Sudan University of Science and Technology
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A Comparative Study of Different Methods for Determination of Selenium in Some Sudanese Fruits, Soil and Water

دراسة مقارنة لطرق مختلفة لتقدير السلينيوم في بعض الثمار السودانية والتربة والماء

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry

By

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إستهلال

قال تعالى : { إِنَّا كُلَّ شَيْءٍ خَلَقْنَٰهُ بِقَدَر}

صدق الله العظيم ,,,,,,,, سورة القمر الآية (49)

Dedication

This work is dedicated to my

parents,

husband,

sisters,

and brothers.

God Bless them All

Acknowledgments

First of all, thank God, who guided me to complete this research and bring it out in this way, which I hope will be of benefit for all.

I would like also to express my sincere gratitude to my distinguished supervisor Professor: Elmugdad Ahmed Ali, to whom I am greatly indebted for providing me with valuable scientific information. I would like also to thank Dr. Mohammed El-Mukhtar, who did not hesitate to help with useful information, to all who have provided information for the completion of my research and to all who spared no effort and time to give me precious advice, remarks and guidance,

Abstract

This study aimed to measure concentration of selenium (Se) in five Sudanese fruits: *Ziziphus spina christi (Nabaque); Adansonia digitata (Tabaldi); Balanites egyptiacea (Laloub); Grewia tenax (Gudeim) and Tamarindus indica (Aradeb).* Se concentration was also measured in soil where the plant grows from different depths (75 cm ,100 cm and 150 cm). Also water sample was taken from nearby (*Hafeer*) to determine Se concentration. All samples were collected according to Standard Method of Sampling. The study area was Alein forest , south east Al-obied city..Se concentration was measured by seven techniques. It was found that the concentration of Se (ppm) increases in the following order: *Gudeim* (0.0140- 0.0348) >*Tabaldi* (0.0138 - 0.0344) >*Laloub* (0.0130- 0.0342) >*Aradeb* (0.0125- 0.0225) > *Nabaque*. (0.0122 – 0.0172). It was also observed that the sensitivity of the techniques applied including parentheses the range of its minimum and maximum results obtained in all fruits increased on the following order: hydride generation atomic absorption spectroscopy "HGAAS" (0.0172 - 0.0348) > graphite furnace atomic absorption spectroscopy " GFAAS" (0.0162 – 0.0338) > energy dispersive x-rays fluorescence "EDXRF" (0.0154 – 0.0188) > scanning electron microscopy + energy dispersive x-ray fluorescence "SEM+EDS" (0.0150 – 0.0185) > mass-mass inductively coupled plasma "ICP MS/MS" (0.0127 – 0.0145) > inductively coupled plasma optical emission spectroscopy "ICP OES" (0.0122 – 0.0140). Also it was found that the sensitivity of the technique applied for determination of Se in all soil depths (cm)75,100 and 150 for all fruits increased in the following order: HGAAS (0.163 - 0.205) > EDXRF (0.150 - 0.202) > GFAAS (0.153 – 0.201) >SEM+EDS (0.1497- 0.1883) > ICP MS/MS (0.055 - 0.088) >ICP OES (0.050 - 0.085). The concentration (ppm) of Se increased in the order : depths 150 cm (0.069 – 0.195) > 100 cm (0.065 – 0.184) > 75 cm (0.050 – 0.183), for *Nabaque* and *Tabaldi*. However, for *Laloub* it increased in the order:

depth 150 cm (0.071 - 0.194) > 75 cm (0.062 – 0.173) > 100 cm (0.060 – 0.169), and for *Gudeim, , Aradeb* in the order: depth 75 cm (0.073– 0.205) > 100 cm (0.075 – 0.200) > 150 cm (0.055 – 0.197). For the concentration ranges (ppm) of Se in soils at all depths increased in the following order: *Gudeim* (0.082 – 0.0205) > *Aradeb* (0.055 – 0.200) >*Nabaque* (0.065 – 0.192) > *Laloub* (0.060 – 0.194) >*Tabaldi* (0.050 – 0.195).Technique wavelength dispersive x-ray fluorescence "WDXRF", however, did not detect Se in both soil and fruits samples owing to the dry ashing pre–treating step. Se concentration(ppm) of water were increased as follows: HGAAS,($0.644x10^{-3}$) > GFAAS ($0.601x10^{-3}$) > ICP MS/MS ($0.498x10^{-3}$) > ICPOES ($0.480x10^{-3}$) respectively. Techniques SEM+EDS, EDXRF, WDXRF were used only to analyze solid samples. The results obtained for fruits, soil and water were consistent with those of (WHO) Standard and some agreed with those of some obtained of global researches. Se concentration was found to increase in the following order: soil > fruits > water.

المستخلص

هدفت هذه الدراسة إلى تقدير تركيز السيلينيوم (Se) في ثمار خمسة نباتات سودانية وهي النبق والتبلدي واللالوب والقضيم والعرديب. كما تم تقدير تركيزعنصر السلينيوم في التربة حيث ينمو النبات من أعماق مختلفة (75 سم ، 100 سم ، 150 سم). كما تم اخذ عينة ماء من مكان مجاور وهو الحفير ، لتحديد تركيز السلينيوم بها. تم جمع جميع العينات وفقًا للطريقة القياسية لأخذ العينات. منطقة الدراسة غابة العين جنوب شرق مدينة الابيض. تم تقدير التركيز بسبع تقنيات. وجد أن تركيز السلينيوم بوحدة (جزء في المليون) يزداد بالترتيب التالي: القضيم (0.0140-0.0348)>التبلدي(0.0138 - 0.0344) > اللالوب (0.0130- 0.0342) > العرديب (0.0125- 0.0225) > النبَق. (0.0122 - 0.0172). كما لوحظ أن حساسية التقنيات المطبقة متضمنة الأقواس في مدى الحد الأدنى والأقصى للنتائج التي تم الحصول عليها لتقدير السلينيوم في جميع الثمار ازدادت بالترتيب التالي: مطيافية الامتصاص الذري لتوليد الهيدريد "HGAAS" (0.0348 - 0.0172)) > مطيافية الامتصاص الذري لفرن الجرافيت "GFAAS" (0.0338 - 0.0162) > مطيافية الفلورة بالاشعة السينية المعتمدة على التشتت بالطاقة "EDXRF " (0.0188 - 0.0154) > الميكروسكوب الالكتروني المعتمد على المسح بالالكترونات والمتصل بتقنية الفلورة بالاشعة السينية المعتمدة على التشتت بالطاقة "SEM + EDS" (0.0150) 0.0185 - > مطيافية البلازما المزدوجة الحثية المعتمدة على الكتلة "ICP MS / MS " 0.0127) (0.0145 - > مطيافية البلازما المزدوجة الحثية المعتمدة على الانبعاث البصري"ICP OES" (0.0140 - 0.0122) كما وجد أن حساسية التقنية المطبقة لتقدير السلينيوم في جميع أعماق التربة (سم) 75و 100 و 150 لجميع النباتات تزداد بالترتيب التالي:HGAAS (0.205 - 0.163) < (0.1497- 0.1883) SEM+ < (0.15 - 0.201 GFAAS< (0.150 - 0.202) EDXRF (0.050 - 0.085) ICP OES < ICP MS / MS (0.055 - 0.088) <EDS . زاد تركيز السلينيوم بوحدة (جزء في المليون) بالترتيب التالي : بالنسبة للنبق والتبلدي العمق (سم) 150(0.069 - 0.195) > 100 (0.065 - 0.184) > 75 (0.050 - 0.183) . اما بالنسبة للالوب العمق (سم) 150 (0.071 - 0.194) > 75 (0.062 - 0.173) > (0.060 - 0.169) , اما بالنسبة للقضيم والعرديب العمق (سم) 75 (0.073 - 0.205) >100(0.075 - 0.200) > 150(0.055 - 0.197) . بالنسبة لتركيزالسلينيوم (جزء في المليون) في التربة في جميع الأعماق ،فقد زاد بالترتيب التالي :القضيم (0.082 - 0.0205) >العرديب (0.055 - 0.200) > النبق (0.065 - 0.192)> اللالوب (0.060 - 0.194) > تبلدي (0.050 - 0.195) ..تقنية تألق الأشعة السينية المشتتة بواسطة الطول الموجي "WDXRF" لم يكتشف وجود السلينيوم في عينات التربة والثمار السودانية بسبب خطوة المعالجة المسبقة للتحليل وهي الترميد الجاف. زيادة تركيز السلينيوم في الماء (جزء في المليون) كان

على النحو التالي: ICP MS/MS <($0.601x10^{-3}$) GFAAS < HGAAS ($0.644x10^{-3}$) ICP OES <($0.498x10^{-3}$) ($0.480x10^{-3}$) على التوالي. تم استخدام تقنيات SEM + EDS و EDXRF و WDXRF فقط لتحليل العينات الصلبة. نتائج تركيز السلينيوم التي تم الحصول عليها للثمار السودانية والتربة والمياه كانت متوافقة مع تلك الخاصة بمعيار منظمة الصحة العالمية (WHO) واتفق البعض مع تلك التي تم الحصول عليها من بعض الأبحاث العالمية. وجد أن التركيز يزداد بالترتيب التالي: التربة > الثمار السودانية > الماء.

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LIST OF ABBREVIATIONS

*AFA = Automated Feature Analysis

* BSE = Backscattered Electrons

*CCT = Collision Cell Technology

* DL = Detection limit

* ED XRF= Energy Dispersive X-rays Fluorescence

*eV = electron Volt

*GFAAS=Graphite Furnace Atomic Absorption Spectroscopy

* GPxs = Glutathione peroxidases

* HGAAS= Hydride Generation Atomic Absorption Spectroscop

*IAEA = International Atomic Energy Agency

* ICPMS= Inductively Coupled Plasma Mass Spectrometry

* ICP OES =Inductively Coupled Plasma Optical Emission Spectroscopy

*keV = kilo electron Volt

* LODs= Limits Of Detection

*MAC = Maximum acceptable concentration

*NBS= National Bureau of Standards

*(REE^{++}) = Doubly Charged ions of Rare Earth Elements

*RF = Radio frequency

*SE = Secondary Electrons

*SEM+EDX= Scanning Electron Microscopy+ Energy Dispersive X-ray

*Si(Li) = Lithium drifted silicon detector

*TE‟s = Trace Elements

*USDA = United State Department of Agriculture

* WD XRF = Wave length Dispersive X-rays Fluorescence

CHAPTER ONE

Introducction and Literature Review

1.1Nutrition and Nutrients

The nutritional status of any living organism, whether it is a human being or an animal is very important in determining the level of response of the immunological defense system to invasion, therefore nutrition play a significant role in any human development program and the optimum expression of human development depends on adequate supply of nutrients. several nutrients have beneficial effects on both antigen specific and non-specific immune responses. Micronutrients, particularly the mineral elements are considered to be inevitable and important for metabolic and physiological processes of animal and human systems. Minerals deficiencies, imbalances, and toxicity have long been held responsible for low production among human-being. Climatic conditions and mineral deficiency widely exist in developing countries and the severity of deficiency depends upon the type of their food (Domy, 2001).

Nutrients are substances present in food which provides energy, promote growth, development and 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. Even though causes of these diseases are often multifactorial, diet is considered as one of the important factors (Niedzielski et al., 2002).

Micronutrients are nutrients required by the body in lesser amounts, but are still essential for carrying out bodily functions. Micronutrients include all the essential minerals and vitamins. In contrast to carbohydrates, lipids, and proteins, micronutrients are not directly used for making energy, but they assist in the process as being part of enzymes. Enzymes are proteins that catalyze chemical reactions in the body and are involved in all aspects of body functions from energy production, digestion and macromolecules building. Minerals are

solid inorganic substances that form crystals and are classified depending on how much of them are needed. 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 (Muller et al., 2007).

1.2 Medicinal plants

Medicinal plants are rich in many minerals and trace elements and suggested that this is important in curative effect of plants According to the World Health Organization (WHO), the use of traditional herbal medicine has spread not only in the developing countries, but also in the industrialized regions, as a complementary way to treat and prevent diseases (Ebrahim et al., 2012).

1.3 Medicinal plants in Africa

Africa has abundant wild medicinal plants and cultivated native species with great agronomic and commercial potential as food crops.However, many of these species, particularly fruits and nuts, have not been promoted or researched and therefore remain under-utilized. Moreover, many of these species face the danger of loss due to increasing human impact on ecosystems (Elamin, 2005).

1.4 Medicinal plants in Sudan

Sudan, as in many other African countries has an immense diversity and variation in vegetation and is one of the richest countries with regard to phytopharmaca ,Although herbal remedies are often perceived as being natural and, therefore, safe, they are not, principally, free from adverse effects.

Sudan is endowed with a range of diverse climatic conditions that favor the growth of many plant species, most of which are adapted to specific ecological zones (Ebrahim et al., 2012). Among these plants:

1.4.1 *Ziziphus spina-christi* (Sidr)

Ziziphus spina-christi (Sidr) known in Sudan as Nabque and belong to the family *Rhamnaceae* . It is found in western and central Sudan, is a species that is highly adapted to the dry and hot conditions which prevail in North Africa. It grows in desert areas with an annual rainfall of 50–300 mm, but is often found in wadis where underground water is available. Fruits Fig 1.1 are consumed either fresh or dryed .In central Sudan fruits used as laxatives, The genus *Ziziphus spina Christi* is known for its medicinal properties as a hypoglycemic, hypotensive, anti-inflammatory, antimicrobial, antioxidant, antitumour, and liver protective agent and as an immune system stimulant (Saied et al., 2007).

Fig 1.1 : *Ziziphus Spina Christi* Fruits

1.4.2 *Adansonia digitata* (Baobab)

Baobab (*Adansonia digitata*), which is a fruit-producing tree belonging to the family *Bombacaceae*.(Gebauer, 2002).The baobab fruit Fig 1.2 has a hard shell (epicarp) with a velvety covering, inside the shell is the seed per carp and seed which are hard and dark coloured and surrounded by dry light/ cream coloured fruit pulp (monocarp) forming lump.Dry slightly darker fibrous material is also contained within the fruit and the pulp (mesocarp). The baobab has an exceedingly wide range of uses ranging from food and beverages to medicinal uses.Tabaldi (*Adansonia digitata*), known locally as Gungulaize, is of awidespread throughout the hot, drier regions of tropical Africa. In Sudan is most frequently found on sandy soils and seasonal streams (khors), in low

grassland Savannas. It forms belts in central Sudan, Kordofan, Darfur, Blue Nile (Gebauer, 2002**)**.

Fig 1.2 : *Adansonia digitata* Fruits

1.4.3 Balanites aegyptiaca (Heglig)

Balanites aegyptiaca (Heglig)**,** known in Sudan as Laloub and belong to the family *Balanitaceae*.The fruit Fig 1.3 is pale- yellow, fine-grained, hard, tough and insect resistant. It is used for turnery, carving, firewood and charcoal. Heglig is found in Africa in most humid areas. It is widespread through the Sudan. Sudan is the main producer of it, is spread all over the country.It considered as a laxative and purgative for stomach, which helps in removing worms from intestine.The oil released from the kernels by boiling, is used for treating headaches (Doughari et al., 2007).

Fig 1.3 : *Balanites aegyptiaca* Fruits

1.4.4 *Grewia tenax* (Godaim)

Grewia tenax(Godaim)Fiori, is a wild fruit species, belonging to the family *Tiliaceae,* with multiple uses in different parts of the Tropics and

Subtropics.G.tenax is highly drought resistant and occurs in the driest savannas at desert margins and regions of higher rainfall, where it grows in thickets on termite mounds in otherwise seasonally flooded country. In the Sahel it grows in rocky places on hills and slopes, in regions with 100-600 mm of rain per annum grown in sandy, rocky and lateritic soils. Fruit Fig 1.4 orange-red at maturity, with 1- 4 spheroid lobes. G. tenax is virtually indistinguishable in fruit**.**The fruits consumed by man contain a large amount of iron and can be made into a refreshing drink and therefore use as anti- anemia **(**Gebauer, 2002)**.**

Fig 1.4 : *Grewia tenax* Fruits

1.4.5 *Tamarindus indica* (Aradeib)

Tamarindus indica (Aradeib) tree is found in the tropical region in most African countries, and is belong to the Family: *Fabaceae* ,the Genus:*Tamarindus* and the Species: *T. indica* . In Sudan, it is located in the central states and extends to the South. The tree grows well in full sun in clay, loam, sandy, and acidic soil types, with a high drought and aerosol salt (wind-borne salt as found in coastal area) resistance.The fruit Fig 1.5 is an indehiscent legume, with a hard, brown shell. The fruit has a fleshy, juicy, acidulous pulp. It is mature when the flesh is coloured brown or reddish-brown. Tamarindus of Asia have longer pods containing 6–12 seeds, whereas African and West Indian varieties have short pods containing 1–6 seeds. The seeds are somewhat flattened, and glossy brown.The tamarind is best described as sweet and sour in taste, and is high in

acid, sugar, vitamin B and, interestingly for a fruit, it is used for the treatment of malaria (Saied et al., 2007).

Fig 1.5 :*Tamarindus indica* Fruits

1.5 Trace elements and their importance

Trace elements (TE‟s) are those nutrients required in extremely small quantities.These elements are released into the environment from the natural weathering of rocks and from various sources related to human activity.Trace elements have many names as well as many definitions, they are known as potentially toxic elements, trace elements, heavy elements, micronutrients, and minor elements. The term "potentially toxic elements" is a recent term, meant to illustrate that while some elements are toxic to humans and plants, not all elements are toxic at all concentrations. In fact, some elements (e.g., selenium) are necessary for life in small amounts (Dungan and Frankenberger 2007).

Trace elements play a very important role in formation of active chemical composition present in medicinal plants and therefore responsible for medicinal as well as toxic properties (Alloway 2009).They are essential for the correct functioning of many plants, animal and human biological systems. Beside their essential functions in every organism on the planet, the interaction between them in biological processes and their role in mediating biological and chemical reactions that are essential to life are still being covered by biologists and scientists every year (Nomita et al., 2008)..

The characteristic properties of a complex system and many interesting problems that arise in different spheres are derived or can be explained by absence or the presence of specific element at low level concentrations. Hence, identification and qualification of these elements become necessary (WHO 2006).The harmful action of different element is different. If it is over the limit, it may develop severe diseases for human, animals, and plants and sometimes may cause death. One very important feature, when considering the health effects of trace elements is their slow accumulation in tissues, even at low doses. Hence, acute effects are very rarely reported, whereas chronic exposure can lead to the build-up of higher concentrations and onset of disease (Herbette et al., 2007).Biomonitoring is a means to detect the deposition, accumulation and distribution of trace elements in ecosystems. Through the use of different types of vegetation, the levels of atmospheric trace metallic concentrations have been successfully monitored (Dungan and Frankenberger,2007).

The essential elements (e.g. the traditional plant micronutrients, Zn, Cu, Mn, Mo and B) and now Ni and Se, are essential for animal and human nutrition (Domy, 2001). Among these nutrients, selenium which is known to have a part to play an important role and necessary for the development of the acquired immune system (Kreps, 2005).

1.6 Selenium element, its chemistry and importance

Selenium (Se) discovered by the Swedish chemist Jacob Berzelius in 1817, is a metalloid in group (6 A) with an atomic weight 78.96 and atomic number of 34. It has five valence states in nature including selenide (HSe^-; oxidation state 2-), elemental Se (Se^0; oxidation state 0) , thioselenate ($SeSO_3^{-2}$, oxidation state 2+), selenite(SeO_3^{2-}; oxidation state 4+) and selenate (SeO_4^{2-}; oxidation state 6+). **(**Yin et al., 2012 **)**.

The fate of selenium 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.The different chemical forms of selenium can control selenium solubility and availability to organisms. Selenate (Se^{+6}) is the most oxidized form of selenium, it is highly soluble in water and generally considered to be the most toxic form. Selenite (Se^{+4}) occurs in oxic to suboxic environments and is less available to organisms because of its affinity to sorption sites of sediment and soil constituents **(**Crompton, 2006**)**.

Selenium has a number of important agricultural and horticultural applications, these include the use of sodium selenite and selenate as additives and dietary supplements in animal feeds. Soil deficiencies are corrected by adding selenium compounds to fertilizers (Conor and Kongkachuichai; 2012). (Nazemi et al., 2010) reported that selenium (Se) is a trace element that, depending on its concentration, is both toxic and an essential part of nutrition. However, the gap between the beneficial and harmful levels of (Se) is quite narrow **(**Hartikainen, 2005).

Selenium (Se) plays a key role in the maintenance of normal health in human population. This micronutrient is a crucial nutrient for human health and enters the food chain through plants and its concentration in foods is determined by a number of geological and geographical factors. The content of Se in food depends on Se content of the soil where plants are grown or animals are raised (Dolph et al., 2012). 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 selenium status (Burk and Fand; 2009).

Glutathione peroxidases (GPxs) are antioxidant selenoenzymes protecting various organisms from oxidative stresses and damage by catalyzing the reduction of hydrogen-peroxide at the expense of GSH.The classical GPx utilizes exclusively GSH as reducing substrate for the reduction of H_2O_2 and a limited number of organic hydrogen-peroxide **(**Schweizer et al., 2005).

The biochemical function of glutathione peroxidase is to reduce lipid hydrogen-peroxide to their corresponding alcohols and to reduce free hydrogen peroxide to water (Muller et al., 2007). An example reaction that glutathione peroxidase catalyzes is:

$$\mathbf{GPx - Se^{-} + H_2O_2 \rightarrow GPx - SeO^{-} + H_2O}$$

As the integrity of the cellular and subcellular membranes depends heavily on glutathione peroxidase, the antioxidative protective system of glutathione peroxidase itself depends heavily on the presence of selenium. Furthermore, selenium is an essential nutrient necessary for normal growth and reproduction in animals and humans (Arscott et al., 2000). Deprivation of Se is associated with impairments in antioxidant protection, redox regulation and energy production as consequences of suboptimal expression of one or more of the Se containing enzymes. These impairments may not cause deficiency signs in the classical sense, but instead contribute to health problems caused by physiological and environmental oxidative stresses and infections. At the same time, supranutritional intakes of Se, i.e. intakes greater than those required for Se lenocysteine enzyme expression, appear to reduce cancer risk. The lower, nutritional, level is greater than the typical intakes of many people in several parts of the world, and few populations have intakes approaching the latter, supranutritional, level. Accordingly, low Se status is likely to contribute to morbidity and mortality due to infectious and chronic diseases. Increasing Se intakes are expected to reduce cancer rates **(**Rayman, 2008**)**.

Food is the main source of selenium Fig 1.6 and the content of this element is related with the food origin.There is a wide spread use of selenium supplementation by enriching commercial foodstuffs **(**Burk and Fand; 2009).

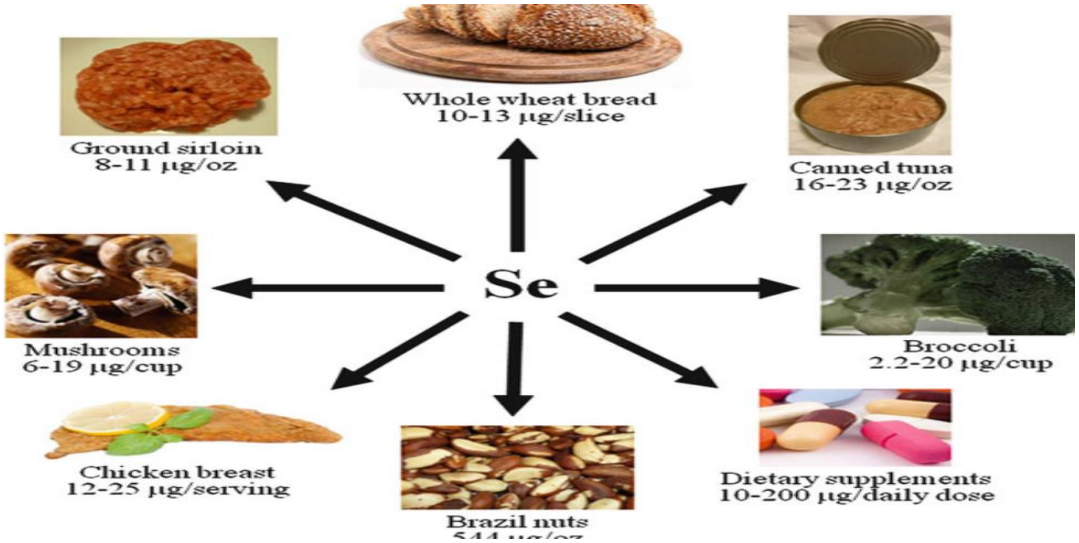

Fig: 1.6 Common food sources of selenium.

1.7 Mobilization of Selenium

Selenium occurs in nearly all materials of the earth's crust and is present in magmatic rocks in high concentrations.In sedimentary rocks, It is also associated with the clay fraction and thus the smallest quantities of Se are in sandstones and limestones. Mobilization of Se from soils is influenced by soil pH. Alkaline conditions favour the conversion of inorganic Se to selenate (Se^{+6}) which is not fixed in the soils, whereas acidic conditions favour selenite (Se^{+4}) which adsorbs to clays and is strongly fixed by iron hydroxides.The availability of Se to plants is also affected by soil moisture, the element is most available in plants under conditions of low precipitation and low soil leaching. Hence the availability of soil Se is affected by soil management procedures as irrigation, aeration and Se fertilization (Gerald and Combs 2009).

1.8 Methods for determination of trace elements

One of Environmental Chemistry's major challenges is the determination of the nature and quantity of specific trace elements in the environment.Thus, chemical analysis is a vital first step in environmental chemistry research. The difficulty of analyzing many environmental trace elements can be awesome. Environmentally significant levels of some trace elements may be only a few parts per trillion.Thus, it is obvious that the chemical analysis used to study some environmental systems require very low detection limit (Manahan, 2000).

The necessary laboratory equipment and analytical units are not only constructed in laboratories, but in ready-made commercial equipment.At present the methods that are used for direct determination of trace elements are based on more or less specific reactions of coloured metal complexes which can be assessed on the basis of the absorption at certain wavelengthes of radiation passing through a solution (Niedzielski et al., 2002).

The significance of knowing the level of Se in environmental, technological and pharmaceutical samples is reflected in the number of devised methods for its determination, ensuring the possibility of optimization for particular cases.The accessibility of a variety of instrumental and methodical solutions permits the choice of a best technique for expected levels of concentration. Analytical methods for determining selenium analysis provide information about its toxicity, bioavailability, migration of element and the impact of industrial technologies has begun replacing simple determinations of total contents in environmental samples. Spectrophotometric methods for determination of trace elements have been very popular but recently have become obsolete, in particular for analyses of environmental samples, as they are time consuming and do not ensure a sufficiently low detection limit **(**Badiadka and Mendalin 2006**)**.The too high detection limits of these methods can be reduced by special sample preparation, e.g. extraction, sorption and chelation, in order to concentrate the metal to be determined. Speciation analyses are possible with the use of different reaction conditions (e.g. different pH) or a combination of different methods (hyphenated techniques that couple separation techniques) with advanced detection systems (Paltridge et al., 2012).

Some errors are, practically, unavoidable in analytical work. Errors are inherent in the methods or instruments employed, or may arise from impurities in reagents and even in distilled or demineralized water.The analyst's skill and general judgment have a direct bearing on the accuracy of the analytical statement. After the chemical analysis of environmental samples has been

completed, the validity of the results can be evaluated by several methods. No one method of checking gives conclusive proof of the accuracy of the results, but the process of checking may reveal some dubious results or some additional constituents of the sample that were not considered in the analysis. The use of reference materials, spiked samples, and samples split between laboratories must constitute at least 15 percent of the workload for any parameter. The percentage for rarely used methods must be considerably higher. Data-review program must provide continual evaluation of the laboratory's performance. To minimize errors and variation in data due to sampling, field personnel must maintain records on sampling, including field measurements (Bahers et al., 2012 **)**.

Appropriate field measure and information peculiar to the sample need to be supplied with the sample to the laboratory. Samples must be preserved, if required, and must be shipped without delay, in bottles and containers appropriate to the determinations.Time-critical determinations need to be performed within the allowable time either in the laboratory or in the field. Analysis performed in the field must be, carefully, monitored. Reference materials must be used. Instruments must be calibrated regularly prior to going to the field (Standard Methods, 2005).

The determination of selenium is of considerable interests, because of its contrasting biological functions. A comparison of performance of the analytical methods used for the determination of Se in environmental samples (Ca´ssia.et al., 2002).There are numerous procedures for determination and quantification of selenium levels in environmental samples, especially in plants, soil and water, these methods include:

1.8.1 Hydride generation atomic absorption spectrometry (HG AAS)

The most sensitive, commonly and successfully used in the determination of selenium in environmental samples are hydride generation atomic absorption spectrometry (HGAAS) (manual or continuous). In most cases, continuous

HGAAS is a preferred method due to the quick and reproducible results that can be obtained, coupled with a low DL of less than 2μg/(Standard Methods, 2005).

The choice depends on the matrix composition and selenium species present in the sample. HGAAS response, strongly, depends on the selenium form. Hydride generation (HG) is a very effective analytical technique developed to separate hydride forming metals, such as Se, from a range of matrices and varying acid concentrations. The heated quartz tube atomizer is, particularly, useful for the determination of selenium because the absorption wavelengths for this element is below 200 nm in an area subject to intense interference from flame radicals that can, significantly, affect detection limits, This analytical technique improves detection limits by a factor of approximately 3000 times that of flame detection limits and, typically, have less interference than graphite furnace techniques (Auron, 2012).These techniques use wet-sample digestion (e.g., nitric-perchloric acid) to destroy organic matter.Sample reduction to convert Se^{+6} to Se^{+4} is necessary using sodium borohydride to reduce all selenium present to selenium hydride.The chemical reactions are shown in Equations (1) and (2) as follows:

1- $\mathbf{NaBH_4 + 4H_2O + HCl \rightarrow H_3BO_4 + NaCl + 10H}$

2- $\mathbf{10H + Se^{+4} \rightarrow SeH_2 + 4H_2}$ **(excess)**

Selenium hydride is thermally decomposed and atomized in the sample beam of the atomic absorption spectroscopy **(**Manjusha et al., 2008 **)**.

1.8.2 Graphite furnace atomic absorption spectrometry (GFAAS)

Graphite furnace atomic absorption spectrometry (GFAAS) is adequate for the measurement of selenium. (GFAAS) offers high sensitivity (5×10^{-11} g selenium/g sample).Also a graphite tube for a transversely-heated furnace. GFAAS is an appropriate atomization technique used to determine selenium concentrations in samples with an acceptable limit of precision at parts per billion (ppb), but interference from the matrix can cause significant difficulties. GFAAS methods rely on the fact that numerous metal compounds react with

selenium compounds to form, relatively, refractory metal selenides. Nickel, molybdenum, and platinum are commonly added to the sample to thermally stabilize selenium (unreduced palladium-nitrate modifier was used in all cases). However, in case of samples that are known to contain sulfur reduced palladium was used as modifier. Organic materials are destroyed by high temperature prior to atomization of the sample (2,700 ^{0}C). All selenium species in the sample are converted to selenates with modifiers and by pyrolysis which in turn must be reduced to selenite (Bouchard et al., 2011).

One advantage of GFAAS techniques is that; the material in the graphite sample cell is chemically treated in situ to reduce chemical interference. GFAAS techniques require correction for background absorption. Correction techniques include deuterium continuum light source method and the Zeeman splitting of the absorption line. A Zeeman-effect system, which applies a magnetic field to the atomizer, allows the background correction to be performed at the exact analyte wavelength without the use of auxiliary light sources (Badiadka and Mendalin; 2006). It was also realized, at an early stage, that concomitants would cause interferences, hence the analyte addition technique was recommended for their control.This, however, makes GFAAS time consuming and, hence, less attractive for routine purposes. Another drawback of the early graphite furnaces was that the sensitivity was not adequate for the determination of trace elements of interest at concentrations <1 pg / l (Bahers et al., 2012). GFAAS was applied soon after its introduction to analytical chemistry to the analysis of natural water for direct determination of numerous trace elements without pre-concentration. (Niedzielski et al., 2002).

1.8.3 The plasma spectrometric methods (inductively coupled plasma - optical emission spectrometry (ICP OES)) and (inductively coupled plasma - mass spectrometry(ICPMS))

Inductively coupled plasma - optical emission spectrometry (ICP OES) is based on the unprompted emission of photons from atoms and ions that have been

excited in a radiofrequency (RF) discharge at high temperature. Each element emits radiation of characteristic wavelengths (allowing qualitative analysis) and intensity proportional (at a given temperature) to the concentration of this element thus, allowing quantitative analysis. Emission of radiation by an atom of a given element is related to changes in the energy states of electrons from the outer electronic shell **(**Xiandeng and Bradley; 2000**)**.

A sample to be studied is transformed into aerosol by nebulizer or in a spark ablation system in which a fragment of the sample is evaporated from its surface and introduced into plasma. In such a form, it is introduced into a stream of neutral gas (e.g. argon) to which a high frequency signal (27-90 MHz) is applied through inductive coupling (electrodeless). The energy of the signal heats up the gas (argon + the sample) to 10,000 K, the state of plasma, in which most atoms are ionized (formation of Me^{+}, Me^{2+} etc.) and (potential of Se volatilization). The excited atoms emit radiation and on the basis of this emission spectrum, the chemical composition quantitative and qualitative can be established.The method based on plasma excitation and analysis of the emission spectrum is known as ICP-AES (inductively coupled plasma - atomic emission spectrometry). The ICP methods, both OES and MS, methods allow the determination of few elements in a wide range of concentrations, in the same sample in a few minutes, so they are superior in performance. The radiation coming from a powerful source (electrodeless lamp EDL, laser) is absorbed by plasma generated by flame or an electro thermal atomizer **(**Ekmek et al., 2004). The absorption causes excitation of atoms, which emit radiation (fluorescence) on coming back to ground level. The wavelength of the emitted fluorescence can be the same as that of the excitation radiation (resonance fluorescence), higher (transition through an intermediate state) or lower (thermo luminescence).The intensity of the fluorescence radiation is proportional to the concentration of atoms in the plasma, which allows quantitative analysis. The intensity of the fluorescence radiation is measured at an angle to the path of the

excitation radiation coming from the source, which eliminates the effect of the excitation radiation on the fluorescence intensity. However, the result can still be affected by scattering of the radiation by the sample. (Hartikainen, 2005).

The ICP-MS method overcomes the sensitivity limitations of inductively coupled plasma–atomic emission spectrometry (ICP-AES) and time consumption of electro-thermal atomic absorption spectro-photometry by providing multi-elements measurement capability in a single run. In the past fifteen years, it has become a widely, used technique in environmental monitoring. Therefore, the more recent instruments propose a wide dynamic range of 109 units, allowing the theoretical determination of elements present at the ng/l level and others at the g/l level. Methods combining the use of selective analytical methods such as ICP-MS, ICP-OES with chromatographic separation of different species containing a given element, have become increasingly available. Gas chromatography coupled with ICP-MS has been used to separate and detect volatile Se species, sequential extraction protocols are commonly used to assess selenium fractionation in samples.The interference due to transition metals can be eliminated by removing metals by, on line, ion-exchange (cationic) resin, having removed the transition metals and applied hydride generation, the method of ICP-AES ensured the detection limit of 0.4 ng /ml (Dolph et al., 2012).

Methods of HG-ICP-MS or HG-ICP-OES are characterized by a similar performance to HG AAS, provided that generation of hydrides is applied and necessary in each of them in order to avoid interferences. When the injection sample supply is used with generation of hydrides, the limits of detection of Se^{+4} and Se^{+6} are 0.012 ng/ml and 0.016 ng/ml low detection limits and the speed of (ICP-MS) make the technique well suited to analyze trace elements including Se in environmental samples. The sensitivity of the method of ICP-MS is the same or higher than that of GFAAS (Bahers et al., 2012). Selenium is weakly ionised in argon plasma and most of the six selenium

isotopes (^{74}Se 0.89%; ^{76}Se 9.36%; ^{77}Se 7.63%; ^{78}Se23.78%; ^{80}Se 49.61% and ^{82}Se 8.73%), undergo significant isobaric or polyatomic interferences, the most important being Ar_2 in^{76}Se, ^{78}Se and ^{80}Se. ArCl interference with^{77}Se is possible in chlorine matrices, while ^{82}Se suffers BrH interference in matrices containing Br. Broadly speaking, interferences could be classed as Spectraum (caused by atomic or molecular fine structure in the spectrum), background (caused by broad features or electronic baseline), matrix effects (primarily caused by physical or chemical influences),,volatile of selenium caused by high temperature instrument, unstable selenium(caused by the presence of some tetravalent selenium at higher temperature about 10000 k for ICP technique which did not generate a measurable signal in any situation) (Badiadika and Mendalin; 2006).

1.8.4 X-ray Fluorescence "XRF" (EDXRF and WDXRF)

There has been a growing interest in the determination of many elements, among them, selenium, by x-ray induced fluorescence. X-ray fluorescence (XRF) are the methods suitable for determination of elements heavier than sodium (elements of smaller atomic mass show very small fluorescence).In XRF method the sample is excited by X-ray radiation where it emits secondary X-ray radiation (the so-called X-ray fluorescence).Each element emits a characteristic spectrum of radiation (qualitative analysis) and spectral line intensities are proportional to the content of a given element in the sample (quantitative analysis)**.**The energy and intensity of the characteristic X-ray is measured either in wavelength or energy mode: the first mode is called wavelength dispersive (WDXRF) mode, which measures elemental concentration ranging from few ppm to nearly 100%.. In order to analyze the photon atom interaction, each and every part of the technique like detection system, exciting source, low noise preamplifier and amplifier system and processing system is well known (Margui et al., 2014).The second one is energy dispersive (EDXRF) mode.With the advent of semiconductor detectors and advances in associated electronics,

energy dispersive and wavelength dispersive XRF analyses have become powerful analytical methods for multi-element analysis. On the other hand (EDXRF) spectroscopy also provides higher sample throughput with better reliable results than the current methods allow. EDXRF spectroscopy offers an alternative to digestive methods, in the determination of total elements such as key nutrients, trace elements and heavy metals across a range of sample types. For the elaboration of the calibration standards, cellulose was used to simulate the matrix of the plants that were analyzed with the EDXRF methodology (Vessman et al., 2001). The emitted characteristic X-rays are measured by a high resolution X-ray detector (liquid nitrogen, cooled Si/Li). Recently (XRF) spectroscopy is proved to be a more powerful and accurate method used to study the unknown concentration, chemical effects, intensity ratio's, cross-sections, thickness and other parameters of the compounds and elements. It has proved to be aversatile tool to measure geological, archaeological, biological, material science, powder, solid and liquid samples **(**Demir et al., 2014).

1.8.5 Scanning Electron Microscope +Energy Dispersive XRF (SEM + EDX)

A SEM is an electron microscopy that uses an electron beam to scan the sample. The electrons that are back scattered, as well as the ones that are knocked off the near-surface region of the object, were detected and used to create high-resolution images. Imaging with a Scanning Electron Microscope (SEM) is a powerful tool.

The applications of (EDX+EDS) are:1-topography to show the surface features ("how it looks"), and texture.2- morphology to explain the shape, size and arrangement of the particles.3- composition to explain the elements present in the sample (David and Paul, 2012). Ascanning electron microscope (SEM) is an instrument that allows the observation and characterization of materials within a mm to nm scale. In most cases, SEM is connected to an energy-dispersive spectrometer (EDS), so there is the possibility of an elemental analysis showing chemical characteristics of the samples.The SEM+EDS

method is applied in various fields, also have been used to investigate nanomaterials in ,plants and soils (Clapera, 2006). Compared to other methods of investigation scanning electron microscopy and energy dispersive spectrometry (SEM-EDS) method is significantly less frequently used in the study of the samples related to food and textile technologies. Scanning electron microscope coupled with energy dispersive X-ray spectrometer (SEM/EDS) is an apparatus for qualitative and semi-quantitative chemical analysis at micro level (David and Paul; 2012).

1.9 Importance of samples preparation for selenium determination

The initial step in analytical methods involving environmental materials usually involves destruction of the sample and conversion of the elements to forms suitable for analysis. In the case of selenium this is a very critical stage in the analysis for it is an element which is very readily volatilized, for this reason dry ashing is not favored **(**Benton and Jones; 2001).

For any technique and for less error, sample preparation is very important to get better results. During preparation of the samples containing selenium, the possibility of complete loss of selenium will be occurred due to the volatility of selenium, although dry ashing in the presence of magnesium nitrate is successful. Even oxygen flask combustion procedures are not recommended except in experienced hands but the method is slow. Most of the useful methods for the destruction of organic matters in the sample involve wet digestion procedures but even so, extreme care and strictly controlled procedures are required as it is well established that any significant charring may lead to loss of selenium **(**Maurizio et al., 2015**)**. Favored digestion mixtures involve combinations such as nitric and perchloric acids, nitric, sulphuric and perchloric or nitric acid and phosphoric acid with hydrogen peroxide. Excellent recoveries will be obtained when a preliminary digestion is carried out with a mixture of nitric and perchloric acid before final digestion following the addition of sulphuric acid to eliminate excess nitric and perchloric acids.

A mixture of hydrochloric, perchloric and nitric acids using an optimized temperature time programmed digestion has also been shown to give excellent recoveries. The essential common factor for all methods is that oxidizing conditions are maintained throughout (Ray and Campbell; 2013).

Although glass is normally satisfactory for the digestion vessels, quartz Kjeldahl flasks have been used for determination of selenium in environmental materials avoid possible absorption of selenium on glass. New glassware should be checked to ensure that selenium is not removed from the glass during the digestion. Many attention should be taken to investigate the influence of sample preparation strategy of vegetables on the electro-thermal. Behavior of Se without and with chemical modifiers such as $Pd(NO_3)_2$, $Pd(NO_3)_2$ +$Mg(NO_3)_2$, $Pd(NO_3)_2$ + $Cd(NO_3)_2$, pre-reduced Pd, $Mg(NO_3)_2$, and $Ni(NO_3)_2$, was investigated (Lyly et al., 2012).The low concentration of Se in the aquatic environment makes it difficult to determine this element by traditional detection methods. Use of a preconcentration step allows improved LODs, but at the same time requires larger volumes of sample and more handling steps (Benton and Jones; 2001).

Methods for determining trace elements should involve minimal sample handling, some procedures have been attempted to improve sample digestion by combining the use of reagents with ultraviolet or microwave radiation. This results in additional steps, which may lead to inconveniences such as sampling errors, contamination and losses during handling. In addition, acids might interfere in HGAAS and GFAAS measurements and, therefore, inaccurate results for the determination of selenium can be attributed to the resistance of selenium compounds to oxidation and the volatility of selenium species present or formed (Hartikainen, 2005)**.** An UV-oxidation procedure has been developed to completely digest environmental samples (soil and plants) for the determination of trace levels of selenium. A combined use of UV photolysis and hydrogen peroxide in the presence of an oxidant (HNO_3) results in the complete

oxidation of the organic matter. This method is simpler and requires fewer reagents when compared with other sample pre-treatment procedures. The clear solution obtained is analyze for the selenium content **(**Kumar et al., 2013**)**.

1.10 Selenium in soil and plants

The global average concentration of Se in soil is 0.4 mg kg^{-1}, ranging from 0.01 to 2 mg kg^{-1} (Dungan and Frankenberger, 2007). (Dolph et al., 2012) reported that: Se concentration in (mg kg^{-1}) as < 0.125 Deficient , 0.125 - 0.175 Marginal, 0.175- 3 Moderate – High, >3 Excessive and > 5 Seleniferous. Se concentrations in most soils are within the range 0.01–2.0 mg kg^{-1} (Fordyce, 2007). The average concentration of total Se in soils from Dashan Region was almost 2 times higher than that in Hong Kong (0.76 mg kg^{-1}) 4 times higher than that in Guizhou Province (0.37 mg kg^{-1}) and Shanxi Province (0.32 mg kg^{-1}),which was named the second most Se-enriched region in China; 5 times higher than that in the mainland of China (Zhang et al., 2005)**.**

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 (Beld et al., 2007)..In high precipitation areas in the west of Norway, soil Se concentration has risen from 0.2 to 1.4 mg kg^{-1} (Wu et al., 2005).

Soil Se concentrations in the UK range from 0.1 to 4 mg Se kg^{-1}, with > 95% of soils containing < 1 mg Se kg^{-1} (Broadley et al., 2006). In Germany, mean values of 0.123 mg Se kg^{-1} for 195 agricultural soils and 0.158 mg Se kg^{-1} for 304 grassland soils have been reported (Bahers et al., 2012). Selenium concentrations of 0.24-0.55 mg kg^{-1} have been reported in India (Yadav et al. 2005).The mean total Se content was 0.112 mg kg^{-1}(range 0.059- 0.190 mg kg^{-1}) in the low-Se area of China where Keshan disease is endemic, whereas the corresponding values for high-Se areas in China ranged from 6.39 - 10.66 mg Se kg^{-1}(Alland, 2011)

Nazemi (2010) reported that the concentration of total Se in soils from Iran was ranged between 0.4 – 0.045 mg Se kg^{-1} and according to his

classification (< 0.05 ppm total soil Se = deficiency),(0.175- 0.40 ppm = moderate values),(0.15 ppm = low value).(Kenz and Graham, 2012) reported that not all soil types and conditions across the growing areas are the same. Soil's Se levels vary from high, in the Menaus to Belem region of the lower Amazon, to low, in the Acre- Rondonia region on the upper Amazon, resulting in high variability in Se content. Types of soil in California (dark clay shale with red concentration with Se value 0.2 ppm , sandy shale with Se value 0.6 ppm , botten gray shale with Se value 0.4 ppm**. (**Gerald and Combs, 2009**)** said that food systems need to produce enough of the essential trace element Se to provide regular adult intakes of at least 40 mg/d to support the maximal expression of the Se enzymes, and perhaps as much as 300 mg/d to reduce risks of cancer. **(**Rayman 2008**)** confirmed that. Brazilian nuts Fig1.7 are the richest source of food Se, but the content is very variable, ranging from 0.03 to 0.512 mg/kg fresh weight.

Fig 1.7 :Brazilian nuts

Brazilian nuts are harvested from an enormous area of the Amazon basin, in Brazil, Bolivia, and neighboring countries. In contrast, nuts from the Acre–Rondonia region, on the upper Amazon, where soil selenium levels are low, contain on average of 3.06 ± 4.01 µg/g, with a range of 0.03 - 3.17 µg/g (Reilly 2006). Brazil nuts can be considered as a good, if somewhat variable, source of dietary selenium and have been advocated as an ideal dietary supplement, it is well to recognize that, there could also be a health hazard **(**Rayman, 2008**)**.

Selenium levels (mg/kg) in peanuts from different countries UK (0.030), USA (0.075),Australia (0.140),Thailand (0.032 – 0.186), NewZealand

(0.046-0.150) (Cashman, 2001). Certain types of tree nut, especially those of tropical origin, can contain, relatively, high levels of selenium. A UK study found levels of 0.17 to 0.39 µg/g in cashew nuts, 0.049 to 0.08 in coconut, and 0.034 to 0.087 in Macademia nuts, all three imported, compared to 0.008 to 0.036 in locally grown hazelnut (Dolph et al., 2012) .None of these nuts approached the levels of 0.85 to 6.86, with a mean of 2.54 µg/g found in the same study in Brazil nuts. Brazil nuts have been reported to be the richest natural source of dietary selenium In the US nuts purchased in supermarkets averaged 36 ± 50 µg /g, with the extraordinarily high level of 512 µg /g in an individual nut.However, not all brazil nuts contain such high levels, and concentrations, even in batches of nuts from the same source, can be highly variable (Rayman, 2008).

USDA reported that, the average of Se concentration in pistachio was 0.07 ppm in some parts of the world (Motsara, and Roy; 2008). Se concentration in Cow pea (*V. unguiculata*) 0.11ppm, groundnut (*V. subterranean*) 0.22 ppm, maize (*Z. Mays*) 0.20 ppm, guineacorn (S.bicolor) 0.82 ppm, groundnut (A.hypogea) 0.09 ppm from in Udege tin/columbite mining area of Nasarawa State, Nigeria (Aremu et al., 2010). In a study carried out by (Ebrahim et al. 2012) among some popular Sudanese medicinal plants in order to determine their Se content ,the results was as follows; *Adansonia digitata L* 0.06 ppm, *Balanites aegyptiaca (L.)* 0.04ppm , *Grewia tenax (Forssk.) Fiori*, 0.05 ppm , *Tamarindus indica L* 0.02ppm , *Ziziphus spina-christi (L.)* 0.0114 ppm and reported that a possible correlation between it's curative effects and their selenium content. (Van and Gericke; 2000) illustrated that in Pretoria the nutritional value (Se) recorded by USDA in *Tamarindus indica* was 0.013 ppm. In a report published in 2000 the World Health Organization (WHO) summarized worldwide data on selenium levels in different food groups as follows: cereals and grains; < 0.1 to > 0.8 µg/g;; fruits and vegetables; < 0.1 µg/g (W H O, 2001).

A Japanese study of rice grown in different regions of the country found that its selenium content ranged from 0.011 - 0.182 µg/g and that levels were related to soil selenium content, not to differences between cultivars (Rayman 2008). (Nazemi et al., 2010) reported that the concentration of total Se in rice (0.95 ppm) , wheat (0.74 ppm), date (0.46 ppm) and pistachio (0.40ppm) for the samples collected from some areas of Iran. Se concentration in mustard from California was 0.1ppm (Mahbobeh, 2010). The average levels of selenium in wheat, mustard and rice grown in Rohtak and Haryana districts in India was found to be 0.107 ppm, 0.129 ppm, 0.104 ppm respectively (Parmila, 2012).

In some areas of Saudi Arabia Se content in the date was ranged from 1.48 – 2.96 ppm (Al-Ahmary, 2009). A wide range of selenium levels had also been reported in rice sold for domestic consumption in several countries worldwide, mean levels that had been reported, as µg/g, are 0.05 in Thailand , 0.02 in China, 0.073 in New Zealand, 0.319 in the USA, and 0.10 in the UK While levels of 0.03 to 0.25 µg selenium/g have been reported in garlic grown on normal soil, this was increased to 68 µg/g when the soil was enriched in selenium. Similarly onion, with about 0.002 to 0.01 µg/g under normal growing conditions , has had its selenium content increased up to 96 µg/g when grown on selenium-enriched soil. In both garlic and onion the increased levels of selenium are attributed to the production of organoselenium compounds, including Se-methyl selenocysteine (Sirichakwal et al., 2005).

In a similar manner, though to a lesser extent, leafy members of the *Brassica* family, such as broccoli (*B. oleracea*), which also contain organic compounds of selenium, can accumulate high levels of selenium.When grown on enriched soil they have been shown to accumulate up to 15 µg/g of selenium (Finley, 2006).

In Australia, for instance, average selenium levels in wheat of 0.15µg /g have been reported, compared to North American levels of 0.33 µg/g (Beld et al., 2007) .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 (Reilly, 2006). Mushrooms have also been shown to be able to provide a not insignificant amount of selenium to the diet, even without being grown on enriched soil or compost, levels of 0.08 to 0.1 µg/g (wet wt.) have been reported in the UK and 0.13 µg/g (wet wt.) in ordinary store-bought mushrooms (*Agaricus spp.*) in the USA (Fordyce, 2007).

AFinnish study of several different species of wild mushroom used for human consumption, found considerable differences in selenium levels between species, with 17 µg/g (dry wt.) in *Boletus edulis*, compared to 2.1 µg/g (dry wt.) in *Agaricus spp.***M**ushrooms grown on enriched growing medium can contain more than 100 µg/g (dry wt.) of selenium (Mahbobeh, 2010) .

1.11 Selenium in water

The Irrigation of agricultural lands with Se enriched groundwater has led to enrichment of topsoils with Se in Northwest India (Lin et al., 2002).

(Deverel et al., 2012) reported that selenium is readily mobilized to groundwater and surface water by rainwater or irrigation of selenium rich soils and bedrock. Se enriched water is sometimes used for irrigation of farmlands which could lead to Se enrichment of soils.

WHO (2006) reported that , levels of selenium in groundwater and surface water ranged from 0.00006 mg/l to about 0.4mg/l in some areas, selenium levels in groundwater may approach 6 mg/l. Concentrations of Se increase at high and low pH as a result of conversion into compounds of greater solubility in water. Levels of selenium in tap water samples from public water supplies around the world are usually much less than 0.10 mg/l but may exceed 0.050 mg/l. Drinking-water from a high soil selenium area in China was reported to naturally occurring selenium concentrations in groundwater are generally low, typically much less than 0.001 mg/l. WHO data on levels of selenium in groundwater and surface water are limited mostly to a few reports of Copper ores from Tenzania and Algeria are relatively rich in selenium content. (Chapman and Wang; 2000)

reported that Selenium concentrations were determined as part of a study of groundwater conditions of the Columbia Valley Aquifer, a rural and agricultural area south-west of Chilliwak near Cultus Lake, British Columbia groundwater samples collected from different locations were tested for “total selenium” and five samples contained selenium levels above , MAC, with values ranging from 0.070 to .0.170 mg/l.

Zubel (2000) reported that, the U.S Environmental Protection Agency (USEPA) drinking water standard for selenium is 0.050 mg/l, the World Health Organization (WHO) guideline is 0.010 mg/l and the Ambient Water Quality Criterion for Se is 0.005 mg / l. Some reporting elevated levels of Se, in the Prairie Provinces (excluding Manitoba) and from British Columbia, especially in shallow wells. Lower Se concentrations, although above the Guideline for Canadian Drinking Water Quality (Maximum Acceptable Concentration (MAC) of 0.010 mg/l (Lena and Mrittunjai; 2005).

Health Canada (2006) have reported Se concentration in deeper wells supplying drinking water to the municipality of Ontario 0.050–0.160 mg/l. (Deverel et al., 2012) reported that, Se concentration in irrigation water of different areas in Iran was less than 0.01 mg/l, In the area of Saskatchewan, shallow wells (depth ≤ 30.5 m) tested in 2004 for trace metals contained between < 0.0001 and 0.039 mg Se/l,with mean and median values of 0.006 and 0.001mg Se/l respectively; 18.7% of the wells exceeded the MAC (0.010 mg/l) (Saskatchewan Watershed Authority, 2005).

In comparison to the province as a whole, shallow wells (depth ≤ 30.5 m) sampled throughout the province under the Rural Water Quality Advisory Program of the Saskatchewan Watershed Authority contained comparable selenium levels, with values ranging from < 0.001 to 0.0410 mg/l and a mean of 0.010 mg/l Selenium rich areas(≥ 0.005 mg/g soil)in Canada include the southern Prairies and Ontario.Naturally occurring selenium concentrations in

ground - water and surface water are generally low, typically much less than 0.001 mg/l (Kenz and Graham, 2012).

1. 12 Statement of the problem

Human body suffers from several diseases caused by the lack of selenium element and this element cannot be availed except through, vegetables, fruits, cereal and food , on the other hand despite the significant role of Se in preventing certain degenerative diseases such as cancer and arthritis rheumatoid, the Se contents of soil, water, and the staple food such as Sudanese fruits produced in Sudan have not been evaluated.There is no national soil-Se geochemistry database in Sudan, capable of identifying areas of relatively high or low soil-Se concentrations, therefore, this research is designed to establish the links between the underlying geology and the concentrations of Se, both in the overlying soils and in the Sudanese fruits produced on them. Although only a feasibility study, therefore, the study will focus on this aims as the problem of the study. We will concentrate on the best possible ways to avail the selenium element for the human being and will endeavor to conclude to constructive recommendations in this regard. We hope that this information will assist with the development of informed food policy in Sudan**.** At the outset of this research, no data were available regarding the Se concentration in Sudan soils (Alein forest) and the commodities grown on them. Therefore, a number of assumptions/decisions will be made.Also the determination of Se in natural waters is difficult due to various factors, particularly it's low concentration and matrix effects. Preconcentration and separation can solve these problems and lead to a higher confidence level and easy determination of Se element.

1.13 The importance of the thesis

The importance of this thesis stems from the importance of the selenium element itself for the human body.The increase and decrease of this element have greatly

affected human life on earth.Therefore, it is a matter of great concern to make available source of soil and fruits which is rich of selenium.

1.14 Research Hypotheses

In these research studies the following hypotheses will be tested:

1- Different techniques: hydride generation Atomic Absorption pectroscopy, Graphite Furnace Atomic Absorption Spectroscopy, Inductively Coupled Plasma, X-ray Fluorescence by energy,X-ray Fluorescence by wavelength and Energy Dispersive X-ray "EDX" Linked with scanning electron microscopy "SEM") may exert different concentrations of Se in the samples under study.

2- ICP (Inductively Coupled Plasma) techniques may be the most preferable for selenium determination than the other techniques mentioned above.

3- The variation for Se concentration determined by different mentioned techniques in water , plants and soil under the study (separetly) may be low.

4- Concentration of Se in soil may be highest than that in the water and plants.

5- Concentration of Se in surface layers of soil may be less than in deep layers.

6- Variation can be occur in soils layingjv[lm [,[g only afew meters from each

1.15 Target of the study

This study targets scientists, academics, researchers, specialists and officials in the fields of: sciences, medicine, geography, environment and other social aspects.

1.16 Data collection methods

The researcher will collect the data and the basic information from the elementary sources like the reports; books; pictures; maps; and the internet in order to create the structural and theoretical framework of the study. As for other data and information concerned with the chemical experimental studies, it is based on collecting samples from the nature on what is called the natural environment or site environment (field) and thereafter conducting chemical analysis and lab, determination as experimental method.

1.17 The objectives of the study

The objectives of this study can be summed in the following:

1- To determine selenium concentration in Hafeer water, some Sudanese fruits and their soil from Alein forest

2- To compare the different results obtained by various analytical instrumen techniques.

3- To evaluate an accuracy of analytical methods for determination of Se in environmental materials including water, soil and Sudanese fruits.

CHAPTER TWO

Materials and Methods

2.1 Materials

2.1.1 The study area

A natural reserved forest called Alein Fig 2.1 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 Alobied city altitude (°30 14 21 ,°30 26 06) N , and longitude (30 20 13^0 , 41 50 12^0) E and is bordered to the north by Mountain Kordofan and to the east by Mountain Al ein 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 and Mohammed, 2015).

The climate of the Alein forest is a continental climate with two seasons, one dry and the other rainy and belongs to the poor savanna climate. There are several types of soil in Alein forest (Fig 2.2):

i) Brown gardud cover most of the forest area.

ii) Red- gardud covers a different area between the eastern and north-eastern sides as well as the western side of the forest and the largest area of this soil from the western plateau.(The soil of algardud is originally fixed soil but it was covered with sand dunes and the soil of the alqardud is the most ancient soil in the area around the mountains).

iii) Crocking Clay is the sedimentary soils that include the soils of Albagara creek and Nile creek and are located in small area on the southern side of Alein forest and eastern areas.

iv) Antisol are soils that have been heavily affected by sand, gravel and stone these soils are a newly formed soil of modern materials.

Fig 2.1: Location of Alein forest

Fig 2.2 :The topography in Alein forest

There are some tree species in the Khiran streams, which are an extension of the natural cover in the area making vegetation of the area which consist of many plants like *Adansonia digitata- Grewia tenax – Balanites egyptiaca - Ziziphus spina Christi- Tamarindus indica* and etc. Al Khairan Circle in the Alein Forest are the areas that are located around the watercourses (Khiran), which include the Bagara Creek and the Nile Creek as sources of water and often trees present a broad transaction eg *adansonia digitata and Tamarindus indica* (sedimentary soil).and prevents any cutting activity in this circle (Ibrahim and Mohammed, 2015).

2.1.2 Samples

Sample of water from *Al-hafeer*, five types of Sudanese fruits (*Adansonia digitata - Tamarindus indica - Grewia tenax - Balanites aegyptiaca - Ziziphus spina christi*) and their soils at three different depths(75 cm - 100 cm - 150 cm).

2.1.3 Equipment

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-

an electrical plate - steel ring - Minipress machine (aton / cm^2 hydraulic press) Fig 2.3) - Herzog Ht 40 pressing machine (Fig 2.4).

Fig 2.3 Philips(Minipress) pressing machine for EDXRF

Fig2.4: Herzog Ht 40 pressing machine for WDXRF

2.1.4 Reagents

All reagents used were of analytical grade (Merck, Darmstadt Germany):

1- Extraction solution which contain ammonium acetate + acetic acid + ethelene diamine tetraaceticacid (0.5 M NH_4Ac + 0.5 M HAc + 0.02 M EDTA).

2- Se^{+4} (SeO_2) stock solution (1000 µg/mL).

3- Sodium boroterahydrate ($NaBH_4$) (0.2 g).

4- Sodium hydroxid (NaOH) 0.5%

5- Hydrochloric acid (12 M HCl).

5- Perchloric acid (11 M $HClO_4$).

6- Hydrochloric acid (6 M HCl).

6- Nickel nitrate as a modifier.

7- Nitric acid (15 M HNO_3).

8-Deionized water was used.

9- H_2O_2 (9.8 M).

10- HF(6 M).

11- cellulose**.**

12- Starch.

2.1.5 Instruments

Various analytical techniques were applied for determination of trace amounts of Se (mg/k:ppm) in collected samples. These techniques were:

- Hydride Genration Atomic Absorption Jena, Spectroscopy (HGAAS): model contr AA 700 P Hydride Analytik Jena AG, Germany (Fig 2.5.

Fig 2.5 : Contr AA700 P Hydride Analytik Jena

- Grafite Furnace Atomic Absorption Spectroscopy (GFAAS), model contr AA 700 high- resolution continuum source atomic absorption spectrometer (Analytik Jen AG, Jena, Germany) (Fig 2.6).

Fig 2.6 : ContrAA® 700 High-Resolution Continuum Source AAS for,Graphite furnace and Hydride generation

- Inductively Coupled Plasma Mass Spectrometry (ICP MS/MS), model Aglient 8800 Triple quad (Fig 2.7).

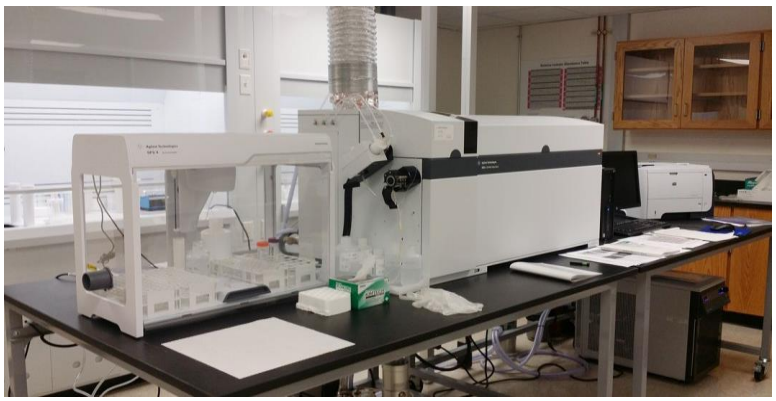

Fig 2.7 : ICP MS/MS (Aglient 8800 Triple quad Germany)

- Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES model 2000 DV Perken Elemer, Germany (Fig 2.8).

Fig 2.8 :ICP OES (2000 DV Perken Elemer Germany)

- Energy dispersive x-rays fluorescence (EDXRF) model CANBERRA SERIES 35 PLUS .USA (Fig 2.9).

Fig 2.9 :(EDXRF) machine model (CANBERRA SERIES 35 PLUS ,USA)

- Wavelength dispersive x-rays fluorescence (WDXRF), model PAN- analytical Axios^{mAX} 2.4 kw Holland (Fig 2.10).

Fig 2.10 : (WDXRF) machine PANanalytical model Axios^{mAX} 2.4 kw Holland

- Scanning Electron Microscopy linked with Energy Dispersive X-ray Fluorescence (SEM+EDX),model Super-scan SSX-550-Shimadzu Corporation Energy –dispersive X-ray EDS (Oxford instruments)The computer-assisted SEM/EDS analysis is performed SEM-EDS system using the Automated Feature Analysis(AFA) program (within the Shimadzu Corporation Perception software.) (Fig 2.11).

Fig 2.11 : (SEM-EDX) model(Super-scan SSX-550) Shimadzu Corporation

2.2 Methods

2.2.1 Collection of samples

Samples under the study (Sudanese fruits and their soils) were collected according to Standaard Methods of Sample Collecting in cleaned paper bags until arrived at the laboratories Water from Alhafeer was collected in very clean bottle, washed by the water sample several times and acidified by drops of HNO_3.

2.2.2 Preparations

(a)Preparation of selenium standard solution

Selenium standard solution was prepared by dissolving 0.14053 g of SeO_2 in 100-ml volumetric flask.Aminimum 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.

(b) Preparation of the samples (fruits – soil)

The pulp of Sudanese fruits were manually separated from the seeds (except *Grewia tenax*) using ceramic knife and then aliquots of them were cut into small pieces and were put at room temperature in small dishes for a day for drying, then grounded , homogenized using a mortar , sieved and 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. The resulting dry and fine powder was used for further digestion to be ready for analysis by various techniques.

Aliquots of the soil materials were put at room temperature in small dishes for a day for dryness , then grounded , homogenized ,using a mortar , sieved , shaked mechanically and 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. The resulting dry and fine powder was used for further digestion to be ready for analysis by various techniques.

2/1 Destructive method (Wet digestion) for HGAAS – GFAAS techniques

(a)Sudanese fruits

1.0 g of dried and homoginized fruits pulp were weighed by sensitive balance 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 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. 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} and kept for about 4 h; then the contents of the digestion flask were transferred in to 50 - ml flask and then dilluted to the volume with deionized water.

(b)Soil

0.5 g of the dried and homogenised 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 5ml 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}, and kept 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 destroy organic matters). Extracted solvent (extraction solution (ammonium acetate + EDTA)) was used after wet digestion (acid digestion) (in case of preparation of fruits and the soil) to concentrate selenium that was present in low concentration; each fraction resulting from the

extraction was subjected to reduction with 3 ml concentrated HCl (12 M) [Se^{+6} passes into Se^{+4}] by heating them to 80 °C for 30 minutes on a sand bath,. The addition of HCl acid to the sample solution reduse the oxidation state of Se from +4 to +2); 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 $NaBH_4$ to form selenium hydride decreased the interferences from 24 ions. To minimize interferences with transition metals at selenium dosage, optimal concentrations of the reagents were established: (0.5% m/v sodium borohydride in 0.5% m/v sodium hydroxide and 50 % v/v hydrochloric acid). For total selenium determination, $NaBH_4$ and hydrochloric acid were added to the real samples to reduce all selenium to selenium (+4) since selenium(+4) could only produce the hydride form. Appropriate amount of concentrated hydrochloric acid was added to the solutions to give 4mol/l as final acid concentration.After reduction of Se (+6) to Se (+4),in case of GFAAS , modifier of (Ni $(NO_{3)})_2$) was used.

2/2 Destructive method (Wet digestion for analysis by ICP OES –ICP MS/MS)

Digestion was performed by accurately weighing (range: 0.0800 - 0.2200 g) Sudanese fruits and their soils material into digestion flask, to which 4 mLof 14 M HNO_3 and 1 ml of 9.8 M H_2O_2 was added. In case of soil samples, an additional 1 mL of 6 M HF was added to the material to fully digest the sample (to digest the silicates present in the material).The mineralization was carried out overnight at 110 °C on a hot plate. Furthermore, in each set of digestions, blanks were included. The samples were stored at 5 °C until analysis. For the determination of Se a 20-fold dilution of the samples was performed to reduce the concentration of the concentrated acids and the matrix elements. For Se a lower dilution factor was preferred because of the lower sensitivity for this element.The use HCl or $HClO_4$ however, as an oxidants in preparation of the

samples to be analyzed by ICP MS/MS is not recommeded since the polyatomic ions $^{40}Ar^{37}Cl^{+}$ interfere with the determination of ^{77}Se.

(3)Non-destructive method for samples of Sudanese fruits and soil

In general the pressed powder method (non-destructive method**)** was used for routine elemental determinations (XRF techniques) in plant and soil especially when simpler and faster preparation was required. Also the sample preparation was quick and easy. It consisted of the following preparations and its technique.

(a) Samples preparation for EDXRF technique

1.0 g of each dry, soft and homogenous sample powder (plant and soil) was weighed accurately and after adding a binding material (cellulose) to the powder to improve the quality of the tablets it was pressed using minipress machine (aton/cm^2 hydraulic press) and made it into pellets of 13 mm diameter and about 2 mm thickness (Fig 2.12).

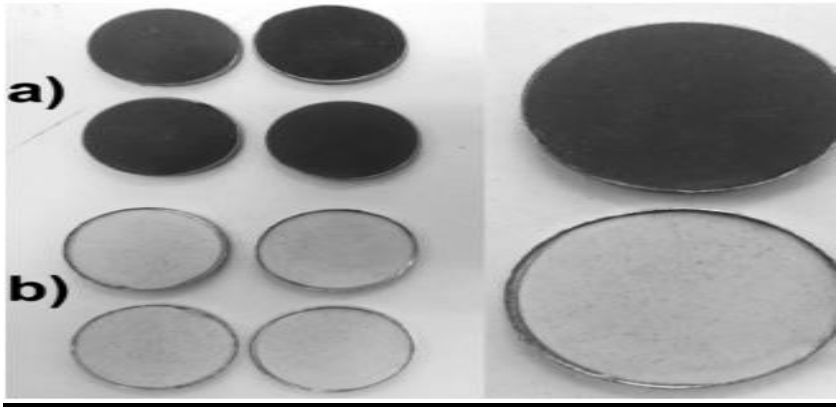

Fig 2.12 : Pressed pellets of samples (a) and standard (b) perior to their analysis by EDXRF

The pellets were homogenized , then pressed and used as targets for the EDXRF experiment. For quality control, two types of Standard Reference Materials were used, and treated in the same manner (IAEA – SOIL- 7 and IAEA – V – 10 - Hay Powder).

(b) Sample preparation for WDXRF technique (Non –destructive method)

12.0 g of the dry, soft and homogenous powder sample was weighed accurately and burned at a temperature of 450 ^{0}C (dry ashing) to get rid of the organic matter that might be present in the sample and could cause cracks on the top

surface of the examined sample during exposure to source of the x-ray. Therefore 9 g was weighed of the burned sample (ash) and pressed in a steel ring on a starch(binder) for mechanical stability. (as pressed samples give higher intensities than loose powder samples). The pellets were pressed with a Herzog Ht 40 pressing machine. The maximum pressing force of the machine was 400 kN.Then the sample swere homogenized; then burned, pressed (pellets) Fig 2.13 and used as targets for the WDXRF experiment.

Fig 2.13 : Pressed pellets of samples perior to their analysis by (WDXRF)

(c) Sample preparation for SEM + EDXRF technique (non- destructive method)

0.05 g of the dry and soft sample powder was accurately weighed. For conventional imaging, the sample ought to be electrically conductive (at least the surface) and electrically grounded to prevent the accumulation of electrostatic charge at the surface. Non-conducting specimens accumulate charge under electron bombardment though an electron beam couldnot scan a charged object. On this account, samples ought to be coated with a conducting layer (with gold) before being analyzed with the SEM. Resin mounted samples had a perfectly prepared flat top surface, which is a requirement for quantitative EDS results. However, the bottom surface could be very rough or even skewed. Typically, these resin samples have a few standard size diameters 32mm or 1 ¼ inch was the diameter most commonly used for optical, as well as electron

optical, (SEM) investigation.Samples were placed in a container and covered in epoxy-resin.since the resin was solid, a cross sectional cut was made through the sample and the exposed surface was polished and coated with gold..

(C) Preparation of water sample

5 ml of water was acidified by 1 ml of concentrated HNO_3 and was heated to about 70 °C for 3 min and was transferred to 100 - ml volumetric flask and diluted to the mark with deionized water. It should be mentioned that for the analysis of these real samples, appropriate amount of concentrated hydrochloric acid was added to the solution to convert Se^{+6} to Se^{+4} and gave 4 mol/l as a final acid concentration**.**

2.2.3 Experimental

(A) Atomic Absorption Spectroscopy (AAS) method

Two types of AAS were used

A.1 Hydride generation atomic absorption spectroscopic technique (HGAAS)

Selenium determination was based on the injection flow using, the peristaltic pumps which were used simultaneously for transporting the reducing agent and the sample, and removing waste. 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 (11 ml was sucked in three batches within two minutes) 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) which was then transported to a gas-liquid separator , 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 was then sent to the atomizer. Atomic absorption measurements were performed in a quartz atomization cell in nitrous oxide - acetylene flame at 196 nm and background correction was made using wavelength correction; the wavelength

stability was also a novel parameter for AAS. The use of an integrated neon correction during wavelength setting offered a unique wavelength stability, where selenium was separated and, after passing through the filter of tetra-fluorine-ethylene, was 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 absorbed radiation from xenon lamp of the spectrophotometer.The instrument was set and the conditions for analysis of selenium by HGAAS were determined as shown in table 2.1 . 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.

Table 2.1: Instrument settings and analytical conditions for determination of Se by HGAAS

Lamp current (mA) (xenon)	10.0
Main line(Wavelength) (nm)	196.0 (100%)
Slit width (nm)	1.2
Inert purge gas	Ar
instrument Mode	Absorbance
BC on Sampling Mode	Automated sampling
Flame type	Nitous oxide(N_2O)-Acetylene
Acetylene flow (L/min)	2.00
Nitous oxide(N_2O)Flow (L/min)	10.0
Measurement mode	Peak Area
Carrier gas	N_2
Gas Flow (N_2L//h)	12
Temperature (ºC) in hydride unit	38
Temperature (ºC) in the system	2600

A.2 Graphite furnace atomic absorption spectroscopy (GFAAS)

A graphite furnace analysis Fig 2.14 consisted of measuring and dispensing a known volume of sample into the furnace.The sample was then subjected to a multi-steps temperature program. In this technique, a tube of graphite Fig 2.15 was located in the sample compartment of the AA spectrometer, with the light path passing through it. A small volume of digested sample solution was

quantitatively (20 µl from the sample was required for analysis) mixed with nickel nitrate salt (1 g/l) as chemical modifier for the determination of selenium in aqueous medium prior to the atomization processes. The solution mixture was placed into the tube through a sample injection hole located in the center of the tube wall (atomizer) programmed to be heated in series of steps, number of steps within each program was variable,; 6 steps made up the typical graphite furnace program Fig 2.16 . These steps included : drying (to remove solvent usually water from the sample at 100-500 ^{0}C) , pyrolysis (to remove organic and other volatile materials in the sample before the analyte is vaporized to volatilize and remove the solvent and matrix components from the sample), Cooling down (optional), atomization (in which the analyte was atomized and the integrated absorbance recorded) ,Cleaning out (to remove any residual material from the graphite tube at 2500-2700 ^{0}C) and Cooling down (to allow the furnace to reach ambient temperature at 200 ^{0}C) , pyrolysis and atomization optimization temperatures were investigated for samples spiked with each species separately ; from 900 up to 1500 ^{0}C were tested for pyrolysis and from 2400 up to 2700 ^{0}C for atomization. After these steps the remaining sample was atomized. Finally the selenium present in the sample was dissociated into atoms, and thus atomic absorption occurred. As atoms were created and diffused out of the tube, the absorbance raised and fall in a peak-shaped signal. The peak height or integrated peak area was used as the analytical signal for quantitation. The sample was atomized in a very short period of time, concentrating the available atoms in the heated cell and resulting in the observed increased sensitivity. The small sample size was compensated by long atom residence times in the light path. The instrument was set and the conditions for the analysis by GFA AS were determined as shown on table 2.2.On the computer screen, the adequate selenium vapor absorbance was recorded as peaks whose height was proportional to the selenium concentration in the analyze sample. .

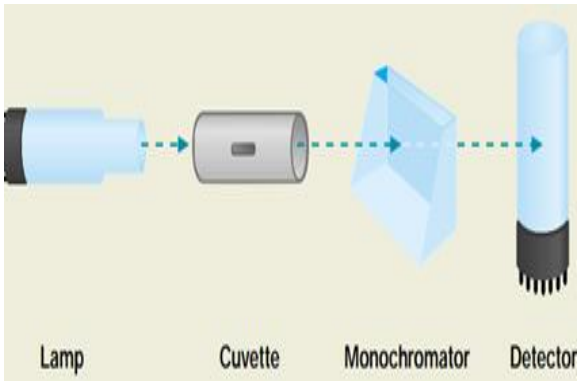

Fig 2.14 : Main parts of GFAAS device

Fig 2.15: photos of a graphit tube before and after injection

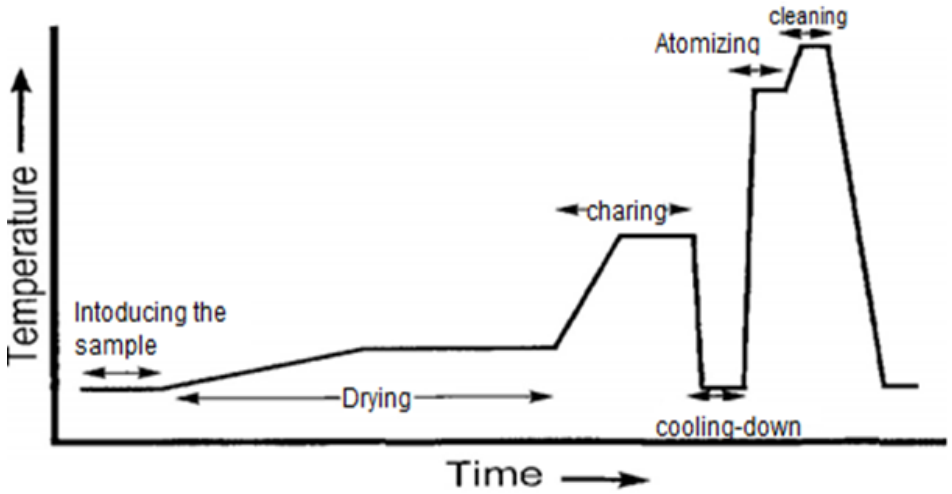

Fig 2.16 :A schematic diagram of a furnace programe

Table 2.2 : Instrument settings and analytical conditions for determination of selenium by **GFAAS**

selenium Wavelength (nm)	196
Slit width (nm)	1
Xenon Lamp current (mA)	15
The purge argon flow rate (L/min)	3
Reading time (s)	5
Background correction	Neon
Concentration unit	ng/mL
Injection volume for sample (µL)	20

(B) Inductively coupled plasma (ICP) techniques

Two types of ICP techniques were used

B.1 Inductively Coupled Plasma Mass-Mass spectrometry (ICP MS-MS):

A representative part of the sample was introduced into the system. Liquid samples were split into aerosol formation and droplet selection. A liquid sample was transported to the nebulizer using a peristaltic pump resulting in a pressure drop in the nebulizer, created by following of the nebulizer gas through a narrow hole at the tip of the nebulizer. When the sample reached the nebulizer, pneumatic action of the gas flow broke the sample into a fine aerosol by mechanical force. The typical gas used was argon. The particular design of concentric nebulizer gave good stability and sensitivity. After the nebulization process, the tiny droplets entered the spray chamber, where the droplet selection took place. Only the smallest droplets were sent to the plasma torch Fig 2.17 for further analysis in order to limit the solvent load of the plasma. In the design of the Scott-type double-pass spray chamber which was used, the aerosol from the nebulizer was guided into a central tube and the droplets then passed through the entire length of the tube, where, the larger droplets (larger than ~ 10 μm in diameter), were dropped out owing gravitational forces and they were removed through a drain tube, which was located at the end of the spray chamber. The smaller droplets (< 10 μm in diameter) would, however, continue passing between the central tube and the outer wall because of a positive pressure and from there, going to the plasma source. This selection of only the smaller droplets could, however, also be seen as a weak point of the instrumentation, since only 2 -5% of the sample was introduced into the plasma source. A second feature of the spray chamber was to smoothen out the nebulization pulses produced by the peristaltic pump if used. Furthermore, the spray chamber could be externally cooled to reduce the introduction of solvent going to the plasma source, which was often required when dealing with organic solvents. ICPMS-MS used an argon plasma source to dissociate the sample into its basic atoms or ions, combined with very low backgrounds, these ions then pass through the interface and the ion lens into the mass spectrometer, where they were isolated

according to their atomic mass-to-charge ratio by a quadrupole analyzer. In this circumstance, metal ions were detected rather than the light that they emit. After being focused by the ion lens, and the ions were separated by their mass-to-charge ratio in the mass spectrometer (quadruple mass filter, Fig 2.18) then were directed to a detector that determined the number of ions present. Once the detector measured the ions, the computerized data system was used to convert the measured signal intensities into concentrations of each element and generated a report of the results. Fig 2.19 .

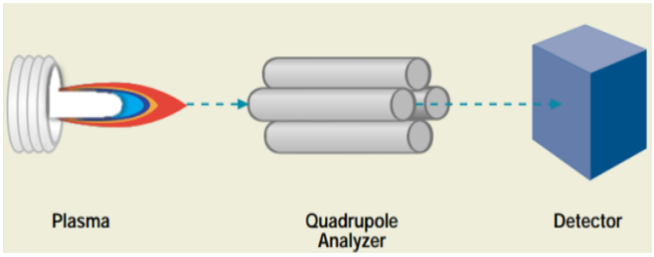

Fig 2.17 : Some parts of (ICP-MS) device

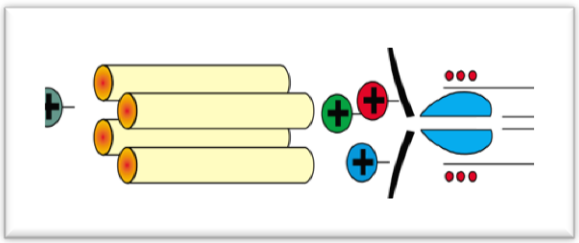

Fig 2.18 : Quadrupole mass filter

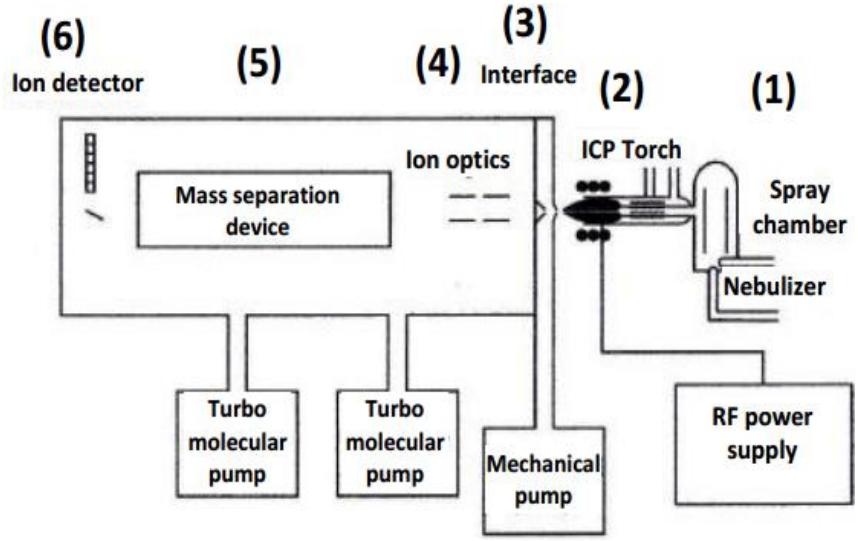

Fig 2.19 : Schematic overview of an ICP-MS instrument: (1) Sample introduction system, (2) ICP torch, (3) Interface region, (4) Ion focusing system, (5) Mass separation device and (6) Ion detector

Table 2.3 shows the operating conditions and parameters of the inductively coupled plasma mass spectrometer.

Table 2.3 : Set – up and operating parameters of inductivelycoupled plasma mass spectroscopy (ICP MS-MS) instrument

Wavelength range	160 – 782 nm
RF generator	40 MHz
Detector	Segment - array Charged – Coupled deviced Detector ,CCD
RF power	1400 W
Plasma gas flow rate	14 L min^{1-}
Nebulizer gas flow rate	0.8 L min^{-1}
Auxiliary gas flow rate	0.95 Lmin^{-1}
Analogue detector	2500 V
Sample introduction rate	1 cm^3 min^{-1}
Extraction	-118 V
PC detector	3850 V
CCT gas (H_2: He) (7% H2+ 93% He) flow rate	5.9 ml min^{-1}
Integration time	0.1 s
Stabilization time	35 s
Sample pump tube(white – white) (Anachem Ltd . Anglia)	1.02 mm

B.2 Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES)

Sample was usually introduced into the plasma in liquid form after wet digestion processes, and injected directly into the instrument. A glass gas–liquid separator of argon (nebulizer) was employed as the sample introduction part to the ICP torch. The sample to be analyzed solution was converted to an aerosol via which maintained high atomization temperature of around 10,000 K allowing the nebulization process, then sent into the centre of the plasma As the plasma free atoms in the gaseous state were generated and adequate energy was often available to convert the atoms to ions, then promoted the ions to excited states, the ionic excited state species may then returned to the ground state via emission of photons. The measured emission intensities of photons by detector Fig 2.20 ,

were then compared to the intensities of standards of known concentration to obtain the elemental concentration in the unknown sample. (Specific wavelength of the photons can be used to identify the elements and the number of photons was directly proportional to the concentration of the element in the sample).Once the detector measured the emission intensities of photons, the computerized data system was used to convert the measured signal intensities into concentrations of each element and generated a report of the results.

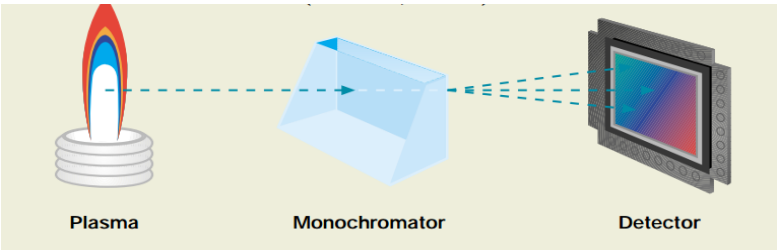

Fig 2.20 : Some parts of (ICP-OES)device

Table 2.4 shows the operating conditions and parameters of inductively coupled plasma optical emission spectroscopy**.**

Table 2.4: Set – up and operating parameters of inductively coupled plasma optical emission spectroscopy (ICP-OES) instrument

Optical system	Echelle grating – based, Ar flushed
Se wavelength	196.0 nm
RF generator	40 MHz
Detector	Segment- array Charged – Coupled deviced Detector ,CCD
Plasma view	Axial
Nebulizer type	Meinhard Type A
Type of peristalitic pump tube	black – black
Optical system resolving power	Normal
Spectral band-pass	0.007 nm
Rf power	1300 W
Nebulizer gas flow rate	0.95 dm^3 min^{-1}
Cooling gas flow rate	15 dm^3 min^{-1}
plasma gas flow rate	15 L Ar/min
Axiliary gas flow rate	1.0 dm^3 min^{-1}
Sample introduction rate	1 cm^3 min^{-1}

(C) X-ray fluorescence (XRF) techniques

Two types of XRF techniques were used:

C.1 Energy dispersive X-ray fluorescence (EDXRF) technique

Energy dispersive XRF spectroscopy technique equipped with Si (Li) detector with a Cd^{109} resolution of 180 keV. Radioisotope Cd^{109} with energy 22.1 KeV was used as a primary source For instance in small disk represents the contents,the sample must be representative of the entire material, and so must be taken very carefully.Once taken, it must also be handeled carefully. 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, Each pellet was placed in a cup and the cup was placed in the spectrometer, and was measured for 1000 seconds.When a primary x-ray excitation source from an x-ray tube source strikes a sample, 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 creating a vacancy 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, These vacancies present an unstable condition for the atom.

As the atom Fig 2. 21 "A ,B"

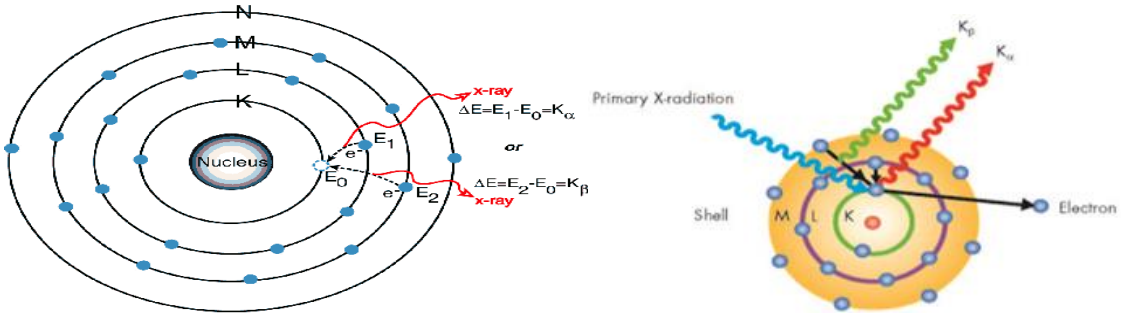

Fig 2. 21 X-ray fluorescence radiation

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. 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 adetector 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 transferred to a computer and then were analyzed.The concentration of Se present in the samples were obtained using AXIL - XRF software available in the computer. For quality control, a standard reference of Hay powder and soil were used and treated in the same manner.Table 2.5.shows the operating conditions and parameters EDXRF

Table 2.5: The operating parameters and optimal conditions of the EDXRF spectrometer for elements determination (including selenium)

voltage	50 Kv
current	300 μA
detector	Si (Li)
a primary source	Cd-109 x-ray source
Gas to cool Si(Li) detector	liquid nitrogen
a resolution	180 keV.
Radioisotope	Cd^{109}
Radioisotope energy	22.1 KeV
real integration time	1000 s
detector dead time	< 1%,
atmosphere vacuum	Pressure < 30Pa
The analytical lines considered for Se	K_{α}
collimator	10 mm

C.2 Wavelength dispersive X-ray fluorescence (WDXRF) measurement

Wavelength Dispersive X-ray Fluorescence (WDXRF) analysis was based on the sample excitation by X-rays and the detection of the characteristic X-rays. The X-ray source, the tube, creates the X-ray beam. Before the X-ray beam penetrates the sample, it can be filtered. Poly-capillaries focusing optics collected X-rays from the divergent X-ray source and directed them to a small focused beam at the sample surface with diameters as small as tens of micrometers. The resulting increased intensity delivered to the sample in a small focal spot, allows for enhanced spatial resolution for small feature analysis and enhanced performance for measurement of trace elements (e.g selenium). The X-ray beam from the tube is referred to as primary radiation and the X-rays emitted by the sample as secondary X-rays. The secondary X-rays were collected by the collimator and directed to the analyzing crystal. WDXRF uses crystals to disperse the fluorescence spectrum into individual wavelengths of each element, providing high resolution and low background spectra for accurate determination of elements concentrations (Fig 2.21). The fluorescent radiation from a sample can be generated by emitting external X-ray radiation into the sample, referred to as primary radiation. The primary radiation can eject an inner shell electron of an atom in the sample, if a primary photon energy is equal or higher than the binding energy of the electron, which will create an unstable intermediate ion. To fix the inner shell electron deficit, an outer shell electron moves to the created empty gap. Because the electron transmitted energy is released as fluorescence radiation whose energy is equal to the energy difference between the electron shells. The energies that release the electrons from the energy shells are determined by the atomic number of the element, which makes the fluorescence characteristic. Elements with lower atomic number require less energy and are referred to as light elements. In WDXRF spectrometers, all of the elements in the sample were excited simultaneously.The different energies of the characteristic radiation emitted

from the sample were diffracted into different directions by an analyzing crystal. By placing the detector at a certain angle, the intensity of X-rays with a certain wavelength can be measured. The fluorescent X-rays were physically separated before detection using diffraction crystals. After analyzing crystal the X-rays go to the detector and to the pulse height analyzer to be measured. X-ray fluorescence is produced by transitions of inner shell electrons of an atom and activates the atom to emit X-ray photons with a characteristic wavelength determined by the atomic number of the element. For a particular energy (wavelength) of fluorescent light emitted by an element, the number of photons per unit time (generally referred to as peak intensity or count rate) was related to the amount of that analytes in the sample. The counting rates for all detectable elements within a sample were usually calculated by counting (In gas proportional counter detectors which use argon as counting gas, Ar- K_{α} of argon may fluorescent), for a set amount of time, the number of photons that were detected for the various analytes' characteristic X-ray energy lines. An X-ray detector converts X-ray photons coming from the crystals into a measurable energy form, voltage pulses. The pulse distribution data from detectors can be evaluated and modified in the pulse height analyzer.The spectra analyzed and then transferred to a computer, concentration of elements present in the samples obtained using AXIL- XRF software available in the computer (Fig 2.22).

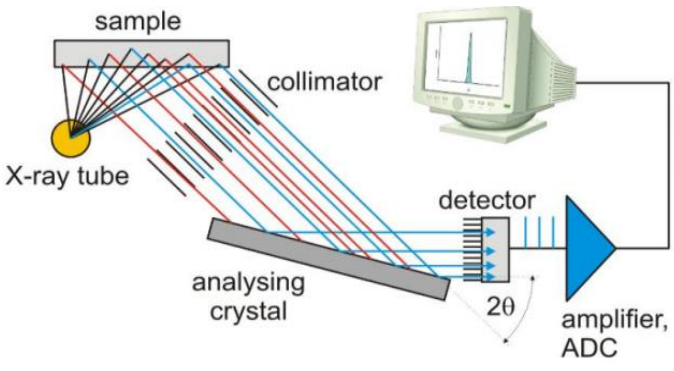

Fig 2.22 : Scheme of WDXRF

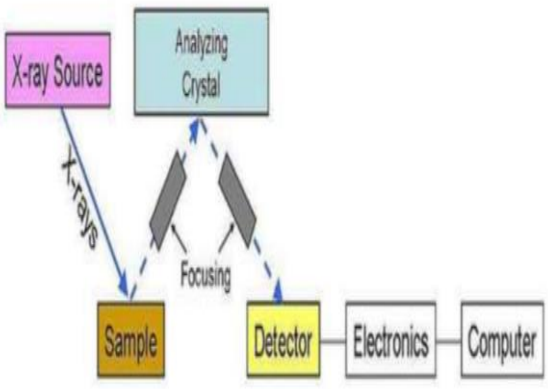

Fig 2.23 : The optical path in WDXRF. The X-ray source and the optics above the sample

(D) Scanning electron microscopy + energy dispersive X-ray fluorescence (SEM/EDXRF) technique

Analysis is conducted on a total of 20 samples, along with two process blanks.Particles from the sample are coated with conducting gold layer , placed on a sample holder , introduced into the device and then are bombarded with electrons from electron gun. The signals are generated through detection of the backscattered electrons, secondary electrons, X- rays, the topographic pictures were generated by analysis of the secondary electrons which give topographic information, the backscattered electrons which can serve as signal to build up the final image (generating a picture) based on the mass of the elements, cathode-luminescence can give information on the electronic structure and the chemical composition of materials; and transmitted electrons can describe the sample's inner structure and crystallography. Another type of signal that is widely used in SEMs is X-rays, X-ray was used for identifying the elemental composition and gives the spectrum, permitted the detection and identification of the X-rays produced by the impact of the focused electron beam on the sample surface.The electrons interact with the atoms that make up the sample, producing signals that contain information about the sample's surface topography, composition, and other properties such as electrical conductivity (Fig 2.23) . The electron beam of SEM was used to excite the atoms in the surface of the sample.These excited atoms produced characteristic X-rays, which could be readily detected. By utilizing the scanning feature of the SEM, a spatial distribution of elements on the sample surface could be obtained. The generation of the X-rays in a SEM is a two-steps process, in the first step, the electron beam hits the sample and transfers part of its energy to the atoms of the sample, this energy can be used by the electrons of the atoms to "jump" to an energy shell with higher energy or be knocked-off from the atom. If such a transition occurs, the electron leaves behind a hole. Holes have a positive charge and, in the second step of the process, attract the negatively-charged

electrons from higher-energy shells. When an electron from such a higher-energy shell fills the hole of the lower-energy shell, the energy difference of this transition can be released in the form of an X-ray, this X-ray has energy which is characteristic of the energy difference between these two shells. It depends on the atomic number, which is a unique property of every element. In this way, X-rays are a "fingerprint" of each element and can be used to identify the type of elements that exist in a sample. SEM images represent the morphology of a sample and can also reconstruct quasi-three-dimensional views of the sample surface (Fig 2.24). After localization of trace elements in the sample with BSE imaging, qualitative and semi-quantitative chemical composition of selected sample was measured using energy dispersive X-ray spectrometer (EDS). EDS detects and processes X-rays that were emitted from constituent elements and were characteristic of each element, dependent on its atomic number. Combination of SEM and EDS was used for single particle analysis. The computerized data system was used to convert the measured signal intensities into concentrations of each element and generated a report of the results.

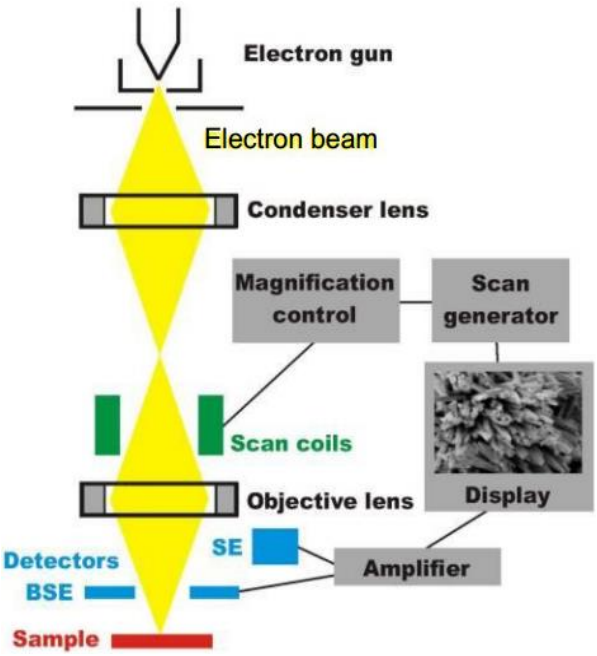

Fig 2.24 : Processes of the SEM technique

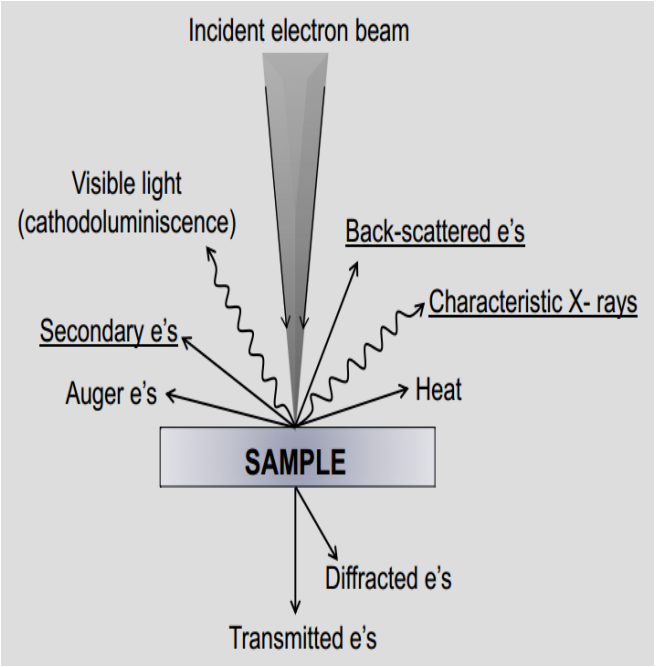

Fig 2.25 : Interaction of the incident electrons with the sample in SEM+EDS technique

Table 2.6 shows the operating conditions and parameters of the Scanning electron microscopy + energy dispersive X-ray technique.

Table 2.6 : The operating parameters and optimal conditions of the SED+XRF technique for elements determination(including selenium)

conditions	under high vacuum conditions
accelerating voltage	20.0kV
Detector of SEM	Backscattered electron(BSE)
working distance	approximately 15mm-17mm
the magnification for the analysis	2,000X
the number of electronic fields	set at a maximum of 5 x 5 nuten/colom
A grid dimension	256 x 256
a dwell time per pixel for searching	8µs
a dwell time per pixel for measuring.	16µs
The size criteria for analysis were a minimum	0.3µm
The size criteria for analysis were a maximum	50.0µm
The maximum number of particles analyzed,	4000 particles
EDS parameters a nominal duration, minimum	3s
EDS parameters had a nominal duration a maximum	6s
a minimum count	300
a target count	2500

CHAPTER THREE

Results and discussion, conclusion and recommendations

3.1 Results and discussion

The results of selenium concentration " ppm" in the analyzed samples by seven techniques are shown in the Tables 3.1, 3.2, 3.3**,** 3.4 and 3.5

Table 3.1: Results of selenium concentration in "ppm" in some Sudanese fruits by various techniques

plants ↓ → technique	HGAAS	GFAAS	ICP – MS/MS	ICP - OES	SEM+ EDX	EDXRF	WDXRF
Ziziphus spina christi	0.0172	0.0162	0.0127	0.0122	0.0150	0.0154	Nd
Adansonia digitata	0.0344	0.0324	0.0143	0.0138	0.0175	0.0188	Nd
Balanites eygptiaca	0.0342	0.0318	0.0137	0.0130	0.0162	0.0170	Nd
Grewia tenax	0.0348	0.0338	0.0145	0.0140	0.0185	0.0188	Nd
Tamarindus indica	0.0225	0.0211	0.0133	0.0125	0.0153	0.0164	Nd

Table 3.2 : Results of selenium concentration in "ppm" in some Sudanese fruits soils (depth 75 cm) by various techniques

Soil depth of the plants↓ techniques →	HG AAS	GF AAS	ICP MS/MS	ICP OES	SEM + EDX	EDXRF	WDXRF
Ziziphus spina Christi (soil depth at 75 cm)	0.183	0.165	0.075	0.070	0.1605	0.165	Nd
Adansonia digitata (soil depth at 75 cm)	0.163	0.153	0.055	0.050	0.1497	0.150	Nd
Balanites eygptiaca (soil depth at 75 cm)	0.173	0.161	0.069	0.062	0.1580	0.165	Nd
Grewia tenax (soil depth at 75 cm)	0.205	0.201	0.088	0.085	0.1883	0.202	Nd
Tamarindus indica (soil depth at 75 cm)	0.200	0.180	0.076	0.073	0.1840	0.191	Nd

Table 3.3 : Results of selenium concentration in "ppm" in some Sudanese fruits soils (depth 100 cm) by various techniques

Soil depth of the plants ↓ / techniques →	HG AAS	GF AAS	ICP MS/MS	ICP OES	SEM + EDX	EDXRF	WDXRF
Ziziphus spina Christi (soil depth at 100 cm)	0.174	0.162	0.070	0.065	0.1629	0.162	Nd
Adansonia digitata (soil depth at 100 cm)	0.184	0.165	0.079	0.070	0.1797	0.182	Nd
Balanites eygptiaca (soil depth at 100 cm)	0.169	0.160	0.065	0.060	0.1610	0.162	Nd
Grewia tenax (soil depth at 100 cm)	0.200	0.188	0.084	0.082	0.1810	0.191	Nd
Tamarindus indica (soil depth at 100 cm)	0.191	0.170	0.080	0.075	0.1806	0.187	Nd

Table 3.4 : Results of selenium concentration in ppm in some Sudanese fruits soils (depth 150 cm) by various techniques

Soil depth of the plants ↓ / techniques →	HG AAS	GF AAS	ICP MS-MS	ICP OES	SEM + EDX	EDX RF	WDX RF
Ziziphus spina Christi (soil depth at 150 cm)	0.192	0.172	0.072	0.069	0.1708	0.170	Nd
Adansonia digitata (soil depth at 150 cm)	0.195	0.177	0.082	0.079	0.1793	0.190	Nd
Balanites eygptiaca (soil depth at 150 cm)	0.194	0.175	0.073	0.071	0.1763	0.189	Nd
Grewia tenax (soil depth at 150 cm)	0.197	0.183	0.083	0.080	0.1830	0.195	Nd
Tamarindus indica (soil depth at 150 cm)	0.167	0.155	0.060	0.055	0.1650	0.161	Nd

Table 3.5 : Results of selenium concentration in ppm in water sample from (*Hafeer*) by various techniques

Name of sample ↓ / techniques →	HG AAS	GFAAS	ICPMS/MS	ICP OES
Hafeer water	0.000644	0.000601	0.000498	0.000480

All samples analyzed by the technique wavelength dispersive x-rays fluorescent (WEDXRF), procedure, however did not detect selenium. This might be due to the preparation method of the samples that adopted the dry ashing at aburning temperature of 450 ^{0}C. At this high temperature all selenium was volatilized.It became evident that selenium could not be detected when the dry a shing was used for preparation of the samples. concentrations in Sudanese nuts and soil determined by HGAAS, GFAAS, ICP-MS/MS, ICP-OES, SEM-EDS and EDXRF are compared in Figure 3.1and 3.2,respectively.

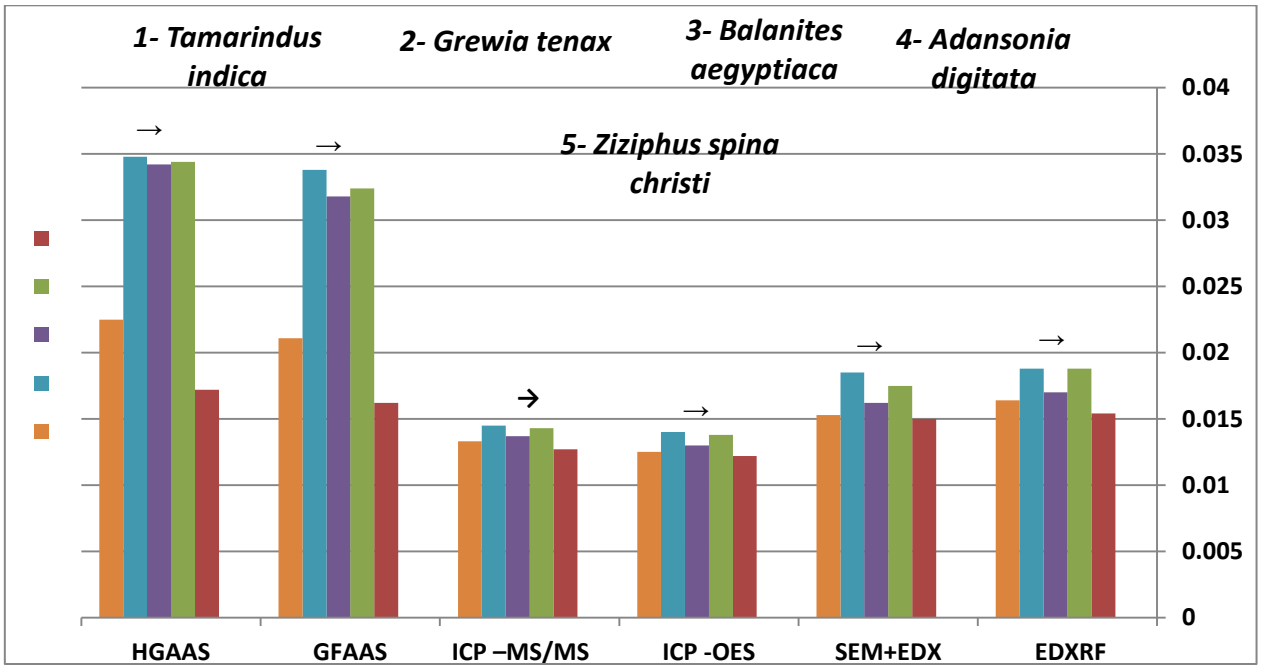

Fig 3.1 : Comparison between Se concentrations in the 5 Sudanese fruits measured, by using the 6 techniques

Figure 3.1 shows that the concentration of selenium in all plants studied increase in this order: *Grewia tenax* (compared to) > *Adansonia digitata* > *Balanites egyptiacea* >*Tamar indusindica* > *ziziphus spina christi* , this might be attributed to the type of the plant , the mechanism of absorption of selenium and the type of the soil in which the plant grew. Figure 3.1 shows that HGAAS technique was more sensitive than the other techniques because all selenium concentration in the sample were reduced to the hydride SeH_2 gas before it was atomized in the flame to give selenium atoms. The figure also shows the sensitivity of the techniques increased in this order: (GHAAS) > (GFAAS) >

(EDXRF) > (SEM + EDS) > (ICP MS/MS) > (ICP OES). In contrast to the hypothesis that ICP techniqual is most sensitive than other techniques for the determination of Se in the plant and soil samples, that obtained in our findings was better than that expected for ICP.

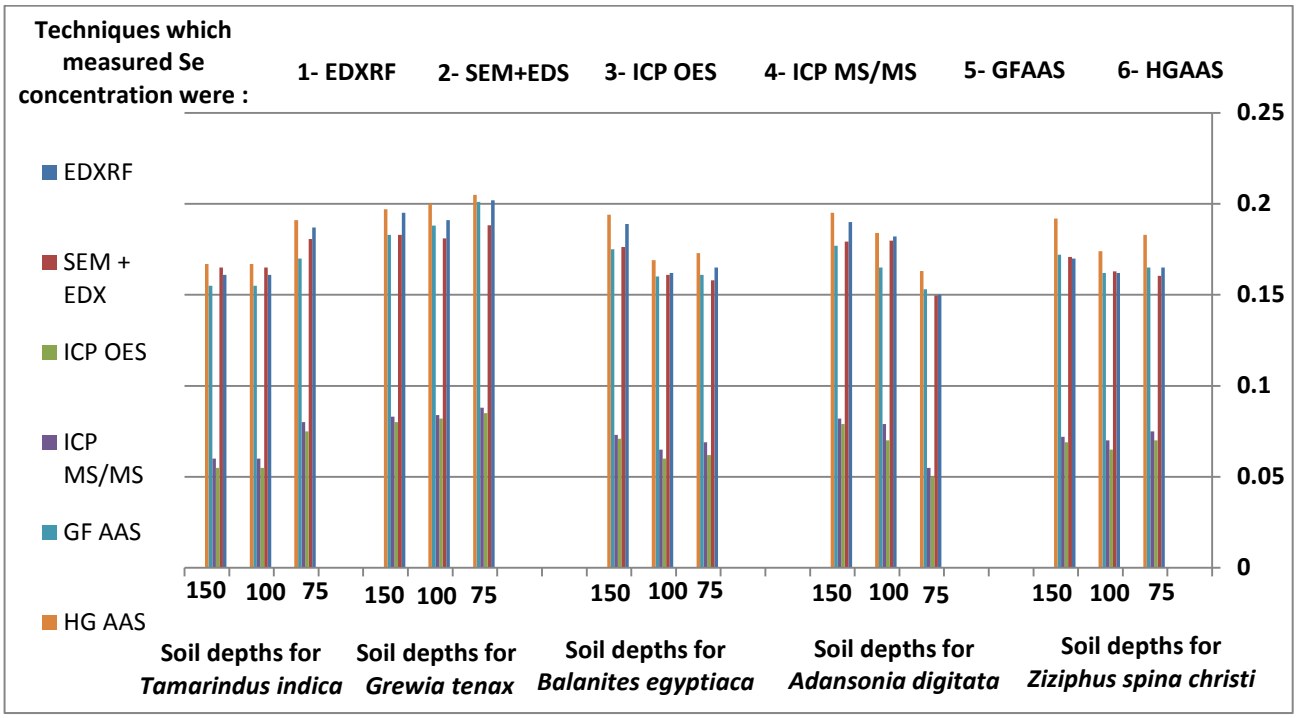

Fig 3.2: Comparison between Se concentration in soil depths for the 5 Sudanese fruits, measured by using the 6 techniques

Figure 3.2 shows that soil depth 150 cm contains high concentration of Se (compare to) > depth 100 cm > depth 75 cm for *ziziphus spina christi* and *Adansonia digitata* fruits. The order of the concentration of Se might be attributed to the type of the soil and type of the plant. Also it was found that the increase of Se at the different depths of the soil for *Balanites egyptiaca* plant followed the following order ;the depth 150 cm > depth 75 cm > depth100 cm . The increase of the concentration of Se in the soil for *Grewia tenax* and *Tamerindus indica* plants followed the following order:depth 75 cm > depth 100 cm > 150 cm.This is also might be attributed both to type of soil and plant. In general; the increase of the concentration of Se in the soil for all plants followed the following order: depth 150 cm > depth 100 cm > 75cm.This order

strengthen the hypothesis that concentration of selenium in surface layers of soil might be less than in deep layers. This figure shows the order of the increase of the concentration of Se in soils for *Grewia tenax* soils > *Tamarindus indica* soils > *ziziphus spina christi* soils > *Balanites* egyptiaca soils, > *Adansonia* digitata soils.This order ensures the hypothesis that variations could occur in soils lying only a few meters by each other.

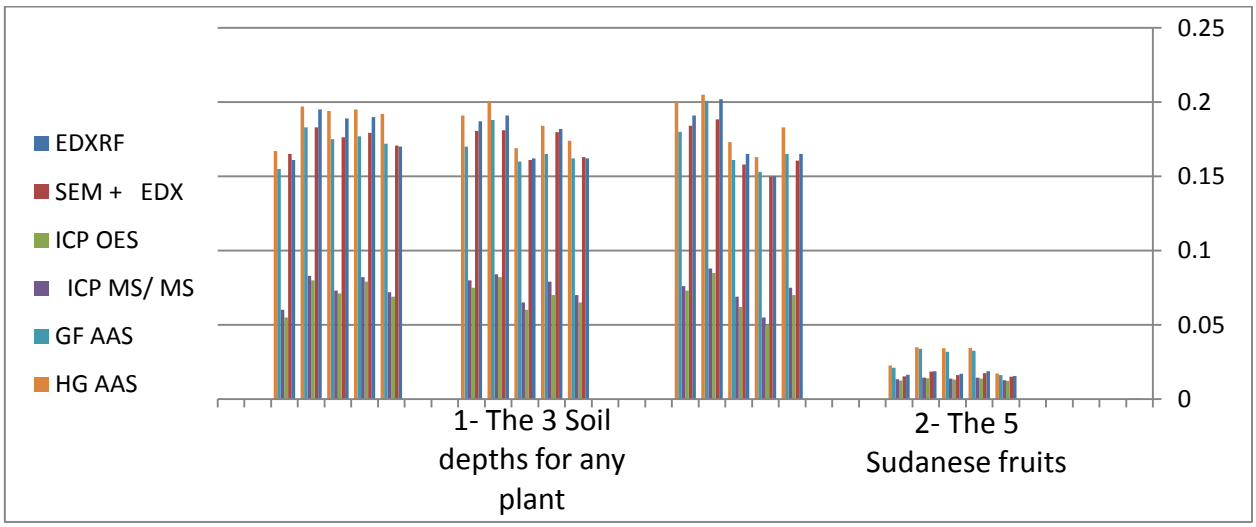

Fig 3.3: Comparison between Se concentration in soil depths samples and the 5 Sudanese fruits, measured by using the 6 techniques

Figure 3.3 shows that values of Se concentration in plants fruits were less than in soil depths. These findings are an agreement with the hypothesis that selenium concentration in soil is higher than that in the plants.

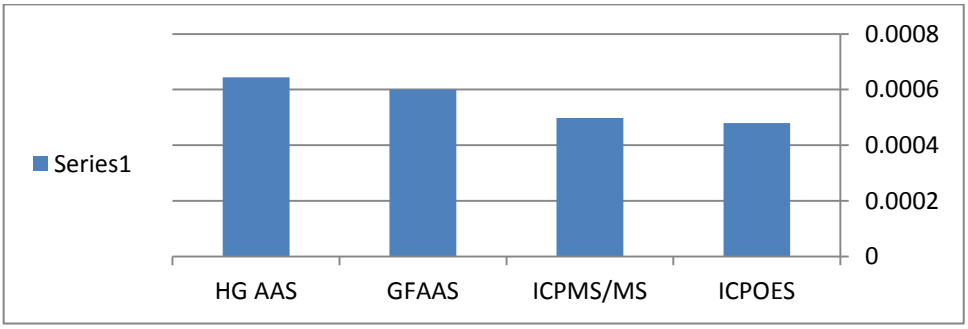

Fig 3.4 : Comparison between Se concentration in water sample measured by using the 4 techniques

Figure 3.4 indicates that HGAAS was more sensitive > GFAAS > ICPMS/MS > ICPOES. It was found that selenium levels in water from (*Hafeer*) in the the study area, in the absence of contamination from industry or other sources, were

very low obtained by all techniques. These results are also in an agreement of the hypothesis that Se concentration in water is less than in the plants.

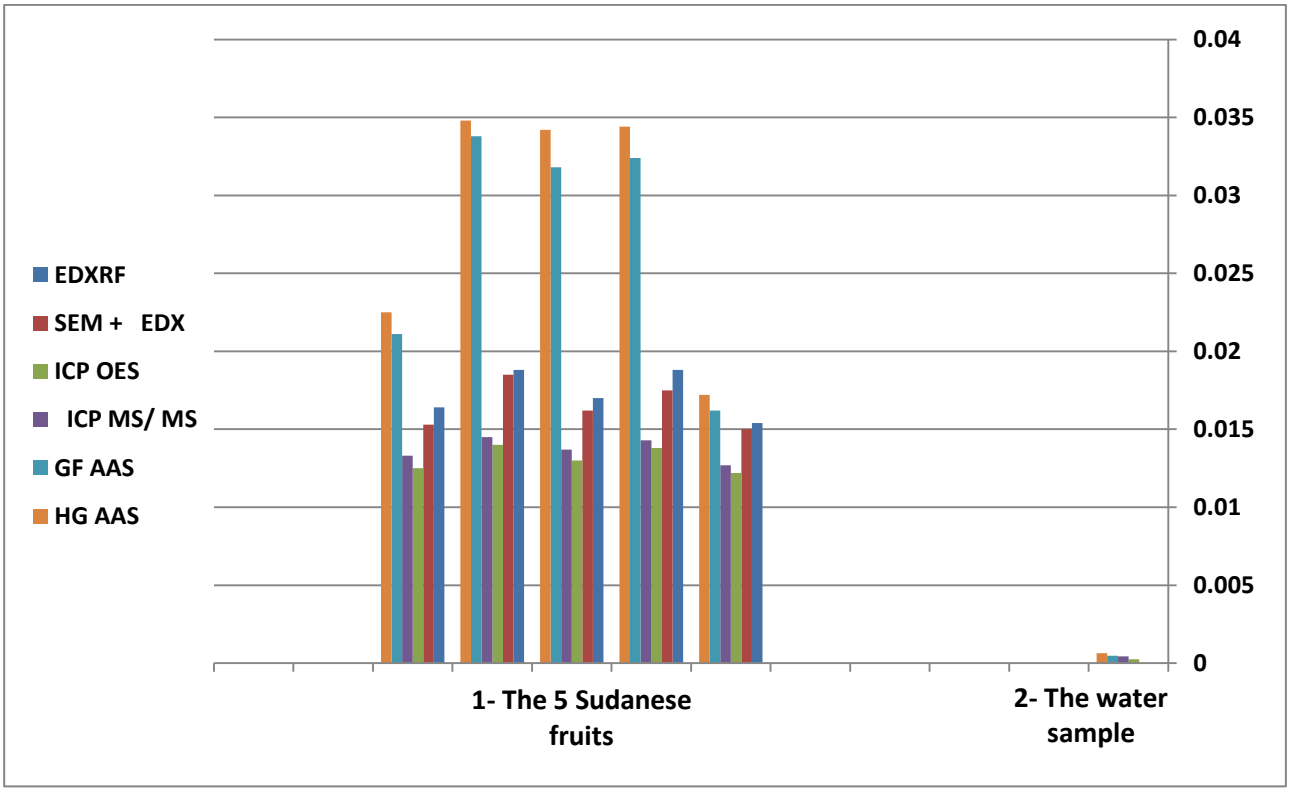

Fig 3.5: Comparison between Se concentration in the 5 Sudanese fruits and the water sample measured by using the 6 techniques

Figure 3.5 shows that selenium concentration in water was less than that in plants fruits.It was found that Se concentration, measured by all techniques, in fruits, soil and water increased in the order of soil >,fruits > water. The determination of selenium in these variations in the results obtained for nuts, soil and water samples applying different spectroscopic techniques (HGAAS, GFAAS,ICP MS/MS ,SEM+EDS,EDXRF,WEDXRF) were in agreement with those expected by the hypothesis.However, the relatively high levels obtained, were in contrast with that assuming low levels.

3.2 Conclusion

- Sudan, like most African countries, is endowed with a range of diverse climatic conditions that favor the establishment of many plants species, most of which are adapted to specific ecological zones.
- A wide range of factors are worth considering when evaluating the suitability of a technique for a particular environmental application; therefore determination of environmental samples such as soil, plants and water is difficult owing to the unavailability of certified standards, researches, instruments especially in Sudan.
- Selenium (Se) is an essential micronutrient that can be deficient in the diet as Se-poor soils yield Se-poor food crops. Accurate quantification of Se in food is necessary to assess nutrient status.
- The determination of selenium is of considerable interest because it would appear to be an essential trace element but it is also toxic at relatively low levels. Recent developments in Se work emanated mainly from its implications to animal and human health, and for this reason, procedures have been developed mainly to suit animal science purposes.
- The significance of knowing the level of selenium in environmental samples was reflected in the number of devised methods of its determination, ensuring the possibility of optimization for particular cases.
- HGAAS gave high concentration of selenium with high efficiency because it depended on a chemical reduction using sodium borotetrahydride to reduce and separate selenium from the digested samples. Unlike other applied analytical techniques, it was free from interferances common technique therefore has the advantage of being able to isolate selenium from complex samples. Therefore, for this cause the HGAAS was accepted as standard method for determination of selenium in plants, soil and water.

- Better sensitivity had been obtained also by using (GFAAS) because this method avoided the problems associated with wet digestion by employing high temperature oxidation in graphite furnace (the suitable temperature 2600 C^0), graphite furnace AAS (GFAA) replaces the flame with an electrically heated graphite furnace.
- EDXRF was, however, a non-destructive sample analysis with good analytical performance, offered excellent signal-to- noise ratio and peak separation leading to very low background noise and high resolution and made satisfactory results in selenium concentration for samples under study.
- (SEM + EDS) gave better sensitivity using Gold coating, provided better resolution, was more frequently used in SEM studies and made satisfactory results in selenium concentration for samples under study.
- ICP MS/MS and ICP OES techniques suffered from various interference effects. The severity of these effects caused a big variation on the results of the samples under study.

3.3 Recommendations

1. Initially, attention must be given to the adequacy of the methods used to analyze selenium in Sudanese fruits , their soils and water
2. Greater attention should be given to the improvement of continuous complementary research in the future. Further detailed information regarding appropriate sample selection for evaluation of environmental toxicity of Se is presented.
3. The sample collected must be representative of the material being studied, and must be protected from either contamination or loss of selenium during analysis. Equally important is the proper collection and treatment of samples before analysis.
4. It should be recognized that soil sampling to a depth of 1 m is more meaningful than shallower sampling, especially where soil-selenium levels are excessively high.
5. Further research is needed to study the on correlation between the level of selenium intake and the incidence of cancer..
6. Two or more analytical techniques must be interfaced to enhance analytical capabilities. In several of these, high-performance liquid chromatography (HPLC) is hyphenated to ICPMS, Also HG is hyphenated to ICPOES, these are powerful analytical tools.
7. Amodel must be prescripted to manage deficiency of Se in body of the human being.

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Appendix (1): Comparison of results obtained by all techniques for selenium concentration in Sudanese fruits with results obtained by the same techniques in various global fruits and crops, which agree and which dis-agree as below:

Appendix (1.a) : by HGAAS

Name of the fruit	**The country**	**Se concentration(ppm)**	**Technique**	**Reference**	**Which agree and which disagree**
(Zizphus spina Christi) *(Adansonia digitate)* *(Balanites eygptiaca)* *(Grewia tenax)* *(Tamarindus indica)*	Sudan	0.0172 0.0344 0.0342 0.0348 0.0225	HGAAS	The author	-
(Peanut)	USA	0.03	HGAAS	William et al., 2009	Agree
(Brazilnuts)	Brazil	38	HGAAS	Manjusha 2007	Disagree
(Brazilnuts)	Peru	1.26	HGAAS	Thavarith 2004	Disagree
(Brazilnuts)	Amazon region	0.02	HGAAS	Junior 2017	agree
(Brazilnuts) (Almond)	Brazil Brazil	0.101 0.26	HGAAS	Daniela 2015	Disagree
(Brazilnuts)	Poland	0.2 - 512	HGAAS	Pilarczyk 2010	Disagree
(Coconut)	Saudi-Arabia	0.093	HGAAS	Al-Ahmary 2009	Disagree

The reason of disagree, may be due to the type of soil which plant grows or type of plant or other factors

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Appendix (1.b): by GFAAS

Name of the fruit	The country	Se concentration (ppm)	Technique	Reference	Which agree and which disagree
(Zizphus spina Christi) *(Adansonia digitate)* *(Balanites eygptiaca)* *(Grewia tenax)* *(Tamarindus indica)*	Sudan	0.0162 0.0324 0.0318 0.0338 0.0211	GFAAS	The author	-
Peanut) (Soybean) (Chestnuts)	Korea	0.146 0.008 0.002	GFAAS	Choi 2009	Disagree
(Peanut)	USA	0.075	GFAAS	Junior 2017	agree
(Peanut) (Brazilnuts) (Almond)	Brazil	2.51 36.1 0.37	GFAAS	Egea 2017	Disagree
(Groundnut:vigna subterranean) (Groundnut:arachis hypogea)	Nigeria (in mining area) Nigeria (in mining area)	0.22 0.11	GFAAS	Aremu 2010	Disagree
(Groundnut:vigna subterranean) (Groundnut:arachis hypogea)	Nigeria (in non -mining area) Nigeria (in non- mining area)	0.21 0.07	GFAAS	Aremu 2010	Disagree Agree
(Peanut) (Peanut) (Peanut) (Peanut)	UK Australia Thailand New Zealand	0.030 0.140 0.032– 0.186 0.046– 0.150	GFAAS	Cashman 2001	Agree Disagree Agree Agree

The reason of disagree may be due to the type of soil which the plant grews or due to the type of plant or due to other factors

Appendix (1.c): by ICPMS/MS

Name of the fruit	The country	Se concentration (ppm)	Technique	Reference	Which agree and which disagree
(Zizphus spina christi) *(Adansonia digitata)* *(Balanites eygptiaca)* *(Grewia tenax)* *(Tamarindus indica*	Sudan	0.0127 0.0143 0.0137 0.0145 0.0133	ICP MS/MS	The author	-
Grass and old trees	Egypt (Nuclear Research Center (NRC) and different locations	0.002- 0.88	ICP MS/MS	Abd - alateef 2004	agree
Tamarindus indica	Pretoria	0.013	ICP MS/MS	Van, 2000	agree

The reason of disagree may be due to the type of soil which the plant grows or due to the type of plant or due to other factors.

Appendix (1.d) : by ICP OES

Name ofthe fruit	The country	Se concentration (ppm)	Technique	Reference	Which agree and which disagree
Zizphus spina Chrisit *(Adansonia digitata)* *(Balanites eygptiaca)* *Grewia tenax)* *(Tamarindus indica*	Sudan	0.0122 0.0138 0.0130 0.0140 0.0125	ICP OES	The author	-
(Cashew) (Pistachio)	USA	0.052 0.070	ICP OES	Motsara 2008	Disagree
(Peanut) (Soybean)	Belgium	0.32 0.40	ICP OES	Jean 2012	Disagree
(Pistachio)	Iran	0.40	ICP OES	Nazemi. 2010	Disagree

(cashew nuts) (coconut) (Macademia nuts) (Hazelnut)	UK	0.17 - 0.39 0.049 - 0.08 0.034-0.087 0.008-0.036	ICP OES	Dolph et al .(2012)	disagree disagree agree agree
(Zizphus spina christi) *Adansonia digitata* *(Balanites eygptiaca)* *Grewia tenax* *(Tamarindus indica)*	Sudan	0.0114 0.06 0.04 0.05 0.02	HG -ICP OES	Ebrahim et al 2012	Agree Disagree Disagree Disagree agree

The reason of disagree may be due to the type of soil which the plant grows or due to the type of plant or due to hyphenated technique or other factors

Appendix (1.e): by EDXRF

Name of the fruit	The country	Se concentration (ppm)	Technique	Reference	Which agree and which disagree
(Zizphus spina christi) *Adansonia diigitate* *(Balanites eygptiaca)* *(Grewia tenax)* *(Tamarindus indica)*	Sudan	0.0150 0.0188 0.0170 0.0188 0.0164	EDXRF	The author	-
(Hay powder)	Belgium	0.034	EDXRF	Jean . (2013)	Disagree
indian medicinal plants(Leaves , Ariel and Flower) NIST(1515)((Apple leaves)	India	1.31 – 0.10 0.05	EDXRF	Giridhar . (2015)	Disagree

The reason of disagree may be due to the type of soil which the plant grows or due to the type of plant or due to other factors.

Appendix 1.1: Comparison of results for Se concentration in Sudanese fruits with international standards values in different types of fruits

Name of the fruit	The country	Se concentration (ppm)	Technique	Reference	Which agree and which disagree
(Zizphus spina Christi) *(Adansonia digitate)* *(Balanites eygptiaca)* *(Grewia tenax)* *(Tamarindus indica)*	Sudan	0.0172 0.0344 0.0342 0.0348 0.0225	HGAAS	The author	-
(Zizphus spina christi) *(Adansonia digitate)* *(Balanites eygptiaca)* *(Grewia tenax)* *(Tamarinduindica*	Sudan	0.0162 0.0324 0.0318 0.0338 0.0211	GFAAS	The author	-
(Zizphus spina Christi) *(Adansonia digitata)* *(Balanites eygptiaca)* *Grewia tenax)* *(Tamarindu indic*	Sudan	0.0122 0.0138 0.0130 0.0140 0.0125	ICP OES	The author	-
Zizphus spina christi *(Adansonia digitata)* *(Balanites eygptiaca)* *Grewia tenax* *(Tamarindusindica)*	Sudan	0.0127 0.0143 0.0137 0.0145 0.0133	ICP MS/MS	The author	-
(Zizphus spina christi) *(Adansonia digitate)* *(Balanites eygptiaca)* *Grewia tenax)* *(Tamarindusindica)*	Sudan	0.0154 0.0188 0.0170 0.0188 0.0164	EDXRF	The author	-
(Zizphus spina christi) *(Adansonia diigitate)* *(Balanites eygptiaca)* *(Grewia tenax)* *(Tamarindus indica*	Sudan	0.0150 0.0175 0.0162 0.0185 0.0153	SEM+EDX	The auther	-
cereals and grains	-	< 0.1-> 0.8		WHO 2007	agree

Hay powder	The world	0.022		International Atomic Energy Agency recommended 2000	Agrree

The reason of disagree may be due to the type of soil which the plant grows or due to the type of plant or due to other factors

Appendix 2 : Comparison of results obtained by various techniques for selenium concentration in soil of Sudanese fruits with results obtained by the same techniques in various global soil ,which agree and which disagree

Appendix (2 - a): by HGAAS technique:

Type of the soil	The country	Se concentration (ppm)	Technique	Reference	Which agree and which disagree
Sudanese nuts soil(Clay,gardod eand and precipitated soil)	Sudan (Alaien forest)	0.163– 0.205	HGAAS	The author	-
	Canada	0.218	HGAAS	Bahers 2012	Agree
Yellow soil	China	0.31 – 7.65	HGAAS	Kun. 2015	Disagree
Clay surface soil and sandy soil	Libya	0.1 – 5	HGAAS	Busheina 2016	Some agree
Igneous and sedimentary soil Certain shales	Turkey	0.05 – 0.08 Up to 0.6	HGAAS	Nicholas 2009	Disagree
	Romania	o.o20 – 0.100	HGAAS	Broadley 2006	Disagree
	India	0.207 – 0.552	HGAAS	Sanjiv 2005	Some agree
	China	4.11 – 33.1	HGAAS	Wang 2012	Disagree
	China	0.21 – 4.08	HGAAS	Huang 2009	Some agree

Dark clay shale with red concentration Sandy shale Gray – brown clay Botten- gray shale	California	0.2 0.6 0.1 0.4	HGAAS	Gerald and combs (2009)	Agree Disagree

The reason of disagree may be due to the type of the soil or other factors.

Appendix (2 - b): by GFAAS technique:

Type of the soil	The country	Se concentration(ppm)	Technique	Reference	Which agree and which disagree
Sudanese nuts soil (Clay ,gardodean d and precipitated soil)	Sudan (Alein forest)	0.153– 0.201	GFAAS	The author	-
Soil in mining area	Nigeria	1.07 – 1.19	GFAAS	Aremu 2010	Disagree
	China	2.2 – 22.2	GFAAS	Han 2013	Disagree
	India	0.13– 2.85	GFAAS	Sharma 2014	Some agree
	Australia Finland	0.11 – 0.41 0.21	GFAAS	Beld 2007	Some agree Agree
	Hong-Kong	0.76	GFAAS	Shataheri 2006	Disagree
	India	6.8 – 13.1	GFAAS	Eiche 2015	Disagree

The reason of disagree may be due to the type of the soil or other factors.

Appendix (2- c): by ICPMS/MS technique

Type of the soil	The country	Se concentration (ppm)	Technique	Reference	Which agree and which disagree
Sudanese nuts soil (Clay , gardode and precipitated soil)	Sudan (Alaien forest)	0.055– 0.088	ICP MS/MS	The author	-
Agricultural soil Mud soil and sand soil	Egypt	0.02 – 2.5 0.020 – 0.032	ICP MS/MS	Abd-alatif 2004	Some agree Agree
Top soil (alkaline)	Jordan	1.6 – 17.5	ICP MS/MS	Asma 2017	Disagree
Topsoil	China	0.00014– 0.002	ICP MS/MS	Huang 2009	Disagree
Lower river basin soil	Nigeria	0.0001 – 0.0097	ICP MS/MS	Ahola et.al 2017	Disagree

The reason of disagree may be due to the type of the soil or other factors.

Appendix (2 - d): by ICP OES technique

Type of the soil	The country	Se concentration(ppm)	Technique	Reference	Which agree and which disagree
Sudanese nuts soil ,(Clay ,gardode and precipitated soil)	Sudan (Alaien forest)	0.050– 0.085	ICP OES	The author	-
	Iran	0.04 - 0.4	ICP OES	Nazemi 2010	Some agree
	U.k	0.1 - 4	ICP OES	Dolph 2012	disagree
	Norway	0.2 – 1.4	ICP OES	Wu 2005	Some agree
	China	0.32 – 0.37	ICP OES	Zhang 2005	disagree
	Georgia	0.01 – 2.0	ICP OES	Uttam. 2017	Some agree
	India	0.207 - 0.552	ICP OES	Sanjiv 2005	disagree

The reason of disagree may be due to the type of the soil or other factors

Appendix (2 - e): by EDXRF technique

Type of the soil	The country	Se concentration (ppm)	Techniqu e	Reference	Which agree and which disagree
precipitat ed soil)	Sudan (Alaien forest)	0.150 – 0.202	EDXRF	The author	-
	china	0.07 – 0.17	EDXRF	Biao 2009	Agree
	India	0.24 – 0.55	EDXRF	Yadav 2005	Some agree
Agricult ural soil Grass- land soil	Germany	0.123 0.158	EDXRF	Baheres 2012	Semi – agree Agree
Low Se area High Se area	China	0.059 – 0.190 0.39 – 10.66	EDXRF	Alland 2011	Some agree

The reason of disagree may be due to the type of the soil or other factors

Appendix (2.1): Comparison of results of Se concentration obtained by various techniques in Sudanese fruits soils with international standards values of different types of soils.

Type of the soil	The country	Se concentration(ppm)	Techniq ue	Referenc e	Which agree and which dis-agree	The reason
Sudane se fruits soil(Clay , gardode and precipit ated soil)	Sudan (Alaien forest)	0.163 – 0.205 0.153 – 0.201 0.055 – 0.088 0.050 – 0.085 0.1497 – 0.1903 0.150 – 0.202	HGAA S GFAA S ICP MS/MS ICP OES SEM+E DS EDXRF	The auther	-	-
Clay soil Sandsto ne Limesto ne	The world	0.4 0.050 – 0.080 0.030 – 0.100		W H O standards Kabata and Pendias (2001)	Disagree by all instruments Disagree by all instruments except ICP MS/MS, ICP OES (agree) Disagree by all	May be due to the type of the soil or other factors May be due to the type of the soil or other factors Also this method does not give accurate result with

Normal rocks		0.1 – 0.4			instruments except ICP MS/MS, ICP OES (agree)	regard to Selenium concentration
Dry rocks		0.01 – 0.2			agree by all instruments except ICP MS/MS, ICP OES (disagree)	May be due to the type of the soil or other factors
Seleniferrous		40.0 – 80.0			Agree by all instruments disagree by all instruments	
	The world	<0.05 (deficiency)		W H O standards and classification (2007)	Not deficient by all instruments	-
	The world	0.175 – 0.40(moderate values)			moderate by all instruments except ICP MS/MS, ICP OES	-
		<0.15 (low values)		EPA standard Theodore (2005)	Not low values by all instruments except ICP MS/MS, ICP OES(low values	this method does not give accurate result with regard to Selenium concentration

		0.5 (toxic) >3 (excessive) >5 (seleniferrous) < 3.2 (hazardous)			Not toxic by all instruments Not excessive by all instruments Not seleniferrous by all instruments Not hazard by all instruments	
MRG-1 silicated rocks	Canada	0.194		(Burean certified reference) Canada center for minerals and energy technology (Yash 2002)	agree by all instruments except ICP MS/MS, ICP OES (disagree)	May be due to the type of the soil or other factors Also this method does not give accurate result with regard to Selenium concentration
Most non-contaminated of the world soil	The world	0.01 – 2		Fordyce (2005)	agree by all instruments	

Appendix (3): Comparison of results obtained for selenium concentration by various techniques in water of Al-hafeer with results obtained by the same techniques in various global water, which agree and which disagree:

Appendix (3- a): by HGAAS technique

Name of the source of water	The country	Se concentration (ppm)	Techniq ue	Reference	Which agree and which dis-agree
Hafeer	Sudan(Alei n forest)	0.000644	HGAAS	The auther	-
Tap water	U.S.A	0.050	HGAAS	Auron 2012	Dis-agree
Tap water	Libya	0.001- 0.005	HGAAS	Busheina 2016	Some agree

The reasons of disagree may be due to the source of water or other factors.

Appendix (3- b): by GFAAS technique:

Name of the source of water	The country	Se concentration (ppm)	Techniqu e	Reference	Which agree and which disagree
Hafeer	Sudan(Alein forest)	0.000601	GFAAS	The auther	-
Tap water	U.S.A(California)	0.050	GFAAS	Elarina 2013	Disagree
Ground water	China	0.00018 –0.000576	GFAAS	Zhang 2005	Agree
Tap water	U.S.A(Los-angelos)	0.001	GFAAS	Jason 2009	Agree

The reasons of disagree may be due to the source of water or other factors.

Appendix (3- c): by ICP MS/MS technique

Hafeer	Sudan(Alei n forest)	0.000498	ICP MS/MS	The auther	-
Pond water	Nigeria	<0.007	ICP MS/MS	Ahola 2017	Agree
Underground	Egypt	3.175 – 0.020	ICP MS/MS		Disagree
Sea water Underground	Belgium	40 - 120 0.00012	ICP MS/MS	Bisson 2012	Disagree Disagree
Tap water	U.S.A(Los-angelos)	0.00001	ICP MS/MS	Jason 2009	Disagree

Appendix (3- d): by ICP OES technique

Type of the water	Reference	Technique	Se concentration(ppm)	The country	Which agree and which dis-agree
Hafeer	Sudan(Ale in forest)	0.000480	ICP OES	The auther	-
Tap water	Greece	0.00006	ICP OES	Pappa 2006	Disagree
Fresh water Irrigation water	Iran	0.01 – 0.0004 <0.01	ICP OES	Lyly 2012	Agree Agree
Tap water Ground water	France	0.010 0.002 – 0.0405	ICP OES	Echlin 2013	Disagree
Sea water	India	<0.6	HG-ICP OES	Ranal 2016	Disagree May be due to the hyphenated technique or other factors

Appendix (3.1): Comparison oresults for Se concentration obtained by various techniques in water of Al-hafeer with results of international standards of different types of water:

Hafeer		0.000644 0.000601 0.000498 0.000480	HG AAS GFAAS ICPMS/MS ICPOES	The author	-
Domestic water	The world	<0.01		W H O standards Kabaita and Pendias (2001)	Agree
Domestic water	The world	0.01		European standards Theodore (2005)	Disagree
Domestic water Well water	The world	0.05 0.0041		USEPA Theodore (2005)	Disagree
Tap water	The world	0.0127		NIST- 1643c WHO 2011	Disagree

The reasons of disagree may be due to the source of water or other factors.

A ppendix (4)

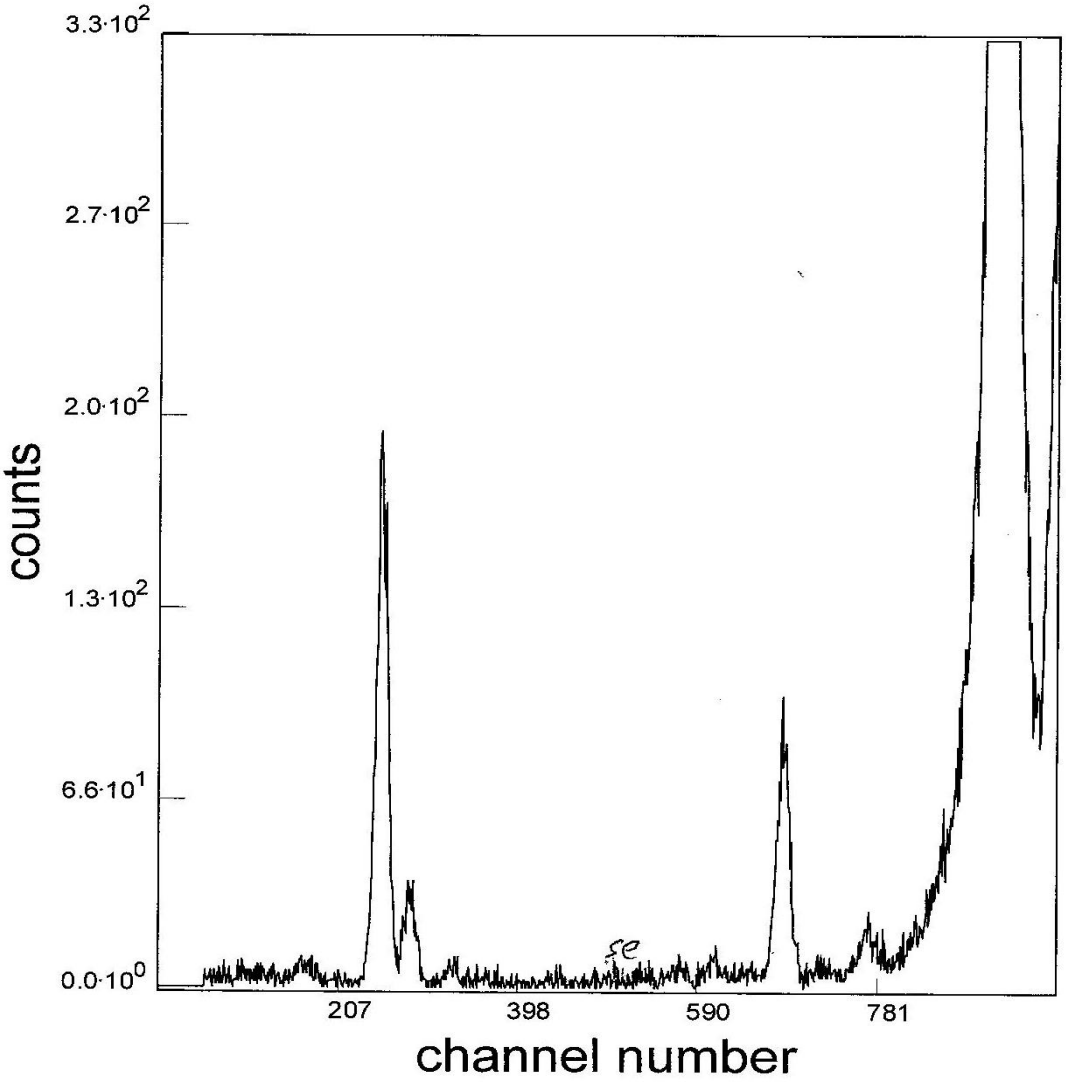
QXas for Windows ... SO-AD100 Sun Jun 10 15:08:02 2018

Measurement date: 5-24-2018 Measurement time: 12h:18m:0s

Live time : 1000 s Real time : 1002 s Dead time : 0.2 %

ZERO = 0.00 eV GAIN = 20000.00 eV/ch

FANO = 0.11 NOISE = 120.00 eV



Appendix (4) : Spectrum of selenium in plant of *Adansonia digitata* soil obtained by EDXRF

A ppendix (5)

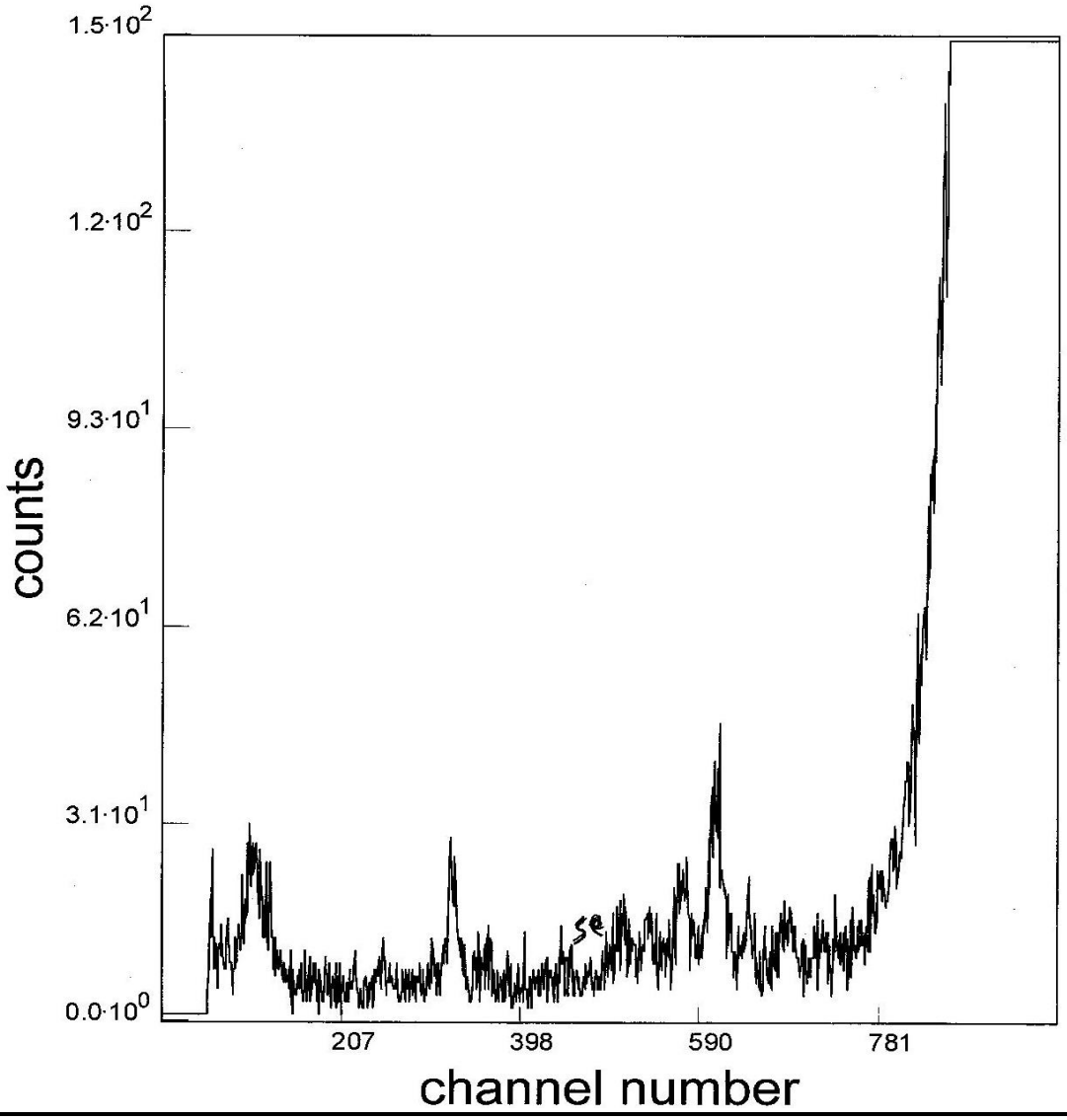
QXas for Windows ... PL-AD Sun Jun 10 15:09:01 2018

Measurement date: 5-28-2018 Measurement time: 10h:35m:0s

Live time : 2000 s Real time : 2005 s Dead time : 0.2 %

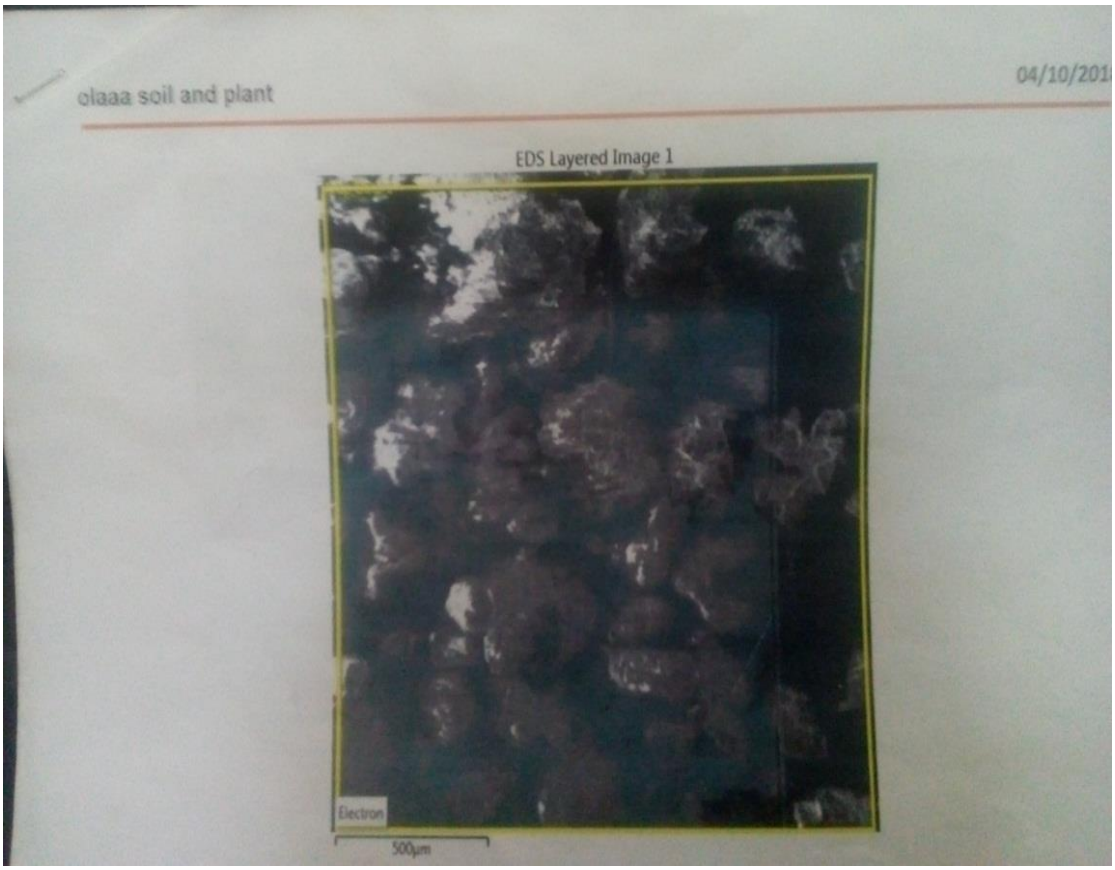
ZERO = 0.00 eV GAIN = 20000.00 eV/ch

FANO = 0.11 NOISE = 120.00 eV



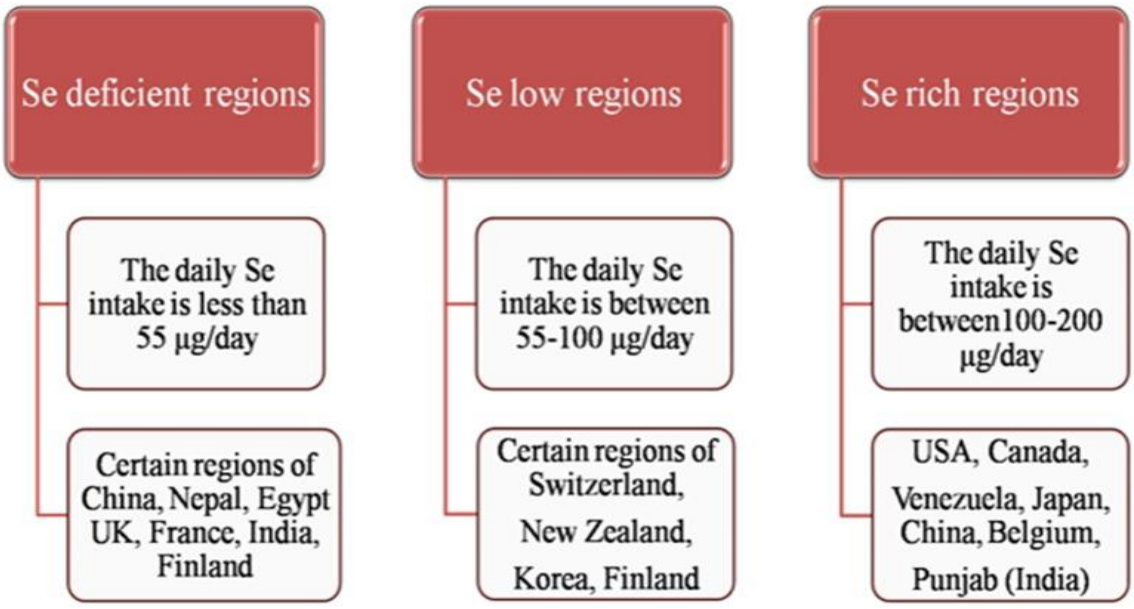
Appendix (5) : Spectrum of selenium in plant of *Adansonia digitata* obtained by EDXRF

Appendix (6) :



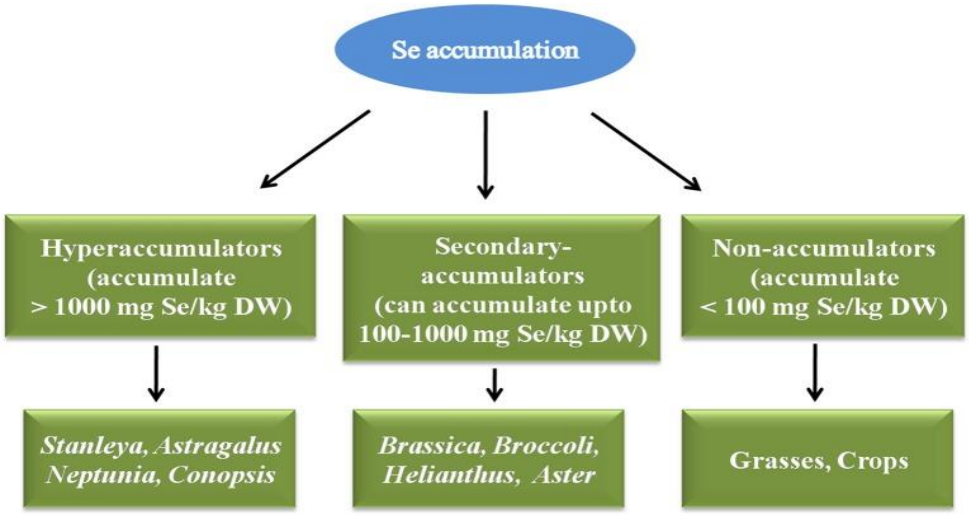
Appendix (6) : Shape of selenium in *Grewia tenax*(godaim) by SEM +EDS

Appendix (7) :



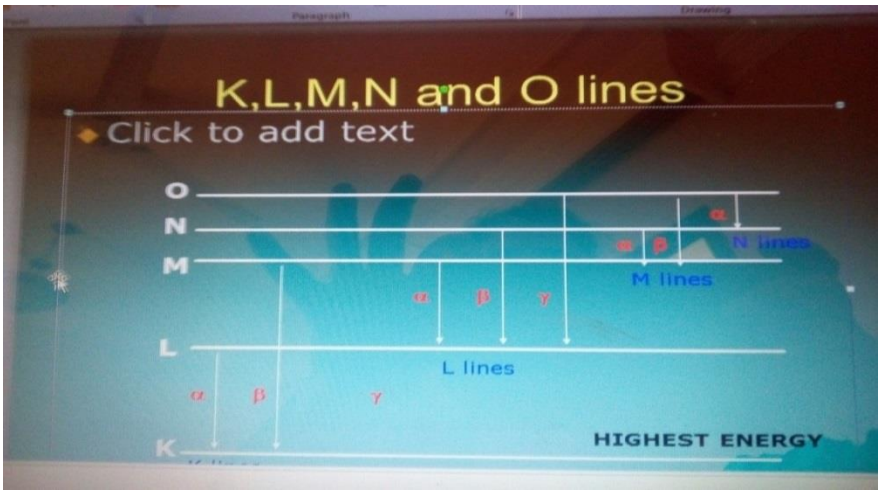
Appendix (7) : Selenium daily intake according to Se status in various regions of the world

Appendix (8):



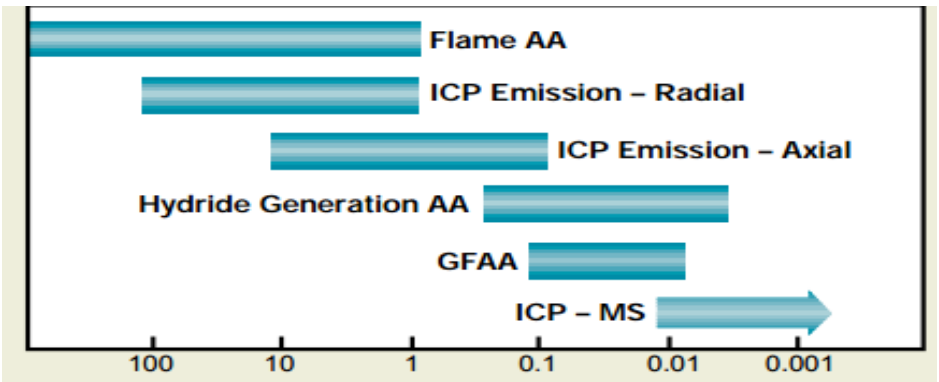
Appendix (8) : Selenium accumulation

Appendix (9):



Appendix (9) : K,L,M.N and O electronic energy lines

Appendix (10):



Appendix (10):Typical detection of atomic absorbtion and emission limit ranges, µg/l

Appendix (11):

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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).

Appendix (11) : paper published from the thesis (comparative study on two methods for determination of selenium in some Sudanese plants fruits and their soils)