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Determining the Mobility of some Essential Elements in Saffron (*Crocus sativus* L.) by the Neutron Activation Analysis

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Abstract:

The main purpose of this investigation is to evaluate the concentrations of six essential metals (Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+}) in saffron and a farm soil using the neutron activation analysis (NAA) as a nuclear spectrometry method. The stratified random sampling method was used here. The NAA results showed the well uptake of Mg^{2+}, K^+, Ca^{2+}, Fe^{2+}, and Zn^{2+} in saffron, which is lower than the toxicity range. Based on the contamination factor and geoaccumulation index, soil contamination levels were determined uncontaminated by Zn, moderately contaminated by Na^+ and Fe^{2+}, and strongly contaminated by Ca^{2+}, K^+, and Mg^{2+}. Results of the contamination degree and pollution load index indicated moderately/strongly soil contamination and a moderate geometric mean of the contamination index. The Na^+ enrichment factor (EF) showed a minimal man-made impact on sodium enrichment. Saffron cultivation has likely caused more accumulations of Mg^{2+}, K^+, Ca^{2+}, and Fe^{2+}, as well as a considerable deficiency of Zn^{2+} in the soil, based on EFs. The biological concentration factor showed a significant zinc accumulation by the corm of saffron. There was well translocation from corm to all the aerial tissues for K^+. Also, sodium adsorption ratio, exchangeable sodium percentage, pH, and electrical conductivity evaluated the non-salinity level of soil in all saffron farms.

Keywords: Biological concentration factor, Contamination, Crocus sativus L., Enrichment factor, Neutron Activation Analysis, Salinity soil, Translocation Factor.

Introduction:

Saffron, Crocus sativus L., is a perennial herbaceous plant, which has been categorized as a monocot flowering and stemless herb in the Iridaceae family from Asparagales order that usually grows up to 35 cm. Saffron has been cultivated on more than 100,000 hectares of Iranian farms, producing about 340 tons of saffron annually. Accordingly, Iran produces over 90% of saffron worldwide [1]. Although saffron farms spread over 20 provinces of Iran, the great Khorasan region (including North, South, and Khorasan Razavi provinces) has produced above 90% of saffron in Iran [2]. In the great Khorasan region, Torbat Heydarieh County plays a significant role in the production of saffron. Zaveh has been separated from Torbat Heydarieh County since 2008. Therefore, Torbat Heydarieh (including Zaveh) is an exceptional area in the production of Iranian saffron [3].

Tracking trace elements in environmental samples plays a significant role in the health of plants, animals, and humans [4, 5]. Plants are always the first living component of a food chain. Therefore, evaluations of the concentrations of trace elements and their translocation abilities from soil to crops play an essential role in human health [6]. Atomic absorption spectroscopy (AAS), inductively coupled plasma (ICP) [7], neutron activation analysis (NAA) [8,9], and X-ray fluorescence (XRF) [10, 11] have been widely reported as the most commonly elemental analytical techniques for the measurement of heavy metals and trace elements in environmental samples [12]. NAA has been classified as a nuclear analytical method (NAMs), which is non-destructive and independent of chemical processes [9].

All living organisms need food to continue their lives. Nutrients are sorted into micro and macro. Macronutrients include carbohydrates, fats, proteins, fiber, and water, which are needed in large amounts and their most essential function is to supply energy and growth processes. Micronutrients, which are needed in a small amount, play considerable roles in many chemical reactions of living organisms [13].

Micronutrients are classified into two groups: vitamins and minerals. In addition, minerals can also be divided into micro- and macro-minerals [14]. Around 20 minerals are required in various biological species to live and grow [15]. In this investigation, six metals were chosen as required minerals. Four various metals are merely included in the macro-mineral group, viz. sodium (Na^{+}), magnesium (Mg^{2+}), potassium (K^{+}), and calcium (Ca^{2+}). Besides, iron (Fe^{2+}) and zinc (Zn^{2+}) are the most essential metals as micro-minerals for living systems.

Mineral nutrients are constantly circulating between living organisms and their habitat. The uptake and assimilation of minerals by plants are the main step in the rotation of minerals within the biosphere. Therefore, the biggest root area expansion and its ability to absorb different concentrations of soil mineral nutrients are very effective in the process of nutrient uptake. The movement of ions within tissues, the movement of water, takes place from the vacuole of one cell to the that of another cell, and this trajectory, which is the main path for ions to move, is called the pathway within the cytoplasm. Of course, ions from the cytoplasm and their entry into the cytoplasm of adjacent cells should not be overlooked. Another side path is the extra cytoplasmic pathway that passes through the skeletal wall of cells and intercellular spaces.

Selection is the property by which a cell absorbs and even stores some of the elements in the environment, or vice versa, absorbs some in very small amounts. If we compare anions and cations in terms of permeability in plant roots, we see that their penetration rate is basically a function of the conditions and especially its pH. The rate of penetration of the material in the cells of the lethal fibers is inversely proportional to the coarseness of the adsorbed particles. The non-uniform rate of penetration of different substances into the cell causes the pH of the vacuolar sap to be compromised and altered, in which case the cell solves this problem by increasing organic acids.

Internal factors affect material absorption. The semi-permeability of the plasma membrane of the cells as well as the ability of the cells to accumulate or select materials are obvious signs of cell viability and change after death. Wound formation in plant cells alters their permeability. Cell permeability varies according to the concentration of minerals in the cells, even if the cell does not belong to a root but belongs to a tissue isolated from a plant. Inside the cells, there is a balance between cations and anions. The connections thus observed between intracellular factors and the permeability of substances in the cell link metabolism and absorption of substances.

The main purpose of this investigation is to evaluate the concentrations of six essential metals in saffron and a farm using the neutron activation analysis as a nuclear spectrometry method. Then, the contamination, enrichment, and translocation of metals, and soil salinity were estimated by statistical analyses and contamination, enrichment, translocation, and salinity indexes.

Material and Methods:

Collection and Preparation of Samples:

Two types of samples should be collected: (a) soil samples, and (b) plant samples. The organs of saffron plant can be classified into three main categories. The corm is the first part of the plant located under the soil. The herbaceous organs include leaves, petal, and stem. The stigmas (red threads) are the last organ and the only edible part of saffron plant. Therefore, saffron plant samples included three main categories in this study: (a) corm, (b) herbaceous specimens, and (c) stigmas.

Saffron corms were located at a soil depth of 15–20 cm. Hence, the soil in cultivated farms was plowed up to a depth of 30 cm. However, the soil was highly tough at a depth of more than 30 cm, where it could not easily absorb water [16]. Accordingly, soil samples were collected from two types: (a) topsoil (up to 15 cm of depth), and (b) soil around of the saffron corm (a depth of 15–30 cm). The map of Iranian provinces and the locations of Torbat Heydarieh and Zaveh counties in Khorasan Razavi province are illustrated in Fig. 1 A and B, respectively. The geographic coordinates of Torbat Heydarieh and Zaveh are shown in Table 1. Torbat Heydarieh County is placed in the south-west of Mashhad (the capital of Khorasan Razavi).

Table 1. The locations of Torbat Heydarieh and Zaveh counties.

Name of counties	Longitude	Latitude	Area (km^2)
Torbat Heydarieh	35°:16'	59°:13'	6175
Zaveh	35°:08'	59°:51'	2437

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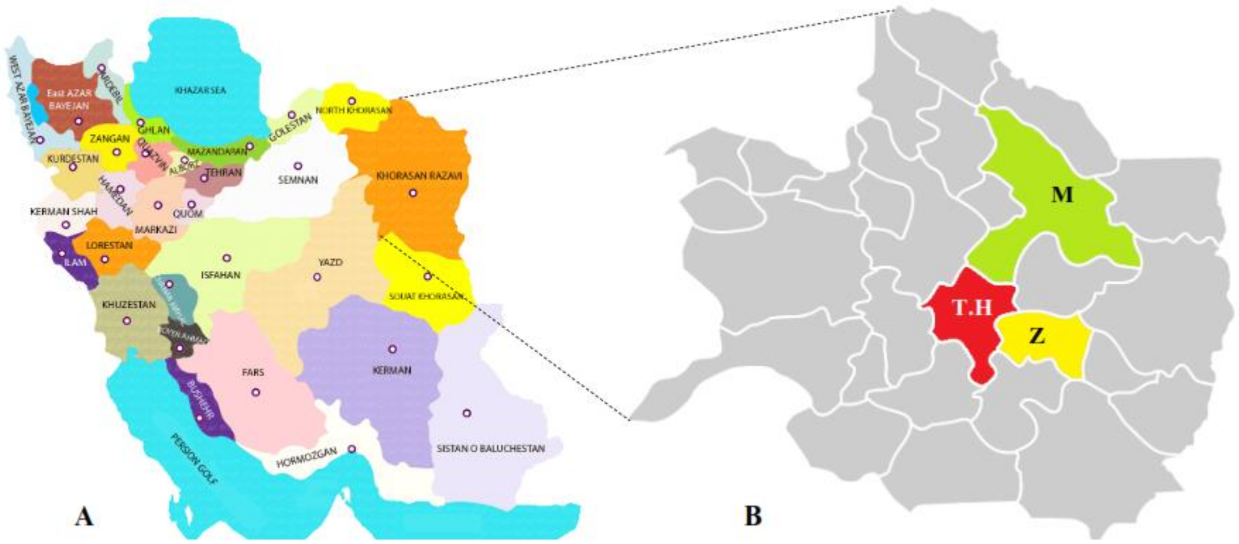


Figure 1. (A): Provinces of Iran; (B): Mashhad (M) capital of the Razavi Khorasan, Torbat Heydarieh (T.H), and Zaveh (Z).

The stratified random sampling method was used according to the literature review. Torbat Heydarieh and Zaveh counties have vertical and horizontal shapes, respectively. Each county was divided into three same zones. Therefore, three zones of Torbat Heydarieh were named north, center, and south. Also, west, center, and east were zones of Zaveh. Then, 100 of approximately equal parts were segregated for sampling from each zone. Therefore, the areas of each part of Torbat Heydarieh and Zaveh counties were around 2000 and 700 hectares, respectively. Then, each part was finally isolated into nine same sections, as shown in Fig. 2.

Around 50–100 farms were selected in each part and 200 farms were accordingly chosen in each county. A saffron farm with the greatest cultivation and uppermost saffron production was sampled at five various spots. Then, a combined sample of each type of sample was prepared for each farm. Afterward, a combined sample was made from several farms of nine sections for each part. Samples of 25 parts were homogeneously mixed to create a mixture sample for each type of sample. A total of 120 samples were prepared for Torbat Heydarieh and Zaveh counties in five sample types [17].

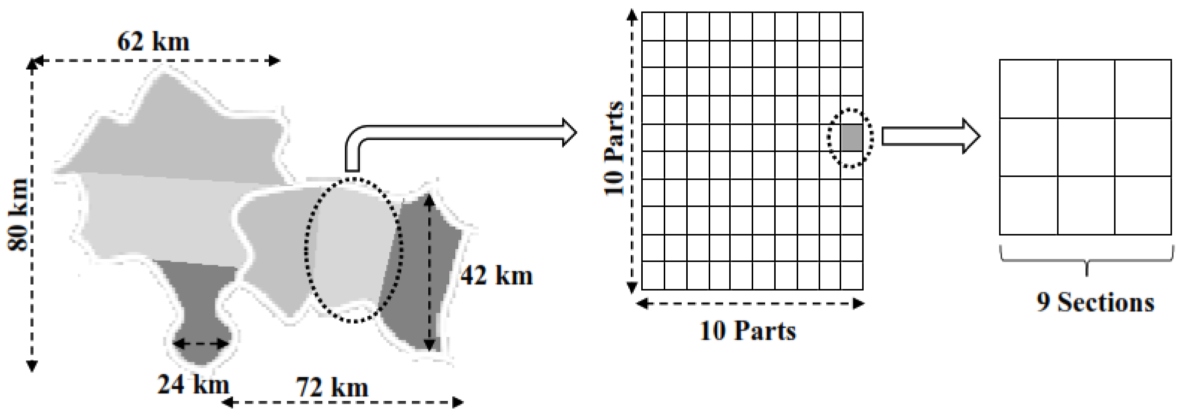


Figure 2. Proceeding of selecting farms to collect the samples.

A garden shovel, scissors, and a manual earth auger were used to collect the plant and soil samples. The plant samples were washed with distilled water. Then, the humidity of all samples was reduced with dry air within approximately a week. The soil and plant samples were crushed firstly by an electric grinder. Then, the samples powdered finely using a ceramic mortar and pestle. The samples were finally sifted by a lab sieve shaker and packaged in special plastic cylinders.

Measuring the element concentration using NAA:

Neutrons with zero electric charges bombard the target in the neutron activation analysis (NAA). Therefore, they can pass through the Coulomb potential barrier and can excite the target nucleus. Accordingly, NAA is one of the most accurate types of analysis methods. The reactive specification of an exciting target nucleus is affected by the detector efficiency, neutron energy, and neutron cross-section. Therefore, the activity of the sample was compared with that of a standard sample to avoid some practical limitations, which is called the relative NAA method. The main equations of relative NAA are presented in Eq. 1 [9]. Table 2 shows the symbols in the relative NAA.

$$\frac{A_{sam}}{A_{st}} = \frac{N_{sam}}{N_{st}} = \frac{W_{sam}}{W_{st}} \Rightarrow D = \frac{W_{sam}}{G} = \frac{W_{st}}{G}\frac{A_{sam}}{A_{st}} \quad (1)$$

Table 2. Symbols of relative neutron activation analysis.

Unknown element	Sample	Standard
Activity	A_{sam}	A_{st}
Mass	W_{sam}	W_{st}
Number of nuclei	N_{sam}	N_{st}
Total Mass	G	
Weight Concentration	D	

The source of particles or photon is the most essential and considerable instrument in all spectrometry systems. Neutron sources are merely in three main groups with different applications, namely (a) radioisotope, (b) neutron generator, and (c) nuclear reactor [8]. The nuclear reactor is the most reliable and best source of neutrons [18]. There are seven irradiation channels with various advantages and usages in different shapes and lengths at the stall end of the TRR pool. A, D, E, and G channels are 6-inch cylinders used in this research. F and B are 8-inch cylindrical and 12-inch square shapes, respectively. The longest channel, named C, is a 6-inch cylinder. Moreover, there are graphite blocks in two thermal columns at the stall end of the TRR pool [19]. In this study, the relative standard deviation (RSD) was used to evaluate the precision of NAA method and equipment, which is measured by Eq. 2:

$$\mathrm{RSD} = \frac{\delta}{\text{Mean of Data}} \times 100 \quad (2)$$

where δ is a standard deviation of the repeated results of three known standard samples [20]. The accuracy of the NAA results was evaluated by a standard method. Thus, the concentrations of elements in the reference samples were measured by the NAA. Afterward, the NAA results of standard samples were compared with their confirmed values [21, 22].

Statistical evaluation of the NAA results:

Paired t-test was used to evaluate the similarity of an element concentration in various soil depths, which shows the element displacement ability in the soil [23]. The difference between the measured values of an element in two sample types would be zero, which was called the null hypothesis (H_0) [24]. P-values were measured by the SPSS 16 statistical computer code. The H_0 was accepted when the p-value was more than 0.05, which means that concentrations of an element were statistically similar in these two various sample types [11]. The linear correlation coefficient (r_{xy}) was used to determine the relation of two elements in a type of sample, which is computed by Eq. 3 [9]:

$$r_{XY} = \frac{Cov_{(XY)}}{\sigma_X \sigma_Y} = \frac{\sum_{i=1}^{n}(X_i-\bar{X})(Y_i-\bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i-\bar{X})^2 \sum_{i=1}^{n}(Y_i-\bar{Y})^2}} \quad (3)$$

where X and Y are the measured concentrations of two elements in the soil depth. Also, $Cov_{(XY)}$ and n are the correlation coefficient and the number of measurements, respectively. Besides, σ_x and σ_y symbols are the square root of the variances, and X_i is a measured value. A positive correlation coefficient illustrates that the increasing value of one variable means a rise in the other. A r_{xy} of 1 shows the strongest positive relation. Moreover, a negative r_{xy} proves an increase in one variable and a decrease in the other. Therefore, a correlation coefficient of −1 shows the strongest inverse relation [25].

Measurement of contamination:

In this study, the contamination levels were estimated by four environmental indexes, which have been widely used as useful tools for a comprehensive assessment of environmental contamination rates [26]. The contamination factor (CF) is a simple and effective parameter for monitoring metal pollution in environmental samples. CF is calculated by Eq. 4, where C_i is the average concentration of an i^{th} element in soil samples of an area and GB_i is the background value of this element in sediments and soil or the geochemical background value in fossil argillaceous sediment and world average shale as background values [27]. GB_i values used in this study are shown in Table 3 [28].

$$\mathrm{CF_i} = \frac{\mathrm{C_i}}{\mathrm{GB_i}} \quad (4)$$

Table 3. Background values of Na, Mg, K, Ca, Fe, and Zn in Earth's shale.

GB (mg/kg) [28]					
Na	Mg	K	Ca	Fe	Zn
11000	8000	18000	20000	32000	60

The geo-accumulation index (I-geo) is recommended to evaluate the sedimentation intensity (increasing concentration), especially for metals in the natural context of topsoil. Although this increasing concentration is caused by various variables, it has been seriously affected by human activities. I-geo is calculated by Eq. 5. A coefficient of 1.5 is a correction factor, which has been suggested for lithologic variations.

$$\mathrm{Igeo_i} = \log_2 \frac{\mathrm{C_i}}{1.5 \times \mathrm{GB_i}} \quad (5)$$

On the other hand, contamination degree (CD) determines the total contamination of soil in an area. CD is defined as the sum of CF values of all studied elements in soil samples of an area, which is shown in Eq. 6 [26]:

$$\mathrm{CD} = \sum_{i=1}^{n} \mathrm{CF_i} \quad (6)$$

where n is the number of studied elements. Table 4 shows the contamination levels based on CF, I-geo, and CD values. The pollution load index (PLI) is also used to evaluate the total pollution of environmental samples in an area. PLI is the geometric mean of the contamination index of a region and is calculated from Eq. 7, where n is the number of analyzed elements. PLI is an easy way to prove the deterioration of soil conditions. Additionally, the extent of contamination can be overall estimated by PLI, which is also marked in Table 4 [29].

$$\mathrm{PLI} = \sqrt[n]{\mathrm{CF_1} \times \mathrm{CF_2} \times \cdots \mathrm{CF_n}} \quad (7)$$

Table 4. Classification of contamination indexes.

Class	Contamination Rate	CF [30]	I-geo [30]	CD [31]	PLI [29]
0	Uncontaminated	Cf <1	I-geo≤0	Cd <6	0≤PLI <1
1	None/Moderately pollution		0 <I-geo≤1		1≤PLI <2
2	Moderately pollution	1≤Cf <3	1 <I-geo≤2	6 ≤Cd <12	2≤PLI <3
3	Moderate-Strongly pollution		2 <I-geo≤3		3≤PLI <4
4	Strongly pollution	3≤Cf <6	3 <I-geo≤4	12 ≤Cd <24	4≤PLI <5
5	Strong-Extremely pollution		4 <I-geo≤5		5≤PLI <6
6	Extremely pollution	6≤Cf	5 <I-geo	24 ≤Cd	6≤PLI

Enrichment factor:

Enrichment factor (EF) measures element sedimentation in the soil of an area. Hence, EF compares the ratio of the element weight per that of an immobile element in the soil sample and Earth's shell [27]. Eq. 8 calculates EF of the i^{th} element, where C_i, GB_i, and CF_i are the sample parameters of the i^{th} element in Eq. 3. Ref also indicates the reference element [32].

$$\mathrm{EF_i} = \frac{\mathrm{C_i/C_{ref}}}{\mathrm{GB_i/GB_{ref}}} = \frac{\mathrm{C_i/GB_i}}{\mathrm{C_{ref}/GB_{ref}}} = \frac{\mathrm{CF_i}}{\mathrm{CF_{ref}}} \quad (8)$$

Immobile elements, such as Al and Fe, can be chosen as reference elements [31]. In this study, Fe was examined as an essential element. Therefore, Al was used for measuring the EF. The measurement background usually causes EF values of < 10; therefore, the determination of a certain enrichment origin is not almost easy [33]. Nevertheless, values of $0.5 < EF \leq 1.5$ indicate that changes in the environmental elemental distribution are caused by natural activities without any anthropogenic influence. Therefore, chemical distribution in an area with an EF > 1.5 was affected by other sources. $EF < 0.5$, $0.5 \leq EF < 2$, $2 \leq EF < 5$, $5 \leq EF < 20$, $20 \leq EF < 40$, and $40 \leq EF$ dictate deficiency, minimal, moderate, significant, very high, and extremely high enrichment, respectively [34].

Translocation of elements:

Two factors have been widely used to evaluate the phytoremediation ability for absorbing an elemental from the soil [35]. The first is the biological concentration factor (BCF), which can measure the translocation ability of an element from soil to a plant's root. BCF is calculated by Eq.9 [29]:

$$\mathrm{BCF} = \frac{\text{Element in root of plant}}{\text{Element in soil}} \quad (9)$$

The biological accumulation factor (BAF) is the second index for the evaluation of element translocation ability from soil to a plant, which is calculated by Eq. 10. Although BAF of a plant indicates the uptake of a given element from all exposure paths (such as soil, water, and air), the plant BCF refers to the specified element uptake only from the soil. A plant can be classified as a good accumulator of a trace element when BCF > 1 (or BAF for all exposure paths).

$$\mathrm{BAF} = \frac{\text{Element in aerial parts}}{\text{Element in soil}} \quad (10)$$

On the other hand, a BCF ≤ 1 infers a poor accumulator of a certain element from the soil. Furthermore, the translocation ability of an element inside a plant from root to aerial parts is estimated by the translocation factor (TF) value, which increases with a higher TF. Eq. 11 calculates TF [36, 37].

$$\mathrm{TF} = \frac{\text{Element in aerial parts}}{\text{Element in root}} \quad (11)$$

Soil Salinity Test:

In this study, four factors were employed to estimate the quality and salinity of the soils of saffron farms. Sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP) were used to evaluate soil salinity. The altogether sodicity of soil, soil solution, and irrigation water are estimated by SAR. However, ESP is only calculated to determine soil salinity. SAR and ESP indexes are calculated by Eq.s 12 and 13, respectively [38]:

$$SAR = \frac{Na}{\sqrt{\frac{(Ca+Mg)}{2}}} \quad (12)$$

$$ESP = \frac{Na}{(Na+Mg+K+Ca)} \times 100 \quad (13)$$

The pH values of the solution of soil and distilled water (1:5) were measured by a pH meter (Model-AZ 86502). Besides, an electrical conductivity (EC) meter (Model: AZ-86503) was used to evaluate the EC values of this solution. Table 5 represents the grouping of farm soil based on EC, SAR, ESP, and pH values.

Table 5. Classification of soils' Salinity.

Soil Salinity	EC	SAR	ESP	pH
None	<4	<13	<15	<8.5
Saline	>4	<13	<15	<8.5
Sodic	<4	>13	>15	8.5-10
Saline-sodic	>4	>13	>15	<8.5

EC: Electrical conductivity (dS/m);ESP: Exchangeable sodium percentage (%);SAR: Sodium adsorption ratio (mEq/100 g)

Results and Discussion:

Determination of the precision and accuracy of NAA results:

Three known multi-element samples (standard samples of TRR reactor) were chosen to evaluate the accuracy and precision of NAA results, and their elements were measured repeatedly at least 10 times by the use of a TRR reactor. RSD and average relative standard deviation (ARSD) of each element were calculated for the studied six minerals (Table 6).

The precision of the results of a method increase by decreasing RSD. The precision of results is typically acceptable with an RSD less than 20% [21]. Also, acceptable RSD values for results of neutron activation analysis have been recommended less than 10% in the fields of biomedical and biophysics by the International Atomic Energy Agency (IAEA) [39].

Table 6. Results of Relative Standard Deviation.

Elements	RSD 1	RSD 2	RSD 3	ARSD
Na^+	3.95	5.38	4.58	4.64
Mg^{2+}	3.43	5.06	5.01	4.49
K^+	1.39	3.42	1.43	2.08
Ca^{2+}	2.54	2.37	1.91	2.27
Fe^{2+}	3.22	4.15	4.94	4.11
Zn^{2+}	3.26	2.15	3.73	3.05

Calculated ARSDs for all measured elements were found less than 5%, which indicates a high precision of NAA to determine the concentrations of Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} in saffron plant and soil. The accuracy of the NAA results was evaluated by comparing measured and confirmed concentrations of elements in the reference samples (multi-element standard). The calculated standard deviations and relative errors of elemental concentrations were very small between measured and confirmed values. Therefore, the accuracy of the NAA results was acceptable to determine the essential metals in bio-samples of saffron farms. Likewise, previous investigations proved a high accuracy of NAA to determine trace elements in biological samples [22].

Measured Concentrations of Minerals Using NAA

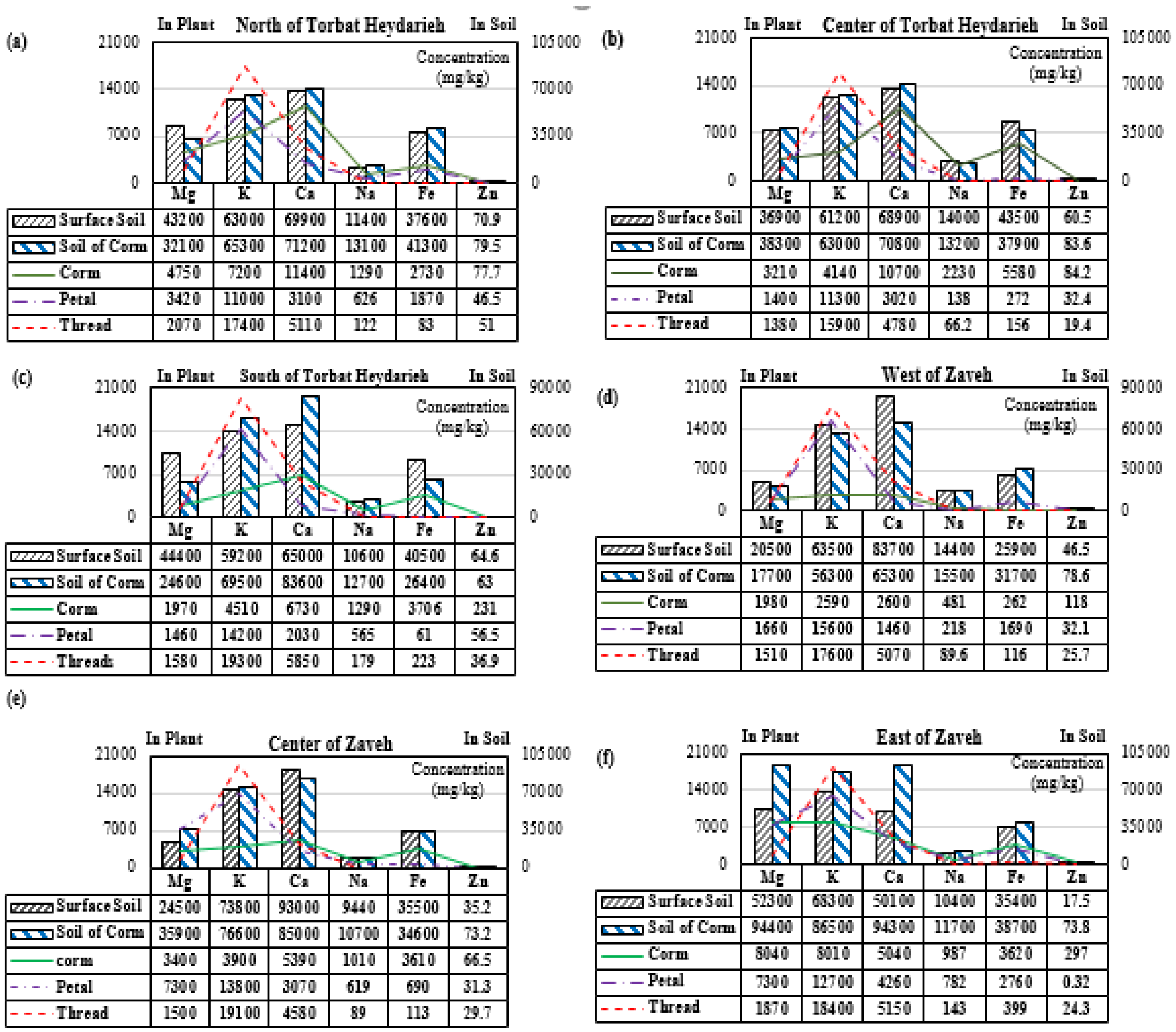
The samples and known standards were simultaneously sent into the TRR reactor core. Afterward, the concentrations of the elements were calculated by Equation 1. This cycle was repeated at least three times for each sample. Figure 3 shows the mean concentrations of Mg^{2+}, K^+, and Ca^{2+} (three macro-minerals), and those of Na^+, Fe^{2+}, and Zn^{2+} (three micro-minerals) separately for each site.

Minerals play a significant role in the growth of plants, and accordingly in the health of crops. Some researchers have investigated the sufficient and toxic levels of mineral concentrations in various plants. Sodium in the salt form generally plays a role in the environment and agriculture. Researchers have usually focused on salinity and salinity stress from sodium concentrations in agriculture and biological samples. Therefore, sodium levels in saffron would be accordingly discussed in soil salinity. Table 7 shows the ranges of sufficiency and toxicity values of Mg^{2+}, K^+, Ca^{2+}, Fe^{2+}, and Zn^{2+} in typical plants [40].

Table 7. Sufficiency and toxicity range of Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Fe^{2+}, and Zn^{2+} of typical plants.

Elements	Concentrations (mg/kg)	
	Sufficiency [40]	Toxicity [40]
Mg^{2+}	1500–3500	15000
K^{+}	5000–40000	50000
Ca^{2+}	500–10000	100000
Fe^{2+}	50–6000 [41]	---*
Zn^{2+}	15–30	100 - 300

* FAO/WHO Fe limit not yet been established for medicinal plants



(a) North of Torbat Heydarieh

	Mg	K	Ca	Na	Fe	Zn
Surface Soil	43200	63000	69900	11400	37600	70.9
Soil of Corm	32100	65300	71200	13100	41300	79.5
Corm	4750	7200	11400	1290	2730	77.7
Petal	3420	11000	3100	626	1870	46.5
Thread	2070	17400	5110	122	83	51

(b) Center of Torbat Heydarieh

	Mg	K	Ca	Na	Fe	Zn
Surface Soil	36900	61200	68900	14000	43500	60.5
Soil of Corm	38300	63000	70800	13200	37900	83.6
Corm	3210	4140	10700	2230	5580	84.2
Petal	1400	11300	3020	138	272	32.4
Thread	1380	15900	4780	66.2	156	19.4

(c) South of Torbat Heydarieh

	Mg	K	Ca	Na	Fe	Zn
Surface Soil	44400	59200	65000	10600	40500	64.6
Soil of Corm	24600	69500	83600	12700	26400	63
Corm	1970	4510	6730	1290	3706	231
Petal	1460	14200	2030	565	61	56.5
Threads	1580	19300	5850	179	223	36.9

(d) West of Zaveh

	Mg	K	Ca	Na	Fe	Zn
Surface Soil	20500	63500	83700	14400	25900	46.5
Soil of Corm	17700	56300	65300	15500	31700	78.6
Corm	1980	2590	2600	481	262	118
Petal	1660	15600	1460	218	1690	32.1
Thread	1510	17600	5070	89.6	116	25.7

(e) Center of Zaveh

	Mg	K	Ca	Na	Fe	Zn
Surface Soil	24500	73800	93000	9440	35500	35.2
Soil of Corm	35900	76600	85000	10700	34600	73.2
corm	3400	3900	5390	1010	3610	66.5
Petal	7300	13800	3070	619	690	31.3
Thread	1500	19100	4580	89	113	29.7

(f) East of Zaveh

	Mg	K	Ca	Na	Fe	Zn
Surface Soil	52300	68300	50100	10400	35400	17.5
Soil of Corm	94400	86500	94300	11700	38700	73.8
Corm	8040	8010	5040	987	3620	297
Petal	7300	12700	4260	782	2760	0.32
Thread	1870	18400	5150	143	399	24.3

Figure. 3- Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Fe^{2+}, and Zn^{2+}concentrations in (a) North, (b) Center, and (c) South of Torbat Heydarieh; (d): West, (e) center, and (f) East of Zaveh.

Minimum, maximum, and average concentrations of Mg^{2+}, K^{+}, Ca^{2+}, Fe^{2+}, and Zn^{2+} in three main parts of saffron plant in Torbat Heydarieh and Zaveh counties are shown in Table 8. The concentrations of all the five elements in the corm and aerial parts of saffron were approximately within the sufficient range for plant growth, according to Tables 7 and 8. In addition, Mg^{2+}, K^{+}, Ca^{2+}, Fe^{2+}, and Zn^{2+} concentrations were found to have lower than toxicity levels in all parts of saffron plant; therefore, saffron can uptake sufficient values of required metals. Measurements of the soil contamination indexes, AFs and EFs, and TFs of the minerals were required to investigate the reaction between soil and saffron.

Table 8. Element concentrations in the saffron plant in Torbat Heydarieh and Zaveh.

Elements	Corm			Petal			Threads		
(mg/kg)	Min	Max	Avg.	Min	Max	Avg.	Min	Max	Avg.
Mg^{2+}	1970	8040	3491	1460	7300	3531	1380	2070	1876
K^{+}	2590	8010	4225	11000	15600	14100	15900	19300	14090
Ca^{2+}	2600	11400	6976	1460	4260	3158	4580	5860	4755
Fe^{2+}	262	5580	3251	61	2760	926	83	399	479
Zn^{2+}	66.5	297.0	145.0	0.3	56.5	39.0	19.4	51.0	30.0

Determination of soil contamination indicators:

Table 9 shows mean values of pollution factors, CDs, and pollution load indexes of the soil samples calculated by Eq. s 4, 6, and 7. Furthermore, the geo-accumulation indexes of saffron farm soil calculated by Eq. 4 are shown in Table 10. Although the soils of all farms in both Torbat Heydarieh and Zaveh counties were uncontaminated by Na^{+}, Fe^{2+}, and Zn^{2+} based on the geo-accumulation index, they were merely unpolluted by Zn according to its CF. The use of CF also revealed that the soils were moderately contaminated by Na^{+} and Fe^{2+}, except in the center and west of Zaveh, which were unpolluted by Na^{+} and Fe^{2+}, respectively.

Table 9. Soil pollution factors, contamination degrees, and pollution load indexes.

Elements	GB [28] (mg/kg)	Contamination Factor (CF) TN	TC	TS	ZW	ZC	ZE
Zn^{2+}	60	0.03	0.02	0.02	0.02	0.02	0.02
Na^{+}	11000	1.11	1.24	1.06	1.37	0.92	1.00
Fe^{2+}	32000	1.23	1.27	1.05	0.91	1.10	1.16
K^{+}	18000	3.56	3.45	3.58	3.33	4.18	4.30
Ca^{2+}	20000	3.53	3.49	3.72	3.73	4.45	3.61
Mg^{2+}	8000	4.71	4.70	4.31	3.39	3.78	9.17
PLI		1.13	1.14	1.05	1.01	1.04	1.17
CD		14.17	14.17	13.73	12.00	14.43	19.26

Contamination Level: 0 1 2 3 4 5 6

GB: Global average in the soil; PLI: Pollution Load Index; CD: Contamination Degree; TN: North of T. Heydarieh; TC: Center of T. Heydarieh; TS: South of T. Heydarieh; ZW: West of Zaveh, ZC: Center of Zaveh, ZE: East of Zaveh

Table 10. Indexes of geo-accumulation of saffron farms soil.

Elements	Index of Geo-accumulation (I-geo) TN	TC	TS	ZW	ZC	ZE
Zn^{2+}	-5.90	-5.96	-6.14	-6.23	-6.38	-6.62
Na^{+}	-0.43	-0.28	-0.50	-0.14	-0.71	-0.58
Fe^{2+}	-0.28	-0.24	-0.52	-0.74	-0.45	-0.37
K^{+}	1.25	1.20	1.25	1.15	1.48	1.52
Ca^{2+}	1.23	1.22	1.31	1.31	1.57	1.27
Mg^{2+}	1.65	1.65	1.52	1.18	1.33	2.61

Contamination Level: 0 1 2 3 4 5 6

ZW: West of Zaveh, ZE: East of Zaveh, ZC: Center of Zaveh, TS: South of T. Heydarieh, TN: North of T. Heydarieh, TC: Center of T. Heydarieh

The results of I-geo evaluated moderate contamination of soils by Mg^{2+}, K^{+}, and Ca^{2+}. However, the results of CF showed the soils were moderately to strongly contaminated by Mg^{2+}, K^{+}, and Ca^{2+}. On the other hand, the results of both CF and I-geo showed that the contamination level of soil by Mg^{2+} was one degree higher in the farms of eastern Zaveh, where the soil was therefore more contaminated by Mg^{2+}.

The contamination level was estimated by CF at one level higher than that of I-geo, which could be caused by a coefficient of 1.5 as the lithologic correction factor in the I-geo formula. The results of both CF and I-geo showed that the pollution level increased in the order of Zn^{2+} <Na^{+} <Fe^{2+} <K^{+} <Ca^{2+} <Mg^{2+}. This proves that both CF and I-geo can be used for essential micro- and macro-minerals in plant and soil samples. The CDs of the soils (Table 9) are in the range of 12–24. Therefore, the soils of all farms in Torbat Heydarieh and Zaveh were found at moderate/strong levels based on the total contamination by the studied six elements. Also, the results of the PLI (Table 9) showed the pollution status of sediments in terms of the elements measured in the studied areas. The geometric mean of contamination index indicated moderate contaminations in the two regions based on the PLI.

Measuring enrichment factor:

In this research, aluminum was chosen as a reference element [31]. The average background value of aluminum in Earth's shale (GB_{ref}) is 67000 mg/kg [28]. Table 11 shows the concentrations and CFs of aluminum in Torbat Heydarieh and Zaveh counties. The table also presents the measured EF values of Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} in both counties based on aluminum as a standard element. The results of EF showed that the enrichment factor increased in the order of Zn^{2+} <Na^+ <Fe^{2+} <K^+ <Ca^{2+} <Mg^{2+}, which is perfectly consistent with those of the calculated pollution factors.

An origin of enrichment of $0.5 < EF \leq 1.5$ is exclusively from natural activities. Therefore, the saffron farms were found with minimal enrichments of Fe^{2+} and Na^+. Also, human activities had a minimal impact on the enrichment of Na^+ and Fe^{2+} in saffron farms. Besides, EF values of Zn^{2+} were measured less than 0.5 in all the farms. Hence, there was a considerable deficiency of Zn^{2+} in all the saffron farms, which can arise from agricultural activities. This Zn^{2+} deficiency can be solved with enriched fertilizers [34].

Table 11. Enrichment factor of saffron farm soil.

		TN	TC	TS	ZW	ZC	ZE
Al (mg/kg)		55250	56200	48000	35300	45500	65700
CF_{Al}		0.82	0.84	0.72	0.53	0.68	0.98
Enrichment Factor (EF)	Zn^{2+}	0.03	0.03	0.03	0.04	0.03	0.02
	Na^+	1.35	1.47	1.48	2.58	1.35	1.02
	Fe^{2+}	1.49	1.49	1.46	1.71	1.61	1.18
	K^+	4.32	4.11	4.99	6.32	6.15	4.39
	Ca^{2+}	4.28	4.16	5.19	7.07	6.55	3.68
	Mg^{2+}	5.71	5.60	6.02	4.53	5.56	9.35

EF < 0.5: Deficiency; EF < 2: Minimal; 2 ≤ EF <5: Moderate; 5 ≤ EF <20: Significant; CF_{Al}: Contamination Factor of Aluminum; ZW: West of Zaveh, ZE: East of Zaveh, ZC: Center of Zaveh, TS: South of T. Heydarieh, TN: North of T. Heydarieh, TC: Center of T. Heydarieh

The EFs were in the range of $2 \leq EF < 5$ for Na^+ and Mg^{2+} in the west of Zaveh and Ca^{2+} and K^+ in Torbat Heydarieh and east of Zaveh, indicating a moderate enrichment of these elements. Additionally, a range of $5 \leq EF < 10$ was found in some farms for Mg^{2+}, K^+, and Ca^{2+}, which proves a significant enrichment. The cultivation of saffron has likely caused more accumulations of Mg^{2+}, K^+, Ca^{2+}, and Fe^{2+} in the soil. Therefore, it is recommended to use fewer fertilizers enriched with the mentioned elements in saffron farms.

Evaluation of translocation indexes:

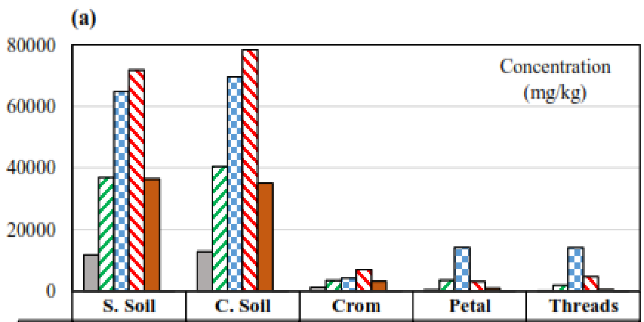
Paired t-test was used to investigate the movement ability of each mineral at various soil depths. Therefore, a statistical pair was made of two various soil depths for each element. The calculated results of p-values (Table 12) for paired t-test showed that the overall translocation ability of elements in the farm soil increased in the order of Na^+ <K^+ <Ca^{2+} <Mg^{2+} <Fe^{2+} <Zn^{2+}. Accordingly, the concentrations of Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} were statistically equivalent in the topsoil and surrounding soil of the saffron corm. It indicates the good translocation ability of Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} in throughout the soil. Nevertheless, Na concentrations were not statistically equivalent in different soil depths, which is caused by a high reactivity of Na with air.

Table 12. Results of paired t-test.

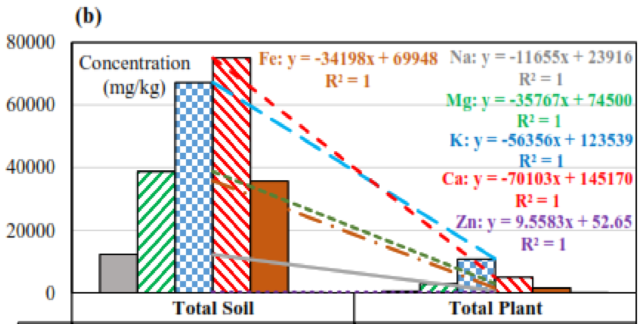
Pair	P-Value					
Name	Na^+	K^+	Ca^{2+}	Mg^{2+}	Fe^{2+}	Zn^{2+}
Ground & Corm Ground	0.02	**0.21**	**0.38**	**0.51**	**0.78**	**0.87**

Mean concentrations of Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} were firstly calculated in all the five types of samples to determine the coefficients and equations of motion from soil to saffron plant. Fig. 4A shows average values of mineral concentrations in topsoil (S. soil), surrounding soil of corm (C. soil), corm, petal, and threads of saffron, which would be used to determine the BCFs, BAFs, and TFs of Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} in Table 13.

In this study, three indicators were used to assess the absorption routes of Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} from the environment to saffron plant. The first was the BCF, which was applied to evaluate metal absorption by the root (corm of saffron). The BF was secondly used to estimate metal intake by aerial parts of saffron plant. Finally, the curves of metal concentrations were drawn from the soil to saffron plant, the slope of which indicated average metal absorption by the whole saffron plant.



	S. Soil	C. Soil	Crom	Petal	Threads
Na	11706.67	12816.67	1214.67	407.33	198.80
Mg	36966.67	40500.00	3491.67	3531.67	1876.67
K	64833.33	69533.33	4225.00	14166.67	14090.00
Ca	71766.67	78366.67	6976.67	3158.33	4755.00
Fe	36400.00	35100.00	3251.33	926.00	479.50
Zn	49.13	75.28	145.72	39.17	30.42



	Total Soil	Total Plant
Na	12261.67	606.93
Mg	38733.33	2966.67
K	67183.33	10827.22
Ca	75066.67	4963.33
Fe	35750.00	1552.28
Zn	62.21	71.77

Figure 4. Average concentrations of Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} (A): in topsoil, corm's surrounding soil, corm, petal, and threads of saffron; (B): in all depths of soil and whole of saffron plant.

Table 13. Biological concentration factors (BCF), bioaccumulation factors (BAF_P and BAF_T), and translocation factors of petal and threads (TF_P and TF_T) for Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+}.

Elements	BCF	BAF_P	BAF_T	TF_P	TF_T
Mg^{2+}	0.10	0.10	0.05	0.97	0.48
K^+	0.07	0.20	0.27	**3.09**	**3.50**
Ca^{2+}	0.10	0.04	0.07	0.47	**1.04**
Na^+	0.10	0.04	0.01	0.47	0.12
Fe^{2+}	0.09	0.03	0.01	0.39	0.14
Zn^{2+}	**2.57**	0.51	0.50	0.33	0.38

The BCF grade increased as K^+ <Fe^{2+} <Mg^{2+}=Na^+=Ca^{2+} <Zn^{2+}. Also, the BCF value was greater than 1 for zinc only. Na^+, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} BCF values were less than 0.10. Therefore, saffron plant can be merely classified as a good accumulator of Zn^{2+}. BAF_P and BAF_T are the BAFs of petals and threads of saffron, respectively. All the calculated BAFs were less than 0.51 for the aerial parts of saffron plant, suggesting that the aerial parts of saffron are poor accumulators of these six essential minerals directly from the soil.

Furthermore, average concentrations of Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} in the mixed soil and the whole plant samples are shown in Fig. 4B. A linear equation can significantly explain the displacement of each element from the soil to the saffron plant with the highest R-squared (r^2=1), as shown in Fig. 4B. The curve slope shows the absorption ability of a particular metal by all components of saffron plant. The Zn^{2+} curve slope was merely positive, which proves that saffron is a good accumulator of zinc. According to the results of BCF, BAF, and the concentration curve slope, the examined metals were merely absorbed by the corm of saffron.

TF_P and TF_T are respectively the TFs of petal and threads, which display the movement ability of minerals from the root to the other parts of saffron plant. The TF grades of petals and threads increased as Na^+ <Fe^{2+} <Zn^{2+} <Mg^{2+} <Ca^{2+} <K^+ and Zn^{2+} <Fe^{2+} <Na^+=Ca^{2+} <Mg^{2+} <K^+, respectively. TF of K^+ was greater than that for the entire aerial tissue. In addition, TF > 1 in the threads was merely measured for Ca^{2+}. Accordingly, the uptake and accumulation of K^+ were higher in the aerial tissues (including shoot, petal, and threads) than the corm of saffron. Ca^{2+} is also accumulated in the threads (crop of saffron).

Assessment of soil salinity:

The concentration units of Na^+, Mg^{2+}, K^+, and Ca^{2+} were firstly changed from SI unit (mg/kg) to the traditional unit (meq/100 g), which is necessary to calculate SAR and ESP in Eqs. 12 and 13, and Table 5. The NAA results (Fig. 3) are in the unit of mg/kg, which can be converted to meq/100 g by Eq. 14:

$$\left[{}^{meq}/_{100g}\right] = \frac{\left[{}^{mg}/_{kg}\right]}{\frac{Atomic\ Weight}{Charge} \times \frac{1000}{100}} \qquad (14)$$

Therefore, a unit of meq/100 g was calculated via dividing the unit of mg/kg by 230, 122, 391, and 200 for Na^+, Mg^{2+}, K^+, and Ca^{2+}, respectively [42]. Table 14 shows the concentrations of Na^+, Mg^{2+}, K^+, and Ca^{2+} in the unit of meq/100 g for soil samples in the six studied zones. Moreover, SAR and ESP were calculated by Eqs. 12 and 13 (Table 14). The pH and EC values of soil and water solution (1:5) were measured by a pH meter and an EC meter, respectively (Table 14). The soils of all the studied saffron farms were accordingly estimated to be at non-salinity levels in Torbat Heydarieh and Zaveh counties. Also, the results of soil salinity factors are completely equal to those of the Na^+ enrichment factor.

Table 14. Average values of pH, EC, ESP, and SAR.

	Elements	TN	TC	TS	ZW	ZC	ZE
meq/100 g	Na^+	53.26	59.13	50.65	65.00	43.78	48.04
	Mg^{2+}	308.61	308.20	282.79	156.56	247.54	601.23
	K^+	164.07	158.82	164.58	153.20	192.33	197.95
	Ca^{2+}	352.75	349.25	371.50	372.50	445.00	361.00
pH		6.53	6.86	7.13	7.12	7.09	7.27
EC (dS/m)		8.19×10^{-3}	8.10×10^{-3}	8.65×10^{-3}	7.59×10^{-3}	823×10^{-3}	5.53×10^{-3}
ESP (%)		6.64	7.43	6.30	8.88	4.92	4.72
SAR($\sqrt{\frac{meq}{100g}}$)		2.93	3.26	2.80	4.00	2.35	2.19

TN: North of Torbat Heydarieh, TC: Center of Torbat Heydarieh, TS: South of Torbat Heydarieh, ZW: West of Zaveh, ZE: East of Zaveh, ZC: Center of Zaveh; EC: Electrical conductivity; ESP: Exchangeable sodium percentage; pH: Alkalinity per Acidity; SAR: Sodium adsorption ration

Conclusion:

In this research, the NAA is used to determine the concentrations of Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} in saffron plant and a farm. The great Khorasan region produces above 90% of produced saffron in Iran, which accounts for over 90% of saffron production in the world. In this study, therefore, Torbat Heydarieh and Zaveh counties are chosen for sampling as the main regions of saffron production in the great Khorasan region. The results of the NAA show that the concentrations of Mg^{2+}, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+} in the corm, and aerial parts of saffron are approximately within the sufficient ranges for plant growth. Additionally, concentrations of these elements are lower than toxicity levels in all parts of saffron plant. Based on CF measurements, soil contamination levels are determined uncontaminated by Zn^{2+}, moderately contaminated by Na^+ and Fe^{2+}, and strongly contaminated by Mg^{2+}, K^+, and Ca^{2+}. The contamination level is estimated at one level lower by the I-geo than the CF, which could be caused by the use of the lithologic correction factor in the I-geo formula. The results of both CF and I-geo show that the pollution levels increase in the order of $Zn^{2+} < Na^+ < Fe^{2+} < K^+ < Ca^{2+} < Mg^{2+}$, indicating that both CF and I-geo can be used for essential micro- and macro-minerals in the plant and soil samples. The geometric mean of the contamination index is moderately contaminated in all examined regions based on the pollution load index (PLI). In this research, Al is chosen as a reference element to calculate the EF, showing increased EFs in the order of $Zn^{2+} < Na^+ < Fe^{2+} < K^+ < Ca^{2+} < Mg^{2+}$. Also, human activities have a minimal impact on the enrichment of Na^+ and Fe^{2+} in saffron farms. EF values of Zn^{2+} are also measured less than 0.5 in all the studied farms, suggesting a considerable deficiency of Zn in all the saffron farms. A moderate enrichment is found for Na^+ and Mg^{2+} in the west of Zaveh and K^+, and Ca^{2+} in Torbat Heydarieh and east of Zaveh. The EFs of Mg^{2+}, K^+, and Ca^{2+} prove a significant enrichment. The cultivation of saffron has likely caused more accumulations of Mg^{2+}, K^+, and Ca^{2+} Mg^{2+}, K^+, Ca^{2+}, and Fe^{2+} in the soil. Therefore, it is recommended to use fewer fertilizers enriched with these elements in saffron farms. Saffron plant can be merely classified as a good accumulator of Zn^{2+}. All calculated BAFs are > 0.51 in aerial parts of saffron plant, implying that these organs are poor accumulators of the six essential minerals directly from the soil. TF of K^+ in petals and threads of the corm to all the aerial tissue and merely that of Ca^{2+} in the threads of saffron are found greater than one. Accordingly, the uptake and accumulation of K^+ are higher in the aerial tissues (including shoots, petals, and threads) than the corm of saffron. Ca^{2+} is accumulated in the threads as well. Soil EC and pH values estimated non-salinity levels in soils of all saffron farms in Torbat Heydarieh and Zaveh counties. The results of soil salinity factors are completely compatible with those of EFs. Human-made activities have a minimal effect on Na^+ and Fe^{2+} enrichments in the whole Torbat Heydarieh, and that of Ca^{2+} in the east of Zaveh. Based on EF values, there is a considerable deficiency of Zn^{2+} in all the sites, and deficiencies of Na^+ and Fe^{2+} in the east of Zaveh, which could arise from agriculture activities, especially saffron cultivation. Furthermore, SAR, ESP, pH, and EC estimate non-salinity levels in all the examined soils.

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Author's declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Besides, the Figures and images, which are not mine, have been

given the permission for re-publication attached with the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee in University of Torbat Heydarieh.

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تحديد تنقل بعض العناصر الأساسية في الزعفران (Crocus sativus L.) عن طريق تحليل التنشيط النيوتروني

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الخلاصة:
الغرض الرئيسي من الدراسة هو تقييم تركيزات ستة معادن أساسية (Na^{+}، Mg^{2+}، K^{+}، Ca^{2+}، Fe^{+2} و Zn^{+2}) في الزعفران وتربة المزرعة باستخدام تحليل التنشيط النيوتروني (NAA) كطريقة قياس الطيف النووي. تم استخدام طريقة أخذ العينات العشوائية الطبقية هنا. وأظهرت نتائج NAA امتصاص البئر لـ Mg^{+2} و K^{+} و Ca^{+2} و Fe^{+2} و Zn^{+2} في الزعفران ، وهو أقل من نطاق السمية. بناءً على عامل التلوث ومؤشر التراكم الجغرافي ، تم تحديد مستويات تلوث التربة غير الملوثة بالزنك ، وملوثة بشكل معتدل بـ Na^{+} و Fe^{+2} ، وملوثة بشدة بـ Ca^{+2} و K^{+} و Mg^{+2}. أشارت نتائج درجة التلوث ومؤشر حمل التلوث إلى تلوث متوسط / شديد للتربة ومتوسط هندسي معتدل لمؤشر التلوث. أظهر عامل التخصيب (EF) Na^{+} تأثيرًا ضئيلًا من صنع الإنسان على تخصيب الصوديوم. ومن المحتمل أن تكون زراعة الزعفران قد تسببت في تراكم المزيد من Mg^{+2} و K^{+} و Ca^{+2} و Fe^{+2} ، بالإضافة إلى نقص كبير في Zn^{+2} في التربة ، بالاعتماد على EFs. أظهر عامل التركيز البيولوجي تراكم الزنك بشكل كبير بواسطة قرم الزعفران. كان هناك انتقال جيد من القرم إلى جميع الأنسجة الهوائية لـ K^{+}. كما قيمت نسبة امتصاص الصوديوم ، ونسبة الصوديوم القابلة للتبديل ، ودرجة الحموضة ، والتوصيل الكهربائي مستوى عدم ملوحة التربة في جميع مزارع الزعفران.

الكلمات المفتاحية: عامل التركيز البيولوجي ، التلوث ،الزعفران ، عامل الإثراء ، تحليل التنشيط النيوتروني ، ملوحة التربة ، عامل النقل.