A Comparative Study of Heavy Metals and Trace Elements Concentration in Milk Samples Consumed in Iraq

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Abstract:

The measurements of major and trace elements in different brands of milk powder selected from the Iraqis market via the X-ray fluorescence (XRF) Technique have been studied in the present work. The result of the measurements reveals the high concentrations of sodium, phosphorus, sulfur, chlorine, potassium, calcium and magnesium. Furthermore, low concentrations of aluminum, silicon, iron, bromine, molybdenum, iodine, barium, titanium, manganese, cobalt, chrome, nickel, copper, zinc and lead were detected. Neutron activation analysis (NAA) and Kjeldahl technique were also employed to determine the concentrations of nitrogen. It was found that the nitrogen concentration was in the range of (1.96 - 3.23) % which is within the permissible limits. The concentrations of the trace elements (copper, manganese, zinc, lead) were verified via the atomic absorption spectrometer (AAS) technique. The results are compared with the authorized limit by the food and agriculture organization and the world health organization (FAO/WHO) standards.

Keywords: Concentration elements, Kjeldahl technique, Neutron activation analysis (NAA), X-Ray fluorescence (XRF).

Introduction:

Milk is a high nutritional food; it is the main source of several vitamins and many minerals that are essential for mammals. It is the most versatile for natural food in terms of configuration and determines the necessary components for a healthy life. The milk and its products are the most important source of calcium (Ca) in our diet. It mostly contains potassium (K), magnesium (Mg) and phosphorus (P) (1,2). Furthermore, it contains more than twenty elements of different trace most of them are necessary and significant, such as copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) (3). These metals are involved with the enzymes to play an important role in many cell functions (4). It was reported that the lack of these metals cause disturbances and pathological conditions (5).

Furthermore, it was reported that the increasing of heavy elements such as chromium (Cr), cadmium (Cd), lead (Pb) and mercury (Hg) in the milk samples are due to several environmental

factors that affect nutrition as well as the manufacturing and the packaging process (6,7). Several damages in the human's organs were noticed due to the absorption of these elements, such as kidney disorder, liver and anemia (8,9). The minor and trace elements in the milk samples are lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), tin (Sn), zinc (Zn) and titanium (Ti). Previous reports indicated the presence of heavy metals in milk samples (10). Metals such as lead, cadmium and mercury were reported in milk samples. This raises a concern, since milk is largely consumed by infants and children (11). On the other hand, the nitrogen (N) contents and the proteins in the milk samples were also studied (11). Protein contains a unique nitrogen ratio and these studies revealed that the amount of nitrogen and milk protein is important in the formation of amino acids responsible for growth and development (12). Different techniques were employed to determine the elements contents in milk samples such as X-ray fluorescence (XRF) (13), Atomic Absorption Spectroscopy (AAS) (14,15), Neutron Activation Analysis (NAA) (16) and Plasma Atomic Emission Spectrometry (17). The important elements and their concentrations needed by the children body as

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recommended by the World Health Organization (WHO) are shown in Table 1 (18). The aim of this study is to determine the concentration of elements in selected milks powder that are consumed widely in Iraq. The samples were prepared and analyzed

through the X-rays fluorescence, neutron activation analysis, atomic absorption spectrometer and Kjeldahl technology are employed. The results are compared with the international organizations regulations.

Table 1. The elements necessary by the Infants and children with recommended amounts by the WHO.

Elements	Infants Age (year) 0.0-0.5	Infants Age (year) 0.5-1	Children Age (year) 1-3	Children Age (year) 4-8
Calcium (mg)	200	260	700	1000
Magnesium(mg)	30	75	80	130
Phosphorus(mg)	100	275	468	500
Potassium (g)	0.4	0.7	3.0	3.8
Sodium (g)	0.12	0.37	1.0	1.2
Chloride(g)	0.18	0.57	1.5	1.9
Manganese(mg)	0.003	0.6	1.2	1.5
Selenium (µg)	15	20	20	50
Molybdenum(µg)	2	3	17	22
Chromium (µg)	0.2	5.5	11	15

Materials and Method:
Sample Collection and Preparation

Eight samples were selected referring to the most consumed ones by the Iraqi population. The samples collected from the Iraqi markets are Anchor, Nan, Similac, Nido, Gold, Guigoz /1, Guigoz /2 and Novalac. Initially, the samples were dried for 24 hr in a drying oven. An equal mass of four grams of each samples was pressed via hydraulic piston with pressure of 15 Ton/cm^2. The result tablets were in a diameter of 32 mm and were kept in a vacuum dissector for later measurements.

The Instrumentations
The X-Ray Florescence (XRF)

In this work, XRF spectrometry (Spectro Xepos, SPECTRO Analytical Instruments) unit was

employed to measure the main and the trace elements of the milk samples. The unit utilized a silicone drift lithium detector, with an energy resolution 45 eV at photon energy 5.9 keV of iron (Fe-55) isotope. The detector was cooled using Peltier effect and the thickness of beryllium window was approximately 0.076 mm. Several targets were used to generate different X – ray energies. The targets are the highly oriented pyrolytic graphite (HOPG), alumina (Al$_2$0$_3$) and Molybdenum. Precision and accuracy of the results were tested by normal standard reference analysis in XRF unit. The results imply that the unit is credible for such measurements. Table 2 proves the ability of X-ray fluorescence technique to analyze a diverse collection of samples with good precision.

Table 2. shows the comparison of results between experimental data and the results published in certificate data to [PCC-1]

No	A. N	E.S	Con*	Con**	Error%	No.	A. N.	E.S.	Con.*	Con.**	Error%
1	11	Na	0.34	0.36	5.55	10	25	Mn	0.517	0.5	3.4
2	12	Mg	28.80	26.19	9.96	11	26	Fe	1.804	1.25	44.32
3	13	Al	0.32	0.35	8.57	12	27	Co	0.0066	0.0062	6.4
4	14	Si	20.69	19.48	6.21	13	28	Ni	0.349	0.372	5.94
5	15	p	0.38	0.40	5.00	14	29	Cu	0.0001	0.0001	0.0
5	16	S	0.145	0.244	26.64	15	30	Zn	1.64	1.87	12.42
6	17	Cl	0.761	0.875	13.03	16	34	Se	0.002	0.0031	35.48
7	19	K	8.012	10.00	19.88	17	42	Mo	0.004	0.0031	29
8	20	Ca	0.22	0.272	26.47	18	80	Hg	0.0013	0.0013	0.0
9	24	Cr	0.021	0.031	32.25	19	82	Pb	0.0001	0.0001	0.0

A.N.: atomic number, E.S.: element symbol, Con*: The experimental concentration (%), Con**: The certificate concentration (%).

Neutron Activation Analyses (NAA)

Neutron activation analysis was employed to determine the concentration of nitrogen. The samples were irradiated with the standard samples by a neutron source with a flux of 4×10^4 n/cm^2.s at

irradiation time 1 min. The result of the gamma emitted from the (n, gamma) reaction was measured via a gamma spectroscopy unit. The system consisted of a NaI(TI) detector with energy resolution (FWHM) at [137]CS is 8.5% connected to a

personal computer via Integrated Computer Spectrometer (ICS-PCI-4k) card. The resulted spectrums were analyzed via the gamma spectroscopy software.

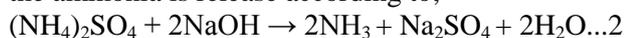*Atomic Absorption Spectrometry (AAS)*

The atomic absorption spectrometry (AAS) technique is widely employed to determine the quantitative and qualitative elements in a sample (19). This technique is considered the best to measure the concentrations of trace elements with the detection limit less than part per billion (ppb) (20). For samples to be analyzed using this technique, several chemical processes need to be done before using the AAS unit. Initially, the milk samples were digested using acids nitric, sulfuric and perchlorate at 80°C. The samples then were gradually brought to the boiling temperature for four hours, cooled, filtered and diluted to 100 ml volume with deionized water.
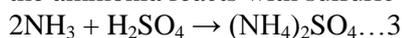
Kjeldahl method

The Kjeldahl method is recognized widely in the detection of the nitrogen and then calculating the protein. This method consists of several basic steps. The sample is inserted in a test tube and digested with the (anhydrous potassium sulfate (K_2SO_4), Selenium (Se), concentrated sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2)). The sample is digested using the following reactions (21).

$N + H_2SO_4 \rightarrow CO_2 + H_2O + (NH_4)_2SO_4$ …1

the ammonia is release according to;

$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$...2

the ammonia reacts with sulfuric acid;

$2NH_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4$…3

The prepared tube had heated at 420 °C for 30 min and then cooled to 50-60 °C. The distillation of the ammonia into trapping solution and the quantification of the ammonic by titration with a standard solution concentration (22). The Kjeldahl method dose not measure the concentration of protein directly. The protein concentrations are estimated by multiplying the measured nitrogen concentration with the conversion factor (F) that equals to 6.3 (21).

Results and Discussions:
Concentration elements using XRF and AAS

The results of the milk samples consumed widely by the Iraqi population was measured through the XRF and were verified through AAS. The results can be classified to major elements which are Na, P, S, Cl, K, Ca and Mg, covering the vast range of elements. Beside the major elements, trace elements were found in the selected milk samples. These are Al, Si, Fe, Zn, Br, Rb, Mo, I, Ba, Ti, Mn, Co, Cu, Mn, Pb.

Major Elements

Fig 1 shows the results of the major elements in the milk samples, the results reveal that there is a small variation in the measured concentrations of the major elements Na, S, Ca, K, Mg, and P for most samples which distinguish the quality of the milk (23). It was found that the Nido contains the highest concentration of essential elements. The average range concentrations are as follows: Ca (0.750 - 1.766) %, K (1.00-1.87) %, Mg (0.047- 0.138) %, Na (0.206 -0.417) %, S (0.23 - 0.527) % and P (0.62 -1.23)%. In the measured samples containing Ca, P, Mg, Na, Al, and K, the concentrations of these elements are within the permissible limits of World Health Organization (WHO) (24) as shown in Table 3.

Table 3. Identify concentrations of certain elements and compare them to limits allowed by WHO

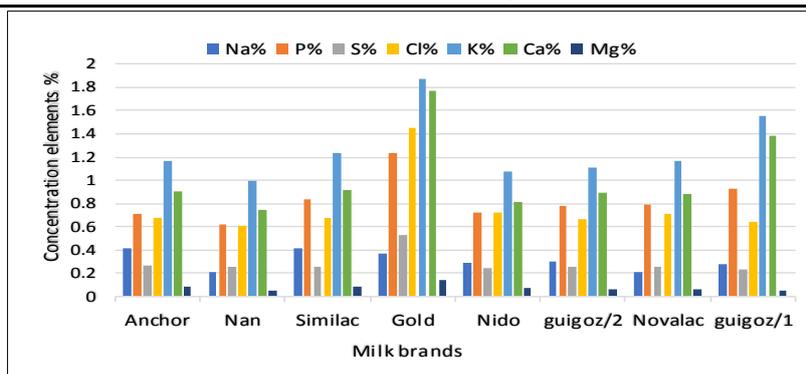| Elements	Minimum mg/kg	Maximum mg/kg	WHO Limits mg/kg
Ca	7500	17660	4400
P	6200	12300	4400
Mg	470	1380	4400
Na	2060	4170	5000
K	10000	18700	5000

Figure 1. Major Concentration Elements (%) using XRF technique

Trace Elements

The large differences in the group of trace elements Al, Si, Fe, Br, Mo, I, Ba and Ti, Mn, Co, Cr, Ni were revealed in Fig 2 and 3 respectively. Fig 4 also shows a different concentration of Zn for all samples. The different concentrations of these elements depend on the diet of cows (25). The results showed the inverse correlation among Fe, Si and Zn in most of the measured samples. The differences in the concentration of Al, Zn, Mo, I was insignificant for most of the measured samples. Furthermore, it was found that there is a direct relationship correlated between Cu, Fe and inverse correlation with Mn. Copper deficiency is very dangerous due to the relationship between the copper and iron and associated diseases with iron deficiency. Copper is essentially stimulating the ferroxidase enzymes responsible for the movement of iron in tissues, therefore insufficient copper will probably cause iron deficiency, consequently, this will probably cause diseases that are related to the lack of these two elements. An increase in the proportion of manganese weakens the activity of copper and therefore affects the proportions of iron. It is usually the prevention of mineral deficiency in milk by using cows diet rich in minerals by licking minerals (26). It was reported that several external factors such as environmental pollution, packaging and storage probably might increase the concentration of lead and cadmium (27), the mean value of lead and cadmium is leas then the limit of World Health Organization (WHO) (1 mg/kg , 0.03 mg/kg) (28). In the measured samples also containing Cr, Cu, Fe, Mn, Ni, and Zn, the concentrations of these elements are within the permissible limits of World Health Organization (WHO) (29). as shown in Table4.

Table 4. Identify concentrations of certain elements and compare them to limits allowed by WHO

Elements	Minimum mg/kg	Maximum mg/kg	WHO Limits mg/kg
Cr	3.8	8.7	1.61
Cu	1.5	3.4	24.2
Fe	10	490	0.5
Mn	0.1	6.4	55.5
Ni	0.5	1.5	0.43
Zn	20	100	121

The concentrations of Al, Si, Fe, Zn, Br, Rb, Mo, I, Ba, Ti, Mn, Co, Cu, Mn, Cr, Ni and Pb for milk samples were measured and their range were (46 - 99) ppm, (20-930) ppm, (10-490) ppm, (20-60) ppm, (10-50) ppm, (10-470) ppm, (10-20) ppm, (20-70) ppm, (19-120) ppm, (0.7-6.7) ppm, (0.1-9.9) ppm, (1.0-2.1) ppm, (1.5-3.4) ppm, (0.1-9.9) ppm (3.8 -8.7) ppm , (0.5-1.5) ppm and (0.5-1.3)ppm, respectively.
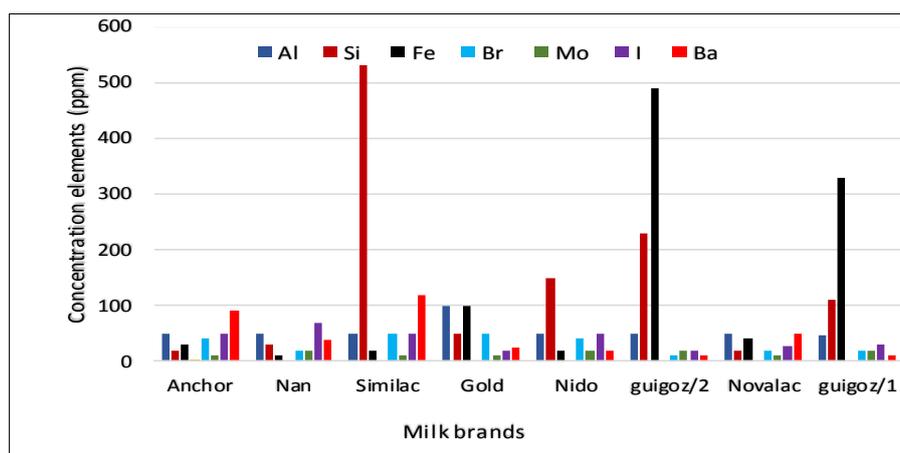

Figure 2. Trace concentration elements (ppm) using XRF technique
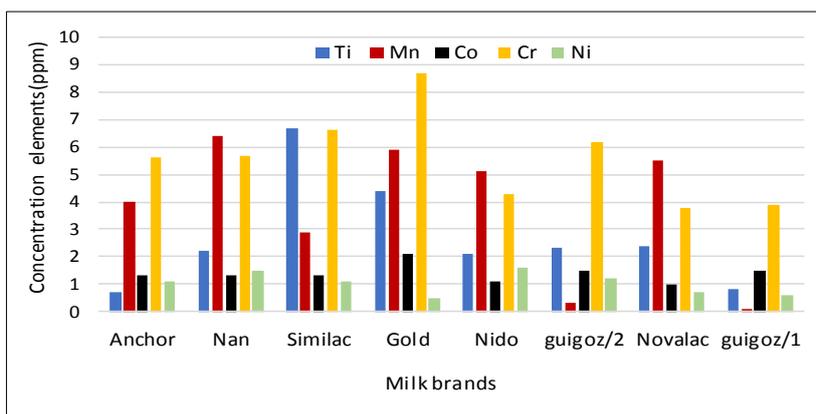
Figure 3. Trace concentration elements Ti, Mn, Co, Cr and Ni (ppm) using XRF technique

The results of selective elements were verified via the AAS. Fig 4 and 5 reveal the elements Cu, Mn, Pb, Zn concentration in the eight samples of the milk. The concentrations of elements were (0.61-3.5) ppm, (1.5-7.8) ppm, (10.01-50.2) ppm, (0.53-1.3) ppm, respectively. Compared with the results obtained from the XRF technique, Milk is a poor source of Cu (30). In all the samples measured, lead contents less than authorized limit by FAO/WHO standard (Pb: 20 mg/kg) (31). The concentration of lead is based on feeding the cows method of packaging and storage (27).
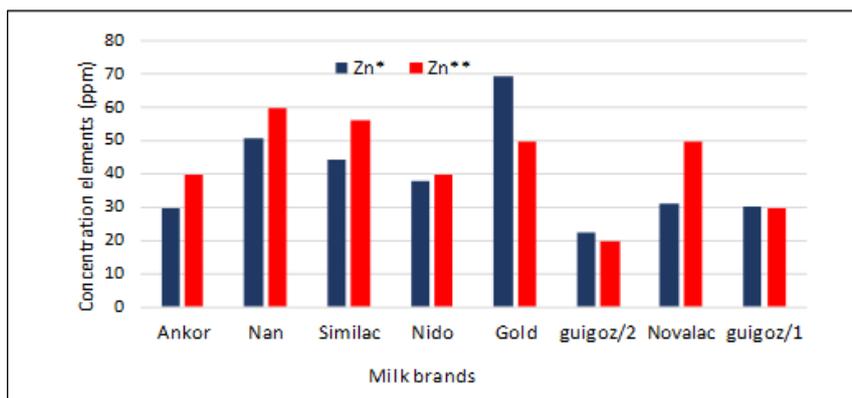

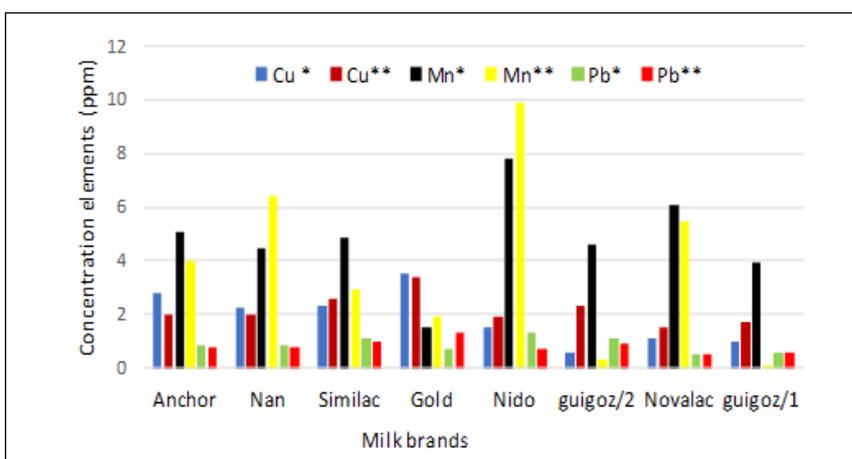**Figure 4. Concentration of Zinc using AAS*technique and XRF** technique**

Figure 5. Concentration elements (ppm) using AAS*technique and compare with XRF**

Concentration of nitrogen and protein

From Fig 6 it can be noticed that the highest concentration of protein is 22.41% in Nido and less concentration is 12.82% in Similac. The value of protein is 6.38 multiplied by the total nitrogen determined by the kjeldahl method. Diet affects the proportions of milk protein and is an effective tool for evaluating nutrition programs. Increasing the proportion of protein has severe consequences (32). Calculating the protein in this way represents the

raw protein because the measured nitrogen is derived from protein and from non-protein sources. When using melamine, a type of plastic, rich in nitrogen, illegally added with animal feed to

increase the proportion of nitrogen causes a significant risk to human health, for example causes renal failure and kidney stones (33).
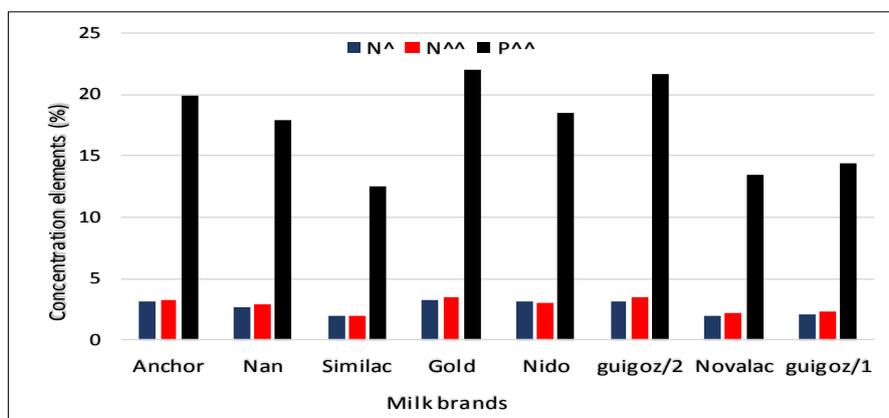

Figure 6. Concentration of nitrogen and protein using NAA$^\wedge$ technique and Kjeldahl^^ method

The World Health Organization (WHO) does not approve the addition of melamine to animal feed. The methods used to calculate the level of protein fail to distinguish between the nitrogen output from the melamine or amino acids, making the level of protein not real (34). The World Health Organization (WHO) has identified the protein level 34 % in the solid milk segment depending on the country producing the milk, how it is treated, and the withdrawal or addition of components to milk (24).

Conclusions:

In summary, XRF, NAA, AAS were used to analyze the minerals in several milk powder samples. The samples were collected from the Iraqi markets which are used frequently by the local populations. The result of the measurements reveals two main groups of samples, one with high concentrations namely Na, P, S, Cl, K, Ca and Mg. The second group with low concentrations namely, Al, Si, Fe, Br, Mo, I, Ba, Ti, Mn and Co. Furthermore, selected elements concentrations were verified via XRF and AAS methods.

The results of this study show that the milk content has an effect on the nutritional quality of milk, where the level of heavy elements is less than the permissible limits by FAO/WHO as well as determining protein level in milk and its effect on milk quality. It is necessary to make further studies to evaluate the contents of the major and trace elements and ensure the absence of potential toxic risks.

Conflicts of Interest: None.

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دراسة مقارنة للمعادن الثقيلة وتركيز العناصر النادرة في عينات الحليب المستهلكة في العراق

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الخلاصة:

في العمل الحالي تم قياس العناصر الرئيسية والنزرة لنماذج مختلفة من مسحوق الحليب المختار من الاسواق العراقية باستخدام تقنية فلورة الأشعة السينية (XRF). كانت نتيجة القياسات تشير الى احتواء النماذج على تراكيز عالية من الصوديوم ،الفوسفور ،الكبريت ،الكلور ،البوتاسيوم ،الكالسيوم والمغنيسيوم. وعلاوة على ذلك ، تم الكشف عن تراكيز منخفضة من الألمنيوم ،السيليكون ،الحديد ،البروم ،الموليبيدينوم، اليود، الباريوم ،التيتانيوم ،المنغنيز ،الكوبالت ،الكروم ،النيكل ،النحاس ،الزنك والرصاص. تم أيضا استخدام تقنية التحليل بالتنشيط النيوتروني (NAA)وتقنية Kjeldahl لتحديد تراكيز النيتروجين. وجد أن تركيز النيتروجين كان في حدود (1.96 - 3.23)٪ وهو ضمن الحدود المسموح بها. تم التحقق من تراكيز العناصر النزرة (النحاس، المنغنيز، الزنك، الرصاص) عن طريق استخدام تقنية مطيافية الامتصاص الذري (AAS) قورنت النتائج مع الحدود المسموح بها لمعايير منظمة الأغذية والزراعة ومنظمة الصحة العالمية.

الكلمات المفتاحية: تراكيز العناصر، تقنية كيلدال، تحليل التنشيط النيوتروني (NAA) ، فلورة الأشعة السينية (XRF)