Measurement 129 (2018) 256–259

Contents lists available at ScienceDirect

Measurement

journal homepage: www.elsevier.com/locate/measurement

Measurement of selenium in two Algerian chenopods (*Atriplex canescens* (Pursh.) Nutt. and *Suaeda fruticosa* (Linn.) Forssk)

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ARTICLE INFO

Keywords:
Selenium
Atriplex canescens
Suaeda fruticosa
Deficiency
Algeria
NAA measurement

ABSTRACT

Neutron activation analysis laboratory (NAA measurement) was applied for the determination of selenium (Se) contents in two perennial forage species (*Atriplex canescens* (Pursh.) Nutt. and *Suaeda fruticosa* (Linn.) Forssk) used in extensive livestock production systems, in arid rangelands of Algeria. Se content was estimated in leaves and in the soil in which the plants were grown. The vegetal and soil samples were irradiated for 6 h duration with a thermal neutron flux of $4.5 \times 10^{13}\,cm^{-2}\,s^{-1}$ of Es-Salam research reactor (Berine, Algeria). The daily intake of Se was determined and compared with the values recommended by National Research Council (NRC) guidelines. Result showed that the highest contents of Se were found in the rainy period (January–15 winter) for both species. By way of contrast, for the surrounding soil this metalloid was higher during dry season (July–15 summer). The maximum uptake of Se was predominantly recorded in *A. canescens* ($0.16 \pm 0.07\,\mu g\,g^{-1}$) compared to *S. fruticosa* ($0.07 \pm 0.03\,\mu g\,g^{-1}$). The bioaccumulation factor (BF_{Se}) from soil to plant showed a high enrichment of Se especially for *A. canescens*. In conclusion, this study envisages that *A. canescens* can be gazed by livestock in winter season as a source of Se; however the problem of Se deficiency was expected during the other seasons for animals grazing *A. canescens* and *S. fruticosa*.

1. Introduction

Selenium (Se) is an antioxidant essential metalloid involved in the protection against oxidative stress [13]. It helps to regulate thyroid hormone activities and enhances absorption of vitamin E [14]. Se deficiency is one of the major limiting factors for grazing ruminants in the world [19]. White muscle disease (WMD), poor growth rate, immunity dysfunction, infertility and low glutathione peroxidase activity (GPX) were the most recognized diseases of Se deficiency [10].

For small ruminants (sheep and goats), the normal Se level is $0.1\,\mu g\,g^{-1}$, but a more practical content is $0.2\,\mu g\,g^{-1}$ particularly if fodder was deficient in vitamin E or in the case of high level of sulfur (S). The occurrence of WMD in livestock was frequently associated to fodders containing Se less than $0.1\,\mu g\,g^{-1}$ DM [15]. Inorganic selenite (Se^{4+}) or selenates (Se^{6+}) were the two forms of Se largely supplemented to livestock to meet their requirements [11].

Atriplex canescens (Pursh.) Nutt. is the most widespread saltbush in North USA [17]. This halophyte is well adapted to extreme environmental conditions (drought and salinity) and may possess interesting properties for soil rehabilitation. *A. canescens* has been introduced in Algeria since 1987 as a source of fodder in pastoral plantations. *Suaeda fruticosa* (Linn.) Forssk is a perennial shrub which occurs naturally throughout the Algerian salt rangelands and is used widely to provide forage to small ruminants [16].

Neutron activation analysis (NAA) is a nuclear analytical measurement able to determining simultaneously large rang of chemical elements in different matrices with high sensitivity, no spectral interference and good detection limits. This methodology uses neutrons for samples irradiation and measures their radioactivities using γ-ray spectrometry [8].

Information about seasonal dynamics of Se in Algerian grazing fodder species is fairly limited. In the present study we aimed to determine seasonal changes of Se levels during one growing year in two chenopods (*A. canescens* and *S. fruticosa*) mostly grazed by livestock in central Algerian rangelands, in relation to Se requirement of small ruminants.

2. Materials and methods

2.1. Collection and sample preparation

Atriplex canescens, S. fruticosa and topsoil samples were collected from *Mesrane* region (Djelfa province) in the central rangelands of Algeria (3°03′E, 34°36′N and and 830 m a.s.l.). Topsoil samples and aboveground biomass of *A. canescens* and *S. fruticosa* were took place at regular intervals (middle of each seasons) during one growing year

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https://doi.org/10.1016/j.measurement.2018.07.029
Received 18 July 2017; Received in revised form 1 June 2018; Accepted 12 July 2018

(January–15, April–15, July–15 and Oct–15 of 2015), by collecting the shoot biomass (recently expanded leaves) in ten (10) randomly shrubs for each species according to the approach described by Bacha et al. [3]. In order to provide the Se determination in topsoil, three composites of soil samples were randomly collected at each season, from the same site which the plant samples were taken. Soil samples were obtained using a stainless steel sampling auger to a depth of 10–20 cm (rooting zone). Each composite soil sample was obtained by mixing three subsamples. The soil and plant samples were oven-dried at 65 °C/48 h, fine powdered in agate mortar (diameter of 200 μm) and stored in the capped sterilized bottles.

2.2. Sample irradiation by NAA technique

Approximately 120 mg of each sample (vegetal and soil) were placed in an aluminum (Al) irradiation capsules and irradiated for 6 h duration with a thermal flux $4.5 \times 10^{13}\,cm^{-2}\,s^{-1}$ in Es-Salam research reactor, Algeria (15 MW heavy water reactor type). All activities were measured by a coaxial HPGe detector (Canberra), with 1.8 keV resolution at 1332.5 keV γ-peaks of ^{60}Co with 35% RE (relative efficiency). Five replicates (05) from each sample (species and soil) were prepared together with two (02) certified reference materials CRMs [IAEA 336 (Lichen) and WEPAL–ISE 41–868 (Sandy soil)] to determine the accuracy of analytic measurement. The samples were allowed for cooling time of 04 weeks, the γ transition generated by neutron capture ^{74}Se (stable isotope) ^{75}Se ($E\gamma = 264.7$ keV, $t_{1/2} = 120$ days) was used to determine the concentration of Se. This γ-ray is interfered with those at 264.8 keV of ^{75}Ge ($t_{1/2} = 82.78$ min) and 264.4 keV of ^{77}Ge ($t_{1/2} = 11.30$ h). These interferences were eliminated by long cooling periods (04 weeks) necessary for the decay of short-lived activation radionuclides. The commercial software HyperLab (2005) was used for spectral assessment.

2.3. Measurement and statistical analysis

According to Greenberg et al. [8], the concentration of Se was obtained using the formula (1):

$$\rho_{samp.}(\mu g\,g^{-1}) = \frac{[(N_p t_m^{-1})DC^{-1}]_{samp}}{[(N_p t_m^{-1})DC^{-1}]_{stand}} \cdot \frac{[\rho_{stand.} W_{stand.}]}{W_{samp.}} \quad (1)$$

The two indices '*samp.*' and '*stand.*' indicate the sample and the standard respectively, $\rho_{samp.}$: the concentration of the Se in sample, Np: the net photo-peak values, t_m: the decay constant for the (n,)) product radionuclide, D and C: the decay and counting factors respectively, W: the sample masse and $\rho_{stand.}$: the concentration of the Se in standard.

In order to evaluate the precision of the NAA measurement, the three statistical parameters (*Z*-score, *U*-score and accuracy) were determined. These parameters are calculated according to the following formulas (2–4):

$$|U\text{−score}| = \frac{(Se_{mes} - Se_{cert})}{\sqrt{\sigma^2{}_{mes} + \sigma^2{}_{cert}}} \quad (2)$$

where: Se_{mes}, σ_{mes}, Se_{cert} and σ_{cert} are the measured value of Se in the CRM, the standard uncertainty of Se_{mes}, the certified value and the standard uncertainty of Se_{cert}, respectively.

The technique performance is evaluated as satisfactory if *U*-score ≤ 1 and unsatisfactory for *U*-score > 1 [5].

$$|Z\text{−score}| = \frac{Se_{mes} - Se_{cert}}{\sigma_{cert}} \quad (3)$$

The laboratory performance is assessed as adequate if *Z*-score ≤ 2, questionable for 2 < *Z*-score < 3 and inadequate for *Z*-score ≥ 3 [18].

$$Accuracy = \frac{Se_{mes} - Se_{cert}}{Se_{cert}} x100 \quad (4)$$

The precision of measurement is adequate if the accuracy is lower than ± 10% [20].

The Plant capacity to uptake Se (bioaccumulation rate) from its surrounding soil layers was evaluated by the bioaccumulation factor (BF_{Se}), expressed by the following formula [6] (5):

$$BF_{se} = \frac{Se_{plant}}{Se_{soil}} \quad (5)$$

where: Se_{plant} and Se_{soil} are the contents of Se in the plant and soil samples, respectively.

A one-way analysis of variance (ANOVA) was used to test effects of the seasons (sampling date) on the Se contents in both species and their surrounding soil. *Tukey*'s multiple range test ($P < 0.05$) was carried out to determine least significant range between means. A Principal Components Analysis (PCA) was used in order to elucidate possible relationships between season and the bioaccumulation of Se in both species. PCA is a statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components (PCs). The first principal component (PC1) is required to have the largest possible variance. The second component (PC2) is computed under the constraint of being orthogonal to the first component [2]. All statistical calculations were carried out using Software Statistica, version 9.1.

3. Results and discussion

The accuracy of measurement was checked by the analyses of two standard reference materials IAEA 336 (Lichen) and WEPAL–ISE 41–868 (Sandy soil). Table 1 gives a comparison of our results for the CRMs to its certified values and the statistical parameters *Z*-score, *U*-score and accuracy. From this table it can be seen that Se has a *U*-score and *Z*-score less than 1 which shows that our reported results are in good agreement with the certified values of two CRMs. For both standards our results showed accuracy below 10% signifying the reliability and precision of the analytical methodology employed.

Results of Se contents in *A. canescens and S. fruticosa* chenopods are presented in Fig. 1, and the concentrations are given on dry weight basis (i.e., $\mu g\,g^{-1}$) as means of five replicates ± standard error. The seasonal patterns had a significant influence on Se content ($P < 0.05$) for both species (Table 2). For all season, the data show that *A. canescens* exhibits high contents of Se compared to *S. fruticosa*. The maximum values of Se were found in January–15 (winter), with an average of 0.160 ± 0.07 and 0.072 ± 0.03 $\mu g\,g^{-1}$ respectively for *A. canescens and S. fruticosa*. Se contents did not change ($P > 0.05$) for summer and autumn seasons for both species (Fig. 1).

A one-way ANOVA showed that season (sampling date) had a significant effect on concentrations of Se ($P < 0.05$) in surrounding soil in which the plants were grown (Table 2). Fig. 2 displayed the contents of

Table 1
Comparison of measured values of selenium ($\mu g\,g^{-1}$) with certified values in standard reference materials IAEA 336 (Lichen) and WEPAL–ISE 41–868 (Sandy soil). Values represent mean ± SE (n = 5).

Standard reference materials	Certified values	Measured values	|*U*-score|	|*Z*-score|	Accuracy
IAEA 336 (Lichen)	0.22 ± 0.03	0.215 ± 0.03	0.12	0.16	2.27
WEPAL–ISE 41–868 (Sandy soil)	0.053 ± 0.004	0.054 ± 0.008	0.11	0.25	1.89