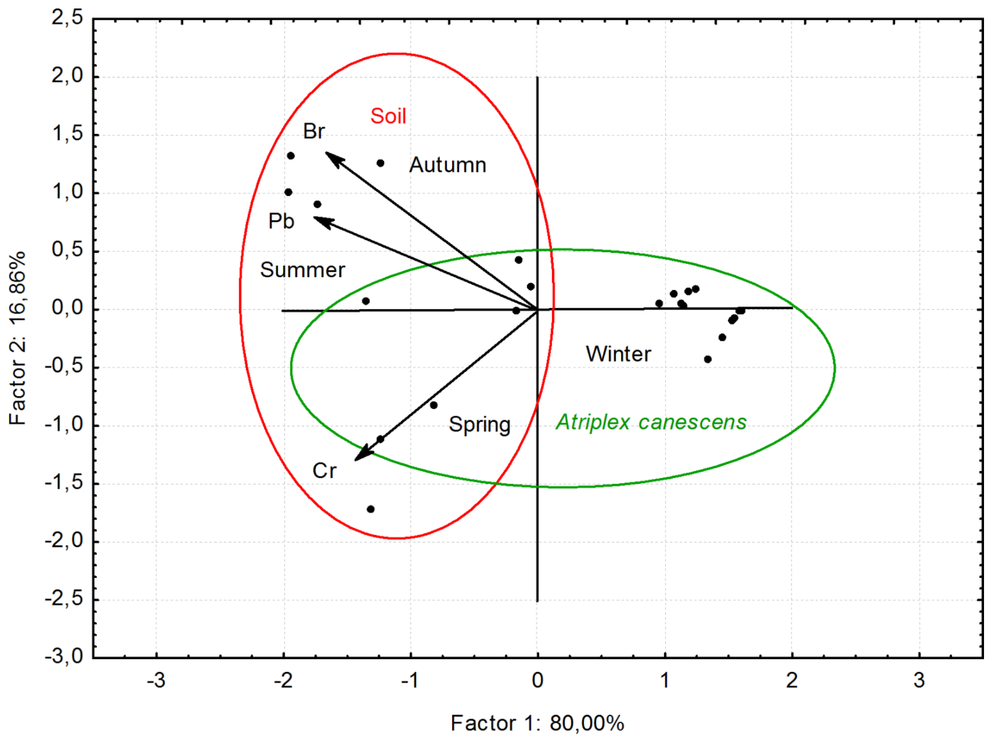

Fig. 2 Principal component analysis (PCA) ordination diagram of *A. canescens* leaves and their soil related to Br, Cr and Pb concentrations

Table 5 Pearson correlation coefficient (*r*) for concentrations of Br, Cr and Pb in *A. canescens* leaves and its related soil

A. canescens	Seasons	Br	Cr	Pb
Seasons	1.00			
Br	-0.76**	1.00		
Cr	0.36 ns	-0.22 ns	1.00	
Pb	0.87***	-0.86***	0.19 ns	1.00
Soil	Seasons	Br	Cr	Pb
Seasons	1.00			
Br	0.86***	1.00		
Cr	-0.04 ns	0.05 ns	1.00	
Pb	0.79**	0.88***	0.13 ns	1.00

Significant correlations at: ** $P<0.01$. *** $P<0.001$. ns: non-significant

Table 6 Potential daily intakes (PDI) of Br, Cr and Pb by small ruminants from *A. canescens* leaves, and its comparison to maximum tolerable level (MTL).

Seasons	PDI ($\mu g\ g^{-1}$) [a]	MTL ($\mu g\ g^{-1}$) [b]
Bromine		
Winter	3.88	200
Spring	4.40	200
Summer	6.63	200
Autumn	6.44	200
Chromium		
Winter	4.04	1000
Spring	11.63	1000
Summer	8.07	1000
Autumn	9.55	1000
Lead		
Winter	0.09	30
Spring	0.09	30
Summer	0.40	30
Autumn	0.39	30

[a] Assumed sheep body weight 45 kg; assumed daily DM intake 1.0 kg

[b] Maximum tolerable level by NRC (2007)

Conclusion

This work was undertaken to determine seasonal changes of some potentially toxic trace elements namely Br, Cr, and Pb in *A. canescens*, which is commonly browsed by livestock living on the Algerian arid steppe, and to know if the contents of these elements were within the safety-limits set by NRC for animal consumption. Results indicated that Br, Cr and Pb accumulation in plant tissues varied significantly among the seasons. Maximum concentrations of Br, Cr and Pb were found in summer and autumn seasons in both plant and soil, while the transfer element factor was < than 1 in all seasons, thus confirming that *A. canescens* has a low tendency to bioaccumulate these metals. The concentrations of Br, Cr and Pb in *A. canescens* plants were found below the permissible limits suggested by NRC. Collectively, the findings of the current study show that *A. canescens* is safe as a forage and does not pose any animal health risk, thus, there is no need to recommend restriction to browsing of this saltbush.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00128-022-03681-6.

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Declarations

Conflict of Interest No potential conflict of interest was reported by the author.

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