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Comparative study on two methods for determination of selenium in some Sudanese plants fruits and their soils

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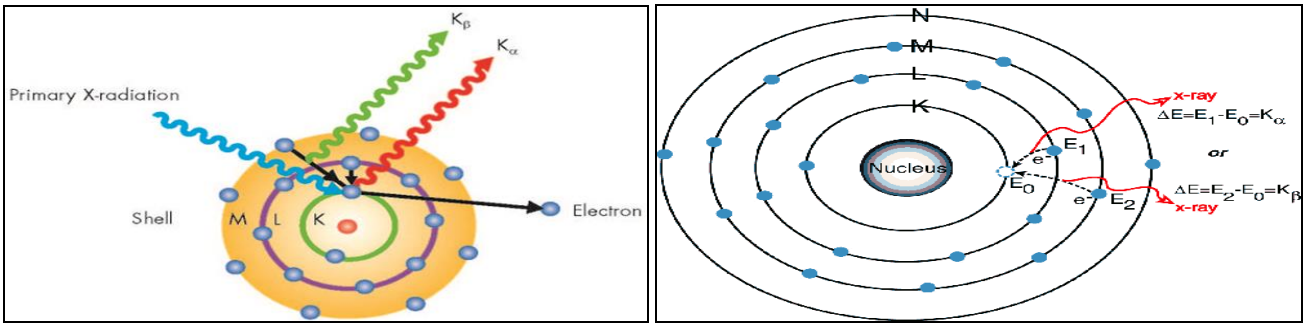
Abstract
This study was undertaken to determine selenium (Se) concentration in some Sudanese plants fruits, *(Ziziphus spina Christi), (Adansonia digitata), (Balanites egyptiaca)* and soil where these plants were planted from different depths (75 cm, 100 cm, and 150 cm) by using two techniques, hydride generation atomic absorption (HGAAS) and energy dispersive x-rays fluorescence (EDXRF) as well as to compare the determined amounts of Se for fruits and their soils by using two techniques. All samples were taken from a forested area called Alein forest, north of Al-obied city in west of Sudan. The results in unit (ppm) was as follows by using the two techniques mentioned respectively, first, with regard to the plants: 0.0172,, 0,0154 for *ziziphus s*, 0.0346, 0,0186 for *Adansonia d*, and 0.0348, 0.0170 for *Balanites e*,. As for the soil depths (75 - 100 – 150 cm) results were as follows: Between 0.163 – 0.195 by (HGAAS) and between 0.150 - 0.190 by (EDXRF).From results it was found that; Se concentration ranged as follows: *Adansonia d*, > *Balanites e*, > *ziziphus s*. From results, for plants and for different soil depths it was observed that (HGAAS) measured highest Se concentration with high efficiency, then (EDXRF). From results, it was found that depth of 150 cm contains high Se concentration > depth 75 cm > depth 100 cm. It was found that soils of *Ziziphus s* hve a higher Se concentration > *Balanites e* soils > *Adansonia d* soils, it was cleared that: soil contain high Se concentration than plants.

Keywords: Sudanese fruits, determination, comparative study, selenium

1. Introduction
Nutrients are substances present in food which can provide energy, promote growth and development as well as maintain normal functions of the body. Deficiency or excessive intake of nutrients may lead to diseases such as heart diseases, diabetes mellitus and certain types of cancer. Micronutrients include all the essential minerals and vitamins. Trace minerals, such as molybdenum, selenium, zinc, iron, and iodine, are only required in a few milligrams or less. Many minerals are critical for enzyme function, others are used to maintain fluid balance, build bone tissue, synthesize hormones, transmit nerve impulses, contract and relax muscles, and protect against harmful free radicals. (Richard et al. 2008) [12]. among these nutrients, selenium (Se) which is known to play an important role and necessary for the development of the acquired immune system. Selenium was shown to be essential for animals and to be an integral part of glutathione peroxidase, an enzyme that catalyzes the breakdown of hydrogen peroxide in cells**.** Glutathione peroxidase activity was found to be a good measure of Se status **(**Burk et al. 2009) [1]. The fate of Se in natural environments such as soils and sediments is affected by a variety of physical, chemical and biological factors which are associated with changes in its oxidation state and as a variety of organic compounds. Se solubility and availability to organisms. Some of the methods for determining Se in different materials have been compared within the same laboratory for accuracy and precision (Niedzielski 2002) [10]. There are numerous procedures for determination of Se in environmental samples and, especially, in plants and soil, such as: ● hydride generation atomic absorption spectroscopy (HGAAS) in this technique Sample reduction to convert Se^{+6} to Se^{+4} is necessary prior to using sodium borohydride ($NaBH_4$) to reduce all Se present to selenium hydride. ● energy dispersive x-rays fluorescence (EDXRF), in this technique, when a primary x-ray excitation source from an x-ray tube or a radioactive source strikes a sample, the x-ray can either be absorbed by the atom or scattered through the material, an x-ray was absorbed by the atom by transferring all of its energy to an innermost electron is called the "photoelectric" effect each element present in the object produces X-rays with different energies. An electron can be ejected from its atomic orbital (K shell) by the absorption of a light wave (photon) of sufficient energy (an external primary excitation x-ray) creating a vacancy (Fig, 1, 2).



(a) **(b)**

Fig 1, 2: X-ray fluorescence radiation

The energy of the photon (hv) must be greater than the energy with which the electron is bound to the nucleus of the atom. if the primary x-ray had sufficient energy, electron when ejected from the inner shells, creating vacancies. These vacancies present an unstable condition for the atom.as the atom returns to its stable condition, An electron from a higher energy level orbital (L shell) would be transferred to the lower energy level orbital (K shell) to fill the vacancy. During this transition a photon may be emitted from the atom. In the process, it emits a characteristic x-ray unique to this element and in turn, produces a vacancy in the L shell (Elamin 2005) [7]. The sensitivity of modern spectrometers is so high that they even detect fingerprints, which can disturb the analysis. Spectrometers only analyze the sample's surface layer, so it must be representive of the whole sample. For instance in small disk represents the contents, the sample must be representative of the intire material, and so must be taken very carefully. Once taken, it must also be handeled carefully (Ebrahim et al. 2012 [6]. Concentration of Se in soil is important to soil scientists not only for environmental purposes, such as quantifying contamination, but also in helping to solve problems associated with human and plant toxicity (Biao & Zhong 2009) [2]. Dolph et al. (2012) [4] reported that high concentrations of Se in irrigated soils and shallow groundwater pose a threat to agricultural production and the health of humans and animals. On the other hand, Conor (2012) studied Se concentrations in some Libyan soils and found that the concentrations in clay surface soil are higher than in sandy soil. The multiple regression analysis confirmed the importance of pH as well as other soil properties such as texture, electrical conductivity and organic matter or carbonates on the behavior of Se. Global Se concentration ranged from 0.01 to 2 mg kg^{-1} (Dungan & Frankenberger 1999) [5]. Se concentration in (mg kg^{-1}) (<0.125 Deficient), (0.125 - 0.175 Marginal), (0.175 -3 Moderate - High), (>3 Excessive), (>5 Seleniferous) (Fordyce 2005) [8]. Se concentrations in most soils are within the range 0.01–2.0 mg Se kg^{-1} and Shanxi Province 0.32 mg kg^{-1}, In Australian soils, Se ranges between 0.11–0.41 mg kg^{-1}., while in Finland, a mean soil Se concentration of 0.21 mg kg^{-1} was found in the plough layer (Shahtaheri et al. 2006) [13]. Se concentrations of 0.24-0.55 mg kg^{-1} have been reported in India (Yadav et al. 2005) [14]. Brazil nuts can be considered as a good, if somewhat variable, source of dietary Se and have been advocated as an ideal dietary supplement, it is well to recognize that there could also be a health hazard. In contrast, nuts from the Acre–Rondonia region, on the upper Amazon, where soil Se levels are low, contain on average of 3.06 ± 4.01 µg/g, with a range of 0.03 to 3.17 µg/g. Reilly 2006) [11], compared to North American levels of 0.33 µg/g. In vegetables and fruits, Australian figures were 0.001 to 0.022 µg/g, somewhat lower than American findings of 0.004 to 0.063 µg/g in similar vegetables and fruits. Se levels (mg/kg) in peanuts from different countries UK (0.030), USA (0.075), Australia (0.140), Thailand (0.032 – 0.186), New Zealand (0.046 – 0.150) (Cashman 2001) [3]. In a study carried out by (Ebrahim, et al 2012) [6] among some popular Sudanese plants fruits, the results of Se concentration was as follows; Adansonia digitata.0.06 ppm, Balanites aegyptiaca 0.04ppm, Ziziphus spina-christi, 0.0114 ppm.

2. Materials and Methods

2.1 Materials

2.1.1 The Study area

A natural reserved forest called Alein which is reserved for the Republic of Sudan by decree No. 867 dated 15/4/1954 and is managed by the National Forestry Commission of the Ministry of Agriculture and Forestry. Alein forest is located in North Kordofan State, 20 km south east of Al- obied city altitude (°30 14 21, °30 26 06) N, and longitude (30 20 130, 41 50 120) E and is bordered to the north by Mountain Kordofan and to the east by Mountain Alein and from the west- north by the villages of Dubeiba, Kallo and Aldabaa and is bounded from the south by Warshal Hafr, Warshal Mudkha and Alhogratt villages, and from South-east of Paduga village The forest area is 44,000 acres (Ibrahim. & Mohammed. 2015) [9].

Fig 3: Location of Alein forest

2.1.2 Samples

New cultivated fruits of three types of Sudanese plants fruits (*Ziziphus spina Christi* - *Adansonia digitata* - *Balanites aegyptiaca*) and their soils at three different depths (75 – 100 – 150 cm)**.**

2.1.3 Equipments

Volumetric flasks **(**100 ml- 50 ml).- ceramic knife-- small

dishes-- oven adjust at 110 ^{0}C - mortar and sieving - sensitive balance - digestion flask- quartz vessels- Minipress machine (a ton/cm^2 hydraulic press) - an electrical plate..

2.1.4 Reagents

Deionized water, Se^{+4} (SeO_2) stock solution (1000 µg/mL), Hydrochloric acid (12 M HCl), Hydrochloric acid (6 M HCl), Nitric acid (15 M HNO_3), Perchloric acid (11 M $HClO_4$), Sodium boroterahydrate ($NaBH_4$) (0.2 g), Extraction solution which contain ammonium acetate + acetic acid + ethelene diamine tetraaceticacid (0.5M NH_4Ac + 0.5 M HAc + 0.02 M EDTA),, sodium hydroxide 0.5%, 6 M HF, 9.8 M H_2O_2, cellulose. All reagents were of analytical reagent grade (Merck, Darmstadt Germany).

2.1.5 Instruments

Two analytical techniques applied for determination of trace amounts of Se in collected samples, these techniques were:

- Hydride Generation Atomic Absorption Spectroscopy (HGAAS) model contrAA700 P Hydride Analytik Jena AG, Jena, Germany.
- Energy Dispersive X-rays Fluorescence (EDXRF) model CANBERRA SERIES 35 PLUS. USA.

2.2 Methods

It consisted of three steps

2.2.1 Sampling (collection & transportation of the samples)

The samples under study were collected according to Standard Methods from the study area (Alein forest) and kept in very cleaned paper bags until arrived at the laboratories.

2.2.2 Preparations

It consisted of two activities

1. **Preparation of selenium standard solution (for HGAAS method)**

Prepared by dissolving 0.14053g SeO_2 in 100 ml volumetric flasks. A minimum amount of NaOH was added to dissolve the selenium dioxide before dilution to the mark with deionized water. The fresh standard solutions were prepared daily by successive dilution from the stock solution.

2. **Preparation of samples**

The preparation of the samples included two steps

(A) Drying and homogenizing

(A_1) Sudanese plants fruits

The pulp of Sudanese plants fruits were manually separated from the seeds using ceramic knife and then aliquots of these pulp were cut into small pieces and were put in room temperature in small dishes for a day for drying and then grounded and homogenized using a mortar and sieving Then transferred into an oven at 110 ^{0}C for one hour to vaporize water from them, The fine powder was packed inside air-tight containers.

(A_2) Soil

Aliquots of the soil materials were put in room temperature in small dishes for a day for dryness and then grounded and homogenized using a mortar and sieving and mechanical shaker, then transferred into an oven at 110 ^{0}C for one hour to vaporize water from them, the fine powder collected was packed inside air-tight containers.

(B) Destructive method (Wet digestion) for HGAAS technique

(B_1) Sudanese plants fruits

1 g of dried, fine and homogenized fruits pulp were weighted and transferred into digestion flask. In the digestion flask, 10 ml of concentrated nitric acid(15 M HNO_3) was added and allowed to stand over-night, then was heated carefully on a hot plate until the production of red NO_2 fumes had ceased, the digestion flask cooled. After cooling a small amount (2-4 ml) of (70%) perchloric acid (11 M $HClO_4$) was added and heated again and allowed to evaporate to a small volume. then the contents of the digestion flask was transferred in to 50 ml flask and then diluted to the volume with deionized water

(B_2) Soil

0.5 g of the dried, fine and homogenized soil was properly weighed into quartz vessels. Subsequently, digested overnight in a 50- mL conical flask by 10 mL of concentrated acid mixture nitric acid (HNO_3) / perchloric acid ($HClO_4$) 4:1 volume/ volume).Then 5 ml of concentrated hydrofluoric acid (6 M HF) was added carefully. The mixture was then heated carefully at 100°C for 1 h, 120°C for 2 h and then 180°C for 1 h, using an electrical plate. The samples were then heated at 210°C until no white fumes appeared. The remaining solution was cooled down to room temperature, and 5 mL of hydrochloric acid (HCl 12 M) was added to convert Se^{+6} to Se^{+4}, for about 4 h. Finally, the solution was adjusted to 25 mL with deionized water. The measurement of total Se was accomplished by the use of previously established methods (wet or acids digestion for the samples to destructive organic matters. Extracted solvent (extraction solution (ammonium acetate + EDTA)) was used after wet digestion (acid digestion to concentrate Se that was present in low concentration, each fraction resulting from the extraction was subjected to reduction with 3 mL concentrated HCl (12M) [Se^{+6} passes into Se^{+4}] by heating them to 80 °C for 30 minutes on a sand bath, then 10 ml of solution was used for analysis. The influence of HCl concentration in the sample solution on the interferences Se passes from the oxidation state IV to II), then 10 ml of that solution was used for analysis. (Notice that: Nitric acid addition was required to contribute to sample dissolution via NO^- radicals and to maintain oxidizing conditions. In case of HGAAS technique (for Sudanese fruits and soil) addition of hydrochloric acid prior to $NaBH_4$ in order to form selenium hydride decrease the interferences from 24 ions. To minimize interferences with transition metals at selenium dosage, optimal concentrations of the reagents have been established: -sodium borohydride 0.5% m/v in sodium hydroxide 0.5% m/v -hydrochloric acid 50 % v/v. For total selenium determination, $NaBH_4$ and hydrochloric acid were added to the real samples to reduce all se to Se^{+4} since Se^{+6} only can produce the hydride form. To reduce Se^{+6} to Se^{+4}, appropriate amount of concentrated hydrochloric acid was added to the solutions to give 4 mol/L as final acid concentration.

(C) Non-destructive method for EDXRF technique

A quantity of 1.0 g of each powdered, dried, soft and homogenized samples (fruits and soil) were weighed by

sensitive balance and after adding a binding material (cellulose) (to improve the quality of the tablets), they were then compressed using mini-press machine (a ton/cm2 hydraulic press), and made it into pellets or tablets of 13 mm diameter and about 2 mm thickness (Fig 4).

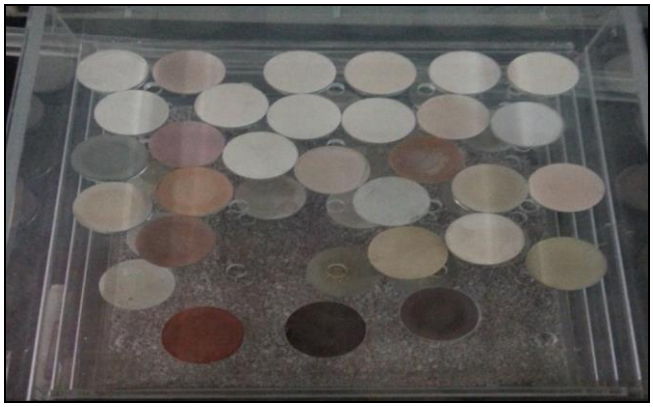


Fig 4: Pressed pellets of samples perior to their analysis by (EDXRF)

The tablets were then after homogenized and pressed used as targets for the EDXRF experiment. For quality control two types of Standard Reference Materials were used, and treated in the same manner.

2.2.3 Experimental

Two instrumental analytical methods may be employed to measure the concentration level of selenium (in ppm) in various samples. (Plants – soil) The most predominant techniques were atomic absorption spectrometry (AAS) including HGAAS, Energy dispersive X-ray fluorescence (EDXRF)

1. Hydride generation atomic absorption spectroscopic (HGAAS) technique

Selenium determination based on the injection flow by using, the peristaltic pumps. Which used simultaneously for transporting the reducing agent and the sample, and for waste removal. when the fill valve was opened, the exact volume of an aqueous sample containing selenite (the only reactive selenium species) was been loaded; when the valve was on the injection position, the sample was introduced into the carrier phase and transported to the reaction section with sodium borohydride in the presence of hydrochloric acid, to generate gaseous selenium hydride (SeH_2) the selenium hydride was then transported to a gas-liquid separator and was thermally decomposed and atomized in the sample beam of the atomic absorption spectrometer. The gas phase was separated from the reaction mixture solution (SeH_2) in the gas/liquid separator via transportation by the carrier gas (N_2) and then they were sent to the atomizer. Atomic absorption measurements were performed in a quartz atomization cell in air-acetylene flame at 196 nm and background correction was made using wavelength correction. When selenium was separated and, after passing through the filter of tetra-fluorine-ethylene and transported in the flame to the absorption cell (located on a metal support mounted above the atomic absorption spectrophotometer burner) by carrier and purge gas "argon" which absorbs radiation from xenon lamp of the spectrophotometer. The spectrophotometer ‘’Thermo Elemental’’ was controlled by computer, and after adding the reducing agent to analyzed solution the absorbance measurement begins. On the computer screen, the adequate selenium vapor absorbance was recorded as peaks whose height was proportional to the selenium concentration in the analyzed sample.

2. Energy Dispersive X-rays Fluorescence (EDXRF) technique

An energy dispersive XRF spectroscopy technique equipped with Si (Li) detector with a 109 resolution of 180 keV. Radioisotope Cd with energy 22.1 K eV was employed as a primary source used for measurement of Se in some Sudanese plants fruits and their soils (which prepared above), were presented to the EDXRF spectrometer system, to analyze. Each of the pellet was placed in a cup and the cup was placed in the spectrometers, and was measured for 1000 seconds. In EDXRF a whole spectrum was measured simultaneously and the area of a peak profile determines the concentration of an element. Measuring of the height of the peak profile was an alternative, but a lot of information would be lost because the area of a peak profile is less sensitive to noise than the height of the same peak. This fluorescent light is called the characteristic X-ray of the element. EDXRF can be seen as beams of photons with associated energies spectrometers have detector that is able to measure the different energies of the characteristic radation coming directly from the sample. The detector can seprate the radiation from the sample into the radiation from the elements in the sample. This separation is called dispersion. Then, the spectra obtained as results of x-ray, excitation using Cd-109 x-ray source) were transferred to a computer. The spectra was then analyzed and concentration of the Se present in the samples was obtained using AXIL - XRF software available in the computer. For quality control, a standard reference of Hay powder was used and treated in the same manner.

3. Results and discussion

Table 1: Results of selenium concentration in ppm in some Sudanese fruits by using two techniques (HGAAS & EDXRF)

Plants ↓ / Techniques →	HGAAS	EDXRF
Ziziphus spina christi	0.0172	0.0154
Adansonia digitata	0.0346	0.0188
Balanites egyptiaca	0.0348	0.0170

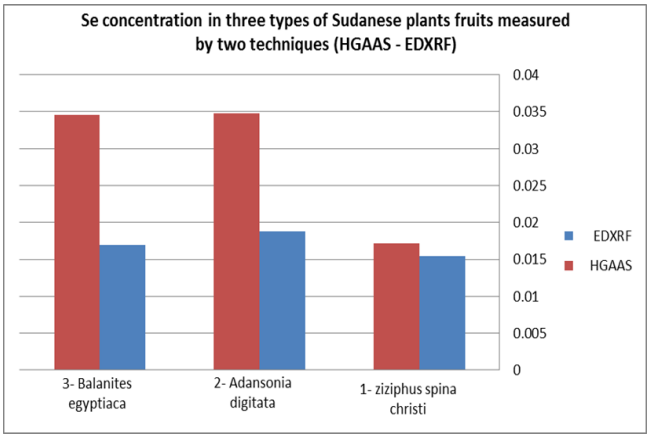


Fig 5: Comparison of Se concentration in the studied plants by using the two techniques (EDXRF & HGAAS)

From this figure it was noted that: results of measuring concentration of selenium in plants under the study was as follows: *Adansonia digitata* > *Balanites egyptiacea* > *ziziphus spina Christi*. Also it was noted that: the (HGAAS) measured the highest concentration of selenium with high efficiency, followed by by (EDXRF).

Table 2: Results of selenium concentration in ppm in some Sudanese fruits soils (75 cm – 100 cm – 150 cm) measured by HGAAS

Plant /soil depth	Soil depth (75 cm)	Soil depth (100 cm)	Soil depth (150 cm)
Ziziphus spina christi	0.183	0.174	0.192
Adansonia digitata	0.163	0.184	0.195
Balanites egyptiaca	0.173	0.169	0.194

Table 3: Results of selenium concentration in ppm in some Sudanese fruits soils (75 cm – 100 cm – 150 cm) measured by EDXRF

Plant/soil depth	Soil depth (75 cm)	Soil depth (100 cm)	Soil depth (150 cm)
Ziziphus spina christi	0.170	0.165	0.162
Adansonia digitata	0.150	0.182	0.190
Balanites egyptiaca	0.165	0.162	0.189

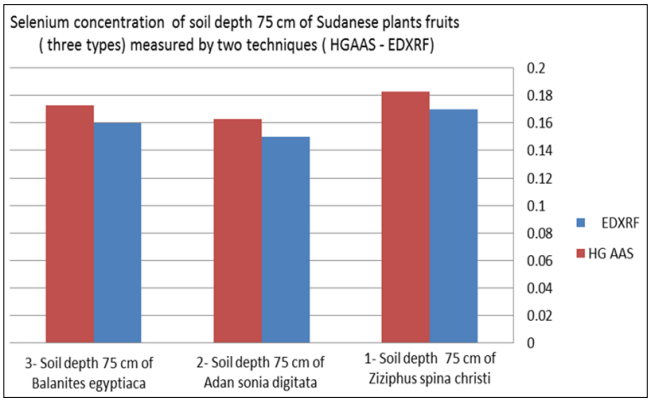


Fig 6: Comparison of Se concentration in the studied plants soil depth 75 cm by using the two techniques (EDXRF & HGAAS)

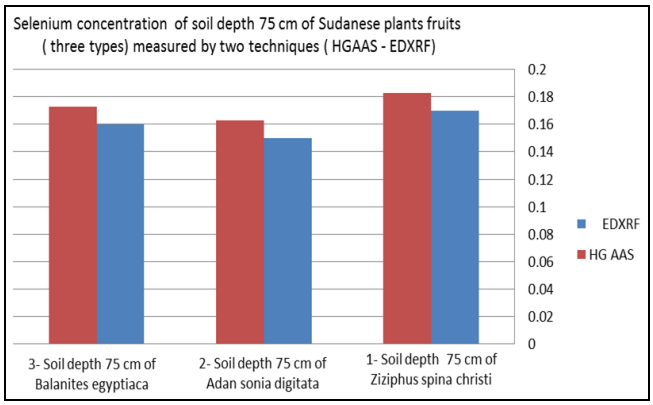


Fig 7: Comparison of Se concentration in the studied plants soil depth 100 cm by using the two techniques (EDXRF & HGAAS)

From this figure it was observed that soil depth 75 cm of *Ziziphus s* contain high Se concentration followed by *Balanites e* followed by *Adansonia d*.

From this figure it was observed that soil depth 100 cm of *Adansonia d* contain high Se concentration > *Ziziphus s* > *Balanites e*.

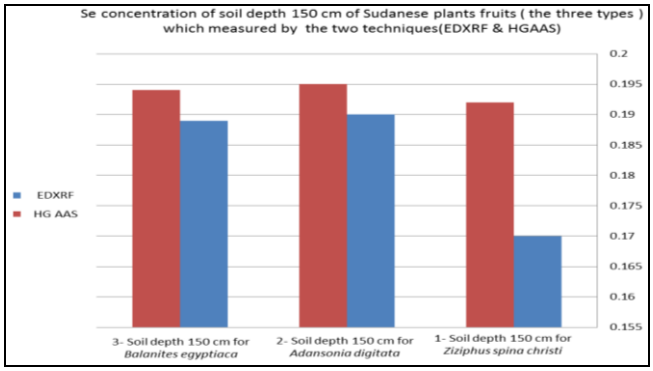


Fig 8: Comparison of Se concentration in the studied plants soil depth 150 cm by using the two techniques (EDXRF & HGAAS)

From this figure it was observed that soil depth 150 cm of *Adansonia d* contain high Se concentration > *Balanites e* > *Ziziphus s*.

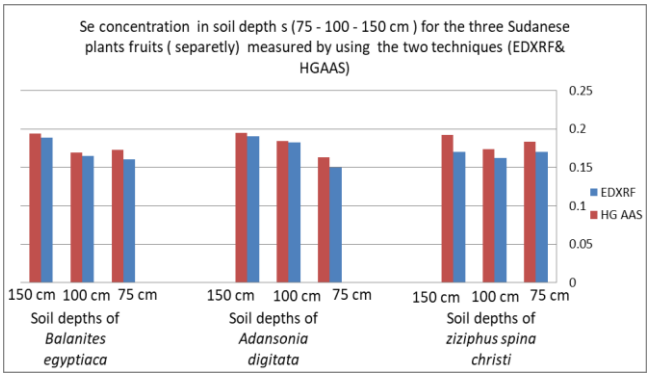


Fig 9: Comparison of Se concentration in the studied plants soil depth (75 cm – 100 cm – 150 cm) for any plan by using the two techniques (EDXRF & HGAAS)

From this figure it was noted that : about *ziziphus s* soil depth 150 cm contain high Se concentration > depth 75 cm > depth 100 cm, also for *Adansonia d*, soil depth 150 cm contain high Se concentration > depth 100 cm > depth 75 cm., also for *Balanites* e, soil depth 150 cm contain high Se concentration > depth 75 cm > depth 100 cm.

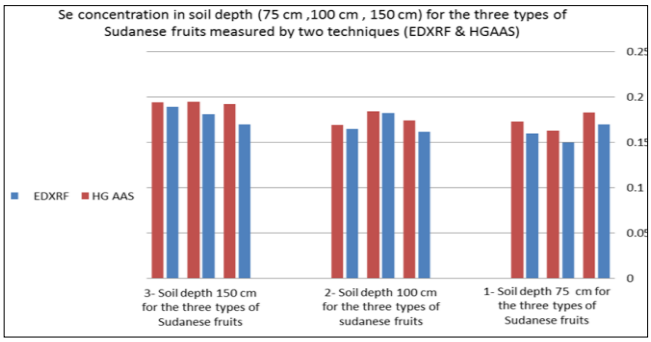


Fig 10: Comparison of Se concentration in the studied plants soil depth (75 cm – 100 cm – 150 cm) by using the two techniques (EDXRF & HGAAS)

From this figure it was observed, (from the average of all soil depths): soil depth 150 cm contains high Se concentration > depth 75 cm > depth 100 cm.

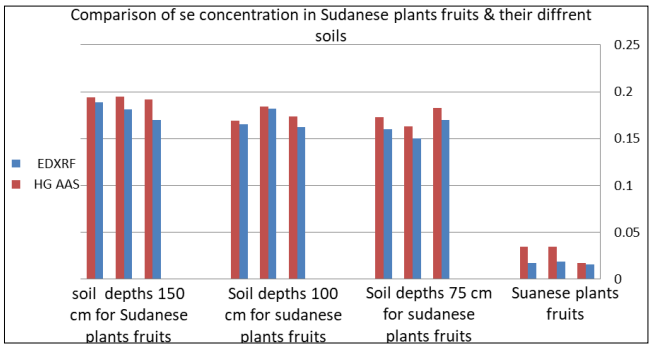


Fig 11: Comparison of Se concentration in the studied plants & soil depths by using the two techniques (EDXRF & HGAAS)

From this figure it was cleared that: the soil contain higher concentration of selenium than the plants**.**

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