Microwave assisted digestion coupled with inductively coupled plasma optical emission spectrometry for determining element concentrations in halophytes

Narges Chamkouri[1*], Masoud Torabpour[2], Farokh Ghafarizadeh[1]

1. Abadan School of Medical Sciences, Abadan, Iran
2. Department of Nursing, Abadan School of Medical Sciences, Abadan, Iran

[*]**Corresponding author:** Tel: +98 6153335358; fax: +98 6153335358
Address: Abadan School of Medical Sciences, Abadan, Iran
E-mail: narges.chamkouri@gmail.com
Received; 2015/03/31 accepted; 2015/04/11

Abstract

Introduction: This study was carried out to determine elements (Li, Na, K, Mg, Ca, Ba, B, Al, Si, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Ag and P) in the Suaeda vermiculata (S. vermiculata) and Suaeda aegyptiaca (S. aegyptiaca) in three different regions of Southern Iran using inductively coupled plasma optical emission spectrometry (ICP-OES) after microwave-assisted acid digestion.
Materials and methods: The present study was carried out the common halophytes, S. vermiculata and S. aegyptiaca in three different region of Dashti in Busher province, Iran, in early summer, 2013. S. vermiculata and S. aegyptiaca are native to the south, southern- east and Persian Gulf coastal and are recognized as Tahma and Kakol in Bushehr province. After drying at 95°C for 1 h to a constant weight, the samples were separated and weighed individually. The dried samples were homogenized and grounded using a mortar.
Results: A new, simple and highly sensitive method was proposed for determination some elements in halophytes by ICP-OES. The limit of detection (LOD) was below 10 $ngkg^{-1}$,and the relative standard deviations (RSD) were below 5%.The recoveries of all the elements were in the range of 88% to 108%, and the linear correlation coefficients (R) were 0.99.
Conclusion: Advantages of this method were low limit of detection (LOD), the relative standard deviations (RSD), recoveries of all the vitamins were in the range of 88% - 108%, and the linear correlation coefficients (R) 0.99. Finally to the best of our knowledge there are no published research studies about determination of some element concentrations in halophytes and the proposed ICP-OES method has been successfully applied for determining element concentrations in halophytes.

Keywords: Suaeda vermiculata, Suaeda aegyptiaca, Inductively coupled plasma optical emission spectrometry (ICP-OES), Microwave digestion

Introduction

Halophytes are plants that can live in highly saline conditions containing approximately 200 mM NaCl (1). Some halophytes are also compatible to live in immersion regions with saline water, for example coastal salt swamps. They store inorganic ions and produce a high osmotic potential. In response, halophytes increase the uptake of Na and decrease the uptake of K to their shoots; in addition, the activity of their fermentative enzymes is particularly high (1-2). Suaeda vermiculata (S. vermiculata) and Suaeda aegyptiaca (S. aegyptiaca) are common species of the genus Chenopodia. There are 26 families of halophytes species in Iran and more than 70% of them has belonged to the Chenopodiaceae family. They grow during

[Downloaded from demo.medilam.ac.ir on 2026-11-14]

both high and low tides from April to October. The growth rates of S. vermiculata and S. aegyptiaca decreased after July and then increased after August. The leaf of halophytes has been used as a medicine for hepatitis and antiviral activity. The young leaf of it often mixed with other vegetable to reduce their saltiness (3-6). Elemental food composition data are important for both consumers and health professionals. Several analytical techniques, such as inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ETAAS), are used to determine element contents in food samples (5-15). ICP-OES can provide a rapid program for multi-element analysis (5). Microwave acid digestion, which is usually very simple and fast, followed by ICP-OES and ICP-MS analyses is a powerful method for determining metals in food samples (6-20). The proposed method was successfully applied to determine some elements in halophyte samples. This is the first study to report ICP-OES determination of elements in S. aegyptiaca and S. vermiculata.

Materials and methods

In this study, concentrations of (Li, Na, K, Mg, Ca, Ba, B, Al, Si, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Ag and P) were determined in samples by ICP-OES (Varian 730-ES Axial ICP-OES) using argon (99.99%) as the carrier gas. Sample digestion was carried out in a microwave oven (Milestone MLS 1200 Mega model (Sorisole, Italy).

Materials and reagents: All reagents were of analytical grade and were purchased from Merck (purity >99%). Water used in all the experiments was ultrapure water. High purity ultrapure water was obtained from Millipore, Milli-Q (Bedford, MA, USA). All stock solutions and working standards were stored at 4°C and brought to room temperature (25 ^{0}C) before use.

Sample preparation and study area: The present study was carried out the common halophytes S. vermiculata and S. aegyptiaca in three different region of Dashti in Busher province, Iran, in early summer, 2013. The area under study includes a part of the coastal areas of the north of Persian Gulf in south of the country that regarding political divisions this area is a part of Bushehr province. S. vermiculata and S. aegyptiaca are native to the south, southern- east and Persian Gulf coastal and are recognized as Tahma and Kakol in Bushehr province. After drying at 95°C for 1 h to a constant weight, the samples were separated and weighed individually. The dried samples were homogenized and grounded using a mortar.

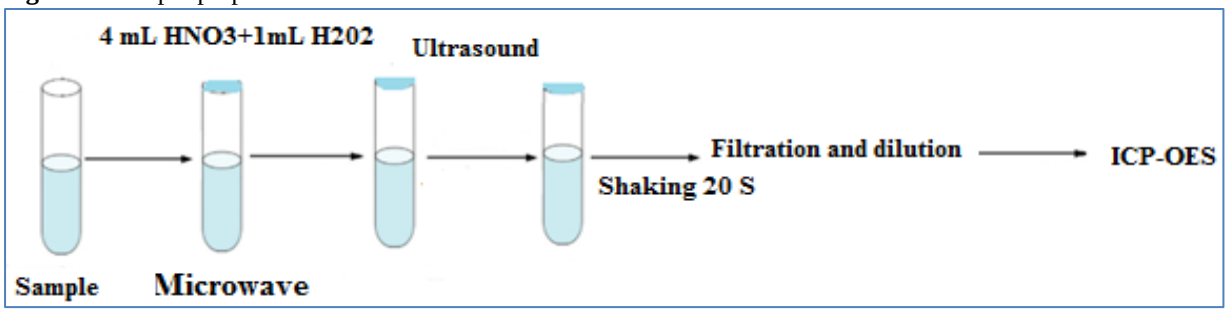
Microwave assisted digestion: For the microwave assisted digestion the samples were macerated and homogenized in an agata mortar. Subsequently, approximately 0.5 g of the dried sample was weighed directly into polytetrafluorethylene (PTFE) flasks. After adding 4 mL HNO_3 (6 M) and 1 mL of H_2O_2 (1 M), the mixture was subjected to the following digestion program: 100 W (3 min), 0 W (2 min), 250 W (2 min), 0 (2 min), 500 W (2 min), 0 W (2 min), 400 W (2 min), 0 W (3 min) and 450 W (2.5 min).

Six different mixtures of reagents using HNO_3 and H_2O_2 were tested. Mixture I is 5 mL HNO_3 (6 M), mixture II is 4 mL HNO_3 (6 M), mixture III is 3 mL HNO_3 (6 M), mixture IV (mixture of 4 mL HNO3 (6 M) and 0.5 mL H_2O_2 (1 M), mixture V (mixture of 4 mL HNO_3 (6 M) and 1 mL H_2O_2 (1 M), mixture VI (5 mL HNO_3 (6 M) and 2 mL H2O2 (1 M). Further dilution to 1:50 v/v ultrapure water was necessary for elements analysis. Features of the sample preparation method include:(a) microwave assisted dissolution of the samples (b) addition of 4 mL HNO_3(6 M) and 1 mL of H_2O_2 (1 M) and

[Downloaded from demo.medilam.ac.ir on 2026-11-14]

(c) ultrasonic 20°C and of 3 min, filtration and dilution of the solution to a predetermined volume before being subjected to analysis by ICP-OES (Figure 1).

Figure 1. Sample preparation of the method.



Results

Optimization of the digestion procedure: For the optimization of the microwave assisted sample preparation six different mixtures of reagents using HNO_3 and H_2O_2 were tested. The recoveries for the elements in samples after microwave digestion in six different mixtures are given in Table 1. The best recoveries were obtained by performing the digestion using 4 mL HNO_3 (6 M) and 1 mL of H_2O_2(1 M) were found to be 88% and 108%. Thus mixture V was chosen for further experiments.

Table 1. Recoveries for the elements determined after microwave digestion using six different mixtures.

	Recoveries (%) of six digestions					
Element	**I**	**II**	**III**	**IV**	**V**	**VI**
Li	93	93	95	97	101	100
Na	79	81	82	84	92	87
K	72	74	76	78	93	93
Mg	85	78	83	85	91	86
Ca	68	92	92	91	95	92
Ba	47	55	67	89	93	91
Al	63	69	73	76	92	83
B	54	59	63	79	95	90
Si	85	86	89	91	93	84
Se	80	83	90	92	93	91
Ag	94	95	96	98	101	99
V	88	86	87	93	94	90
Cr	74	77	80	87	92	88
Mn	81	85	84	84	100	94
Fe	87	89	90	96	103	95
Co	86	89	93	94	96	93
Ni	82	86	87	89	102	101
Cu	85	94	92	93	99	96
Zn	67	69	71	76	89	87
P	77	90	95	91	104	100

[Downloaded from demo.medilam.ac.ir on 2026-11-14]

Method evaluation: The limit of detection (LOD) determined as three times the standard deviation of 10 blank measurements divided by the slope of the calibration curve was less than 10 ng kg^{-1}. The precision estimated from the relative standard deviation (RSD) was less than 5%. The calibration curves for the analytes over the desired concentration ranges exhibited good linearity. The correlation coefficients (R) for all calibration curves were 0.99.The results investigated after microwave assisted digestion of samples using mixture V are listed in Table 2. The accuracy of the proposed method was estimated by determining the recoveries of the analytes by spiking experiments. The recoveries in samples at concentration levels of 5 and 100 mg kg^{-1} were found to be 88% and 108%. The accuracy of the element concentration data for spiked samples is shown in Tables 3 and 4.

Table 2. Figures of merits for elements and method evaluation.

Element	R	LOD ($ng.kg^{-1}$)	RSD (%)
Li	0.9996	9.3	0.8
Na	0.9992	2.5	1.4
K	0.9994	3.8	4.5
Mg	0.9997	2.1	3.9
Ca	0.9998	5.5	3.6
Ba	0.999	7.1	4.8
Al	0.9992	5.2	4.3
B	0.9995	8	1.5
Si	0.9986	7.4	1.1
V	0.9989	7.9	2.4
Cr	0.9991	2.7	2.3
Mn	0.9992	3.4	3.7
Fe	0.9986	3.8	0.5
Co	0.9995	4.3	2.5
Ni	0.9993	4.8	4.9
Cu	0.9991	2.6	3.3
Zn	0.9975	1.3	4.7
Se	0.9973	8.9	1.6
Ag	0.9991	3.4	0.4
P	0.9997	1.9	3.6

[Downloaded from demo.medilam.ac.ir on 2026-11-14]

Table 3. Accuracy data of elements for spiked leaves of S. vermiculata and leaves of S. aegyptiaca.

Element	Added	Found [a]	Recovery[b]	Added	Found[c]	Recovery[d]
Li	5	4.9	98	5	4.7	94
Na	100	97	97	100	93	93
K	5	4.4	88	5	4	80
Mg	100	101	101	100	100	100
Ca	5	5.1	102	5	4.2	84
Ba	100	100	100	100	103	103
Al	5	4.5	90	5	4.7	94
B	100	100	100	100	103	103
Si	100	95	95	100	90	90
V	5	4.7	94	5	4.1	82
Cr	100	91	91	100	94	94
Mn	50	51	102	50	50	100
Fe	100	100	100	100	89	89
Co	5	5.2	104	5	5	100
Ni	100	89	89	100	84	84
Cu	5	5.4	108	5	5	100
Zn	100	93	93	100	91	91
Se	5	4.7	96	5	3.9	78
Ag	5	5	100	5	5	100
P	100	101.9	102	100	99	99

a, c: $ngkg^{-1}$ b:% S. vermiculata d:% S. aegyptiaca

Table 4. Analysis of elementas concentration by ICP-OES (n = 3).

Element	Wavelength(nm)	S. vermiculata ($ngkg^{-1}$)	S. aegyptiaca[b] ($ngkg^{-1}$)
Li	744.542	56±2.01	29±1.93
Na	796.897	95612±1.22	11862±2.14
K	279.809	11478±2.44	11384±1.12
Mg	315.887	1935±2.32	4292±1.39
Ca	396.152	784±0.18	1979±0.11
Ba	267.716	6.2±0.37	6.3±0.14
B	268.197	32±0.65	26±0.65
Al	257.615	2435±0.15	1228±0.19
Si	238.892	1179±0.04	1154±0.01
V	177.434	2±2.65	4±0.49
Cr	267.716	0.93±0.05	0.92±0.12
Mn	257.61	25±0.57	24±1.31
Fe	238.204	549±0.28	238±0.57
Co	238.892	0.01±0.43	0.01±0.79
Ni	231.604	0.02±0.57	0.03±0.44
Zn	213.875	43±0.21	32.4±1.12
Cu	214.675	37±0.18	36.8±1.07
Se	196.026	1.6±0.29	2.3±0.41
Ag	213.65	4.1±0.76	4.7±1.2
P	177.437	1590±6.5	2923±3.2

[Downloaded from demo.medilam.ac.ir on 2026-11-14]

Discussion

To evaluate performance of the proposed method, elements were determined in real samples under the above mentioned conditions. Asri et al. (1997) studied the minerals of halophytes and concluded that the accumulation of Na was higher than those of K, Ca and Mg (20). The Na content in both species was higher than the contents of (Li, K, Mg, Ca, Ba, B, Al, Si, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Ag and P).The Na contents in S. vermiculata were ten times higher than those in S. aegyptiaca. The Mg contents in the plant organs of S. vermiculata were higher than the Ca contents. Ca content decreased with increasing soil salinity, likely due to the competitive effect with Na. The largest Ca concentration was found in S. aegyptiaca.; Ca content was twice that of S. vermiculata in this study. Grattan et al. (1992) indicated that salinity may cause an excessive accumulation of phosphate, inducing P toxicity (21). Silberbush et al. (1989) showed that salinity had no effect on P uptake, and that P content increased with increasing Mg concentration (22). The concentration of Mg, Se and P in this study was greatest in S. aegyptiaca, which had twice the Mg, Se and P content of S. vermiculata.The population of S. vermiculata is more densely distributed in regions with higher contents of Mg, K and Na, and as noted previously, halophytes possess mechanisms that can select these ions from the soil. Form the study of four halophytes, Riasi et al. (2007) found that the levels of Na, K and Cl in these plants were above the critical level, while the Ca and Mg concentrations were below the critical level (23). The role of Si and Ba for growth of salt swamp plants has, as far as we know, not yet been examined. The concentration K, Ba, Si, V, Cr, Mn, Co, Ni and Ag contents in both of them were approximately equal. The amount of Li, Na, Al, Fe and Zn in S. vermiculata twice S. aegyptiaca. The mineral elements in the halophytes were significantly different depending on the weather conditions during growth. Halophytes such as S. vermiculata have mechanisms that can adapt and select the critical ions from the soil. The mineral elements in halophytes are significantly changed during growth in different weather conditions. The element concentrations in samples are shown in Table 5.

Conclusions

In this study, concentrations of some elements (Li, Na, K, Mg, Ca, Ba, B, Al, Si, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Ag and P) were determined in the leaves of S. vermiculata and S. aegyptiaca from three different regions of southern Iran using ICP-OES after microwave-assisted acid digestion. ICP-OES and ICP-MS techniques were suitable for elemental determination in samples following microwave-assisted acid digestion. The samples were conveniently smashed and homogenized in an agata mortar, avoiding oil loss before digestion. Advantages of this method were low limit of detection (LOD), the relative standard deviations (RSD), recoveries of all the vitamins were in the range of 88%-108%, and the linear correlation coefficients (R) 0.99. Finally, the proposed ICP-OES method has been successfully applied for determining element concentrations in halophytes.

References

1. Azzawi JM, Alhdad GM, Seal CE. The effect of combined salinity and waterlogging on the halophyte Suaeda maritima: The role of antioxidants. J Environ Experiment Botany. 2013; 87(1):120–5.
2. Fujita K, Moghaieb REA, Saneoka H. Effect of salinity on osmotic adjustment, glycinebetaine

[Downloaded from demo.medilam.ac.ir on 2026-11-14]

accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, Salicornia europaea and Suaeda maritima. J Plant Sci. 2004; 166 (5):1345–9.

3. Harikrishnan R, Kim JS. Effect of dietary supplementation with Suaeda maritima on blood physiology, innate immune response, and disease resistance in olive flounder against Miamiensis avidus. J Experiment Parasitol. 2012; 131(2):195–203.
4. Paredes E, Prats MS, Maestrem SE, Todolí JL. Rapid analytical method for the determination of organic and inorganic species in tomato samples through HPLC–ICP-AES coupling. J Food Chem. 2008; 111(2): 469–75.
5. Anne M, Colmer OP, Wetson TD. Oxygen dynamics in a salt-marsh soil and in Suaeda maritima during tidal submergence. J Environment Experiment Botany. 2013; 92(1): 73–82.
6. Cvijanovic G, Lenhardt M, Gacic Z, Jaric I, Jovanović L, Skorić S, Višnjic-Jeftic Ž. Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (Acipenser ruthenus) from the Danube River in Serbia by ICP-OES. J Microchem. 2011; 98(1): 77–81 .
7. Da Santil D, De Souza MM, Dos Santos. WPC, Fernandes AP, Korn MGA, Hatje V. Optimization of a centrifugation and ultrasound-assisted procedure for the determination of trace and major elements in marine invertebrates by ICP OES. J Microchem. 2010; 95(2):169–73.
8. Hamilton M, Merchant M, Rode P, Sneddon J. Determination and comparison of heavy metals in selected seafood, water, vegetation and sediments by inductively coupled plasma-optical emission spectrometry from an industrialized and pristine waterway in Southwest Louisiana. J Microchem. 2008; 88(1):52–5.
9. Lofrano BE, Hardaway CJ, Martins. MC, Meche A, Merchant M, Verdade L. Determination of heavy metals by inductively coupled plasma-optical emission spectrometry in fish from the Piracicaba River in Southern Brazil. J Microchem. 2010; 94(2):171–4.
10. Cindric. IJ, Kröppl M, Zeiner M, Stingeder G. Comparison of sample preparation methods for the ICP-AES determination of minor and major elements in clarified apple juices. J Microchem. 2011; 99(2): 364–369.
11. Fallah AA, Nematollahi A, Saei-Dehkordi SS, Jafari T. Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (Oncorhynchus mykiss) using ICP-OES technique. J Microchem.2011; 98(2): 275–9.
12. Castro JT, Costa LM, Korn M, Korn, MGA, Nobrega JA, Santos EC, Santos. WPC. A critical evaluation of digestion procedures for coffee samples using diluted nitric acid in closed vessels for inductively coupled plasma optical emission spectrometry. Talanta. 2009; 78(4):1378–82.
13. Evangelista FS, Nardi. EP, Tormen. L, Saint Pierre TD, Curtitus. AJ, Souzas S, Barbosa F. The use of inductively coupled plasma mass spectrometry (ICP-MS) for the determination of toxic and essential elements in different types of food samples. J Food Chem. 2009; 112(3):727–32.
14. Dominguez A, Fernández-de Córdova ML, Llorent-Martínez. EJ, Ortega-Barrales P, Ruiz M. Investigation by ICP-MS of trace element levels in vegetable edible oils produced in Spain. J Food Chem. 2011; 127(3):1257–62.
15. Demirbas A. Oil, micronutrient and heavy metal contents of tomatoes. J Food Chem. 2010; 118(3):504–7.
16. Oliveira PV, Naozuka J, Marana SR. Water-soluble Cu, Fe, Mn and Zn

[Downloaded from demo.medilam.ac.ir on 2026-11-14]

species in nuts and seeds, J Food Compos An. 2010; 23(1):78–85.
17. Bakkali K, Martos NR, Souhail B,BallesterosE.Characterization of trace metals in vegetables by graphite furnace atomic absorption spectrometry after closed vessel microwave digestion. J Food Chem. 2009; 116(2): 590–4.
18. Barbosa JT, Fernandes AP, Nunes LS, Lemos VA, Santos WN, Korn MG, Teixeira L. Multi-element determination of Cu, Fe, Ni and Zn content in vegetable oils samples by high-resolution continuum source atomic absorption spectrometry and microemulsion sample preparation. J Food Chem. 2011; 127(2): 780-3.
19. Araujo RG, Chaves ES, Curtius AJ, Frescura VL, Oliveira JV, Santos EJ. Metals and phosphorus determination in vegetable seeds used in the production of biodiesel by ICP OES and ICP-MS. J Microchemical. 2010; 96(1):71–6 .
20. Asri Y ,Ghorbanli M. The halophilous vegetation of the Orumieh lake salt marshes. Iran. Plant Ecol. 1997; 132(1):155–70.
21. Grattan SR, Grieve CM. Mineral element acquisition and growth response of plants grown in saline environments. Agriculture, J Ecosystems and Environment. 1992; 38(4):275-300.
22. Ben-Asher J, Ephrath JE, Silberbush M. A model for nutrient and water flow and their uptake by plants grown in a soilless culture. J Plant Soil. 2005; 271(2): 309–19.
23. Danesh M, Riasi A. Stern MD. Chemical composition, in situ ruminal degradability,Kochia ap-rdodmacpfthp, scoparia Ad, Suaeda arcuata and Gamanthus gamacarpus. J Animal Feed Sci Technol. 2008; 141(3):209-19.

[Downloaded from demo.medilam.ac.ir on 2026-11-14]