ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Seasonal Variations of Cu, Zn, Mn and Fe Levels in Soil and *Atriplex halimus* in Arid Zones of South East Algeria.

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ABSTRACT

The present study was conducted to evaluate the trace minerals contents (Cu, Zn, Mn and Fe) in soil and *Atriplex halimus* collected from Essaada area in South-East Algeria. The soil and plant samples were taken from 10 random locations in studied area each season during 2014-2015. Seasonal effects were observed in all soil trace mineral except for manganese. Copper, zinc, manganese and iron concentrations in *A. halimus* were affected by season ($p < 0.05$). Trace mineral soil contents in all seasons were considered sufficiently high to meet requirement for normal plant growth. In *A. halimus* Cu, Zn and Mn levels were ranged from marginal deficient (5.6, 27 and 33 mg/kg DM respectively) to marginally above the range requirements for ruminants (13, 43 and 64 mg/kg DM respectively). High Fe levels in *A. halimus* in all seasons were sufficiently to meet the recommended requirements of ruminants (> to 35- 50 mg/kg DM) and is on agreement with the high soil Fe level. There was a positive correlation ($r = 0.77$, $p<0.001$) between Cu in soil and Cu in *A. halimus*.
Keywords: Arid zones, soil, *Atriplex halimus*, trace mineral, seasonal variation, ruminants.

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INTRODUCTION

The forages are important component of feed for livestock. Under pasture systems, animals depend on forages to satisfy all of their nutritional requirements [1]. The climate variation leads to drought that becomes a constraint for the plant biodiversity mainly in the arid regions of Algeria. Drought exposed the soil to salinization for many years. Salinity is one of the major limiting factors that affects adversely growth and development of plants, agricultural productivity and caused disappearance of some plant species [2]. The most characteristic of wetland in these areas are endorheic Wetlands (Chotts and Sebkha). They are typically seasonal which dry out in summer and re-flood in winter [3]. Local vegetation is mainly composed by halophytic, succulent, and perennial species. They are essential in the contribution to nutritional needs for livestock. Generally, salinity, scarce and irregular precipitations, excessive temperature are critical environmental factors that influence the life cycle of these plants and their incidence on nutritional value of arid browses is not yet established [4]. *Atriplex halimus* (Chenopodiaceae) is a xerohalophyte, which is perennial and native in arid and semi-arid Mediterranean regions. Their various agronomic and nutritional aspects are well-characterized [5].This species tolerate well, harsh conditions such as salinity, light stress, cold and drought. It has been reported that this plant survive under rainfall of 100-400 mm year and can survive between one and several years without rainfall [6-7]. *A. halimus* is very palatable and is a preferred fodder shrub of livestock during dearth periods, endowed with a complex root system and a considerable biomass; it is an efficient cheap barrier against erosion and desertification [3]. *A. halimus* in South-East Algeria reveals a relatively high content in ash (15 % DM) and crude protein (13 % DM) [4]. The mineral contribution of this plant to animal remains poorly investigated in this region. The nutrition of grazing animals is a complicated interaction between soil, plant and animal [1]. Many factors affect the mineral composition of forage such as plant age, species, seasonal variability, grazing pressure, change in the moisture content and salinity of the soil. In arid area of Algeria, trace elements such as Cu, Zn and Mn are likely to be lacking to ruminants production under grazing pasture. Knowledge on these mineral compositions in *A. halimus* would form base-line data on trace mineral status available for small ruminants grazing this plant in these areas. The objective of this study was to evaluate and compare, seasonally during one year, the trace minerals Cu, Zn, Mn and Fe contents in soil and *A. halimus* that grow in South-East Biskra (Algeria), as indicators of minerals contribution, deficiencies or excess during each season, and to determine correlations between levels of these trace mineral in soil and *A. halimus*.

MATERIAL AND METHODS

Location and climate

The study was conducted in Essaada which is located 30km from Biskra in South-East Algeria (34°40' N latitude and 5°52' E longitude). The climate of this grazing area is arid with an average annual rainfall of 147 mm year mainly during the end of autumn to the beginning of spring. The average minimum winter and maximum summer temperature are 7°C in January and 41°C in July.

The natural vegetation is represented by halophytes such as *Atriplex halimus*, *Salsola vermiculata* and *Sueada mollis* [5]. On saline and degraded soils, it is often the dominant plant species, forming mono-specific stands [7]. Those plants mainly *A. halimus* use excluded and included cationic strategies to tolerate the constraint salinity [2]. This halophyte, particularly its leaves, constitute a solution to feed shortages that occur during drought and dormant seasons for small-ruminants.

Samples collection

The soil and plant samples were taken from 10 random locations in studied area each season during the year 2014-2015 (10 October 2014, 10 January 2015, 10 April and 10 June 2015) and distance between a different sampling positions varied from 10 to 15 m . Sampling was performed on a surface about 400 m^2. Plant samples were cut using a stainless steel knife and placed in plastic bags. Plant samples were dried in oven at 55°C for 72 h [8] and subsequently ground, with a 1mm stainless steel sieve and stored in polypropylene bottles for subsequent analysis. The wet digestion with nitric-perchloric acid mixture [8] was made to obtain extracts for the determination of trace minerals.

Ten soil samples were collected each season near the plants along the sampling surface, using a stainless steel sampling auger at a depth of 20 cm. Samples were collected in paper bags, dried at 65°C for 72 h and subsequently ground and passed through a 2 mm sieve [3]. Ground soil was also stored in closed plastic bottles. The soil pH and the grain size distributions were determined according to the technique recommended by ISRIC [9]. The loss on ignition (L.O.I) was determined after calcinations of a dry soil sample (1g) at 520°C during 16 h in furnace. The loss on ignition was expressed as a percentage of the weight loss.Ca, Na and K contents of the soil samples were analyzed using Atomic Absorption Spectrophotometry . To prepare samples for trace mineral determinations, one gram of ground sample of soil was boiled in 10 ml of aqua regia (a mixture of HNO_3 and HCl: 1/3 ratio) for 2 h at 100 °C using the refluxing system. The extract was cooled and filtered using Whatman filter paper (N°540) and diluted to 50 ml with distilled water. Both extract solutions obtained for soil and plant samples were stored at 4°C until analysis. Trace minerals in soil and *A. halimus* were measured using Atomic Absorption Spectrophotometry with air/acetylene flame (Shimadzu model AA6800).

Statistical analysis

The data were analyzed using Medcalc software (12.7.1version) [10]. Season effect for soil and plant samples was determined by one-way analysis of variance. Differences between mean were ranked using the Student Newman Keuls and significance levels was set at $P<0.05$. Correlation coefficients of soil-plant mineral contents were determined after pooling the data for mineral levels of soil and *A. halimus*.

RESULTS AND DISCUSSION

Physico-chemical soil analysis in study area are shown in table 1. Soil texture is silt-clays, organic matter content is relatively high (2.7 %) and the soil pH is basic (8.1). High pH is due to the presence of more base cations and to relatively low precipitation amounts. Therefore, the micronutrients (Cu, Zn, Mn and Fe) availability for plant will be decreased.

Table 1: Soil properties in study area

Clay (%)	Silt (%)	Sand (%)	OM (%)	pH	Na (g/kg)	K (g/kg)	Ca (g/kg DM)
38	44.5	17.5	2.7	8.1	3.3	5.1	159

Calcium concentration (159 g/kg) far exceeded those of sodium and potassium (3.3 and 5 g/kg respectively) (table 1). High Ca level may be a result of soil high in calcium carbonate ($CaCO_3$) and other Ca salt, that are responsible in high pH soil. Na and K contents are the two element responsible for increase in osmotic potential of soil and were most abundant in saline area.

Soil and *Atriplex halimus* analysis

Table 2 and 3 show the trace mineral concentration in soil and *A. halimus*. The mean Cu concentration in soil varied from 8 to 18 mg/kg DM (table 2). The highest ($P<0.05$) mean soil Cu level was recorded in autumn 18 mg/kg DM and followed by spring 13 mg/kg DM (table 2). Cu level remained unchanged in winter and summer. A seasonal effect was observed ($P<0.001$). Cu levels in both seasons winter and summer were lower ($P<0.05$) than those measured in autumn and spring. All soil samples during the year had levels of copper more than adequate for normal growth of plants (table 2). The mean soil Cu concentration was higher than the critical level (table2) of 0.3 mg/kg stated by McDowell *et al*. [11] and Rojas *et al*., [12] and 0.6 mg/kg suggested by Horowitz and Dantas [13].

High Cu levels in the present study were in disagreement with those reported by Khan *et al*. [1] in Pakistan and by Shisia *et al*. [15] in Kenya. Mineral concentration like Cu, Zn, Mo, Co in soil has a great effect on soil pH, which, in turn has a major impact on mineral uptake by plants [16]. Therefore copper availability to plant decreases with increase in soil pH [14-17]. A significant seasonal effect ($P<0.001$) was observed in *A. halimus* Cu concentration. Mean Cu concentration in plant was high in autumn 13 ± 3.2 mg/kg DM, followed by spring 10 ± 1.7 mg/kg DM (table 3). A significant decrease ($P<0.05$) was observed in winter 5.6 mg/kg DM, which could be attributed to leaching due to the *wadi* re-flooding in this area. Cu levels in *A. halimus* are corroborated with the finding by Haddi *et al*. [4] and Arab *et al*. [8] in some forage plants studied in the same area. *A. halimus* Cu levels in all seasons were higher than those reported by Ramirez *et al*. [18] in shrub species in Mexico and lower than to those reported in Pakistan [1]. Cu concentrations in forage plant were considered sufficient to meet ruminants requirements (10 mg/kg DM) [19-20] during autumn and spring. However, the levels in summer and winter (table 2) were only meeting the marginal to deficient requirements of ruminants respectively. As for other ruminants, the copper requirement of goat may be assessed at 8-10 mg/kg DM [21]. The goats, especially young animals seem to be less sensitive to copper toxicity than sheep. Poisoning has been known to occur in areas where the herbage contains copper of the order of 10-20 mg/kg DM and low levels of molybdenum [22]. Chronic copper poisoning results in necrosis of the liver cells, jaundice, loss of appetite and death from hepatic coma. The absorption of Cu by plant can be reduced with high level of Ca and Fe in soil. In our study area, Ca (table 1) and Fe (table 2) in soil were high which may lead to making Cu unavailable for plants. Cu deficiency problems are most likely to occur because of the presence of antagonistic mineral in the diet rather than extremely low intakes of Cu. A positive correlation was observed between soils and plant forage Cu during all seasons (r=0.77; P<0.001) (figure 1), which indicate that Cu concentrations in plant are depending on Cu concentrations in soil.

Table 2: Mean concentration (± SD) of trace minerals in soil during the different seasons

Season	Cu (mg/kg DM)	Zn (mg/kg DM)	Mn (mg/kg DM)	Fe (g/kg DM)
Autumn (n=10)	$18^{a} \pm 4.5$	$53.4^{c} \pm 16$	263 ± 68	$27^{a} \pm 8.9$
Winter (n=10)	$8^{c} \pm 3.4$	$108.3^{a} \pm 28$	250 ± 23	$28^{a} \pm 4$
Spring (n=10)	$13^{b}.1 \pm 1.9$	$65^{b} \pm 13$	238 ± 76	$21^{b} \pm 3$
Summer (n=10)	$8^{c} \pm 1.9$	$111.3^{a} \pm 55.5$	230 ± 43	$27.5^{a} \pm 5.4$
Season effect	***	***	ns	*
Critical value (mg/kg)[1]	0.3	1-2.5	5	2.5[2]

*,**,*** Significant at 0.05, 0.01 and 0.001 levels, respectively. ns: non-significant. Means with different letters in each group, are significantly different (P<0.05). n: sample size.
[1] Rhue and Kidder (1983), [2] Viets and Lindsay (1973).

Table 3: Mean concentration (± SD) of trace minerals in *Atriplex halimus* during the different seasons

Season	Cu (mg/kg DM)	Zn (mg/kg DM)	Mn (mg/kg DM)	Fe (mg/kg DM)
Autumn (n=10)	$13^{a} \pm 3.2$	$26^{c} \pm 3$	$64^{a} \pm 22$	$228^{c} \pm 56$
Winter (n=10)	$5.6^{d} \pm 1.7$	$43^{a} \pm 19.4$	$37.6^{b} \pm 12.5$	$1958^{a} \pm 464$
Spring (n=10)	$10^{b} \pm 1.7$	$27^{c} \pm 4$	$38^{b} \pm 10$	$498^{b} \pm 159$
Summer (n=10)	$7.4^{c} \pm 1.9$	$32^{b} \pm 6.5$	$33^{c} \pm 20.1$	$501^{b} \pm 178$
Season effect	***	**	***	***
Ruminants requirements (mg/kg DM)[1]	10	50-60	50-60	50
Goat requirements (mg/kg DM)[2]	9	30	30	35

*,**,*** Significant at 0.05, 0.01 and 0.001 levels, respectively. ns: non-significant. Means with different letters in each group, are significantly different (P<0.05). n: sample size.
[1] Recommended requirement by INRA (1988) and Meschy (2010).
[2] Recommended requirement by NRC (1981); Kessler (1991) and Ramirez-Orduna and al., 2005).

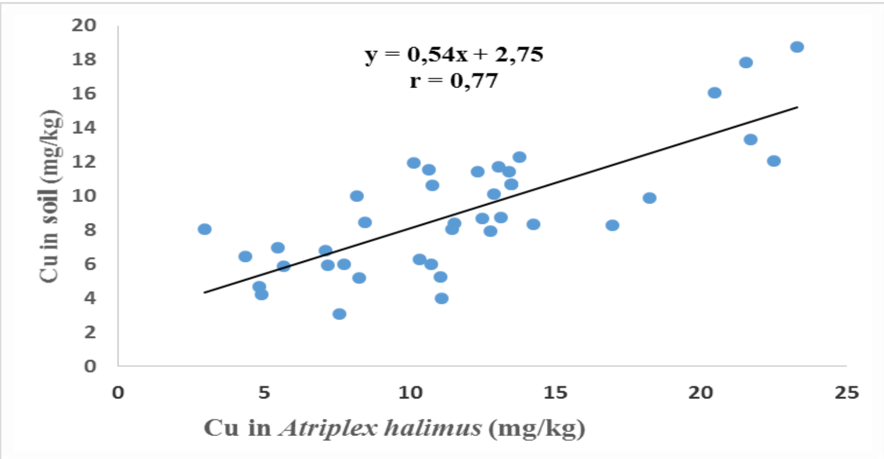

Figure 1: Correlation between copper levels in soil and in *Atriplex halimus*

Mean Zn concentration in soil varied significantly ($P<0.001$) with season (table 2). The high Zn levels in soil ($P<0.05$) occurred in summer 111 mg/kg and winter 108 mg/kg DM. A significant reduction in soil zinc level was observed during autumn and spring 53 and 65 mg/kg DM respectively. The critical level varied from 1 to 2.5 mg/kg of Zn in soil was considered adequate for plant growth [23-1]. Based on this, all mean values of soil Zn level in the present study are higher than critical value. Our results were higher than those found in Kenya (South-east Africa) [15] and in a semi-arid region of Zimbabwe [24]. Soil Zn is usually more available in soils with greater organic matter content and higher proportion of clay [25]. In contrast, Zn extractability from soil is negatively related to phosphate and calcium carbonate content in soil, therefore, low plant availability of Zn can be expected in calcareous soils [25].

The mean Zn concentration in *A. halimus* ranged from 26 to 43 mg/kg DM and a significant season effect was observed ($P<0.01$) (table 3). A high level was observed in winter 43±19.4 mg/kg ($P<0.05$). Similar values were in autumn and spring (table 3). Those concentrations are marginally above the Zn requirements for goat (30 mg) [18-26-27] and slightly deficient for sheep and cattle (50-60mg) [19-20]. Deficiencies Zn in studied plant is probably due to the calcareous soil. It was noted that there was an inverse relationship between Ca and Zn [28-29]. The Zn content of *A. halimus* in South Algeria is higher than that measured in South Africa at Lovedale (11 mg/kg DM) [29], site with an average annual rainfall of approximately 130 mm, similar to our arid area and similar to those obtained in forage collected in late stage of growth in a semi-arid region of Zimbabwe [24].

Besides, the Zn dietary requirement of 30 mg/kg DM was considered sufficient for growing, gestating and lactating for beef cattle according to Olsen [17]. In the present study, Zn levels in *A. halimus* could meet the requirements of ruminants in study area. Increasing soil pH, especially above 6.5, results in decreased extractability and plant availability of soil Zn [17-25], possibly it is among the causes of lower levels of Zn in plants. There was no relationship between Zn levels in soil and Zn levels in plant ($r= 0.24$; $P>0.05$).

Mn levels varied from 230 in summer to 263 mg/kg DM in autumn (table 2). No season effect on soil Mn level was observed ($P>0.05$) (table2). Khan *et al.* [1] reported that generally, soil Mn concentration was higher during summer than that in winter but this seasonal trend is not the case with the present study. Rhue and Kidder [23] suggested that 5 mg/kg of Mn is adequate for normal plant growth. The soil in our study area provide high levels of Mn for plants. Availability of Mn to plants depends on its oxidation state, only the reduced form Mn^{2+} is available to plants [25]. Mn becomes available for plant after release of Mn^{2+} into the soil solution, Mn^{2+} transport to the root surface by mass flow and diffusion, followed by uptake into the root.

A significant seasonal effect ($p<0.01$) was observed in *A. halimus* for Mn. High Mn concentration ($P<0.05$) was recorded in autumn (table 3). A similar values were observed in winter and spring. In all seasons forage Mn levels were marginally above the Mn requirement for goat and deficient for sheep and cattle except in autumn (table 3). The Mn content of *A. halimus* in South Algeria is lower than that measured in the same plant in South Africa (116 mg/kg DM) [29]. At low pH below 5, Mn may become available to plants, and lead to Mn accumulation and toxicity in plants [30]. However, in calcareous soil Mn is insolubilized to oxides manganese, which can generate deficiencies for plant. Soil conditions that impact Mn availability to plant include: pH, Mn can precipitate at high pH leading to low Mn availability, Mn^{2+} is readily chelated by organic molecules and under dry soil conditions, the Mn availability is reduced [30]. Underwood and Suttle [28] reported that Mn concentrations in plants decrease markedly as soil pH increase. It is knowing that silt-clays soil had high level of Ca to lead a low level of Mn in plant. The high pH and Ca content in soil in study area were among possible causes of low levels of Mn in *A. halimus*. As for Zn, no correlation was observed between Mn level in soil and Mn level in *A. halimus* ($r=0.03$; $P>0.05$).

Mean Fe concentration in soil ranged from 21 to 28 g/kg DM (table 2). A significant seasonal effects in soil Fe content was observed at $P<0.05$. Similar levels of soil Fe was observed in winter 28, summer 27.5 and autumn 27 g/kg DM (table 2). A slight decrease was observed in spring 21± 3 g/kg DM. The normal range of Fe for the growth of plants is 2.5 mg/kg [31]. Fe is an element relatively abundant in many cultivated soils, with an average of 20 to 40 g/kg [32]. Solubilization of Fe from soil mineral source is a slow process regulated by pH [32]. Fe availability for plants decrease with increasing soil pH [25]. High pH promotes carbonate or hydroxyl complexes which could decrease the iron availability for plants. High levels of iron in the soil in the present study is probably due to the soil nature which is rich in iron oxides. Ours results support the idea that Fe deficiency is rare in grazing animals due to generally adequate content in soil and forages as reported by Khan *et al.* [14].

Mean Fe levels in *A. halimus* varied from 228 to 1958 mg/kg. The high level (P<0.05) was recorded in winter may be due to contamination of plant with soil. Seasonal effect was significant (P<0.001) (table 3). High level of Fe in *A. halimus* found in this study area is an agreement with the high soil Fe level. Iron levels in *A. halimus* at all seasons were above requirements (>35-50 mg/kg DM) (table 3) for ruminants. Similar findings were reported by several authors who evaluated Fe contents in shrubs species that grow in semiarid regions of North-Eastern Mexico cited by Ramirez-Orduna *et al.* [18].

A. halimus Fe levels in autumn and spring were similar to those reported in the same seasons by Ramirez-Orduna *et al.* [18] in California. The maximum tolerable level of Fe in beef cattle diets was estimated at 1000 mg/kg DM [33-17]. The high concentration of Fe in *A. halimus* during winter can be toxic for ruminants grazing this plant. Iron toxicity is not a common problem in farm animals, but it can result from prolonged oral ingestion of this element [22]. Meschy [20] reported that the high iron content in plants does not seem to represent a significant risk of Cu unavailable for ruminant, except when they are contaminated with soil. However, the excess iron content in drinking water is more reactive with Cu and leads to a secondary copper deficiency. There was no relationship between Fe level in soil and Fe level in *A. halimus* (r =0.20; P>0.05).

CONCLUSION

Our results indicated that trace mineral in soil were above recommended levels for growth plant. The high Fe level in *A. halimus* was probably due to high Fe availability in soil to plant and its ability to accumulate this element. Generally trace mineral levels in *A. halimus* ranging from sufficient to marginal levels in each season. High pH (8.1) and Ca (159 g/kg DM) in soil of studied area were possibly the cause of low levels of Cu, Zn and Mn in *A. halimus.* The deficiencies may be limiting the local production of ruminants that grazing this forage. The knowledge of the translocation of mineral from soil to *A. halimus* and the bioavailability of these elements for ruminants deserve further investigations.

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