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ORIGINAL PAPER

Seasonal variation of selected trace elements in rare endemic species *Thuriferous Juniper* growing in the *Aurès* Mountains of Algeria

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Abstract We used energy dispersive X-ray fluorescence (EDXRF) to determine the seasonal variation of selected trace elements (Cr, Mn, Zn, Cu, Se and Fe) and some potential toxic elements (Cd, Pb and Br) in *Juniperus thurifera* subsp. *africana* Maire (Cupressaceae) a rare medicinal tree, growing indigenously in Aurès Mountains of Algeria. The precision of the results was assessed by analyzing the certified reference material IPE44 (WEPAL) grass leaves. Results showed *J. thurifera* was characterized by high Cr and Mn contents. During autumn and winter mineral concentrations were higher in general. The levels of Cr, Se and Mn were higher during autumn and winter than during spring and summer. Zn contents were higher during summer than in other seasons. Cu content did not vary by season. The potential toxic elements in *J. thurifera* (Pb > Cd > Br) were below the permissible limits recommended by the Joint WHO/FAO guidelines except for Pb in autumn and winter.

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Introduction

Trace elements are important in human health and the biological system through their interaction with hormonal and enzymatic activities. Deficiency of trace elements causes serious physiological and metabolic changes, while surplus can lead to toxicity (Prasad 1993).

Trace elements constitute important components for human body, many enzymes are closely associated with these chemical elements, and many therapeutic effects of medicinal plants are due to the existence of very minor amounts of these elements. On the other hand, it is very important to assessment the elemental composition of medicinal plants for pharmaceutical assays and drugs making (Shirin et al. 2010).

Juniperus thurifera L. (family Cupressaceae) is a long-lived Mediterranean conifer tree of semi-arid areas (Fig. 1). Its geographical distribution extends from Algeria and Morocco over the Iberian Peninsula and the Pyrenees (Spain) to the French and Italian Alps (Adams 2004). Romo and Boratynski (2007) identified two populations of *Thuriferous Juniper* as subspecies; *J. thurifera* subsp. *africana* in North Africa, and *J. thurifera* subsp. *thurifera* in the European province.

In Algeria, *J. thurifera* is a rare endemic species limited to the *Aurès* Mountains (of western Algeria) with a number of scattered and often very large trees that are probably the remains of formerly more extensive stands due to overgrazing and unregulated exploitation (Gauquelin et al. 2012). Despite the international importance of *J. thurifera* in Algeria as a rare endemic species, the main threat to its

Fig. 1 Thuriferous juniper (*Juniperus thurifera* L. subsp. *africana*) on fissured calcareous substrat located at 1300 m of altitude in *Oued Abdi* province (Algeria)

conservation is the devastation of natural stands in addition to the deficient knowledge and consciousness by the local population of the importance of *J. thurifera* forest ecosystems.

J. thurifera trees have their greatest economic potential not so much in contributing to medicinal practices but principally in their uses as wood and forage supply to livestock. *J. thurifera* can provide also non-wood products (cade oil) as well as other benefits are available. Actually the most important opportunities relate to reforestation or replanting to ecological recovery of degraded *J. thurifera* forests.

J. thurifera is a species which has been very little used in forestry research, and nothing is known about its field performance apart from specific instances (Poblador-Soler 1999). This species is considered an important medicinal tree largely used in Algerian folk medicine. Its leaves are widely used in decoctions to treat vomiting and diarrhea. The aromatic berries were chewed to counter bleeding of gums (Bellakhdar 1997). The essential oil of this species has antioxidant, antibacterial and antifungal properties (Mansouri et al. 2010). Its tar (cade oil) is produced by wood distillation and is widely used in traditional medicine (Auclair 1993).

Many authors have reported on the composition of the essential oil from berries and leaves of *Juniperus* species; *J. polycarpus* (El-Sayed 1998); *J. Seravschanica* (Adams 1999); *J. phoenicea* (Ait Ouazzou et al. 2012). However the chemical content of *J. thurifera* has not been studied. Therefore, the present study aimed to estimate for the first time the seasonal variation of selected trace elements in *J. thurifera* leaves using the accurate, precise and reliable measurements of energy dispersive X-ray fluorescence (EDXRF). It seeks to test the hypothesis when the practitioners of traditional medicine use this plant during seasons and to determine the better time during the year to harvest plant parts when concentrations of some essential elements were in optimal levels.

Materials and methods

Plant material and sample preparation

Foliage samples of *J. thurifera* were collected from the area of *Oued Abdi* in *Aurès* Mountains—province of Batna (6°12′ and 6°21′ E, 35°22′ and 35°23′ N and 1300 m.a.s.l., Fig. 2). Vegetation was dominated by *J. thurifera* intermingled with *J. oxycedrus* and *Fraxinus xanthoxyloïdes*. The climate is typically Mediterranean, characterized by wet winters and hot dry summers with mean annual rainfall of 500 mm of which about 80 % is received during July to August. Mean annual temperature is 10.76 °C, with January being the coldest month (mean temperature: −3.05 °C) and July the warmest (mean temperature: 27.61 °C). The soils of this area were predominantly Rendzines and calcareous brown soils. The soil texture varies from sandy to sandy loam with high calcium carbonate (pH 7.5–8.5) and relatively low organic matter (OM) content. In the vicinity of our study area there were

Fig. 2 Location of *J. thurifera* sampling from *Aurès* Mountains, province of Batna (Algeria)

no sources of industrial contamination such as manufacturing, processing or mining.

Five sampling sites were demarcated across the local distribution area of *J. thurifera*. Ten trees from each site were randomly leaves harvested for 4 seasons to measure chemical contents. Subsequently, they were dried in an oven at a temperature of 60 °C. The dried samples were ground to a fine powder (particle size fraction of <200 μm) using agate mortar and pestle.

EDXRF analysis

Plant samples (about 100 mg for each sample) were analyzed using energy dispersive X-ray fluorescence (EDXRF) analysis. The X ray system consisted of source of excitation ^{109}Cd and a Si(Li) type detector with a resolution of 135 keV for the line Kα (5.9 keV) of Mn with active surface of 30 mm^2. Spectra X was collected during a time of 2400 s.

Certified reference plant materials were prepared in a similar manner to the plant samples, and analyzed simultaneously with them for quality control. The reference materials used in this work were IPE44 (WEPAL) grass leaves.

To estimate the precision of the analytical method, we determined the |Z-score| as: $|Z-\text{score}| = \frac{x_{lab} - x_{ref}}{\sigma_{ref}}$, where x_{lab} is the laboratory measured value, x_{ref} is the certified value and σ_{ref} is the standard uncertainty of the certified value.

When the Z-score ≤ 2, the result is in agreement with the certified value;

when $2 < Z$-score ≤ 3, the performance is questionable; and

when the Z-score > 3, the result is not in agreement with the certified value (Siddique and Waheed 2012).

Statistical analysis

The experiment was set up as a completely randomized design, with three replications in each season. Data were analyzed statistically using the SPSS 7.5 software package one-way ANOVA and using Tukey's multiple range test to compare differences between means.

Results and discussion

Nine chemical elements were identified by season in *J. thurifera*. Comparisons of our results for the certified reference material (CRM) to their certified values are listed in Table 1. Measured and certified values were similar for all elements, indicating acceptable performance of the analytical procedure.

Table 1 Comparison of measured values of chemical element mass fraction with certified values in standard reference material CRM—IPE44 (WEPAL) grass leaves

Elements	CRM—IPE44 (WEPAL)		
	Certified value	Measured value	|Z-score|
Br (mg kg^{-1})	24.11 ± 1.2	25.28 ± 2.2	0.98
Cd (μg kg^{-1})	83.9 ± 8.85	88.1 ± 5.14	0.47
Cr (μg kg^{-1})	1844 ± 312	1808 ± 277	0.12
Cu (mg kg^{-1})	7.5 ± 0.38	7.1 ± 0.88	1.05
Fe (mg kg^{-1})	509 ± 27	522 ± 15	0.48
Mn (mg kg^{-1})	79.6 ± 3.4	80.4 ± 7.4	0.24
Pb (μg kg^{-1})	1159 ± 93	1199 ± 68	0.43
Se (μg kg^{-1})	53.04 ± 4.5	53.04 ± 6.2	0
Zn (mg kg^{-1})	33.18 ± 1.33	33.18 ± 3.65	0

Values represent mean ± standard error of mean ($n = 3$)

The Z- score values for all elements were $|Z| < 3$, indicating that the results were within the 99 % confidence interval of the certified values (Table 1).

Season had a significant effect on concentrations of Cr, Se and Mn ($P < 0.001$). Samples collected in autumn and winter had higher concentrations of Cr, Se and Mn than did samples collected in other seasons (Table 2). The lowest Cr, Se and Mn concentrations were recorded in spring and summer.

Season had a significant effect on Fe content of samples ($P < 0.01$). Fe concentrations gradually declined from 754 μg g^{-1} in winter to 460 μg g^{-1} in autumn (Table 2).

Zinc contents varied significantly ($P < 0.01$) by season (Table 2). Zn concentrations were significantly higher during summer and were lower in all other seasons (Table 2). Cu contents did not vary by season (Table 2).

Among assayed trace elements, Cr, Fe and Mn contents were highest (687, 612 and 139 μg g^{-1} respectively) while other essential elements occurred at relatively lower levels, in descending order as Se > Zn > Cu (Table 2).

The highest Cr levels were recorded in autumn and winter seasons (1369 and 1170 μg g^{-1} respectively). In spring and summer, concentrations of Cr were similar. Fe concentrations were lowest in autumn and peaked in winter (754 μg g^{-1}). Mn contents were markedly higher in autumn (368 μg g^{-1}). However, this element was not recorded in spring and summer.

The function of Cr is directly related to the function of insulin, which is important in diabetes treatment (Anderson 1989). Mn is involved in the formation of bone and in specific reactions related to amino acids and metabolism of cholesterol and many metalloenzymes (Crossgrove and

Table 2 Seasonal variation of trace element contents determined in *Juniperus thurifera* growing in *Aurès* Mountains (western of Algeria)

Elements (μg g^{-1})	Seasons				
	Autumn	Winter	Spring	Summer	Seasonal means
Trace elements					
Cr	1369.20 ± 463.95(a)	1170.20 ± 870.04 (a)	168.00 ± 375.66 (b)	41.00 ± 91.68 (b)	687.1
Cu	0.80 ± 0.84 (a)	0.90 ± 0.22 (a)	0.62 ± 1.33 (ab)	0.40 ± 0.89 (ab)	0.68
Fe	460.20 ± 82.66 (c)	753.80 ± 73.49 (a)	659.60 ± 195.10 (ab)	575.20 ± 366.07(b)	612.2
Mn	368.20 ± 368.36 (a)	188.80 ± 327.36 (b)	0.00 ± 0.00 (c)	0.00 ± 0.00 (c)	139.25
Se	29.20 ± 20.22 (a)	31.00 ± 24.36 (a)	5.00 ± 11.18 (b)	1.60 ± 3.58 (b)	16.7
Zn	13.00 ± 10.44 (b)	02.40 ± 2.88 (b)	0.00 ± 0.00 (b)	30.60 ± 68.42 (a)	11.5
Toxic elements					
Br	2.28 ± 1.60 (b)	1.60 ± 1.54 (b)	1.28 ± 1.23 (b)	3.24 ± 2.67 (a)	2.1
Cd	36.60 ± 28.61 (a)	9.20 ± 17.41(b)	4.20 ± 3.83 (b)	43.40 ± 10.45 (a)	23.35
Pb	466.80 ± 212.91 (b)	791.40 ± 108.87 (a)	0.00 ± 0.00 (c)	0.00 ± 0.00 (c)	314.55

Values represent mean ± standard error of mean (n = 3). Values with different letters are significantly different ($P < 0.01$, Tukey's test)

Zheng 2004). Cu is a constituent of several metalloenzymes such as ferroxidases and it is involved in mitochondrial functions (Otten et al. 2006). Selenium is an antioxidant nutrient involved in the defense against oxidative stress. It regulates thyroid hormone actions (Ellis and Salt 2003). Zinc is an important constituent for sperm fertility and is involved in the regulation of gene expression (Prasad 2008).

Br content remained similar during winter, spring and autumn seasons. However a significant ($P < 0.01$) increase in Br was observed during summer (Table 2).

Pb concentrations varied significantly by season ($P < 0.01$). They were higher during autumn and winter than in other seasons (Table 2).

Cd concentrations were significantly ($P < 0.01$) higher in summer and autumn, and the lowest Cd concentrations were recorded in winter and spring (Table 2).

Potentially toxic elements were present in descending concentration as Pb > Cd > Br, at respective values of 315, 23 and 2 μg g^{-1}. All were below the permissible limits recommended by the Joint WHO/FAO (1994) guidelines except for Pb in autumn and winter (Table 3).

Explanations for these results include differential bioavailability of trace elements depending on soil type and soil characteristics (e.g., pH, texture (clay), OM contents, cation exchange capacity (CEC), moisture, root exudates and mycorrhyzea activity) and precipitation reactions and in soil solution (Fotovat et al. 1997).

Table 4 shows comparison of our results with those reported for *Juniperus* species in other countries (Kula et al. 2010; Ozkaya et al. 2013; Maghrabi 2014; Hussain et al. 2013). Our results show that the mass fractions of Fe and Cu were lower than those reported by Maghrabi (2014) and Ozkaya et al. (2013) who evaluated the chemical profile of *J. communis and J. oxycedrus* growing in Saudi Arabia and Turkey, respectively. Our concentrations of Cr, Mn and Se were higher than those reported for Saudi Arabia and Turkey. Results from different countries show large differences for some reported elements, suggesting that chemical composition of species depends on many factors such as soil texture, climatic conditions and vegetal species (Kabata-Pendias and Pendias 1992; Nedjimi et al. 2015).

Globally, the ecology and importance of *J. thurifera* have been largely ignored. In Algeria for instance, there is no restriction and interdiction on villagers to overexploitation and overgrazing. Therefore, continuing degradation by browsing and woody exploitation is subsequent in the loss of *J. thurifera* associations.

The local authorities should consider stricter control on destructive actions to these tree communities such as cutting or grazing. At the same time the participation of local communities and educational programs were needed to

Table 3 *J. thurifera* mean contents, provisional tolerable weekly intake (PTWI) and tolerable daily intake (TDI) of some toxic elements for adult with 70 kg body weight (BW)

Elements	PTWI	TDI*	*J. thurifera* mean content
Pb	50 μg kg BW^{-1} $week^{-1}$	500 μg day^{-1} $person^{-1}$	314.55 μg g^{-1}
Br	7 mg kg BW^{-1} $week^{-1}$	70 mg day^{-1} $person^{-1}$	2.10 μg g^{-1}
Cd	7 μg kg BW^{-1} $week^{-1}$	70 μg $day^{-1}$$person^{-1}$	23.35 μg g^{-1}

* (WHO/FAO 1994)

Table 4 Some chemical elements reported in Juniperus sp. from different regions of the world

Elements (μg g^{-1})	*Juniperus* sp., literature				
	J. communis (Maghrabi 2014) Saudi Arabia	*J. oxycedrus* (Ozkaya et al. 2013) Turkey	*J. foetidissima* (Kula et al. 2010) Turkey	*J. Excelsa* (Hussain et al. 2013) Oman	*J. thurifera* (our study) Algeria
Cr	0.72	2.87	–	–	687.10
Cu	3.48	7.10	–	–	0.68
Fe	315.1	187.95	7.59	0.77	612.20
Mn	29.67	27.79	0.38	–	139.25
Se	1.26	–	–	–	16.7
Zn	4.33	7.70	0.83	0.03	11.5

increase villager awareness and enhance conservation of these ecosystems in the long term.

Conclusion

The data obtained in the analysis of *J. thurifera* samples indicated the existence of essential elements that could enhance the curative process. Significant variations were found between seasons for the studied trace elements. The toxicity of some of the non-essential trace elements in all samples is well below known toxicity limits specified in international guidelines.

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