**REPUBLIC OF TURKEY**
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**INSTITUTE OF SCIENCE**

# PHENOLIC COMPOUNDS, ANTIOXIDANT AND HEAVY METALS IN RHUBARB OF IRAQI KURDISH REGION AND SİİRT

**MASTER DEGREE THESIS**

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## THESIS ACCEPTANCE AND APPROVAL

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## THESIS NOTIFICATION

I declare that all the information in this study prepared in accordance with the thesis writing rules is completely cited to the source of all kinds of information and statements which are obtained and provided in the frame of scientific and academic rules and not belong to me.

Bakhtiyar Mahmood FATTAH

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## PREFACE

Metals impose great influence on various aspects of life at all levels of its organization. Several metals are the essential constituents of biomolecules and participate in many important biological processes. However metals can be harmful when present in excess amounts, while a few of them are toxic to biota even at extremely low concentration.

Plant-metal interactions are complex and depend on many factors. The most important being the plant species exposed, its developmental stage and the chemistry of metal including its concentration plant-metal interactions may have beneficial, harmful or will have no effect on the plant, depending on metal species and concentration. Various physiological and biochemical processes in plants are affected by metals. The contemporary physiological, biochemical and molecular investigations on toxicity and tolerance in metal stressed plants are promoted by the growing metal pollution in the environment. Metal toxicity to plants has great impact and relevance not only for plants but also to the ecosystem in which the plants form an integral component. Plants growing in metal polluted locations exhibit altered metabolism, growth reduction. Lower biomass production and metal accumulation and these functions are of human health concern. Edible plants with high doses of accumulated toxic metals are harmful not only to humans but also for the animals when used as animal feed.

Plants provide a rich source of natural antioxidants. These include tocppherols, vitamin C, carotenoids, and phenolic compounds. Plant phenolics are thought to protect the plants against tissue injuries as they oxidize and combine with proteins and other components. In addition, phenolic compounds in plants may serve as defense systems against herbivore. By-products of photosynthesis may also produce high levels of oxygen, free radicals, and reactive oxygen species (ROS) in profusion. Thus, plants use a myriad of antioxidant compounds to deal with these in order to survive. Many of these compounds have basic molecular similarities in that all have at least one aromatic ring and a hydroxyl group. These include phenolic acids, flavonoids and Gallate esters (hydrolysable tannins).

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## LIST OF ABBREVIATIONS

| Abbreviation | Explanation |
|---|---|
| **CP** | : Chinese Pharmacopoeia |
| **RSD** | : Relative Standard Deviation |
| **RNS** | : Reactive nitrogen species |
| **RNA** | : Ribonucleic acid |
| **DNA** | : Deoxyribonucleic acid |
| **DPPH** | : Radical scavenging activity |
| **FRAP** | : Ferric reducing antioxidant power |
| **ICP-MS** | : Inductively coupled plasma mass spectrometry |
| **HPLC** | : High performance liquid chromatography |
| **SGPT** | : Serum enzymes glutamate private transaminase |
| **SGOT** | : Serum enzymes glutamate oxaloacetate transaminase |
| **ALP** | : Alkaline phosphates |
| **CRF** | : Long-lasting renal failure |
| **ARF** | : Serious renal failure |

# ÖZET

## YÜKSEK LİSANS TEZİ

## IRAK KÜRT BÖLGESİ VE SİİRT YAYLA MUZUNUN ANTİOKSİDAN, TOPLAM FENOLİK MİKTARI VE AĞIR METALLERİN BELİRLENMESİ

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Bu çalışmada yayla muzunda bulunan 30 metalin konsantrasyonları (Li, Be, B, Na, Mg, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, La, Ce, Pt, Tl ve Pb) ve örneklerin alındığı yerler ile ilgili çeşitli kemometrik teknikler kullanılarak birleriyle karşılaştırılmıştır. Örnekler Irak Kürt Bölgesinden (Sor, Rash, Karajar, Gara ve Qalandar) ve Siirt, Türkiye'den (Pervari ve Şirvan) toplanmıştır. Numunelerin mikrodalga ile asit çözünürleştirilmesinden sonra, metal analizi ICP-MS (Termo Scientific ICAP Q ICP-MS) cihazı ile gerçekleştirilmiştir. Kekik içinde bulunan elementler ve kekiklerin alındıklar yerlere ana bileşen analizi (PCA) ve küme analizi (CA) gibi sınıflandırma teknikleri uygulanarak sınıflandırma yapılmıştır.

Bunun yanında, Irak Kürt Bölgesi ve Siirt'ten toplanan yayla muzunun metanol özütündeki toplam fenolik madde miktarı, toplam flavonoid miktarı, antioksidan aktivitesi, ve fenolik madde içeriği incelenmiştir.

Toplam fenolik madde miktarı Folin-Ciocalteu metodu ile değerlendirilmiş olup 84,02-387,53 µg gallic acid $mL^{-1}$ ekstrakt aralığında bulunmuştur. Ayrıca toplam flavonoid miktarı alüminyum klorür kalorimetrik yöntemi ile belirlenerek 69,98-935,75 rutin µg $mL^{-1}$ aralığında tespit edilmiştir. Antioksidan aktivitesi FRAP ve DPPH yöntemleri kullanılarak belirlenmiştir. FRAP yönteminde en yüksek antioksidan aktivitesi ($IC_{50}$) 25,18±0,04 µg $mL^{-1}$; DPPH deneyinde en yüksek inhibisyon yüzdesi 88,11 bulunmuştur. Metal şelatlama aktivitesi Rival ve ark. yöntemi ile belirlenip tüm numunelerde % 70 üzeri olduğu görülmüştür. Son olarak fenolik bileşikler LC-MS/MS cihazı ile belirlenmiştir. Bu aşamada toplam 37 fenolik bileşik analiz edilmiş olup bunlardan sadece 26'sı kalitatif ve kantitatif olarak tespit edilmiştir. Malik asit (15,73±0,53 mg $g^{-1}$ ekstrakt) ve Rutin (76,93 ± 0,03 mg $g^{-1}$ ekstrakt) baskın fenolik maddeler olarak gözlemlenmiştir.

Çalışmanın sonuçları bu örneklerin potansiyel biyolojik aktiviteye sahip olduğunu ve önemli doğal antioksidan kaynağı olarak tanıtılabileceğini göstermiştir.

**Anahtar Kelimeler:** Yayla muzu, metaller, fenolik bileşik, antioksidan aktivite

# ABSTRACT

## M.Sc. THESIS

## PHENOLIC COMPOUNDS, ANTIOXIDANT AND HEAVY METALS IN RHUBARB OF IRAQI KURDISH REGION AND SİİRT

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This study aimed to find out the concentration of thirty metals (Li, Be, B, Na, Mg, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, La, Ce, Pt, Tl and Pb), compare the levels of them and subjected to various chemometric analysis between metal contents and place of cultivated Rhubarb for classification purposes. Samples were collected from Iraqi Kurdish Region (Sor, Rash, Karajar, Gara and Qalandar) and Siirt, Turkey (Pervari and Şirvan). After acid digestion of samples by the microwave, metal analysis was carried out with ICP-MS (Thermo Scientific ICAP Q ICP-MS) instrument. The metals and places of cultivated were classified with principal component analysis (PCA) and cluster analysis (CA) as classification techniques.

In addition, total phenolic content, total flavonoid amount, antioxidant activity, and phenolic profiles of methanol extract of Rheum ribes collected from Iraqi Kurdish Region and Siirt were examined.

Total phenolic content was assessed by Folin Ciocalteau's method and varied between 84.02-387.53 µg gallic acid/mL of extract. Furthermore, total flavonoid content was determined by the aluminum chloride colorimetric method and varied 69.98-935.75 rutin µg $mL^{-1}$ extract. The antioxidant potency was determined by using FRAP and DPPH methods. In FRAP assay, the highest antioxidant activity ($IC_{50}$) was found 25.18±0.04 µg $mL^{-1}$ extract. In DPPH method,the maximum percentage inhibition was found 88.11%. Metal chelating activity was estimated by method of Rival et al, 2001.It was observed that all the samples were over than 70%. Finally phenolic compounds profiles also were determined by LC-MS/MS instrument. In this step total of 37 phenolic compounds in extracts were analyzed, but 26 of them were detected as qualitative and quantitatively. Malic acid (15.72±0.53 mg $g^{-1}$ extract) and Rutin (76.93±0.03 mg $g^{-1}$ extract) were identified as the major phenolic compounds.

The results of the study confirm that these samples have potential biological activities and can be introduced as important sources of natural antioxidants.

**Keywords:** Rhubarb, metals, phenolic compound, antioxidant activity

## 1. INTRODUCTION

Rhubarb (yayla muzu) as known in Turkish that used as edible and natural plant (Sabir, 2000). It is considered as a medical plant and used freshly for human consumption. It can be eaten as fruit and at the same time the leaves are toxic, the stalks are used in pies and other foods for their tart flavor. Rhubarb mostly grows in spring season under the snow of the high mountains in the north and center Asia. Its scientific name is (Rheum ribs) which is a genus of about sixteen perennial plants in the family Polygonaceae. The genus includes the vegetable rhubarb (Rheum rhubarb or Rheum hybrid) (Nieboer and Richardson, 1980).

The species have large somewhat triangular shaped leaves with long, freshly petioles. The flowers are small, greenish-white to rose-red, and grouped in large compound leafy inflorescences, with long stem of about 15-40 centimeters. Through its medical advantages, can be used for the treatment of acidity in stomach and address constipation. Additionally, it kills small worms inside intestine and helps the liquidation in human's liver (Chakravaety and Al-Rawi, 1964)

Also they are used as a purgative, anti-inflammatory agent and herbal medicines for the treatment of constipation and cancer (Xiao P, 1984) (Duck JA, 2002). Most of the published chemical investigation of rhubarb have deal mainly with the substances in the root or with the organic acid present in high proportion in the freshly petiole responsible for the medical properties which held in high esteem to early antiquity (Clupepper CW, 1932).

Rheum ribs has been clinically used for at least 2000 years as laxative agent or antibacterial, the extensive photochemical research on rhubarb isolation and identification of about two hundred chemical compounds. It is identified that environmental and genetic factors and their interactions involve the pharmaceutically important secondary metabolites in medical plants (Hartmann, 2007)

HPLC (high performance liquid chromatography) have been commonly used for the separation and determination of rhubarb that based on the content of biochemical compounds in rhubarb, pharmacologist carried out the research on the effect of different factors, for example precipitation, sunlight and temperature (Ohshima, 1986, Kashiwada,1989, Li L, 2010)

Rhubarb contains more nutrients contain vitamins A and C, thiamin, riboflavin, niacin, potassium and phosphorus. 250 ml (1 cup) raw rhubarb contains 20 kilocalories which have high quality fiber (Chipman, 1974)

Rhubarb is one of the most well-known and widely used traditional Chinese medicines for the treatment of constipation, inflammation and cancer. It derives from the roots and rhizomes of Rheum officinal recorded in the Chinese Pharmacopoeia (CP) (Pharmacopoeia of China, 2005).

### 1.1. Phenolic compounds

Phenolic compounds, in plants are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. These compounds posses an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer.

These compounds play an important role in growth and reproduction, providing protection against pathogens. The antioxidant activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings.

The major effective constituents of rhubarb are phenolic compounds, among which sennosides and anthraquinone glycosides were reported as the main purgative components. In addition, glucose gallates (Kashiwada Y, 1988 & Abe I, 2000) naphthalene's (Tsuboi M, 1977) were also isolated from official rhubarbs, showing significant antioxidant and anticancer activities. However, the unofficial rhubarbs contain varieties of stableness' (Kashiwada Y, 1984 & Shikishima Y, 2001). It was reported that stableness can lower sugar and lipid levels in human blood, and could be used to treat hyper lipid, obesity, and diabetes (Matsuda H, 2001). Also, they exhibit antitumor activities (Jang M, 1997 & Lee SH, 2002).

The rhubarb (Rheum rhubarb) is a perennial vegetable species adapted to cold and temperate climate, known and spread least crop in Romania (Ciofu et al., 2004). Through the application of differentiated technology, the content of organic acids, minerals, carbohydrates, proteins and vitamins differs of the cultivar and the harvesting period (Beceanu, 2002).

Botanically speaking, rhubarb is a vegetable because it has both leaf and stem. Fresh rhubarb is generally available across the country from April to October. Hothouse

rhubarb appears from December through March and may be available year-round in some markets. Rhubarb originated in the Himalayas, where its root was an important medicine believed to purge the body of ill humans.

### 1.2. Heavy metals

The term heavy metal refers to any metallic chemical element that has a relatively high density, atomic weights, or atomic numbers and is toxic or poisonous at low concentrations.

Heavy metal pollution is an issue of global importance which, although associated particularly to intensely industrialized areas. In the same time, heavy metals zinc (Zn), copper (Cu), lead (Pb), cadmium(Cd) mercury(Hg) and Arsenic(As) are most commonly detected are the most significant pollutants of the natural environment, by their negative effects on the plants, animals and human health. The level of heavy metals in the agricultural ecosystem depends by soil pH, type of plant, organic matter, technology applied and vegetation plants period.

Heavy metal pollution is an issue of global importance which, although associated particularly to intensely industrialized areas, has become highly typical for farm land as well (Munteanu et al., 2010). Considering at least 90 pollutant metals, zinc (Zn), copper (Cu) and lead (Pb) are most commonly detected (Hura, 2007). In the same time, heavy metals are the most significant pollutants of the natural environment, by their negative effects on the plants, animals and men health (Butnariu et al., 2005). The level of heavy metals in the agricultural ecosystem depends by soil pH, type of plant, organic matter, technology applied and vegetation plants period.

### 1.3. Antioxidant

Antioxidants are substances that may protect our cells against the effects of free radicals molecules produced when your body breaks down food or is exposed to tobacco smoke and radiation. Free radicals may play a role in heart disease, cancer and other diseases.

Antioxidants come up frequently in discussions about good health and preventing diseases. These powerful substances, are mostly come from the fresh fruits and vegetables we eat. One of the primary factors for the development and progression

of many life threatening diseases and disorders like cancer, atherosclerosis, diabetes, hyper lipids, neural degeneration and hepatic toxicity is oxidative stress.

Aqueous and Methanol extracts of the roots of Rhubarb emodin have been indicated to have anticancer and antioxidant potential.

### 1.3.1 Anticancer activity of antioxidant

One of the primary factors for the development and progression of many life threatening diseases and disorders like cancer, atherosclerosis, diabetes, hyper lipids, neural degeneration and hepato toxicity is oxidative stress.

Due to their undesirable side effects, poisonous and DNA damage commercially accessible and frequently utilized antioxidants are frequently over shadowed. Presently, in modern pharmacopoeia around 25% of drugs are originated from plants (phyto medicines) and several others are synthetic analogues built on the prototype compounds separated from plants. Aqueous and Methanol extracts of the roots of R. emodin have been indicated to have anticancer and antioxidant potential (Rajkumar et al., 2010). In Chinese folk medicine, R. emodin is used in the treatment of cancer, ulcer and liver treatments. Anticancer and antioxidant activity are found from the rhizome/root extracts of *R.* emodin as a result of anthraquinone derivatives (Krenn et al, 2003). The emodin, aloe-emodin and chrysophenol have indicated to perform as anti-angiogenic via averting the formation of blood vessel in zebra-fish embryos (He et al, 2009). The rhubarbs anticancer influence is accredited to the aloe-emodin which not merely subdued the spread however also encouraged apoptosis of two human cancer cell lines (Kuo et al, 2002).

## 1.4. Macro elements

Elements contents of the medicinal plants and their ratios should be checked in accordance with health safety measures and it is imperative to screen for their quality control (QC) (Somer, 1983 and Arceusz et al, 2010). In recent years, several authors across the world have reported in many studies, on the importance of elemental constituents of the herbal drugs which enhanced the awareness of trace elements in the plants (Wong et al, 1993; Sharma et al, 2009; Basgel and Erdemoglu, 2006). Macro elements are usually participate in various metabolism. Other elements such as Ca and Mg are essential macro-elements required for bone structure development and necessary

for carbohydrate and protein metabolism (Adebowale et al, 2007 and Bourne, 1985) Calcium and Mg are also required in fairly large amounts to maintain body electrolytes and tissue homeostasis (Kazi et al, 2011)

### 1.5. Trace elements

Trace metals has an important biological activity role in human, animal and plant health. Trace metals content in medicinal plant research conductingis of great interest because of their effect on the biological active compounds in medicinal plants and may cause serious effects on human health. (Djukic-Cosic et al, 2007) trace elements regulate metabolism as enzymatic cofactors that transport substrates across cell membranes they can lead to serious complications when deficient or in excess (Boullata, 2013 ). Some trace elements such as (Chromium, Copper, Manganese, Selenium, Zinc, Cobalt, Boron, Molybdenum, Chlorine, Nickel. But only at low concentrations. They catalyze many biochemical reactions occurring in the organisms involved in the formation of red blood cells, hormones, and vitamins, as well as take part in the processes of photosynthesis and the creation of pigments, respiration, oxidation, and reduction. Are part of the bone and tissues of living organisms, And participate in the functioning of neural systems. But in too high doses these metals can be toxic (Sattar, 2989: Caldas E.D, 2004; Basgel, 2006: Kara D, 2012). Zinc (Zn) plays a significant role as a co-enzyme for carboxyl peptidase, liver alcohol dehydrogenase, and carbonic anhydrase (Ismail et al, 2011 and Scherz, 2006). Copper (Cu) is required for redox enzymes cytochrome oxidase (Soylak et al, 2009 ). Iron (Fe) is also a trace element found in the heme proteins hemoglobin and myoglobin (Fraga, 2009 and Grayson et al, 2012). A relatively small amount of Ni is required to aid in the absorption of Fe in the body. However, high Ni concentrations can interfere with Zn, magnesium (Mg), and calcium (Ca) utilization and metabolism (Lawrence et al, 1989).

### 1.6. Spectroscopy methods

Spectroscopy was originally the study of the interaction between radiation and matter as a function of wavelength (λ). In fact, historically [1], spectroscopy referred to the use of visible light dispersed according to its wavelength, e.g. by a prism. Later the concept was expanded greatly to comprise any measurement of a quantity as function of either wavelength or frequency. Thus it also can refer to a response to an alternating

field or varying frequency (ν). Spectroscopy often used in physical and analytical chemistry for the identification of substances through the spectrum emitted from or absorbed by them.Common types (Absorption, Fluorescence, X- ray, Flame, Visible, Ultraviolet, Infrared, Near Infrared) (Baianu, 2002: Haupts et al, 1999: Robello and Diaspro, 1999)

## 2. LITERATURE REVIEW

Any of several species of the genus Rheum L. in the family Polygonaceae is called Rhubarb. The genus Rheum L., containing of around 60 herbaceous perennial plants cultivating from thick and short rhizomes, is scattered in the sub-tropical and temperate zone. Accounting for three-quarters of the genus, China is the dissemination center of the genus with more than 41 species and two variations. Mostly, rhubarb is originated in the southwest and northwest zones of China (The Flora of China Editorial Committee, 1998). Conventionally, it is recognized that Qinghai, Sichuan, and provinces of Gansu are the generating zones of rhubarbs in China. A part from china and Himalaya, Kurdistan region is also an important region for producing rhubarb species. In addition to its medical importance, it soon found its way into the kitchen, as in desserts such as rhubarb crumble, as well as in jams, jellies and sauces, its tart flavor became famous. Rhubarb is basically a vegetable but is often thought to be a fruit (Lloyd, J.U 2008) (Newby, L. J, 2005). The stalk of rhubarb are either cooked or eaten raw with particular people dipping raw stalks in sugar so as to eliminate the tartness. Commonly, it caught to be observed that merely the plants stalk is edible, since rhubarbs leaves comprise substantial quantity of potassium oxalate which might occasionally be deadly, particularly for those are susceptible to oxalic acids (Zheng, Qing-xia, et al, 2013).

### 2.1. Bioactivity

In Rhubarb, the composites are separated and predicted to have antiulcer, antioxidant, antifungal, antidiabetic, antimicrobial, wound healing, nephro protective, anticancer, hepatic protective and activities. From an enormous amount of published reports, pharmacological properties of diverse ingredients from Rheum emodin are gathered

### 2.2. Wound healing action

A number of constituents such as emodin, tannins, flavonoids, triterpinoids that extracted and recognized from the herb are the reason behind the wound healing effect of rhubarb. Emodin extracted from rhubarb has been stated to control the TGF-β1 mRNA and it made 2 and 3 protein expressions in a concentration dependant manner

causing primary wound healing in a rabbit wound model (Tang Tin and Yang Jing, 2010). Flavonoids is also recognized as a cause of wound healing potential of rhubarb since it is identified to decrease per oxidation of lipid not merely via averting or decelerating the commencement of cell necrosis but also via secularity improvement. Therefore, the viability of the collagen fibrils is escalated through increasing the collagen fibers strength, circulation increase, cell damage prevention and encouraging DNA synthesis by any drug that inhibits lipid per oxidation (Getie et al, 2002). By primary stimulation of TNF-α, Flavonoids assist in the wound healing acceleration and also act as antioxidant thereby scavenge free radicals (Uma devi et al, 2001) (Goel et al, 2010). Because of flavonoids and tannins astringent and antimicrobial property which seems accountable for the contraction wound and epithelialization escalation rate, tannins and flavonoids promote the process wound healing (Tsuchiya et al, 1996) (Scortichini, M. and Pia Rossi, M., 1991).

### 2.3. Antimicrobial action

Some kinds of fungi and bacteria are resilient to antibiotics. Thus, huge quantity of medicinal herbs and plants are being investigated for their potential activity as antimicrobial. The main anthraquinone derivatives extracted from rhubarb have been stated to possess substantial antimicrobial activity against four strains of methicillin-resistant Staphylococcus aurous are Emodin, aloe-emodin and rheum. Rheum has also been indicated to have antibacterial activity in contradiction of Escherichia coli K12 (Hatano et al, 1999). However, chrysophenol, aloe-emodin, Rheinandphyscion extracted from rhubarb have been stated to have antifungal activity against Cryptococcus neo Farman's, Candida albicans and Aspergillus fumigates (Agarwal et al, 2003). Whereas revandchinone-1 and 3 had moderate antibacterial activity, decent antibacterial activity against some strains of gram negative and gram positive bacteria is exhibited by revandchinone-4. In addition, modest antifungal activity against Rhizopusoryzae and Aspergillusniger is demonstrated by Revandchinone-1, 3 and 4 (Babu et al, 2003). The ethanol that extracted from rhubarb had been stated to have encouraging activity against various strains of H. pylori extracted from gastric biopsy specimens of gastric carcinoma in both in vivo and in vitro examinations (Ibrahim et al, 2006).

## 2.4. Hepatic protective and anti diabetic effect

Liver, which is the biggest organ of the body, is mainly associated with the organism's metabolic activity. It is involved in the detoxification mechanism of the body as it is the central position for the biotransformation of xenobiotic. Liver is responsible for detoxification of the chemical constituents in the blood and in this technique it is exposed to high level of poisonous metabolites and toxicants making it vulnerable to damage (Glaister, J.R, 1996). The impairment of liver resulted from chemical agents and pathogens is of similar nature and an appropriate handling plan or regime is absent for both. In allopathic medicine, the fact that dependable liver defensive drugs are obviously in sufficient (Neha, T. and U.M. Rawal, 2000).encouraged the experts for exploring herbal cures.

The consumption of excessive ethanol results in cellular fibrosis, proliferation, cirrhosis and liver cancer (Wang, X. D, 1999). A vital aspect of alcohol-encouraged liver damage is a compromised vitamin A nutritional condition. Investigation in human Hep G2 cells have indicated that ethanol is cyto toxic to Hep G2 cells, that are trans deuced for expressing P-4502E1 (CYP 2E1) and this poisons results in the cells death particularly in the liver (Wu, D., & Cerebrum, A. I, 1999). The oxidation of hepatic of ethanol for acetaldehyde primarily encompasses the dehydrogenises of alcohol enzyme (Svensson, 1999) which brings the $NAD^+$ to NADH reduction (Lieber, C. S 1997). The severity magnitude of liver via illness or generally hepatic toxins is calculated via the concentration of glutamate oxalic acetate transaminase (SGOT), serum enzymes glutamate private transaminase (SGPT), alkaline phosphates (ALP), and bilirubin, albumin, and all homogenate liver. A big amount of medicinal herbs and plants have a significant role in management of health care globally, and lot of attention is paid to herbal and plant medicines for treating of different illnesses comprising ghepatopathy. India is a rich in the herbal plant and system of medicine, allocates lot of importance for the pharmacological aspects of numerous herbs and plants. A large number of phyto constituents from different herbs and plants are stated to have activity liver protection (Doreswamy, R., & Sharma, D., 1995). Simultaneously, astonishingly, there are limited plant drugs formulations for treating severe liver illnesses. In the recent past, a few outstanding literatures have been published on this subject (Evans, D. A, 2002). Noteworthy hepatic protective activity and refurbishment of all marker enzymes near control concentration against $CCl_4$ and all oxan encouraged liver damage both in vivo

and in vitro utilizing 50 mg/kg and 250 mg/kg, oral dose, the extract from the rhizomes of rhubarb has shown (Ibrahim et al, 2008 & Radhika et al, 2012). In a several investigation it has been indicated that R. emodin rhizome demonstrate activity of ant diabetic not merely via improving the peripheral glucose utilization, via correcting impaired liver and kidney glycolysis and viaconfining its process of gluconeogenic, same as insulin (Radhika et al, 2010), however it also reestablishedthe entire marker enzymes to near control concentration in Alloxan encouraged diabetes in albino proportion (Radhika et al, 2012).

**2.5. Nephro-protective effect**

The toxic influence of metals on the kidney has been recognized for several years. It is one of the major common problems of kidney and happens as the body is exhibited to a toxin or drug (Porter, G.A. and Bennett, W.M, 1981) the kidney can be adversely affected a number of therapeutic agents and antibiotics causing long-lasting interstitial nephritis, severe renal failure and nephritic syndrome. This is due to an escalating amount of powerful therapeutic drugs such as sulphonamides, amino glycoside, acetaminophen, antibiotics comprising penicillin's, acyclovir, pentamidine, sulphadiazine, trimethoprin, tetracyclines, Indomethacin, Aspirin, Ibuprofen, etc. recently, Chemotherapeutic agents have been supplemented to the therapeutic arsenal (Hoitsma, A.J., Wetzels, J.F and Koene, R.A, 1991). Functionally via reduced urine-concentrating capability, tubular protein urea, lysosomalenzymuria, mild glucosuria, reduced ammonium secretion and depressing rate of glomerular filtration, aminoglycoside nephrotoxicity is manifested (Kaloyanides, G. J., &Pastoriza-Munoz, E, 1980). The arsenic are also induced by nephrotoxicity exposure to chemical reagents such as ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals like lead, mercury, cadmium and. Regularly, the merely essential therapy is prompt recognition of the illness and termination of responsible drugs (Paller MS, 1990).

The substances which have protective activity against nephrotoxicity is called Nephroprotective agents. Because of the presence of different complex chemical substances, medicinal plants have curative properties. Different herbs for the therapy of renal disorders are prescribed by early literatures. The retention of nitrogenous waste products of metabolism in the blood is resulting from renal disorder primarily indicated failure of the kidneys excretory function (Herfindal, 2000). Additionally, there is a

regulation failure of fluid and electrolyte balance beside function of endocrine. Fundamentally, the renal failure is classified into long-lasting and severe renal failure (Barry M, 2000). While long-lasting renal failure (CRF) is an irreparable worsening in the renal function which typically grows over years, resulting in loss of excretory metabolic and endocrine functions, serious renal failure (ARF) denotes to the rapid and regularly adjustable damage of renal function which grows during several days or weeks.

The activity of nephroprotective on entire the sections (S1, S2 and S3) of the kidneys proximal tubule against mercury, cadmium and potassium dichromate-induced nephrotoxicity in rats of alcoholic extract of R. emodin has been recognized. Due to the tannins present in the fraction, the impact has been suggested. By utilizing mercuric chloride, cadmium chloride, potassium dichromate and gentamicin in rats and monitoring the levels of urea, nitrogen and creatinine serum, the nephrotoxicity was induced (Alam, 2008). In another research, the induced renal impairment activity nephroprotective in Wistar rats was evaluated in gentamicin, cisplatin and paracetamol. By measuring the levels of body weight, blood urea, and serum creatinine, tissue glutathione and lipid peroxidation the protective property of 70% ethanol extract was suggested. Owing to the presence of saponins, cardiac glycosides and triterpenoids in the fraction, the effect has been proposed (Pramod, 2011).

There are beneficial effects of the rhubarb on the Parkinson's disease, immune system and acute respiratory syndrome. The ethyl acetate extract of rhizome of *R.* emodi has been stated to have activity of immuno-improving on cell lines. The influence is reliant upon the dose leading to a reduction in IL-10 by RAW 264.7 in macrophage cell lines and the escalated discharge of nitric oxide and cytokine TNF-α, IL-12 in the availability of extract alone (Fozia Kounser. and Zargar, M. Afza, 2010; Kounsar, 2011). It has been reported that Emodin to have inhibitory influence on the interaction of SARS-CoV S protein and ACE2. It has been discovered to prevent both the infectivity of S protein-pseudo typed retrovirus to Vero E6 cells the binding of SARS-CoV S protein toACE2. These discovery recommended that emodin is a new anti-SARS-CoV composite and in the treatment of severe acute respiratory syndrome (SARS), it should be regarded as a potential lead therapeutic agent (Ho et al, 2007). It has also been declared that Emodin have inhibitory impact on the monoamine oxidize B on rat brain mitochondria therefore might play an effective role in the prevention and treatment of Parkinson's disease (Kong, 2004).

# 3. MATERIAL AND METHOD

## 3.1. Materiels

The samples of Rheum rib's collected from seven different localities of Siirt and Northern Iraq are the material of this study.

## 3.2. Methods

### 3.2.1. Preparation of plant specimens

The collected plants were dried in the laboratory environment, in a place where the air flow was present and at the room temperature in the shade area, and then powdered with a mixer. Powdered plant samples were placed in glass jars and stored at room temperature.

### 3.2.2. Heavy metals

An ICP-MS instrument (Thermo Scientific İCAP Q ICP-MS) was used for the determination of metal in Rhubarb samples.

### 3.2.3. Digestion procedure

A Bergh of Speed wave MWS-3 model microwave digestion system was used for acid digestion of samples. The microwave acid digestion was carried out as follows: 1.0 g portion of dried sample was weighed and transferred into a pressure- resistant poly tetra fluoro ethylene (PTFE) vessel (volume 100 mL), and the mixture of acids ($HNO_3$ + $H_2O_2$, 2.5: 7.5 mL) was added. Microwave digestion system under the conditions described in Table 1. The power applied in program was 1450 W. The reaction mixture was subjected to an evaporation module in order to remove the acids after the final digestion. Then the residue was dissolved in Milli-Q water and filtered, and the filtrate was diluted to a fixed volume.

**Table 3.1** Operating conditions for digestion by microwave oven.

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **T (ºC)** | 100 | 160 | 180 | 100 |
| **Ta (min)** [a] | 10 | 10 | 10 | 10 |
| **Time (min)** [b] | 5 | 3 | 3 | 3 |

[a] waiting time at desired temperature.

[b]The time between the two sequential temperature

### 3.2.4. Total phenolic compounds

#### 3.2.4.1. LC-MS/MS device and chromatographic conditions

37 LC-MS / MS system used for qualitative and quantitative analysis of phytochemicals; Shimadzu Neexera model UHPLC device and Shimadzu LCMS 8040 model triple quadruple mass spectrometer device. The liquid chromatography system used consists of the LC-30 AD model gradient pump, the DGU-20A3R model degasser, the CTO-10ASvp model column oven and the SIL-30AC model auto sampler. Chromatographic separation was performed on Inertial ODS-4 model C18 (100 mm × 2.1 mm, 2 μm) column. During the analysis the column furnace is set at 35 ºC. In the elution gradient, ultrapure water for mobile phase A and acetonitrile for mobile phase B were used. In addition, 10 mM ammonium format and 0.1% formic acid were added to the water phase to facilitate better chromatographic separation and ionization. After several attempts to achieve optimal separation of analyzes, the most suitable UHPLC gradient profile was obtained with a gradient profile of 5-20% B (0-10 min), 20% B (10-22 min), 20-50% B (22-36 min) % B (36-40), 5% B (40-50 min). The moving phase flow rate was 0.25 mL / min and the injection volume was 4 μL

The triple quadruple mass spectrometer is equipped with an ESI (electro spray ionization) source operating in both a negative and a positive mode. The LC-ESI-MS / MS data were collected and processed by the registered software Lab Solutions (Shimadzu, Kyoto, Japan). The quantitative analysis of the analysts was carried out in the device in multiple reaction monitoring (MRM) mode and the parent ions were combined with one or two product ions (the other was used for qualitative purposes for quantitative purposes). Other parameters that are optimized in the mass spectrometer are: interface temperature; 350 ºC, DL temperature; 250 ºC, heat block temperature; 400 ºC, nebulizer gas (N2) flow; 3 L / min and drying gas ($N_2$) temperature; 15 L / min (Yılmaz, 2015).

**Table 3.2.** Analytical parameters of the LC-MS / MS analysis method ([a]RT: Retention time, [b]Master ion (*m/z):* Molecular ions of standard compounds (m/z rate), [c]$R^2$: Determination coefficient, dRSD: Relative standard deviation, eLOD/LOQ (µg/L): Detection limit/ Assignment limit, f U (%): 95% Relative standard uncertainty at confidence level (k=2)) (Yılmaz, 2015)

| No | Analytes | RT[a] | Main Ion (m/z)[b] | Fragmenation ions | ion mode | Calibration Equation | $R^{2c}$ | RSD%[d] Same day | different days | Linearity December (µg/L) | LOD (µg/L)e | LOQ (µg/L)e | regain (%) Same day | different days | U[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Kumarin | 17.40 | 147.05 | 91.0-103.2 | Poz | y=33.64×-89700 | 0.994 | 0.01306 | 0.01239 | 1000-20000 | 208.4 | 228.4 | 0.99947 | 1.00081 | 0.0237 |
| 2 | Hesperidin | 12.67 | 610.90 | 303.1-465.1 | Poz | y=1340.27×-43769 | 0.998 | 0.00945 | 0.01126 | 25-1000 | 3.4 | 4.2 | 1.01733 | 1.01263 | 0.0262 |
| 3 | p-Kumaric acid | 11.53 | 162.95 | 119.3-93.3 | Neg | y=3199.20×+13002 | 0.992 | 0.01820 | 0.01727 | 25-1000 | 7.3 | 9.1 | 1.00617 | 1.01224 | 0.0516 |
| 4 | o-kumaric acid | 15.45 | 162.95 | 119.4-93.3 | Neg | y=1219.34×-10915 | 0.999 | 0.02730 | 0.02566 | 25-1000 | 24.4 | 31.1 | 0.98344 | 0.99061 | 0.0513 |
| 5 | Gallic acid | 3.00 | 168.85 | 125.2-79.2 | Neg | y=226.76×+38152 | 0.998 | 0.01601 | 0.01443 | 250-10000 | 95.5 | 106.9 | 1.00004 | 1.00454 | 0.0282 |
| 6 | Caffeic acid | 8.80 | 178.95 | 135.2-134.3 | Neg | y=3963.32×+178156 | 0.998 | 0.01454 | 0.01469 | 25-1000 | 18.4 | 22.4 | 1.00917 | 0.98826 | 0.0354 |
| 7 | Vanilic acid | 8.57 | 166.90 | 152.3-108.3 | Neg | y=35.84×-12097 | 0.999 | 0.00528 | 0.00619 | 1000-20000 | 122.2 | 139.7 | 1.00093 | 1.04095 | 0.0508 |
| 8 | Salicylic acid | 11.16 | 136.95 | 93.3-65.3 | Neg | y=5286.26×+309192 | 0.989 | 0.01016 | 0.01242 | 25-1000 | 5.0 | 6.5 | 1.00989 | 0.99013 | 0.0329 |
| 9 | Kynic acid | 1.13 | 190.95 | 85.3-93.3 | Neg | y=41.06×+10671 | 0.996 | 0.00259 | 0.00274 | 250-10000 | 75.8 | 79.4 | 1.00288 | 0.98778 | 0.0082 |
| 10 | 4-OH-Benzoicacid | 7.39 | 136.95 | 93.3-65.3 | Neg | y=409.03×+112079 | 0.998 | 0.01284 | 0.01538 | 250-10000 | 33.2 | 38.1 | 0.99662 | 1.00058 | 0.0289 |
| 11 | Ferulic acid | 12.62 | 192.95 | 178.3 | Neg | y=80.45×-31782 | 0.997 | 0.00708 | 0.00619 | 250-10000 | 36.6 | 42.0 | 0.99987 | 1.00289 | 0.0494 |
| 12 | Chlorogenicacid | 7.13 | 353.15 | 191.2 | Neg | y=781.36×-18697 | 0.998 | 0.00058 | 0.00076 | 25-1000 | 6.2 | 8.1 | 1.00806 | 0.99965 | 0.0069 |
| 13 | Rozmarinic acid | 14.54 | 359.00 | 161.2-197.2 | Neg | y=909.67×-201692 | 0.994 | 0.02014 | 0.01751 | 100-5000 | 6.6 | 8.8 | 0.99206 | 1.03431 | 0.0713 |
| 14 | Protocatechic acid | 4.93 | 152.95 | 108.3 | Neg | y=297.75×+30590 | 0.995 | 0.01236 | 0.01296 | 100-5000 | 28.2 | 31.4 | 0.99404 | 1.01070 | 0.0411 |
| 15 | Cinnamic acid | 25.61 | 147.00 | 103.15-77.3 | Neg | y=9.06×-12403 | 0.996 | 0.00648 | 0.00816 | 5000-20000 | 821.8 | 859.7 | 1.00051 | 0.99927 | 0.0143 |
| 16 | Sinapinic acid | 12.66 | 222.95 | 208.3-149.2 | Neg | y=141.96×-73294 | 0.992 | 0.01446 | 0.01517 | 250-10000 | 78.7 | 86.1 | 1.00164 | 0.99962 | 0.0281 |
| 17 | Fumaric acid | 1.48 | 115.00 | 71.4 | Neg | y=64.99×-11592 | 0.997 | 0.00536 | 0.00460 | 100-5000 | 28.1 | 34.5 | 0.99748 | 0.99867 | 0.0124 |
| 18 | vanillin | 10.87 | 151.00 | 136.3-92.2 | Neg | y=446.10×+70934 | 0.998 | 0.00696 | 0.00793 | 250-10000 | 44.3 | 53.1 | 0.99679 | 0.99611 | 0.0280 |

**Table 3.3**. Analytical parameters of LC-MS / MS analysis method (Continued) (Yılmaz, 2015)

| No | Analytes | RT[a] | Main ion(m/z)[b] | Fragmenation Ions | Ion Mode | Calibration Equation | $R^{2c}$ | RSD %[d] Same Day | RSD %[d] Different Days | Lineerity December (µg/L) | LOD (µg/L)e | LOQ (µg/L)e | regain (%) Same Day | regain (%) Differenet Days | U[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | pyrocatechol | 6.48 | 109.00 | 108.35-91,3 | Neg | y=30.61×+14735 | 0.996 | 0.01313 | 0.01339 | 1000-20000 | 261.1 | 278.4 | 0.99987 | 0.99936 | 0.0235 |
| 20 | Malic acid | 1.23 | 133.00 | 115.2-71,3 | Neg | y=316.95×-42041 | 0.999 | 0.00477 | 0.00527 | 250-10000 | 55.3 | 67.5 | 1.01266 | 0.99836 | 0.0113 |
| 21 | Syringic acid | 9.02 | 196.95 | 182.2-167,3 | Neg | y=42.33×-52547 | 0.996 | 0.01049 | 0.01345 | 1000-20000 | 212.5 | 233.3 | 0.99922 | 0.99977 | 0.0238 |
| 22 | Hesperetin | 31.76 | 300.95 | 164.2-136,2 | Neg | y=876.67×+48916 | 0.997 | 0.03209 | 0.02605 | 25-1000 | 5.6 | 6.9 | 0.98850 | 0.99435 | 0.0562 |
| 23 | Naringenin | 30.68 | 270.95 | 151.2-119,3 | Neg | y=4315.1×+178410 | 0.995 | 0.02054 | 0.02019 | 25-1000 | 5.4 | 6.4 | 0.99883 | 1.01002 | 0.0521 |
| 24 | Routine | 12.61 | 609.05 | 300.1-271,1 | Neg | y=561.91×-16879 | 0.997 | 0.00473 | 0.00624 | 25-1000 | 5.5 | 6.5 | 1.00994 | 0.98017 | 0.0159 |
| 25 | quercetin | 28.17 | 300.90 | 151.2-179,2 | Neg | y=1198.48×+480562 | 0.990 | 0.01589 | 0.01360 | 100-5000 | 23.3 | 28.9 | 0.98470 | 1.00103 | 0.0543 |
| 26 | Kersitr | 16.41 | 447.15 | 301.1-255,1 | Neg | y=339.39×+38910 | 0.999 | 0.01528 | 0.02320 | 100-5000 | 22.0 | 25.2 | 0.99726 | 1.00620 | 2.0079 |
| 27 | Apigenin | 31.43 | 268.95 | 117.3-151,2 | Neg | y=4548.36×+295252 | 0.990 | 0.02304 | 0.02204 | 25-1000 | 5.4 | 6.3 | 1.01444 | 1.01331 | 0.0650 |
| 28 | Chrysin | 36.65 | 252.95 | 143.3-119,4 | Neg | y=2032.13×+95593 | 0.993 | 0.00490 | 0.00630 | 25-1000 | 5.4 | 6.2 | 1.00338 | 1.00437 | 2.0083 |
| 29 | Likiritigenin | 25.62 | 254.95 | 119.3-135,1 | Neg | y=2384.96×+59141 | 0.996 | 0.01849 | 0.01738 | 25-1000 | 5.5 | 6.6 | 1.00333 | 0.99957 | 0.0341 |
| 30 | İzokersitrin | 13.42 | 463.00 | 300.1-271,1 | Neg | y=803.23×+4981 | 0.999 | 0.00682 | 0.00515 | 25-1000 | 5.4 | 6.3 | 1.00594 | 1.00722 | 0.0133 |
| 31 | Apigetrin | 16.59 | 431.00 | 268.2-239,2 | Neg | y=1775.55×+91121 | 0.993 | 0.01797 | 0.01607 | 25-1000 | 5.4 | 6.1 | 1.01394 | 1.00419 | 0.0597 |
| 32 | Roifolin | 16.11 | 577.05 | 269.2-211,1 | Neg | y=237.15×+11887 | 0.999 | 0.00747 | 0.01528 | 100-5000 | 23.1 | 27.9 | 1.01046 | 1.01739 | 0.0941 |
| 33 | Nikotiflorin | 14.68 | 593.05 | 285.1-255,2 | Neg | y=498.38×+79274 | 0.991 | 0.00737 | 0.00875 | 100-5000 | 22.4 | 25.5 | 1.02558 | 1.00970 | 0.0276 |
| 34 | Fisetin | 19.30 | 284.95 | 135.2-121,3 | Neg | y=547.46×+274791 | 0.991 | 0.00557 | 0.00820 | 250-10000 | 54.4 | 61.4 | 0.99877 | 1.00031 | 0.0148 |
| 35 | Luteolin | 28.27 | 284.75 | 133.2-151,2 | Neg | y=3272.65×+150557 | 0.997 | 0.00575 | 0.00696 | 25-1000 | 5.4 | 6.5 | 1.00772 | 0.99524 | 0.0174 |
| 36 | myricetin | 18.72 | 317.00 | 179.2-151,3 | Neg | y=583.55×+205727 | 0.999 | 0.00652 | 0.00711 | 250-10000 | 53.2 | 57.2 | 0.99982 | 1.00042 | 0.0126 |
| 37 | Kamferol | 31.88 | 284.75 | 255.1-117,3 | Neg | y=26.29×+87558 | 0.992 | 0.01436 | 0.01070 | 1000-20000 | 206.6 | 214.3 | 0.99971 | 0.99851 | 0.0209 |

### 3.2.5. Antioxidant

#### 3.2.5.1. Preparation of plant specimens

Collected plants were pulverized, in the laboratory environment, with a sinbo coffee grinder after being dried in a place where the air flow was present and at room temperature in the shade area and after the leaves separated. Powdered plant samples were placed in glass jars and stored at room temperature.

#### 3.2.5.2. Extract preparation

4 g of the dried plant sample was placed in a Beaker and 40 ml methanol with %80 was added. The mixture was left overnight on the Incubated shakers Standard & Cooled - MD13, Range shaked after it was sonicated for 2 minutes on the Wiggin Hauser Homogenizer and 5 minutes on the Sonopuls HD 2070.The extract is then filtered through the filter paper. After the extracts were dried at 38 degrees in an oven, stock concentrations were formed by adding 80% methanol so that the concentration of solid part remaining in the bottom of the tube was 10 mg / ml.

#### 3.2.5.3. Total phenolic analysis

The total phenolic content of the working samples was determined according to the Folin-Ciocalteu reactivity and Gallic acid standard (Slinkard& Singleton, 1977). From the extractsolution, 0.1 ml of Folin-Ciocalteu reactant was added to the flask and the flask was thoroughly shaken. After 3 minutes, 1 ml of a 6% $Na_2CO_3$ solution was added and the mixture was allowed to stand for 1 hour with intermittent agitation.

Absorbance was measured at 760 nm with a Uvmini-1240 Spectrophotometer. The same procedure was repeated in the Gallic acid solutions.

#### 3.2.5.4. Total flavonoid content analysis

Total flavonoid content was determined using the aluminum chloride colorimetric method (Zhishen et al., 1999) (Zou et al., 2004). 0.5 ml of the extract solution was mixed with 2 ml of distilled water and 150 µl of %5 sodium nitrate. After 6 minutes, 150 µl of 10% aluminum chloride and 2 ml of 1 M sodium hydroxide were added and left at room temperature for 15 minutes. The absorbance of the samples was measured at 510 nm with a Shimadzu UV-vis Uvmini-1240 Spectrophotometer. Routine is used as standard.

**Table 3.4.** Total Flavonoid content analysis

| Rutin Standard(mg/ml) | 1 mg/ml of Rutin (µl) | AqueousMethanol% 80 | Total Volume |
|---|---|---|---|
| 1 | 1000 | 0 | 1000 |
| 0.8 | 800 | 200 | 1000 |
| 0.6 | 600 | 400 | 1000 |
| 0.4 | 400 | 600 | 1000 |
| 0.2 | 200 | 800 | 1000 |
| 0.1 | 100 | 900 | 1000 |
| 0.05 | 50 | 950 | 1000 |
| Blank | 0 | 1000 | 1000 |

### 3.2.5.5. Preparation of plant specimens

For DPPH analysis (Villao et al, 2006) 4 ml of 0.01 mM DPPH solution (prepared to 80% methanol) was added to 1 ml of extract, and the precipitate was absorbed at 517 nm wavelength with ShimadzuUv-vis Uvmini-1240 Spectrophotometer after 15 minutes in the dark.

Control: 1 ml of solvent and 4 ml of DPPH

DPPH activities (% incubation) = $\frac{(A_C - A_1)}{A_C} x100$

($A_C$: Control absorbance, $A_1$: Sample Absorbance)

### 3.2.5.6. FRAP analysis

FRAP analysis was performed according to (Benzie & Strain, 1996) 3 ml of FRAP reagent was added onto 100 µl of sample diluted appropriately. The incubation was allowed to proceed for 6 minutes at room temperature in the dark and then absorbed at 593 nm with a Shimadzu UV-vis Uvmini-1240 Spectrophotometer.

FRAP solution: 10 ml Acetate Buffer + 10 ml Iron (III) Chloride-Hexahydrate + 1 ml TPTZ- solution. Acetate Buffer: (300 mmol / L; PH: 3.6) solution preparation; 3.1 g of $NaCH_3COO$ in 16 ml of Acetic Acid / L distilled water. HCL: (40 mmol / L) solution preparation; 400 µl of HCl (32%) / 100 ml of purified water. TPTZ: (10 mmol) Preparation of solution; 31.2 mg TPTZ / 10 mL HC1 40 mmol. $FeCl_3.6H_20$: Solution preparation; 54.1 mg of $FeCl_3 * 6H_2O$ / 10 ml of pure water.

**Table 3.5.** FRAP analysis

| **Frap Standard(μl)** | Dilution | Stock solution | Pure Water |
|---|---|---|---|
| *500* μmol/L | 1:10 | 100 | 900 |
| 625 μmol/L | 1:8 | 100 | 700 |
| 1000 μmol/L | 1:5 | 200 | 800 |
| 1667 μmol/L | 1:3 | 300 | 600 |
| 2500 μmol/L | 1:2 | 500 | 500 |

### 3.2.5.7. Iron chelating

The metal chelating activity of the samples is applied by taking the methods by (Rival et al., 2001& Duh et al., 2001) as basis. Appropriately diluted analysis samples were mixed with 1 ml of 3.7 ml of 95% ethanol. Each sample was incubated with 0.1 ml 2 mM $FeCl_2$ for 60 minutes. After incubation, 0.2 ml of 5 mM ferrozin was added and incubated at room temperature for 10 minutes. The complex in absorbance of iron ions and ferrozin was measured at 562 nm with a ShimadzuUv-vis Uvmini-1240 Spectrophotometer. Controlled sample was prepared under the same conditions as ethanol containing no substance. All experiments were carried out in 3 replicates and the averages of the results were taken.

# 4. RESULTS AND DISCUSSION

## 4.1. Heavy metals analysis

The concentration of heavy metal in rhubarb plants are indicated in Table 4.1. The outcomes exposed obvious differences in the amount of heavy metals produced from rhubarb plant grown at various geographical areas in Kurdistan region/ Iraq and Turkey.

**Table 4.1.** The mean of elements and concentration of rhubarb samples (N=3)

| Label | Mean | | | | | | |
|---|---|---|---|---|---|---|---|
| analyte | pervari | Şirvan | Sor | Rash | Karajar | Gara | Qalandar |
| Li [ ng $g^{-1}$] | 149.16 | 161.90 | 161.82 | 283.04 | 259.84 | 236.22 | 141.45 |
| Be [ ng $g^{-1}$] | 15.69 | 80.89 | 26.95 | n.d. | n.d. | 23.60 | 47.11 |
| B [µg $g^{-1}$] | 26.98 | 38.01 | 31.12 | 33.73 | 43.73 | 42.31 | 34.55 |
| Na [µg $g^{-1}$] | 120.20 | 177.40 | 297.80 | 223.70 | 149.85 | 162.77 | 140.94 |
| Mg [mg $g^{-1}$] | 2.42 | 3.43 | 3.43 | 5.51 | 4.88 | 5.09 | 4.02 |
| P [mg $g^{-1}$] | 3.93 | 5.41 | 7.08 | 6.44 | 5.49 | 4.86 | 6.67 |
| K [mg $g^{-1}$] | 35.20 | 46.30 | 38.80 | 47.77 | 49.74 | 72.70 | 52.20 |
| Ca [µg $g^{-1}$] | 126.38 | 189.32 | 219.91 | 493.05 | 1128.61 | 1929.43 | 187.74 |
| Ti [µg $g^{-1}$] | 5.25 | 7.43 | 4.88 | 12.90 | 26.57 | 49.64 | 4.38 |
| V [ ng $g^{-1}$] | 316.39 | 465.00 | 304.82 | 513.28 | 434.62 | 932.45 | 255.69 |
| Cr [µg $g^{-1}$] | 0.89 | 1.09 | 1.26 | 2.19 | 1.20 | 2.30 | 3.52 |
| Mn [µg $g^{-1}$] | 12.46 | 19.97 | 34.11 | 33.59 | 38.13 | 38.05 | 30.21 |
| Fe [µg $g^{-1}$] | 144.74 | 203.13 | 97.60 | 418.70 | 251.81 | 493.19 | 158.22 |
| Co [µg $g^{-1}$] | 0.29 | 0.43 | 0.16 | 0.36 | 0.28 | 0.42 | 0.37 |
| Ni [µg $g^{-1}$] | 2.11 | 3.30 | 5.31 | 4.09 | 2.99 | 3.94 | 4.67 |
| Cu [µg $g^{-1}$] | 4.96 | 7.11 | 10.67 | 6.94 | 5.31 | 5.58 | 7.34 |
| Zn [µg $g^{-1}$] | 17.83 | 28.20 | 51.62 | 30.77 | 21.23 | 21.25 | 190.65 |
| As [ ng $g^{-1}$] | 68.84 | 113.92 | 67.15 | 117.46 | 71.60 | 161.11 | 51.04 |
| Se [ ng $g^{-1}$] | 243.12 | 626.73 | 208.80 | n.d. | n.d. | 182.89 | 182.52 |
| Sr [µg $g^{-1}$] | 11.00 | 16.09 | 6.94 | 20.85 | 54.71 | 40.78 | 10.27 |
| Mo [µg $g^{-1}$] | 3.08 | 4.91 | 3.30 | 2.83 | 2.35 | 2.12 | 1.93 |
| Cd [ ng $g^{-1}$] | 93.73 | 146.67 | 666.33 | 132.79 | 95.15 | 319.87 | 87.33 |
| Sn [ ng $g^{-1}$] | 382.77 | 382.54 | 341.35 | 468.23 | 226.35 | 186.89 | 249.42 |
| Sb [ ng $g^{-1}$] | 132.93 | 192.03 | 166.17 | 154.98 | 121.99 | 131.16 | 147.73 |
| Ba [µg $g^{-1}$] | 6.07 | 9.14 | 3.49 | 5.20 | 15.64 | 11.91 | 6.65 |
| La [ ng $g^{-1}$] | 275.49 | 203.77 | 67.90 | 76.17 | 94.62 | 315.32 | 109.03 |
| Ce [ ng $g^{-1}$] | 136.66 | 199.22 | 105.90 | 163.89 | 29.19 | 378.03 | 124.33 |
| Pt [ ng $g^{-1}$] | 73.39 | 87.98 | 31.46 | 24.01 | 12.28 | 7.99 | 9.13 |
| Tl [ ng $g^{-1}$] | 38.17 | 70.24 | 22.55 | 23.77 | 16.84 | 19.20 | 19.54 |
| Pb [ ng $g^{-1}$] | 401.53 | 434.52 | 1256.70 | 1097.05 | 546.92 | 624.06 | 595.48 |

**Table 4.2.** The standard deviation of elements and concentration of rhubarb samples (N=3)

| Label | Standard deviation | | | | | | |
|---|---|---|---|---|---|---|---|
| analyte | pervari | Şirvan | Sor | Rash | Karajar | Gara | Qalandar |
| Li [ ng $g^{-1}$] | 54.39 | 80.95 | 161.82 | 40.01 | 178.34 | 81.83 | 70.72 |
| Be [ ng $g^{-1}$] | 23.53 | 123.56 | 46.68 | n.d. | n.d. | 81.77 | 0.00 |
| B [µg $g^{-1}$] | 1.61 | 2.91 | 8.45 | 5.30 | 2.41 | 2.79 | 1.78 |
| Na [µg $g^{-1}$] | 1.99 | 2.85 | 5.15 | 0.62 | 1.70 | 2.40 | 0.65 |
| Mg [mg $g^{-1}$] | 0.03 | 0.08 | 0.03 | 0.14 | 0.05 | 0.06 | 0.07 |
| P [mg $g^{-1}$] | 0.03 | 0.24 | 0.13 | 0.18 | 0.17 | 0.18 | 0.14 |
| K [mg $g^{-1}$] | 0.39 | 1.42 | 0.38 | 1.27 | 0.08 | 2.14 | 0.39 |
| Ca [µg $g^{-1}$] | 1.41 | 2.69 | 6.49 | 12.42 | 23.26 | 31.93 | 0.83 |
| Ti [µg $g^{-1}$] | 0.03 | 0.09 | 0.31 | 0.19 | 0.27 | 0.96 | 0.06 |
| V [ ng $g^{-1}$] | 9.66 | 42.99 | 21.22 | 43.65 | 40.77 | 22.17 | 3.59 |
| Cr [µg $g^{-1}$] | 0.02 | 0.02 | 0.04 | 0.03 | 0.04 | 0.05 | 0.07 |
| Mn [µg $g^{-1}$] | 0.02 | 0.10 | 0.51 | 0.36 | 0.80 | 0.98 | 0.69 |
| Fe [µg $g^{-1}$] | 2.76 | 4.71 | 0.73 | 11.19 | 2.78 | 5.05 | 4.76 |
| Co [µg $g^{-1}$] | 0.01 | 0.01 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 |
| Ni [µg $g^{-1}$] | 0.05 | 0.14 | 0.05 | 0.09 | 0.04 | 0.07 | 0.24 |
| Cu [µg $g^{-1}$] | 0.07 | 0.14 | 0.15 | 0.29 | 0.09 | 0.04 | 0.05 |
| Zn [µg $g^{-1}$] | 0.21 | 0.27 | 1.22 | 0.44 | 0.41 | 0.08 | 1.43 |
| As [ ng $g^{-1}$] | 20.87 | 46.37 | 22.04 | 24.62 | 8.86 | 15.34 | 15.94 |
| Se [ ng $g^{-1}$] | 179.57 | n.d. | n.d. | 116.30 | 0.00 | 316.77 | 316.14 |
| Sr [µg $g^{-1}$] | 0.23 | 0.27 | 0.31 | 0.47 | 0.33 | 0.36 | 0.24 |
| Mo [µg $g^{-1}$] | 0.20 | 0.46 | 0.26 | 0.25 | 0.38 | 0.19 | 0.27 |
| Cd [ ng $g^{-1}$] | 10.04 | 12.58 | 85.77 | 11.47 | 9.27 | 24.71 | 6.51 |
| Sn [ ng $g^{-1}$] | 10.66 | 47.18 | 30.02 | 14.39 | 14.82 | 12.76 | 16.66 |
| Sb [ ng $g^{-1}$] | 1.51 | 19.60 | 13.62 | 4.95 | 8.28 | 8.29 | 14.79 |
| Ba [µg $g^{-1}$] | 0.08 | 0.22 | 0.12 | 0.16 | 0.21 | 0.15 | 0.20 |
| La [ ng $g^{-1}$] | 12.19 | 8.35 | 3.65 | 2.81 | 3.24 | 8.48 | 0.83 |
| Ce [ ng $g^{-1}$] | 4.54 | 9.81 | 6.15 | 7.97 | 0.48 | 8.11 | 3.01 |
| Pt [ ng $g^{-1}$] | 2.14 | 7.29 | 2.39 | 3.39 | 0.44 | 0.25 | 0.75 |
| Tl [ ng $g^{-1}$] | 1.75 | 0.21 | 2.23 | 2.28 | 1.54 | 1.06 | 2.36 |
| Pb [ ng $g^{-1}$] | 9.39 | 5.31 | 36.73 | 10.82 | 8.65 | 16.50 | 16.91 |

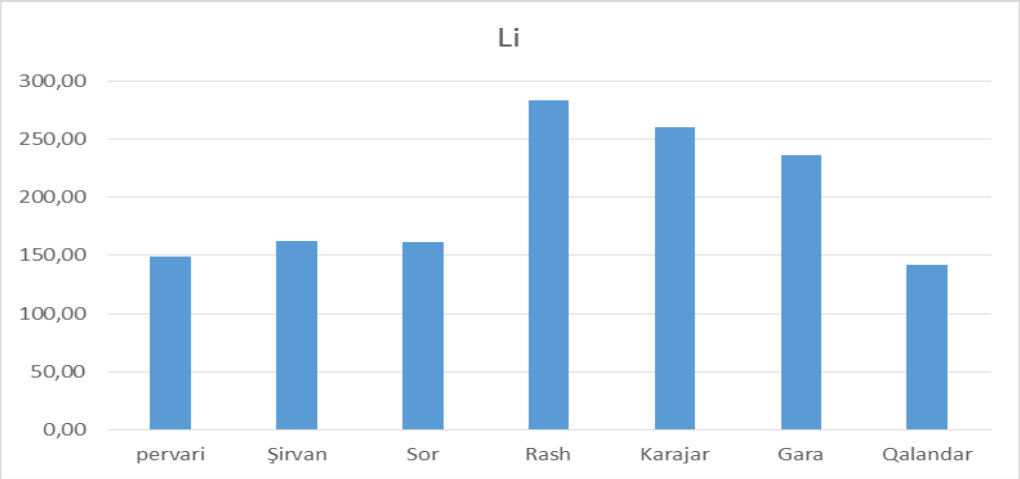



**Figure 4.1.** The concentration of Lithium (Li) in different locations [ng $g^{-1}$]

This figure 4.1 illustrates that there are enormous differences in the concentration of li in different location. It is clear that the highest amount of Li was 283.04±40.01 ng $g^{-1}$ in Rash and the lowest was 141.45±70.72 ng $g^{-1}$ in the region of Qalandar even though there are limited conducted researches in the same field, Similar results were found by (Ipătioaiei(Ipătioaiei D.C et al, 2014) in different location.

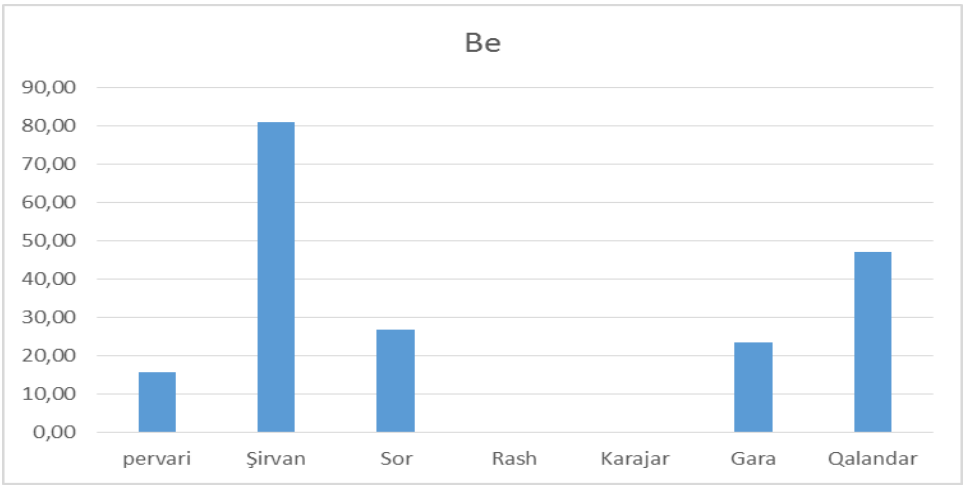



**Figure 4.2.** The concentration of Beryllium (Be) in various sites locations [ng $g^{-1}$]

Figure 4.2 describes the concentration of Be in seven different location in Kurdistan region of Iraq and Turkey. It is revealed that Be is not detected in some locations such as Rash and Korajar. However, the maximum content of Be is found in Shirvan 80.89±123.56 ng $g^{-1}$ and the lowest Be content is in the sample of Pervari 15.96±23.53 ng $g^{-1}$.

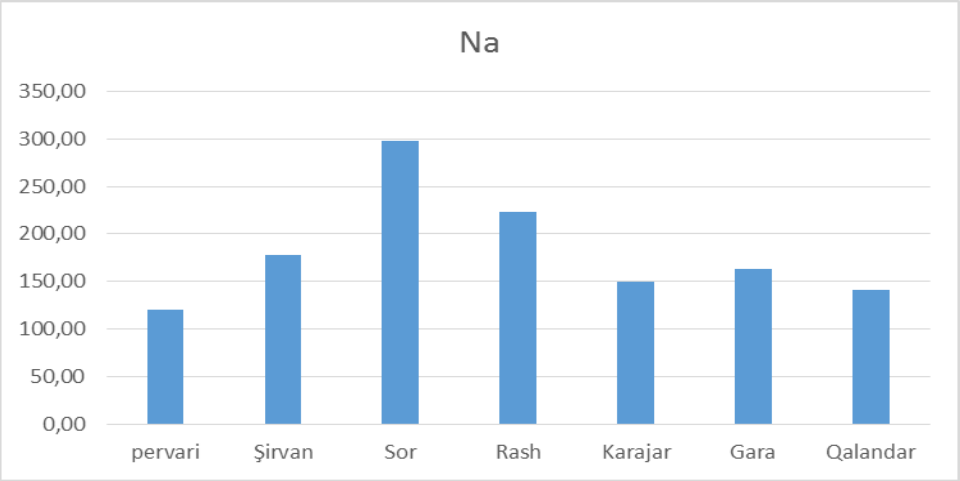



**Figure 4.3. T**he concentration of Sodium (Na) in various sites locations [µg $g^{-1}$]

In this figure 4.3, it can be seen that that there are slight differences in the concentration of Na in different location. It is evident that the largest quantity of Na was 297.80±5.15 µg $g^{-1}$ in Sor and the lowest was 120.20±1.99 µg $g^{-1}$ in the region of pervari. However, (Nazk, 2013) reported that rhubarb in the Sulaimaniye Mountains contain around 84.025 µg $g^{-1}$. This variation might be due the difference in the sample collection site.

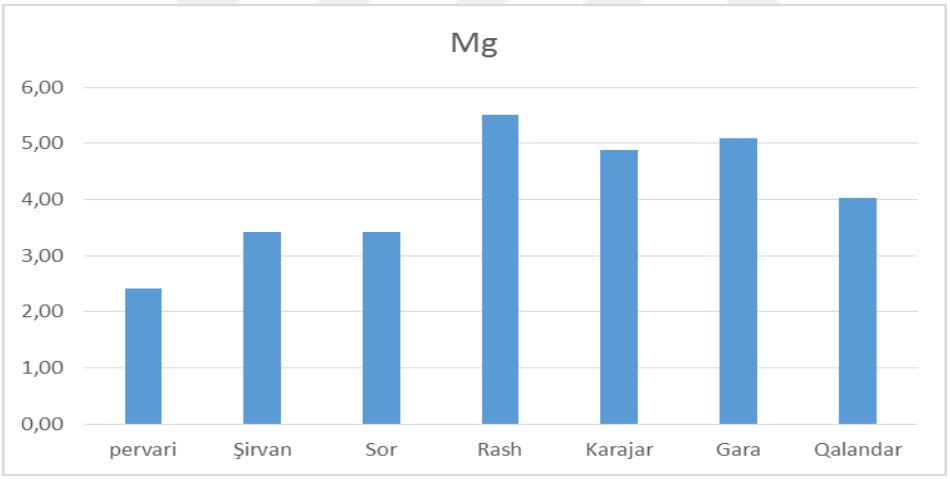



**Figure 4.4.** The concentration of Magnesium (Mg) in various sites locations [mg $g^{-1}$]

It can be seen from figure 4.4 that the variation is slight between the content of Mg in different site. It is obvious that the largest quantity of Mg was 5.51±0.14 mg $g^{-1}$ in Rash and the lowest was 2.42±0.03 mg $g^{-1}$ in parvari. However, (Nazk, 2013) found a content of 73.040 µg $g^{-1}$ in rhubarb collected in the Sulaimaniyah. This variation might be due the difference in the sample collection site.

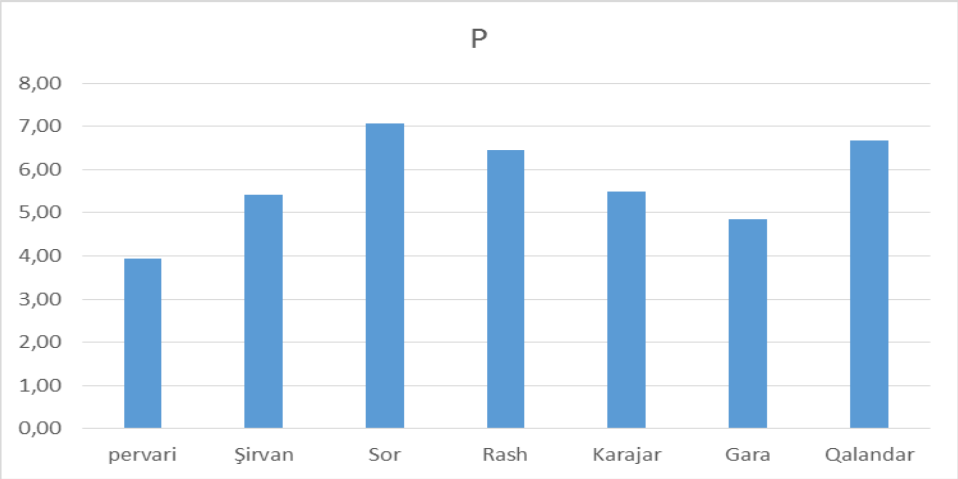



**Figure 4.5.** The concentration of Phosphor (P) in various locations [mg $g^{-1}$]

Figure 4.5 indicates the level of P in seven different locations in Kurdistan region of Iraq and Turkey. It is found that P is that the content of maximum of P is found in Sor 7.08±0.13 mg $g^{-1}$ and the lowest Be content is in the sample of Pervari 3.39±0.03 mg $g^{-1}$.

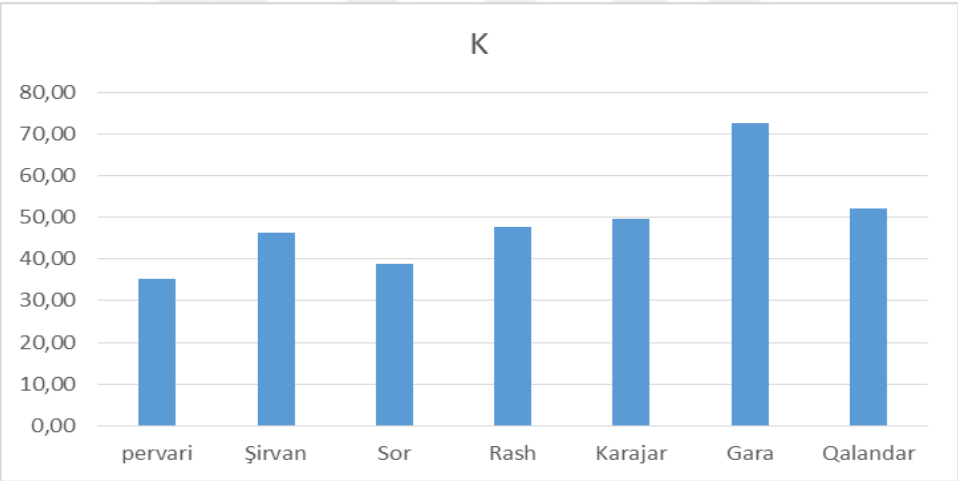



**Figure 4.6.** The concentration of Potassium (K) in various sites [mg $g^{-1}$]

It is discovered that the minimum is 35.20±0.39 mg $g^{-1}$ Parvari, while the maximum content of K is found in Gara 72.70±2.14 mg $g^{-1}$. In the study of (Nazk, 2013) discovered the 1186.5 mg $g^{-1}$, this might be due to the difference in the geographical location and the collection method.

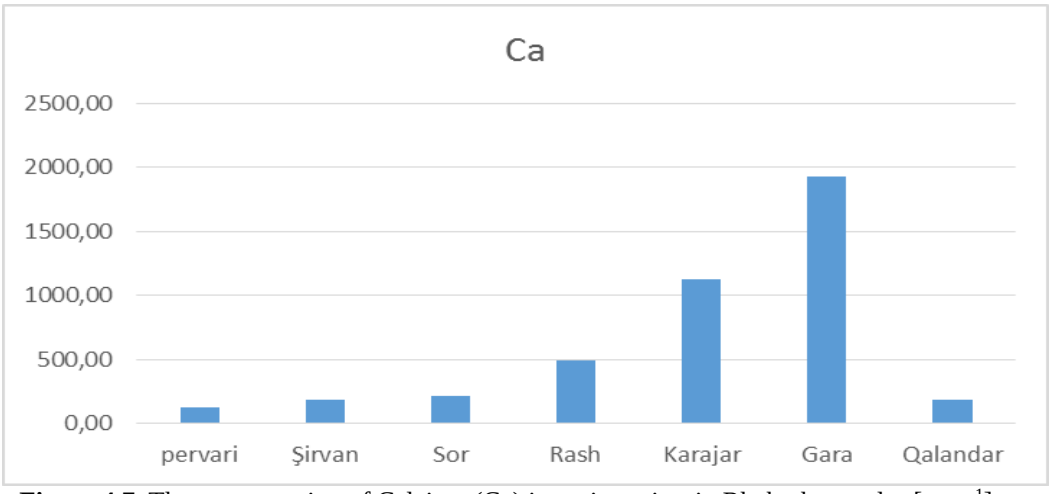



**Figure 4.7.** The concentration of Calcium (Ca) in various sites in Rhubarb samples [ng $g^{-1}$]

In Figure 4.7 revealed that while the maximum content of Ca is found in Gara 1929.43±31.93 ng $g^{-1}$ the minimum Ca content in Pervari region is 126.38±1.41 ng $g^{-1}$. However, (Nazk, 2013), found that Ca content of 65.63 ng $g^{-1}$, this might be due to the difference in the geographical location and the collection approach.

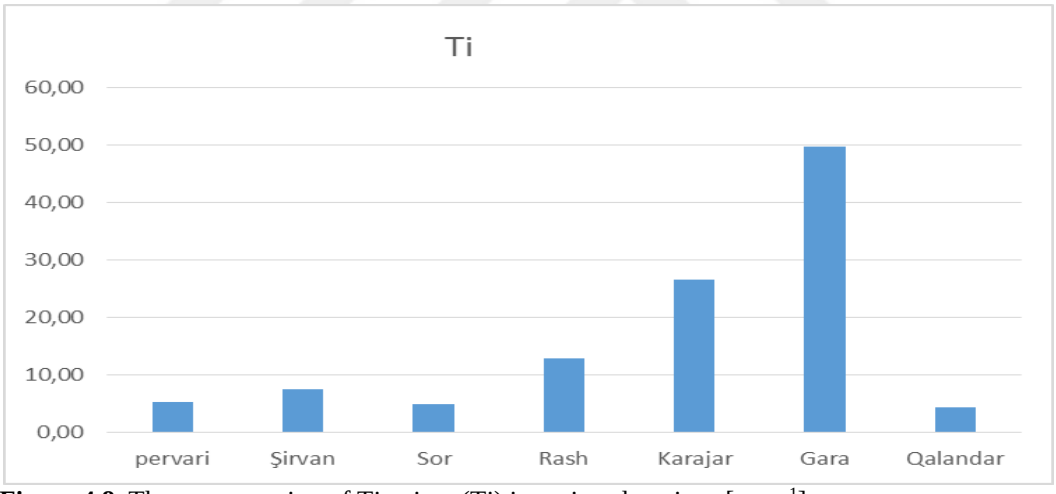



**Figure 4.8.** The concentration of Titanium (Ti) in various locations [µg $g^{-1}$]

It can be seen from figure 4.8., that that the variation are huge between the content of Mg in different site. It is obvious that the lowest quantity of Ti is 4.38±0.06 µg $g^{-1}$ in Qalandar and the biggest is 49.64±0.96 µ $g^{-1}$ in Gara.

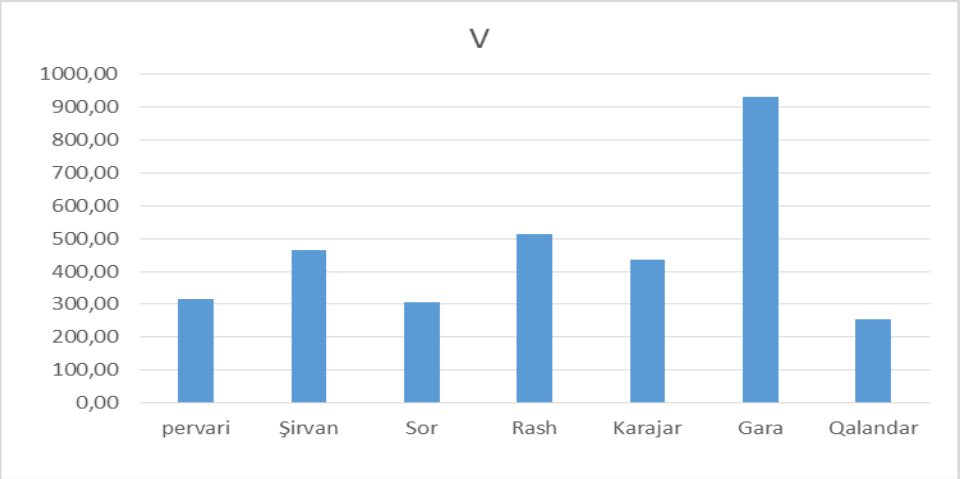



**Figure 4.9.** The concentration of Vanadium (V) in various locations [ng $g^{-1}$]

Figure 4.9 presented that there are variation between the content of V in various site. It is obvious that the lowest quantity of V is 255.69±3.59 ng $g^{-1}$ in Qalanda and the biggest is 932.45±22.17 ng $g^{-1}$ in Gara.

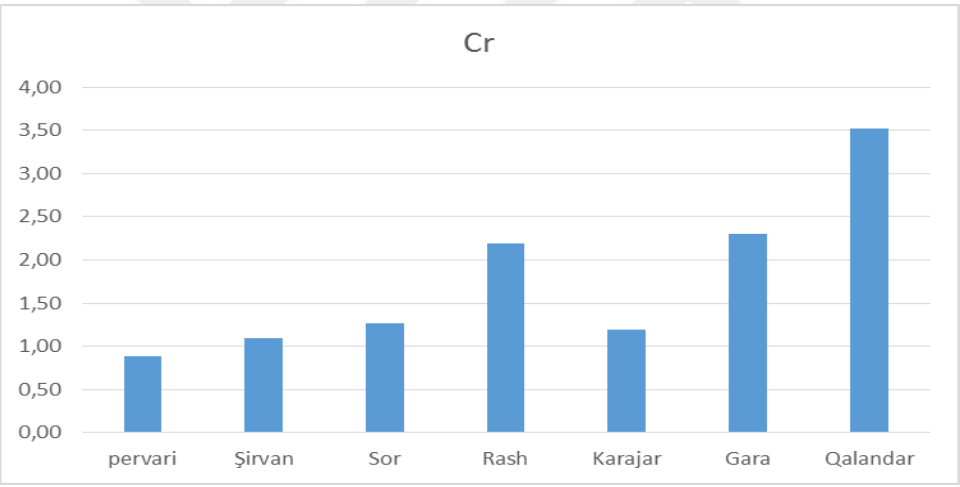



**Figure 4.10.** The level of Chrome (Cr) in various locations [µg $g^{-1}$]

It is uncovered that while the maximum content of Cr in Qalandar is 3.52±0.07 µg $g^{-1}$ the minimum Cr concentration in Parvari region is 0.89±0.02 µg $g^{-1}$ . However, (Nazk, 2013) discovered that Cr content of 0.0026 µg $g^{-1}$, this might be due to the difference in the geographical location and the collection approach.

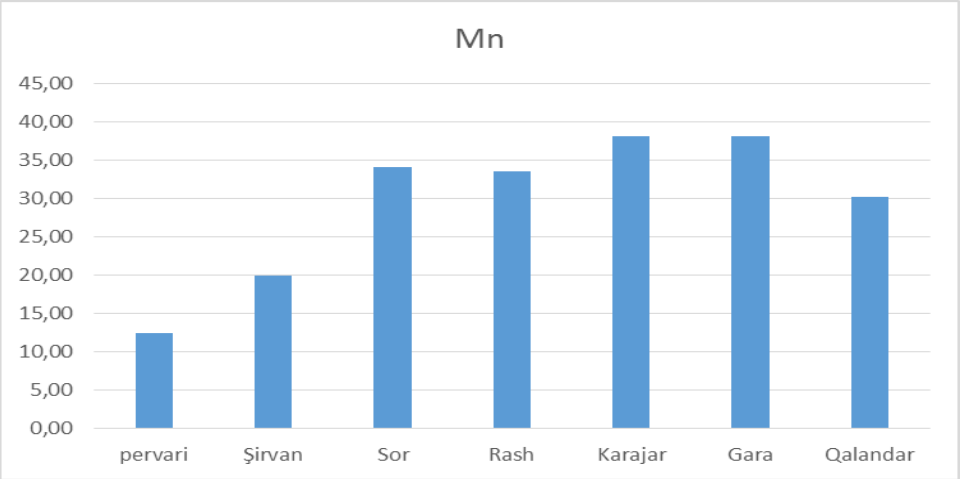



**Figure 4.11.** The Manganese (Mn) in various locations [µg $g^{-1}$]

The concentration of Mn in seven various locations in Kurdistan region of Iraq and Turkey are illustrated In Figure 4.11. It is found that as the maximum content of Mn in Karajar is 38.13±0.80 µg $g^{-1}$ the minimum Mn concentration in Pervari region is 12.46±0.02 µg $g^{-1}$. However, (Nazk, 2013), discovered that Cr content of 0.346 µg $g^{-1}$ in Sulaimaniyah mountains this might be due to the difference in the geographical location and the collection approach.

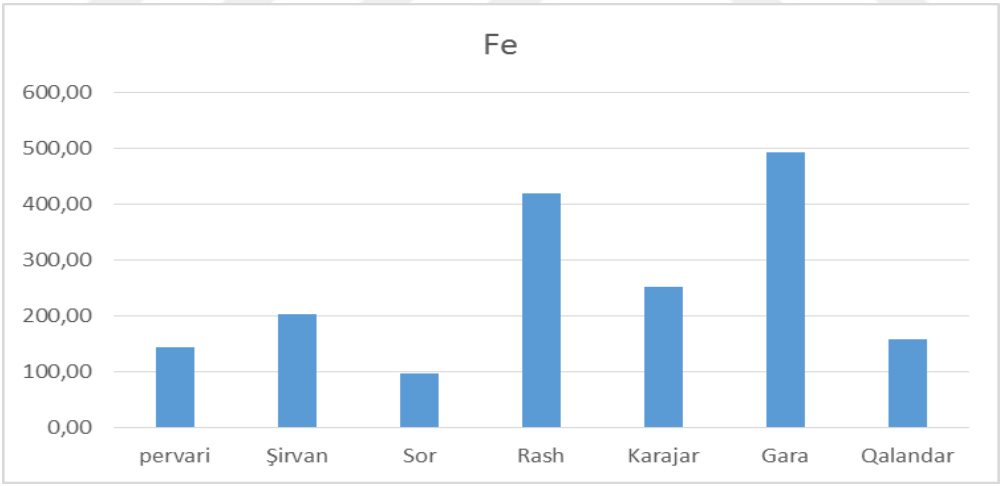



**Figure 4.12.** The Iron (Fe) in various locations [µg $g^{-1}$]

The level of Fe in seven different sites in Kurdistan region of Iraq and Turkey are described in Figure 4.12. The maximum content of Fe 493.19±5.05 µg $g^{-1}$ is found in Gara and the minimum Fe concentration in Sor region is 97.60±0.73 µg $g^{-1}$. However, (Nazk, 2013) discovered that the Fe content is 5.075 µg $g^{-1}$, this might be due to the variation in the geographical location and the collection method.

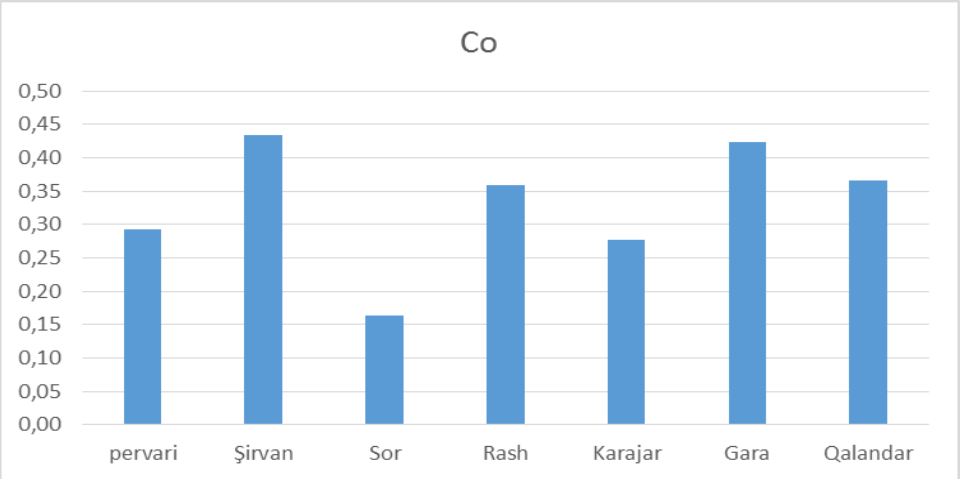



**Figure 4.13.** The Cobalt (Co) in various locations [µg $g^{-1}$]

The highest concentration of Co 0.43±0.01 µg $g^{-1}$ is found in Sirvan and the lowest Co concentration is in Sor region 0.16±0.00 µg $g^{-1}$. However, (Nazk, 2013), discovered that the Co content is 0.006 µg $g^{-1}$ and (Ipatioaiei D.C et al, 2014) also less than 0.01 µg $g^{-1}$, this might be due to the variation in the geographical location and the collection method.

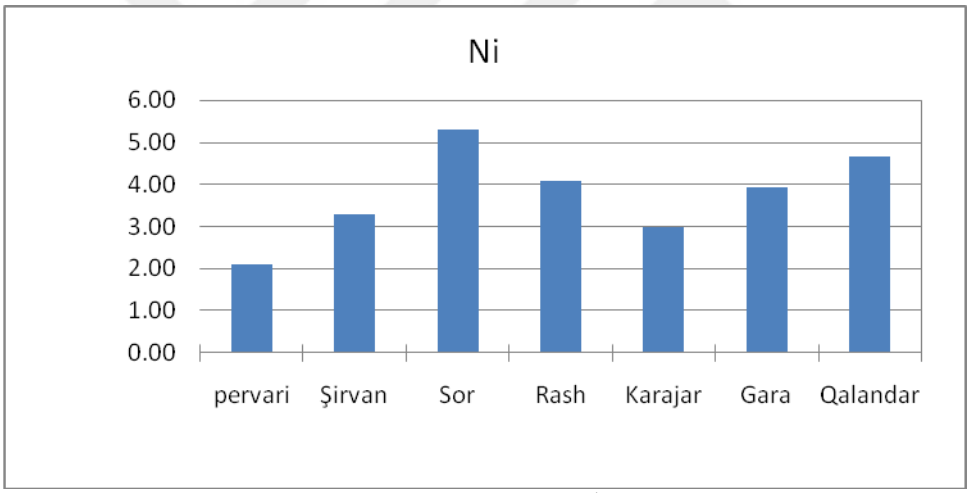



**Figure 4.14.** The nickel (Ni) in various locations [µg $g^{-1}$]

In this figure, it is clear that rhubarb gathered in the Sor region contain the highest concentration of Ni 5.31±0.05 µg $g^{-1}$ and the lowest Ni content is in the Pervari region 2.11±0.05 µg $g^{-1}$. In addition, Shirevan, Rash, kajara, Gara and Qalandar contain 3.30±0.14, 4.09±0.09, 2.99±0.04, 3.94±0.07 and 4.67±0.24 µg $g^{-1}$ respectively.

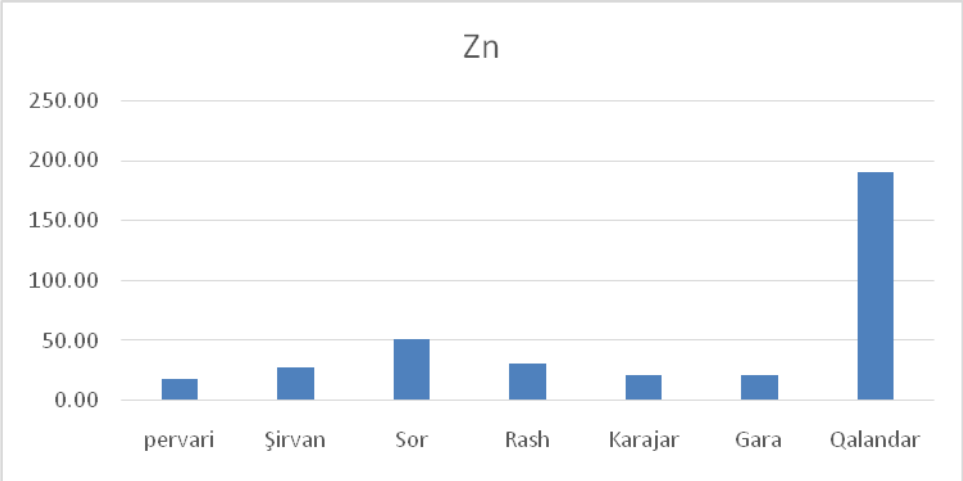



**Figure 4.15.** The zinc (Zn) in various locations [µg $g^{-1}$]

In this figure, the level of metal content in rhubarb samples collected in different sights. It is revealed that the concentration of Zn in Shirevan, Sor, Rash, kajara and Gara are 28.20±0.27, 30.77±0.44, 21.23±0.41, 21.25±0.08 and 190.65±1.43 µg $g^{-1}$ correspondingly. Furthermore, the highest concentration of zinc is in Qalandar while the lowest zinc is in the Pervari sample. However, (Igpatioaiei D.C et al, 2014) found that the average concentration of Zn in rhubarb was 175.81 µg $g^{-1}$ and the (Nazk, 2013) found 0.421 µg $g^{-1}$.

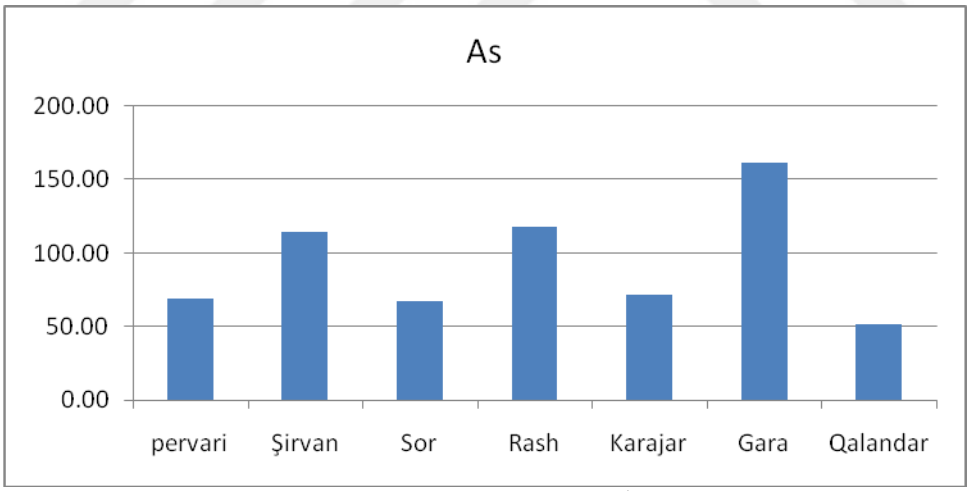



**Figure 4.16.** The Arsenic (As) in various locations [ng $g^{-1}$]

It can be seen that sample collected in Gara contained the maximum amount of As 161.11±15.34 ng $g^{-1}$ compared to the other investigated samples. However, the minimum concentration of As was recorded in rhubarb collected in Qalandar 51.04±15.94 ng $g^{-1}$. Furthermore, the level of As in the pervari, Shirevan, Sor, Rash and kajara were 68.84±20.87, 113.92±46.37, 67.15±22.04, 117.46±24.62 and 71.60±8.86 ng $g^{-1}$ correspondingly.

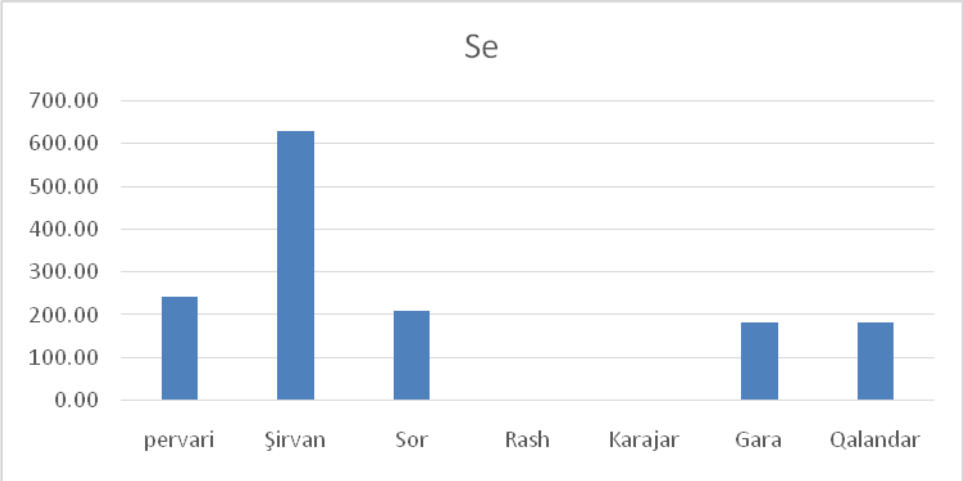



**Figure 4.17.** The Selenium (Se) in various locations [ng $g^{-1}$]

This figure demonstrates the metal content in rhubarb samples gathered in seven different regions. It is uncovered that Se did not detected in samples collected from Rash and Karajar regions.In addition, while samples collected from Shrivan and Qalandar contained the highest and the lowest Se 626.73±n.d. and 182.52±316.14 ng $g^{-1}$ respectively.

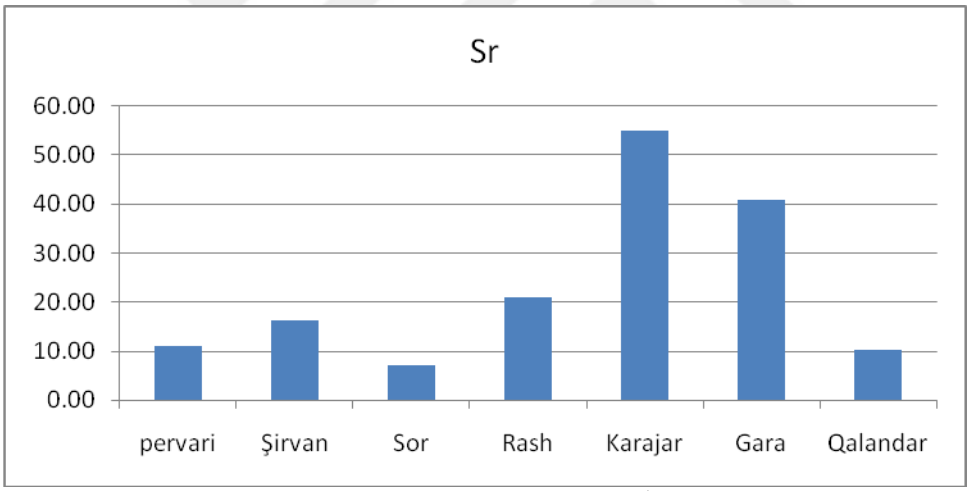



**Figure 4.18.** The Strontium (Sr) in various locations [µg $g^{-1}$]

In the figure 4.18 the Sr concentration in pervari, Şirvan, Sor, Rash, Karajar, Gara and Qalandar regions of Turkey and Kurdistan region of Iraq are examined. It is observed the concentration of Sr varied among the samples. The highest level of Sr was in the Karajar 54.71±0.33 µg $g^{-1}$ while the Sor region contained the lowest level of Sr 6.94±0.31 µg $g^{-1}$.

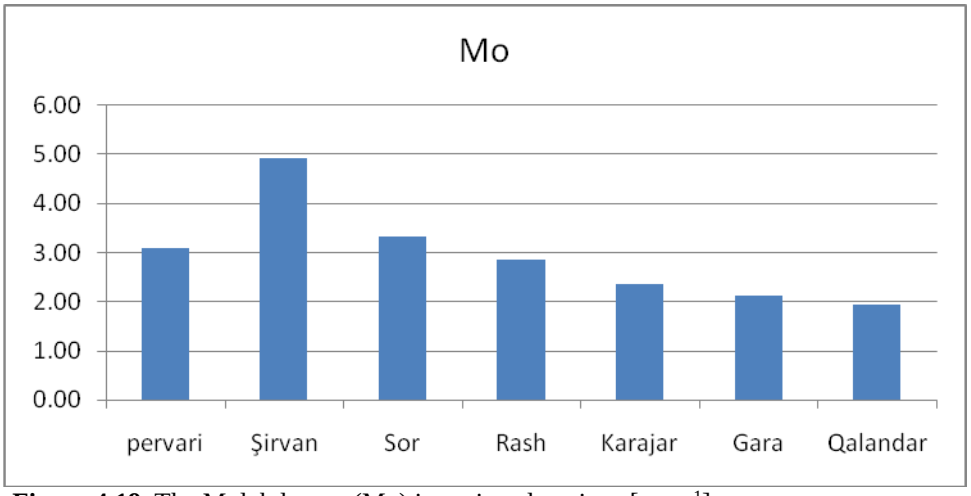



**Figure 4.19.** The Molybdenum (Mo) in various locations [µg $g^{-1}$]

The levels of Mo were varied across the samples collected in different regions. The concentration of Mo were 3.08±0.20, 4.91±0.46, 3.30±0.26, 2.83±0.25, 2.35±0.38, 2.12± 0.19and 1.93±0.27 µg $g^{-1}$ in pervari, Şirvan, Sor, Rash, Karajar, Gara and Qalandar respectively. Moreover, (Nazk, 2013) found that the concentration of Mo was 0.031 µg $g^{-1}$.

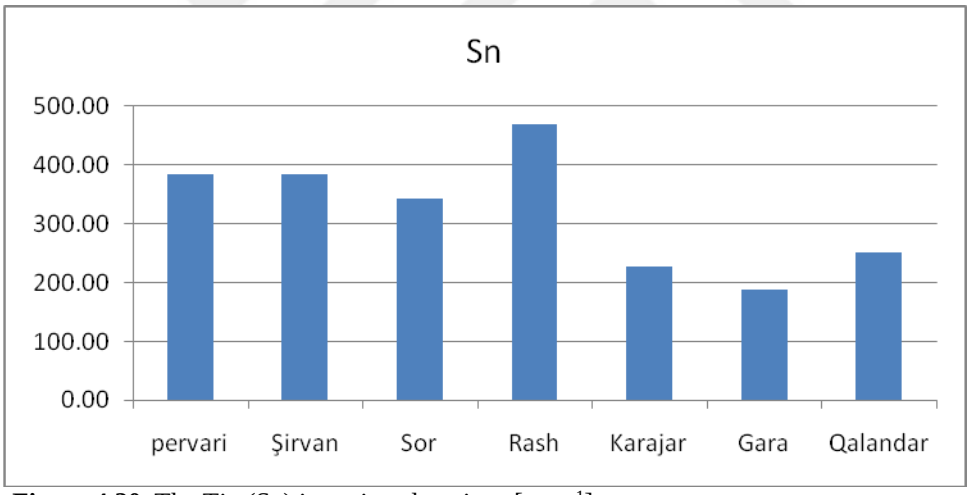



**Figure 4.20.** The Tin (Sn) in various locations [ng $g^{-1}$]

In this figure, the level of Sn in samples of rhubarb collected in pervari, Şirvan, Sor, Rash, Karajar, Gara and Qalandar. The highest concentration of Sn was found in Rash region 468.23±14.39 ng $g^{-1}$. However, the lowest level of Sn was recorded in Gara 186.89±12.76 ng $g^{-1}$. Furthermore, the amounts of Sn were 382.77±10.66, 382.54±47.18, 341.35±30.02, 226.35±14.82 and 249.42±16.66 ng $g^{-1}$ in pervari, Şirvan, Sor, Karajar and Qalandar respectively.

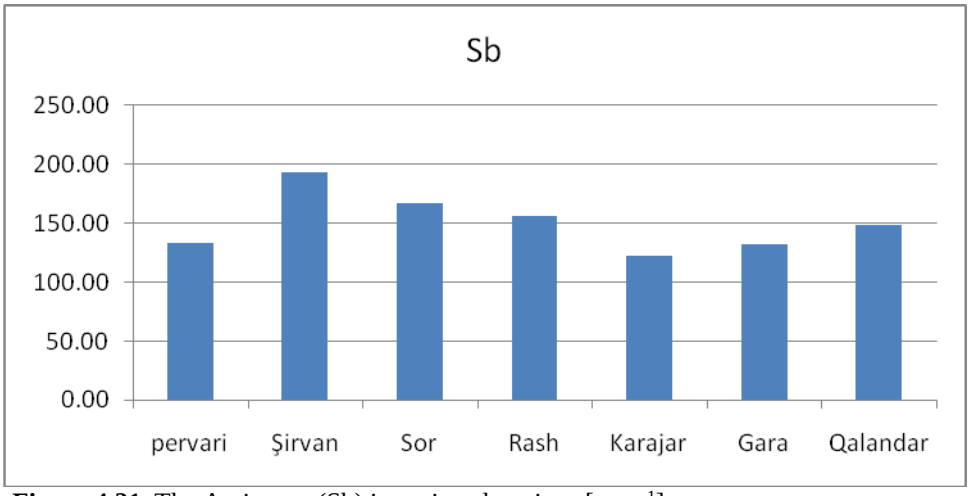



**Figure 4.21.** The Antimony (Sb) in various locations [ng $g^{-1}$]

The content of Sbin the rhubarb sample collected in pervari, Şirvan, Sor, Rash, Karajar, Gara and Qalandar are illustrated in figure 4.21. It is revealed that the rhubarb gathered from Shrivan region had the highest concentration of Sb 192.03±19.60 ng $g^{-1}$. Nonetheless, the lowest concentration of Sb was in Karajar sample 121.99±8.28 ng $g^{-1}$.

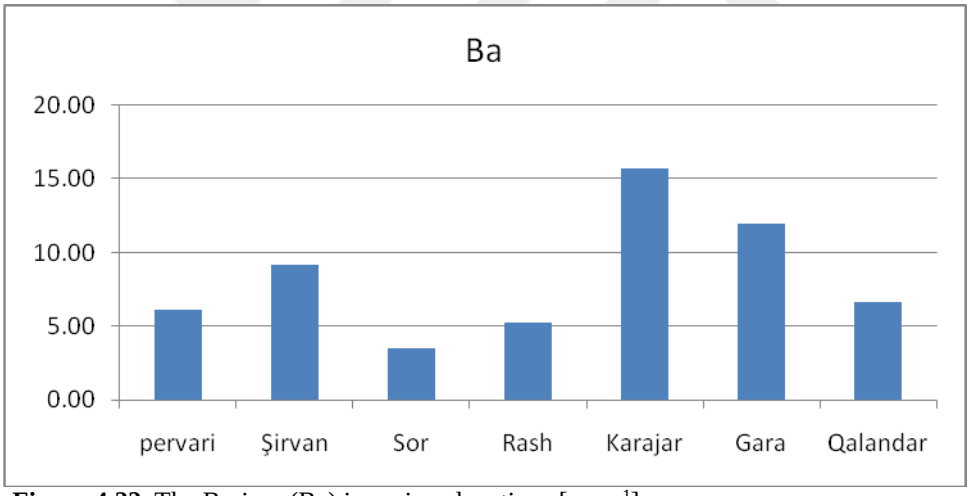



**Figure 4.22.** The Barium (Ba) in various locations [µg $g^{-1}$]

This figure displays the amount of Ba in the rhubarb sample gathered different regions.It is discovered that the rhubarb collected from Karajararea had the biggestlevel of Ba 15.64±0.21 µg $g^{-1}$. Nevertheless, the minimum amount of Ba was in Sor sample 3.49±0.12 µg $g^{-1}$ in addition, the content of Ba in samples from pervari, Şirvan, Rash, Gara and Qalandar were 6.07±0.08, 9.14±0.22, 5.20±0.16, 11.91±0.15 and 6.65±0.20 µg $g^{-1}$ respectively.

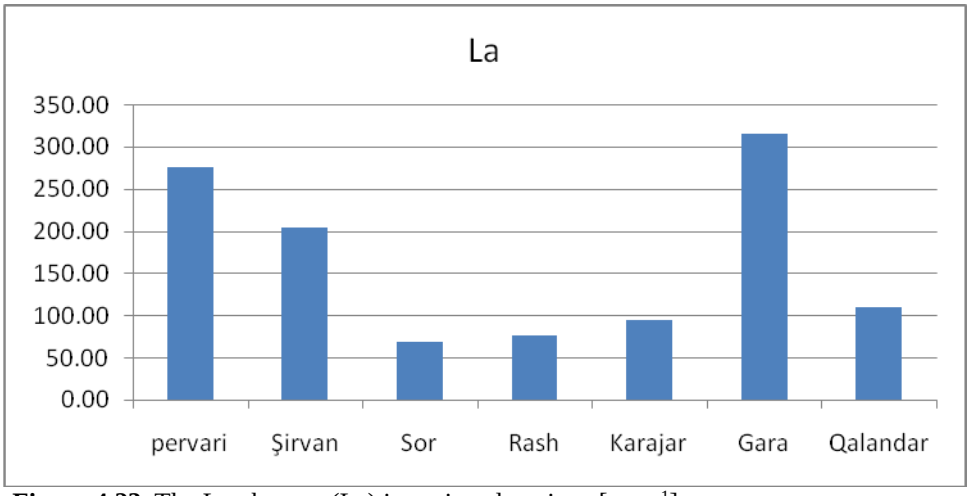



**Figure 4.23.** The Lanthanum (La) in various locations [ng $g^{-1}$]

Figure 4.23, displays the level of La in the rhubarb sample gathered different regions. It is found that the rhubarb sample from Gara area had the highest concentration of La 315.32±8.48 ng $g^{-1}$. However, the lowest level of La was in Sor sample 67.90±3.65 ng $g^{-1}$. Additionally, the concentration of La in samples from pervari, Şirvan, Rash, Kajara and Qalandar were 275.49±12.19, 203.77±8.35, 76.17±2.81, 94.62±3.24 and 109.03±0.83 ng $g^{-1}$ respectively.

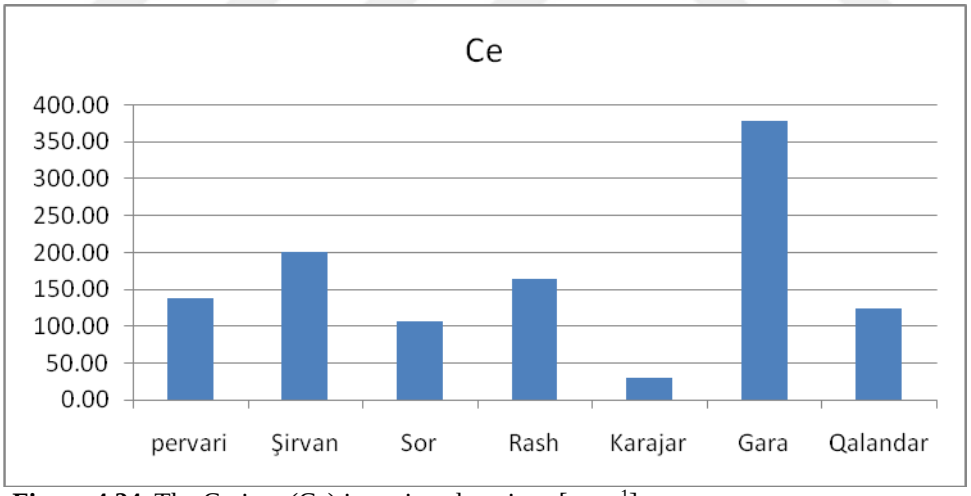



**Figure 4.24.** The Cerium (Ce) in various locations [ng $g^{-1}$]

The level of Ce in the rhubarb samples gathered various areas are described in figure 24. It is revealed that the sample of rhubarb from Gara area had the largest amount of Ce 378.03±8.11 ng $g^{-1}$. However, the lowest level of Ce was in Kajara sample 29.19±0.48 ng $g^{-1}$. Additionally, the concentration of La in samples from pervari, Şirvan, Rash, Sor and Qalandar were 136.66±4.54, 199.22±9.81, 105.90±6.15, 163.89±7.97 and 124.33±3.01 ng $g^{-1}$ respectively.

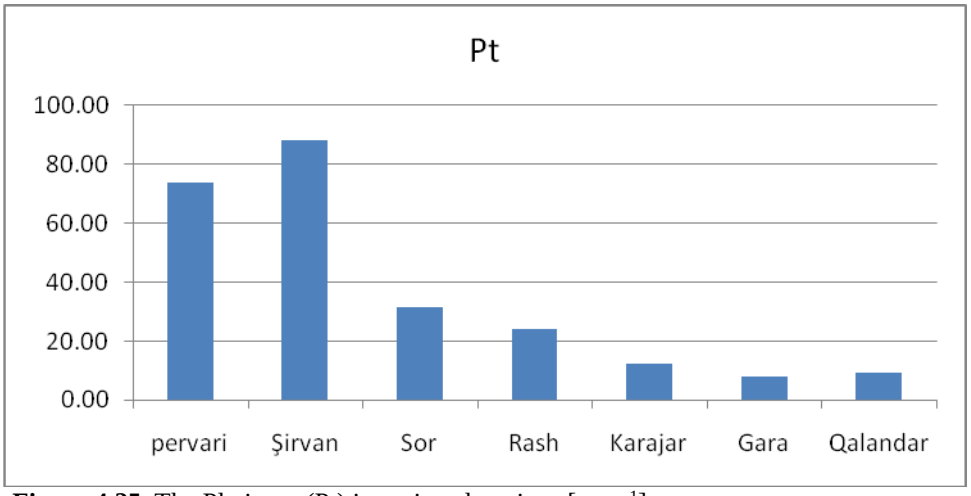



**Figure 4.25.** The Platinum (Pt) in various locations [ng $g^{-1}$]

Figure 4.25 demonstrates the level of Ce in the rhubarb samples gathered various areas. It is found that the sample of rhubarb from Shrivan area had the largest amount of Pt 87.98±7.29 ng $g^{-1}$. However, the lowest level of Pt was in Gara sample 7.99±0.25 ng $g^{-1}$. Additionally, the concentration of La in samples from pervari, Sor, Rash, Karajar, and Qalandar were 73.39±2.14, 31.46±2.39, 24.01±3.39, 12.28±0.44 and 9.13±0.75 ng $g^{-1}$ as a result.

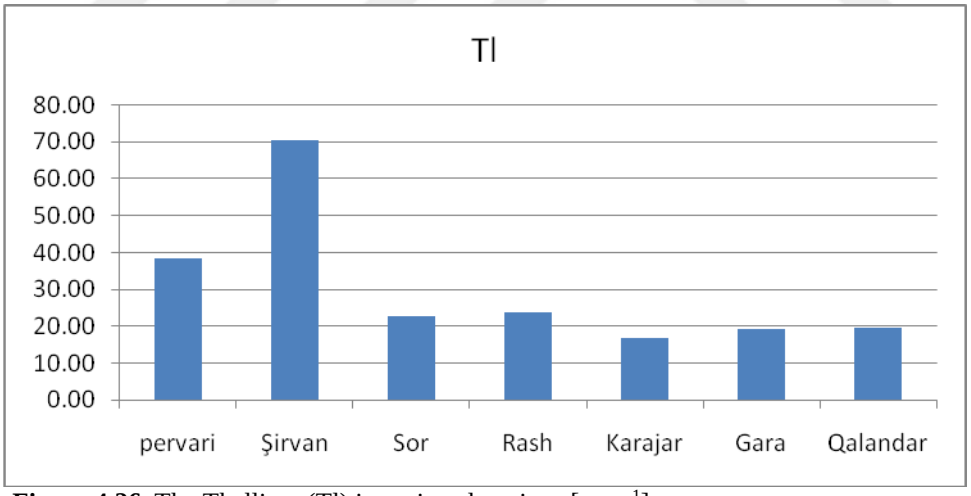



**Figure 4.26.** The Thallium (Tl) in various locations [ng $g^{-1}$]

The amount of Tl in the rhubarb sample gathered in pervari, Şirvan, Sor, Rash, Karajar, Gara and Qalandar are displayed in figure 4.26, the results found that the concentration of Tl in rhubarb from Shrivan region was the highest 70.24±0.21ng $g^{-1}$. Nonetheless, the lowest concentration of Tl was in Karajar sample 16.84±1.54 ng $g^{-1}$.

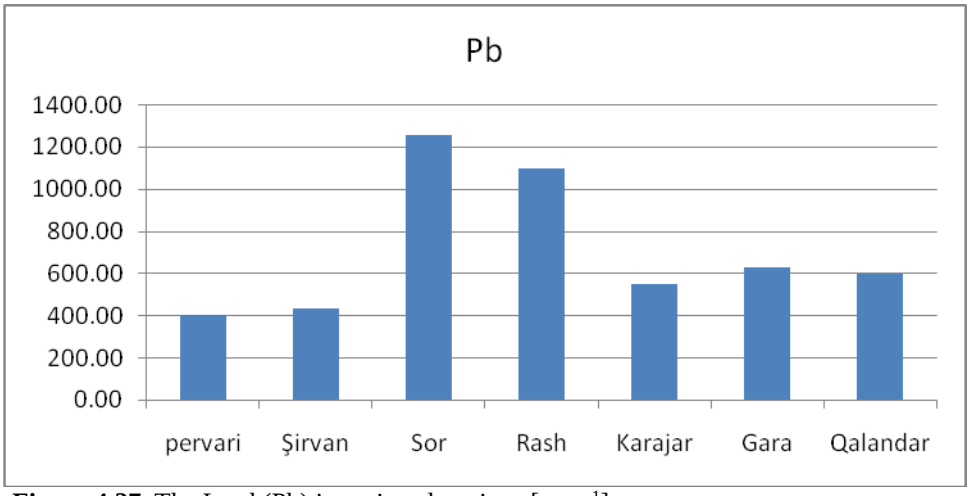



**Figure 4.27.** The Lead (Pb) in various locations [ng $g^{-1}$]

This figure displays the amount of Pb in the rhubarb sample gathered in pervari, Şirvan, Sor, Rash, Karajar, Gara and Qalandar. The results found that while the lowest concentration of Pb was in Pervari sample 401.53±9.39 ng $g^{-1}$, the concentration of Pb in rhubarb gathered from Sor region was the highest 1256.70±36.73 ng $g^{-1}$.

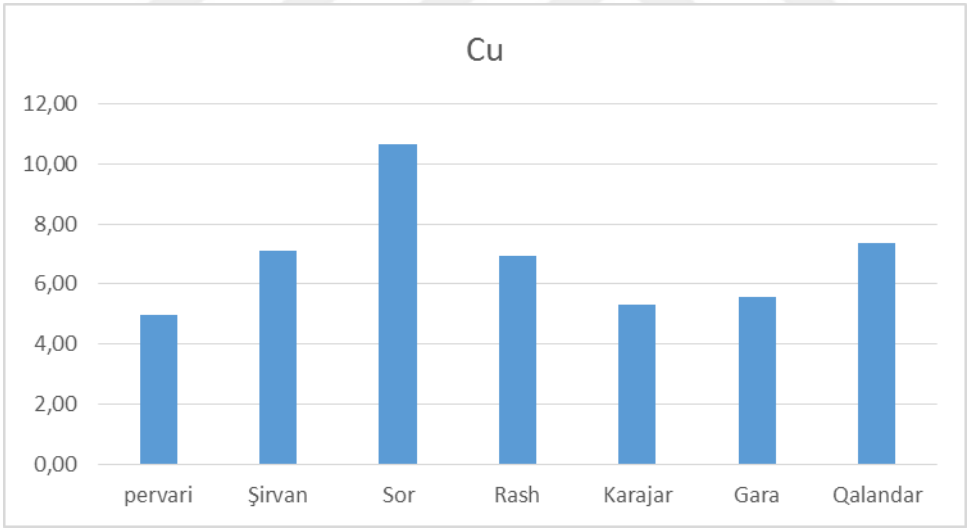



**Figure 4.28.** The Cupper (Cu) in various locations [µg $g^{-1}$]

Figure 4.28 describes the concentration of Cu in seven various sites in Kurdistan region of Iraq and Turkey. The highest concentration of Cu 10.67±0.15 µg $g^{-1}$ is found in Sor and the lowest Cu concentration is in Pavari region 4.96±0.07 µg $g^{-1}$. However, (Nazk, 2013) discovered that the Cu content is 1.69 ppm and (Ipatioaiei D.C et al, 2014) also recorded around 30 ppm, this might be due to the variation in the geographical location and the collection method.

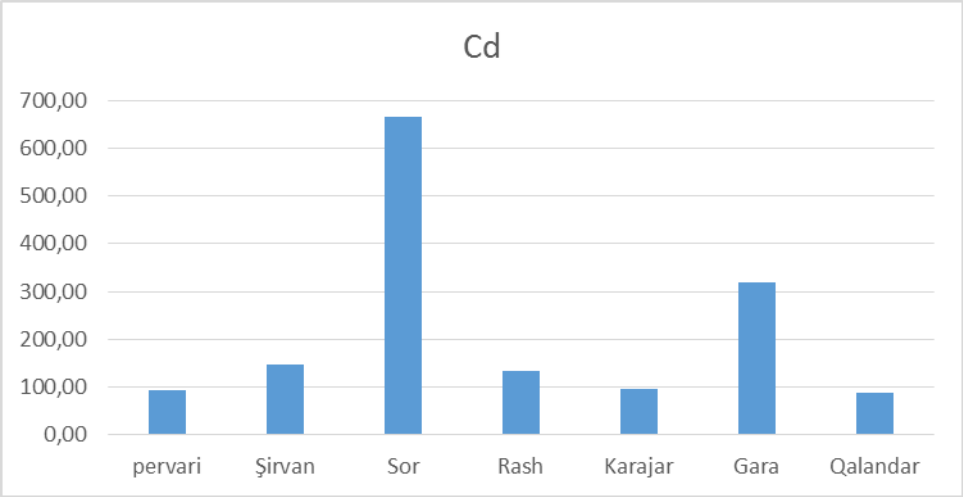



**Figure 4.29.** The Cadmium (Cd) in various locations [ng $g^{-1}$]

The concentration of Cd in seven various sites in Kurdistan region of Iraq and Turkey is illustrated in Figure 4.29. The highest concentration of Cd 666.33±85.77 ng $g^{-1}$ is found in Sor and the lowest Cd concentration is in Qalandar region 87.33±6.51 ng $g^{-1}$. However, (Nazk, 2013), discovered that the Cd content is 1.69 ppm and (Rai, L. C., Gaur, J. P., & Kumar, H. D., 1981) also recorded around 0.016- 0.025 ppm, this might be due to the variation in the geographical location and the collection method.

## 4.2. Antioxidant activity

**Table 4.3**. The average and the Standard deviation (STD) of the antioxidant activity

| Sample (Locality) | Absorbace1 | Absorbace 2 | Absorbace 3 | Average | Standard Deviation | % İnhibition |
|---|---|---|---|---|---|---|
| Gara | 0.344 | 0.351 | 0.0351 | 0.183 | 0.180 | 75.825 |
| Korajar | 0.154 | 0.133 | 0.139 | 0.107 | 0.011 | 85.894 |
| Sor | 0.122 | 0.127 | 0.135 | 0.096 | 0.007 | 87.285 |
| Rash | 0.127 | 0.13 | 0.122 | 0.095 | 0.004 | 87.450 |
| Qalandar | 0.129 | 0.13 | 0.12 | 0.095 | 0.006 | 87.450 |
| Pervari | 0.306 | 0.299 | 0.293 | 0.225 | 0.007 | 70.265 |
| Şirvan | 0.125 | 0.114 1 | 0.12 1 | 0.090 | 0.006 | 88.113 |

It can be seen from the results of analysis; DPPH fractions of 1 ml extract prepared with methanol were given. Accordingly, the highest % inhibition indicated by the sample gathered from Şirvan region (88.113%) and the lowest % inhibition was Pervari sample (70.265%).

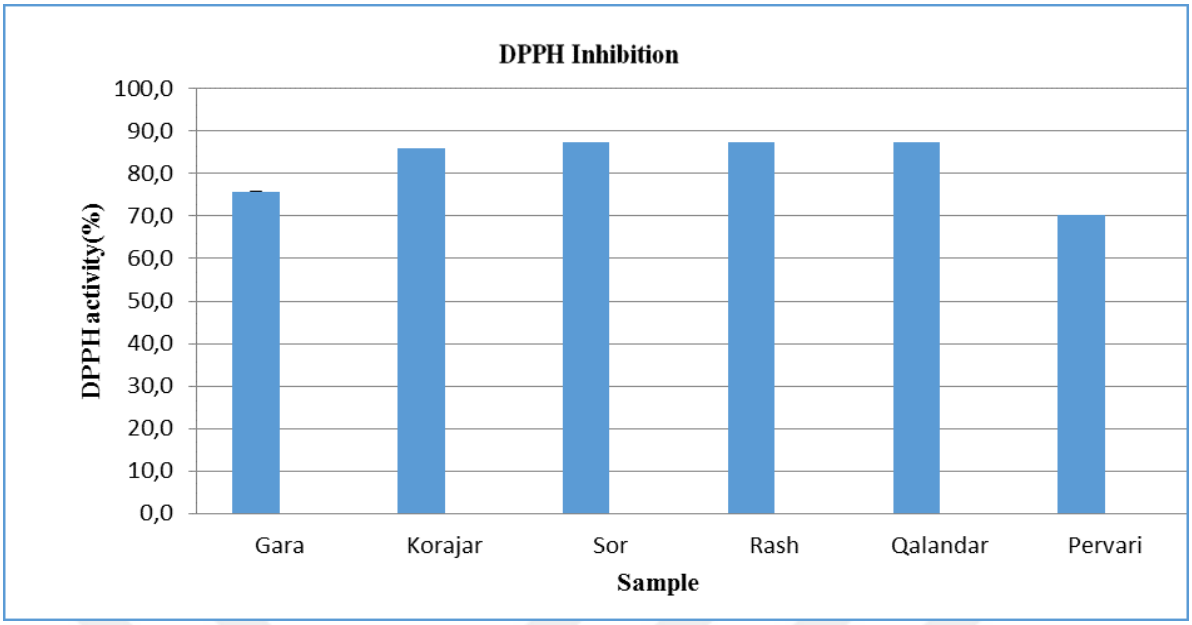



**Figure 4.30.** Activity DPPH of the inhibition.

As shows in figure 4.30, it can be seen that there are a slight different in the antioxidant activity between all the samples. It is also evident that the samples collected from Rash and Qaladar have the highest inhibition percentage among the group. (Öztürk et al, 2007) also stated that the rhubarb has high antioxidant activity.

**Table 4.4.** The average and standard deviation (STD) between Gallic acid equivalent

| Sample (Lokalite) | Absorbance 1 | Absorbance 2 | Absorbance 3 | Average | Gallic acid equivalent mg / ml | Standard Deviation |
|---|---|---|---|---|---|---|
| Gara | 0.088 | 0.092 | 0.09 | 0.090 | 84.015 | 0.002 |
| Korajar | 0.144 | 0.149 | 0.153 | 0.148667 | 128.419 | 0.005 |
| Sor | 0.478 | 0.483 | 0.512 | 0.491 | 387.526 | 0.018 |
| Rash | 0.432 | 0.458 | 0.469 | 0.453 | 358.765 | 0.019 |
| Qalandar | 0.329 | 0.361 | 0.349 | 0.346333 | 278.030 | 0.016 |
| Pervari | 0.089 | 0.092 | 0.094 | 0.091667 | 85.276 | 0.003 |
| Şirvan | 0.147 | 0.158 | 0.166 | 0.157 | 134.726 | 0.010 |

In accordance to the outcomes of analysis, the total phenolic concentration in 1 ml extract was given in the Table 4.4, Total phenolic concentrations were given as gallic acid equivalents. Gallic acid was calculated according to the standard regression curve of gallic acid equivalent. According to these results, the highest value showed sample collected from Sor region (387.526 µg / ml) and lowest value collected from Gara region (84.015µg / ml). Concentration results of the samples are plotted. (Takeoka et al, 2013)

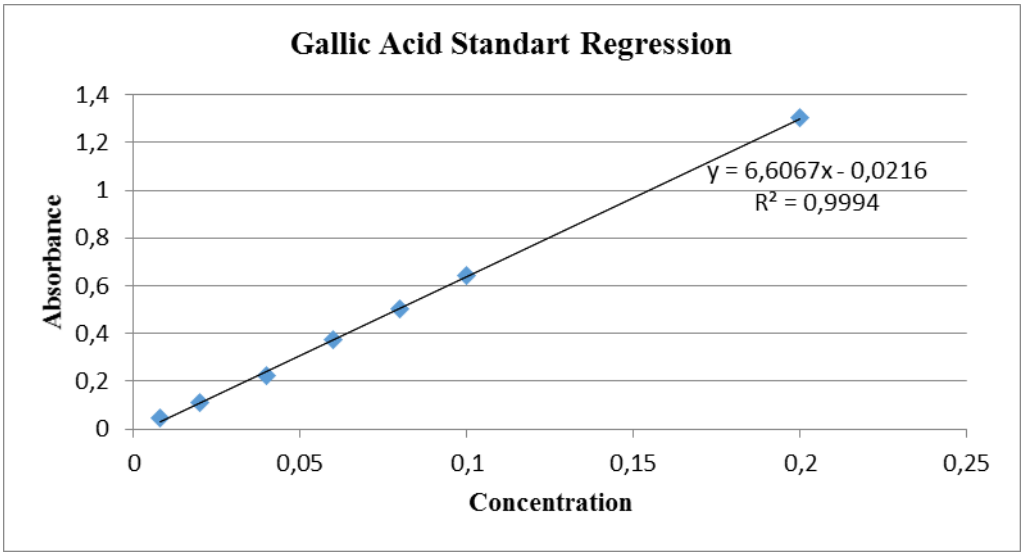



**Figure 4.31.** The relation between concentration of phenolic and the absorbance

As shows figure 4.31, the correlation between the concentration of phenolic and the absorbance is illustrated. It is clear that there is positive correlation between the concentration of phenolic compound and the absorbent activity (Eman et al, 2016).

**Table 4.5.** The total flavonoid analysis

| Sample (Lokalite) | Absorbance 1 | Absorbance 2 | Absorbance 3 | Average | concentration µg / ml | Standard Deviation |
|---|---|---|---|---|---|---|
| Gara | 0.022 | 0.031 | 0.021 | 0.025 | 69.98 | 0.006 |
| Korajar | 0.056 | 0.063 | 0.072 | 0.064 | 246.46 | 0.008 |
| Sor | 0.195 | 0.227 | 0.226 | 0.216 | 935.75 | 0.018 |
| Rash | 0.159 | 0.177 | 0.145 | 0.160 | 683.86 | 0.016 |
| Qalandar | 0.158 | 0.145 | 0.162 | 0.155 | 659.73 | 0.009 |
| Pervari | 0.032 | 0.039 | 0.03 | 0.034 | 110.71 | 0.005 |
| Şirvan | 0.146 | 0.051 | 0.052 | 0.083 | 333.94 | 0.055 |

The results of analysis the total flavonoid concentration in 1 ml extract was given in the Table 4.5, Total flavonoid concentrations are given as routine equivalents. Routine equivalence calculation is based on the routine standard regression curve given. According to these results, the highest value showed the sample collected from Sor region (935.75 µg / ml) and the lowest value collected from Gara region (69.98 µg / ml). Concentration results of the samples are plotted.

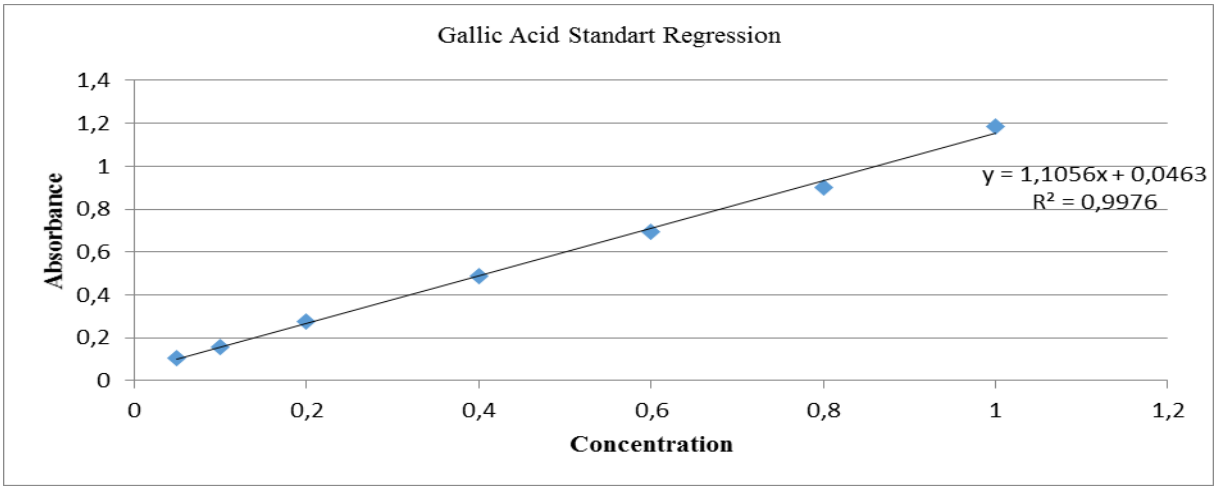



**Figure 4.32.** Gallic Acid Standart Regression

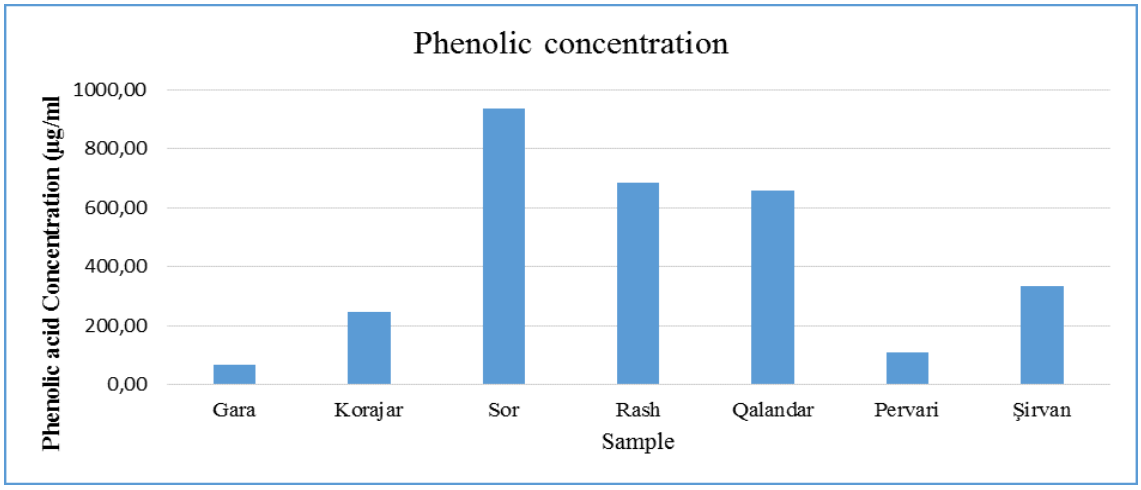



**Figure 4.33.** Phenolic concentration

### 4.2.1. Determination of antioxidant capacity using FRAP method.

**Table 4.6.** The average and Standard deviation of FRAP antioxidant analysis

| Sample (Lokalite) | Absorbance 1 | Absorbance 2 | Absorbance 3 | Average | Standard Deviation | Concentration mg/ml |
|---|---|---|---|---|---|---|
| Gara | 0.231 | 0.25 | 0.24 | 0.240 | 0.010 | 3.121 |
| Korajar | 0.397 | 0.423 | 0.473 | 0.431 | 0.039 | 7.303 |
| Sor | 1.219 | 1.288 | 1.231 | 1.246 | 0.037 | 25.175 |
| Rash | 1.124 | 1.232 | 1.2 | 1.185 | 0.055 | 23.845 |
| Qalandar | 0.892 | 0.92 | 0.912 | 0.908 | 0.014 | 17.763 |
| Pervari | 0.244 | 0.236 | 0.243 | 0.241 | 0.004 | 3.136 |
| Şirvan | 0.467 | 0.504 | 0.493 | 0.488 | 0.019 | 8.553 |

According to the analysis results, FRAP antioxidant capacities (total antioxidant amount) in 1 ml extract were given to Table 4.6, which extracts prepared with methanol. FRAP antioxidant capacities are given as $FeSO_4$ equivalents. The antioxidant capacity results of the samples are given in graphics.

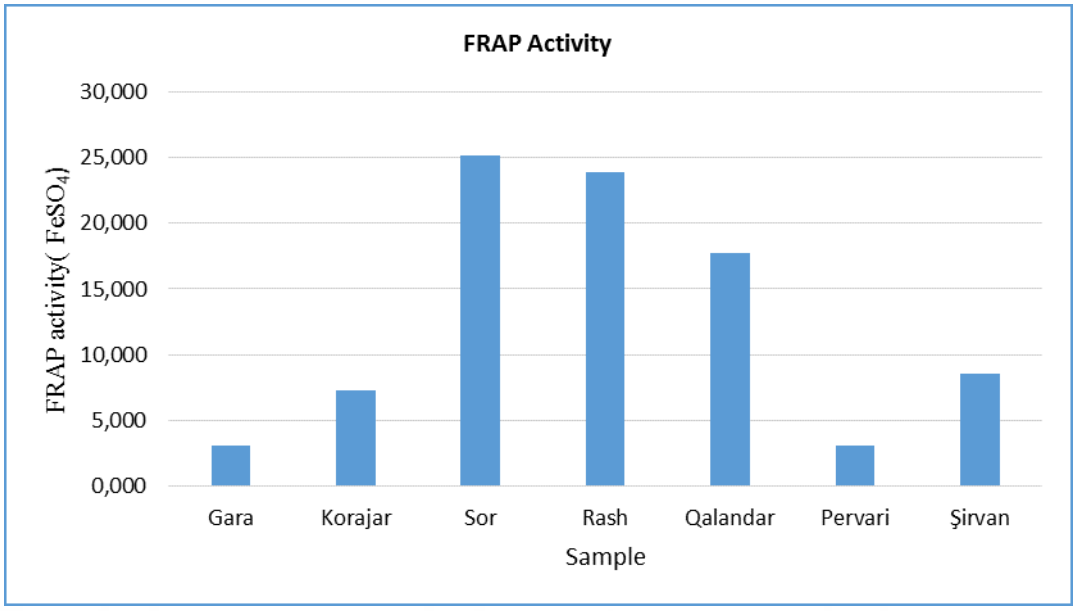



**Figure 4.34.** FRAP activity ($FeSO_4$)

Calculation of the $FeS0_4$ equivalent is based on the FRAP standard regression curve given in figure 4.34. According to these results, the highest antioxidant effect was obtained from the Sor region (25.175 mg / ml) and the lowest antioxidant effect from the Gara region (3.121 mg / ml).

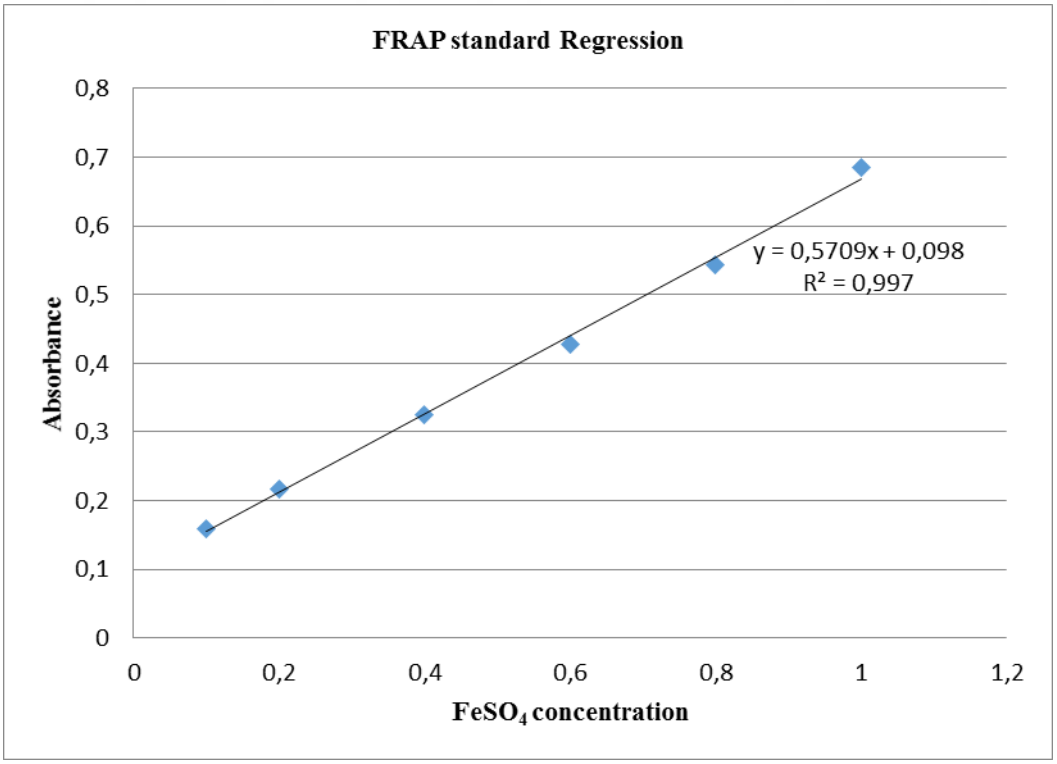



**Figure 4.35.** FRAP Standard regression

### 4.2.2. Iron Chelating

**Table 4.7.** Iron chelating average and standard deviation

| Sample (Lokalite) | Absorbance1 | Absorbance 2 | Absorbance3 | Average | % Iron Chelating | Standard Deviation |
|---|---|---|---|---|---|---|
| Gara | 0.026 | 0.022 | 0.029 | 0.026 | 95.175 | 0.004 |
| Korajar | 0.057 | 0.077 | 0.059 | 0.064 | 90.183 | 0.011 |
| Sor | 0.156 | 0.147 | 0.157 | 0.153 | 73.877 | 0.006 |
| Rash | 0.173 | 0.166 | 0.173 | 0.171 | 71.215 | 0.004 |
| Qalandar | 0.122 | 0.116 | 0.112 | 0.117 | 81.364 | 0.005 |
| Pervari | 0.02 | 0.015 | 0.016 | 0.017 | 97.338 | 0.003 |
| Şirvan | 0.049 | 0.056 | 0.049 | 0.051 | 91.847 | 0.004 |

According to the analysis results, in extracts prepared with methanol, iron chelating capacity of one milliliter extracts given in the table. According to the results, the highest iron chelating capacity (97.338%) collected from the Pervari Region and the lowest iron chelating capacity (71.215%) collected from the Rash region. The iron chelating capacity of the samples is given in graphical form.

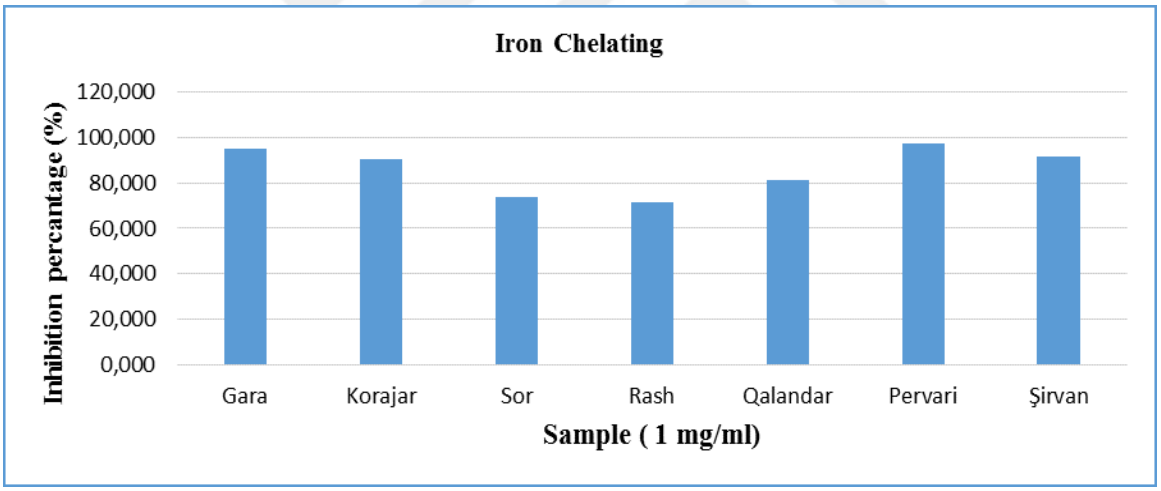



**Figure 4.36.** Iron Chelating

### 4.3. Phenolic compounds

On the basis of the results of quantitative analysis, the major phenolic compounds were garlic acid 834.740, 1079.160, 719.240, 796.100, 410.480, 2025.970 and 972.680in Pervari, Shirvan, Sor, Rash, Korajar, Gara and Qalandar respectively. In addition, several of the mentioned compounds such as fisetin, luteolin, myricetin, kaempferol and pyrocatecholin the table were not detected by the device. In addition, the content of quinic acids 881.300, 1361.860, 1841.990, 541.460, 1487.370, 363.410 and 967.810 in Shirvan, Sor, Rash, Korajar, Gara and Qalandar accordingly. In different research, (Eman et al, 2016) the chemical

composition of Rhubarbs roots was comprehensively and they discovered that, 37 phenolic compounds were recognized.Some of the composites comprisesennosides, anthraquinones, stilbenes, glucose gallates, naphthalenes, and catechins. Furthermore, in (Eman et al, 2016) the highest phenolic compound was vanilic acid while in this study the highest phenolic composites is Gallic acid. As it is seen in table Table 4.8, some phenolic compounds were not detected by the device. The undetected compounds were Fisetin, Luteolin, Myricetin, Kaempferol, o-coumaric acid, Apigenin, Chrysin, Liquiritigenin, Hesperetin and Pyrocatechol. Furthermore, (Krafczyk et al, 2008) studied the phenolic composition by the method of HPLC in rhubarb and discovered 14 following compounds: in the rhubarb found about 14 compounds were detected as follows: two stilbenes (trans-rhapontigenin, trans-desoxyrhapontigenin), Five stilbene glycosides (trans-rhaponticin, cis- and transdesoxyrhaponticin, transresveratrol-4-O-D-glucopyranoside, trans-piceatannol-3- O-D-glucopyranoside) and seven flavonoids [rutin, quercetin-3-O-glucuronide, isovitexin, 6,8-di-C-Dglucosylapigenin, schaftoside, isoschaftoside, (+)- catechin.

**Table 4.8.** The mean value of the phenolic compound ( µg $g^{-1}$)

| Analyte | Pervari | Şirvan | Sor | Rash | Karajar | Gara | Qalandar |
|---|---|---|---|---|---|---|---|
| Coumarin | 8.330 | 5.530 | 9.890 | 7.750 | 6.030 | 4.950 | 9.310 |
| Hesperidin | 24.510 | 2.660 | 23.640 | 6.070 | 1.910 | N.D | N.D |
| p-coumaric acid | 2.660 | 1.760 | 4.040 | 7.660 | 36.870 | 96.960 | 21.400 |
| o-coumaric acid | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Gallic acid | 834.740 | 1079.160 | 719.240 | 796.100 | 410.480 | 2025.970 | 972.680 |
| Caffeic acid | 0.900 | 0.140 | 0.130 | 0.140 | 1.560 | 4.850 | 1.480 |
| Vanillic acid | 8.850 | 12.200 | 6.500 | 10.800 | 10.130 | 12.270 | 127.280 |
| Salicylic acid | N.D | N.D | 1.430 | 0.230 | N.D | N.D | 1.310 |
| Quinic acid | 881.300 | 1361.860 | 1841.990 | 541.460 | 1487.370 | 363.410 | 967.810 |
| 4-OH-Benzoic acid | 4.600 | 1.250 | 1.400 | N.D | N.D | 1.070 | N.D |
| tr-Ferulic acid | 1.160 | 2.920 | 0.950 | 1.060 | 33.270 | 73.910 | 71.170 |
| Chlorogenic acid | 0.020 | N.D | 0.020 | N.D | N.D | N.D | N.D |
| Rosmarinic acid | 73.590 | N.D | 2.380 | N.D | N.D | N.D | N.D |
| Protocatechuic acid | 2.700 | 3.530 | 3.290 | 3.580 | 3.760 | 5.950 | 6.730 |
| Cinnamic acid | 71.840 | 54.270 | 118.750 | 125.480 | 65.660 | 66.800 | 90.400 |
| Sinapinic acid | N.D | N.D | N.D | N.D | N.D | N.D | 3.650 |
| Fumaric acid | 3.500 | 2.210 | 2.640 | 1.820 | 2.790 | 2.720 | 1.690 |
| Vanillin | N.D | N.D | N.D | N.D | N.D | N.D | 1.040 |
| Pyrocatechol | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Malic acid | 3311.690 | 5438.710 | 8495.840 | 3882.010 | 10552.470 | 4656.310 | 15721.900 |
| Syringic acid | N.D | N.D | 5.860 | 3.780 | 2.590 | 6.750 | 3.020 |
| Hesperetin | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Naringenin | 2.480 | N.D | N.D | 1.120 | N.D | 96.720 | 2.060 |
| Rutin | 240.650 | 380.150 | 677.460 | 926.100 | 185.180 | 76927.450 | 545.450 |
| Quercetin | 45.030 | 47.310 | 87.080 | 145.450 | N.D | N.D | N.D |
| Quercitrin | N.D | N.D | N.D | N.D | N.D | 5.200 | N.D |
| Apigenin | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Chrysin | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Liquiritigenin | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Isoquercitrin | 3.100 | 5.960 | 8.130 | 22.060 | 1.980 | 1886.910 | 6.360 |
| Cosmosiin | 1.780 | N.D | 0.190 | N.D | N.D | N.D | N.D |
| Rhoifolin | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Nicotiflorin | 8.780 | 15.270 | 21.330 | N.D | N.D | 17.010 | N.D |
| Fisetin | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Luteolin | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Myricetin | N.D | N.D | N.D | N.D | N.D | N.D | N.D |
| Kaempferol | N.D | N.D | N.D | N.D | N.D | N.D | N.D |

**Table 4.9.** The standard deviation value of the phenolic compound

Phenolic compounds Standart deviation (±)

| Analyte | pervari | Şirvan | Sor | Rash | Karajar | Gara | Qalandar |
|---|---|---|---|---|---|---|---|
| Coumarin | 0.002 | 0.001 | 0.002 | 0.002 | 0.001 | 0.001 | 0.002 |
| Hesperidin | 0.006 | 0.001 | 0.006 | 0.002 | 0.001 | N.D. | N.D. |
| p-coumaric acid | 0.001 | 0.001 | 0.002 | 0.004 | 0.019 | 0.050 | 0.011 |
| o-coumaric acid | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Gallic acid | 0.235 | 0.304 | 0.203 | 0.225 | 0.116 | 0.571 | 0.274 |
| Caffeic acid | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.002 | 0.001 |
| Vanillic acid | 0.004 | 0.006 | 0.003 | 0.005 | 0.005 | 0.006 | 0.065 |
| Salicylic acid | N.D. | N.D. | 0.000 | 0.000 | N.D. | N.D. | 0.000 |
| Quinic acid | 0.072 | 0.112 | 0.151 | 0.044 | 0.122 | 0.030 | 0.079 |
| 4-OH-Benzoic acid | 0.001 | 0.000 | 0.000 | N.D. | N.D. | 0.000 | N.D. |
| tr-Ferulic acid | 0.001 | 0.001 | 0.001 | 0.000 | 0.001 | 0.016 | 0.037 |
| Chlorogenic acid | 0.000 | 0.000 | N.D. | 0.000 | N.D. | N.D. | N.D. |
| Rosmarinic acid | 0.052 | 0.052 | N.D. | 0.002 | N.D. | N.D. | N.D. |
| Protocatechuic acid | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 |
| Cinnamic acid | 0.010 | 0.010 | 0.008 | 0.017 | 0.018 | 0.009 | 0.010 |
| Sinapinic acid | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Fumaric acid | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Vanillin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Pyrocatechol | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Malic acid | 0.374 | 0.374 | 0.615 | 0.960 | 0.439 | 1.192 | 0.526 |
| Syringic acid | N.D. | N.D. | N.D. | 0.001 | 0.001 | 0.001 | 0.002 |
| Hesperetin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Naringenin | 0.001 | 0.001 | N.D. | N.D. | 0.001 | N.D. | 0.050 |
| Rutin | 0.038 | 0.038 | 0.060 | 0.108 | 0.147 | 0.029 | 12.231 |
| Quercetin | 0.020 | 0.020 | 0.021 | 0.039 | 0.064 | N.D. | N.D. |
| Quercitrin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 0.104 |
| Apigenin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Chrysin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Liquiritigenin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Isoquercitrin | 0.000 | 0.000 | 0.001 | 0.001 | 0.003 | 0.000 | 0.251 |
| Cosmosiin | 0.001 | 0.001 | N.D. | 0.000 | N.D. | N.D. | N.D. |
| Rhoifolin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Nicotiflorin | 0.002 | 0.002 | 0.004 | 0.006 | N.D. | N.D. | 0.005 |
| Fisetin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Luteolin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Myricetin | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| Kaempferol | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |

### 4.4. Correlation analysis of elements in rhubarb

Correlation analysis has been found to be very important in some plant surveys conducted by Kara and his colleagues (Kara, 2009; Karadaş & Kara, 2012; Tokalıoğlu, 2012). The correlation analysis was applied to the analysis of the elements in Rhubarb of Kurdish Region of Iraq and Siirt regions of Turkey.

Correlation coefficients between the elements analyzed in Rhubarb plants were calculated in the form of correlation matrix and given in Table 4.10. The element to element correlation data in terms of linear correlation coefficient values that are significant at 95% and 99% confidence level was examined. At 99% confidence level, the pairs of Ti-Ca, (0.918), V-Ti (0.913), As–V (0.938), Se–Be (0.916), Ba-Sr (0.924), Ti-Se (0.904), Ti–Mo (0.917), Ti–Pt (0.916) and Pb–Na (0.918) showed very high and significant correlations. In addition, the pairs of Mg–Li (0.877), V-Ca (0.879), Fe–V (0.889), Ni–P (0.889) and Ba–B (0.879) showed high significant correlations at 99% confidence level. The pairs of Ca–K (0.858), Ti–K (0.866), V-K (0.846), Mn–Mg (0.821), Cu–Na (0.874), Cu–P (0.815), Cu–Ni (0.832), As–Fe (0.862), Sr-B (0.856), Sr–Ca (0.827), Sr–Ti (0.801), Mo–Se (0.806), Cd-Na (0.823), Sb–Mo (0.823), Ce–V (0.844) and Ce–As (0.865)showed high correlations at 95% confidence level. In addition, the pairs of Ca–B (0.763), Fe–Li (0.788), Fe–K (0.780), Fe–Ca (0.778), Fe–Ti (0.798), Zn–Cr (0.798), Sb–Be (0.750) and Pt–Se (0.779) show high correlations at 95% confidence level. Correlation matrix for the element concentrations in Rhubarb samples are shown in Table 1.

**Table 4.10.** Correlation matrix for the element concentrations in spa samples.

| | Li | Be | B | Na | Mg | P | K | Ca | Ti | V | Cr | Mn | Fe | Co | Ni | Cu | Zn | As | Se | Sr | Mo | Cd | Sn | Sb | Ba | La | Ce | Pt | Tl | Pb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Li | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Be | -0.658 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| B | 0.546 | 0.013 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Na | 0.100 | -0.055 | -0.193 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mg | **0.877**$^{**}$ | -0.429 | 0.662 | 0.118 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | | |
| P | 0.026 | 0.065 | -0.034 | 0.706 | 0.302 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | |
| K | 0.424 | 0.004 | 0.741 | -0.222 | 0.674 | -0.084 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | |
| Ca | 0.624 | -0.352 | **0.763**$^{*}$ | -0.174 | 0.653 | -0.282 | **0.858**$^{*}$ | 1.000 | | | | | | | | | | | | | | | | | | | | | | |
| Ti | 0.602 | -0.308 | 0.746 | -0.200 | 0.623 | -0.339 | **0.866**$^{*}$ | **0.996**$^{**}$ | 1.000 | | | | | | | | | | | | | | | | | | | | | |
| V | 0.559 | -0.117 | 0.611 | -0.081 | 0.583 | -0.337 | **0.846**$^{*}$ | **0.879**$^{**}$ | **0.913**$^{**}$ | 1.000 | | | | | | | | | | | | | | | | | | | | |
| Cr | 0.023 | 0.066 | 0.133 | -0.139 | 0.450 | 0.436 | 0.532 | 0.137 | 0.117 | 0.102 | 1.000 | | | | | | | | | | | | | | | | | | | |
| Mn | 0.635 | -0.407 | 0.636 | 0.376 | **0.821**$^{*}$ | 0.526 | 0.596 | 0.642 | 0.582 | 0.418 | 0.394 | 1.000 | | | | | | | | | | | | | | | | | | |
| Fe | **0.788**$^{*}$ | -0.338 | 0.543 | -0.090 | **0.811**$^{*}$ | -0.181 | **0.780**$^{*}$ | **0.778**$^{*}$ | **0.798**$^{*}$ | **0.889**$^{**}$ | 0.286 | 0.491 | 1.000 | | | | | | | | | | | | | | | | | |
| Co | 0.144 | 0.438 | 0.418 | -0.517 | 0.308 | -0.328 | 0.611 | 0.303 | 0.361 | 0.553 | 0.375 | -0.117 | 0.577 | 1.000 | | | | | | | | | | | | | | | | |
| Ni | -0.061 | 0.127 | -0.012 | 0.721 | 0.283 | **0.889**$^{**}$ | 0.185 | -0.045 | -0.085 | -0.047 | 0.534 | 0.584 | -0.028 | -0.238 | 1.000 | | | | | | | | | | | | | | | |
| Cu | -0.321 | 0.267 | -0.315 | **0.874**$^{*}$ | -0.151 | **0.815**$^{*}$ | -0.309 | -0.429 | -0.457 | -0.368 | 0.058 | 0.212 | -0.427 | -0.506 | **0.832**$^{*}$ | 1.000 | | | | | | | | | | | | | | |
| Zn | -0.463 | 0.318 | -0.144 | -0.124 | -0.053 | 0.526 | 0.057 | -0.338 | -0.370 | -0.449 | **0.798**$^{*}$ | 0.080 | -0.343 | 0.054 | 0.509 | 0.289 | 1.000 | | | | | | | | | | | | | |
| As | 0.515 | 0.036 | 0.467 | 0.037 | 0.513 | -0.286 | 0.698 | 0.670 | 0.721 | **0.938**$^{**}$ | 0.024 | 0.246 | **0.862**$^{*}$ | 0.638 | -0.045 | -0.264 | -0.509 | 1.000 | | | | | | | | | | | | |
| Se | -0.634 | **0.916**$^{**}$ | -0.098 | -0.058 | -0.589 | -0.212 | -0.144 | -0.350 | -0.287 | -0.054 | -0.288 | -0.610 | -0.335 | 0.370 | -0.137 | 0.150 | -0.040 | 0.134 | 1.000 | | | | | | | | | | | |
| Sr | 0.725 | -0.438 | **0.856**$^{*}$ | -0.308 | 0.638 | -0.288 | 0.579 | **0.827**$^{*}$ | **0.801**$^{*}$ | 0.579 | -0.082 | 0.576 | 0.576 | 0.183 | -0.306 | -0.567 | -0.396 | 0.374 | -0.424 | 1.000 | | | | | | | | | | |
| Mo | -0.313 | 0.614 | -0.196 | 0.261 | -0.471 | -0.085 | -0.464 | -0.484 | -0.434 | -0.172 | -0.624 | -0.558 | -0.321 | 0.098 | -0.189 | 0.262 | -0.388 | 0.106 | **0.806**$^{*}$ | -0.380 | 1.000 | | | | | | | | | |
| Cd | -0.149 | 0.005 | -0.148 | **0.823**$^{*}$ | -0.106 | 0.432 | -0.042 | 0.059 | 0.041 | 0.073 | -0.176 | 0.341 | -0.157 | -0.567 | 0.657 | **0.777**$^{*}$ | -0.124 | 0.058 | 0.038 | -0.238 | 0.104 | 1.000 | | | | | | | | |
| Sn | -0.010 | 0.013 | -0.642 | 0.351 | -0.226 | 0.108 | -0.664 | -0.670 | -0.640 | -0.361 | -0.326 | -0.500 | -0.153 | -0.088 | -0.102 | 0.230 | -0.253 | -0.052 | 0.174 | -0.575 | 0.586 | -0.063 | 1.000 | | | | | | | |
| Sb | -0.373 | **0.750** | -0.213 | 0.490 | -0.273 | 0.396 | -0.300 | -0.557 | -0.524 | -0.221 | -0.130 | -0.298 | -0.289 | 0.155 | 0.333 | 0.612 | 0.066 | 0.072 | 0.735 | -0.596 | **0.823**$^{*}$ | 0.253 | 0.540 | 1.000 | | | | | | |
| Ba | 0.451 | -0.123 | **0.879**$^{**}$ | -0.511 | 0.408 | -0.404 | 0.543 | 0.708 | 0.695 | 0.475 | -0.109 | 0.356 | 0.383 | 0.320 | -0.428 | -0.628 | -0.278 | 0.280 | -0.124 | **0.924**$^{**}$ | -0.221 | -0.368 | -0.659 | -0.472 | 1.000 | | | | | |
| La | -0.175 | 0.202 | 0.066 | -0.550 | -0.258 | -0.849 | 0.381 | 0.397 | 0.468 | 0.568 | -0.156 | -0.410 | 0.309 | 0.511 | -0.536 | -0.584 | -0.343 | 0.529 | 0.398 | 0.120 | 0.067 | -0.164 | -0.249 | -0.205 | 0.251 | 1.000 | | | | |
| Ce | 0.111 | 0.233 | 0.247 | -0.085 | 0.238 | -0.321 | 0.726 | 0.565 | 0.626 | **0.844**$^{*}$ | 0.245 | 0.079 | 0.676 | 0.652 | 0.065 | -0.194 | -0.192 | **0.865**$^{*}$ | 0.273 | 0.090 | -0.017 | 0.133 | -0.209 | 0.053 | 0.079 | 0.724 | 1.000 | | | |
| Pt | -0.487 | 0.521 | -0.433 | -0.099 | -0.723 | -0.435 | -0.579 | -0.565 | -0.500 | -0.274 | -0.649 | -0.879 | -0.417 | 0.116 | -0.516 | -0.044 | -0.362 | -0.035 | **0.779**$^{*}$ | -0.462 | 0.873$^{*}$ | -0.140 | 0.585 | 0.567 | -0.256 | 0.334 | -0.031 | 1.000 | | |
| Tl | -0.409 | **0.742** | -0.141 | -0.108 | -0.512 | -0.311 | -0.316 | -0.437 | -0.369 | -0.106 | -0.462 | -0.716 | -0.256 | 0.395 | -0.383 | -0.023 | -0.255 | 0.150 | **0.904**$^{**}$ | -0.350 | **0.917**$^{**}$ | -0.198 | 0.462 | 0.724 | -0.098 | 0.313 | 0.121 | **0.916**$^{**}$ | 1.000 | |
| Pb | 0.266 | -0.340 | -0.247 | **0.918**$^{**}$ | 0.308 | 0.750 | -0.148 | -0.105 | -0.145 | -0.075 | 0.100 | 0.502 | 0.056 | -0.531 | 0.754 | 0.759$^{*}$ | 0.004 | -0.007 | -0.398 | -0.257 | -0.074 | 0.697 | 0.331 | 0.226 | -0.553 | -0.628 | -0.115 | -0.364 | -0.415 | 1.000 |

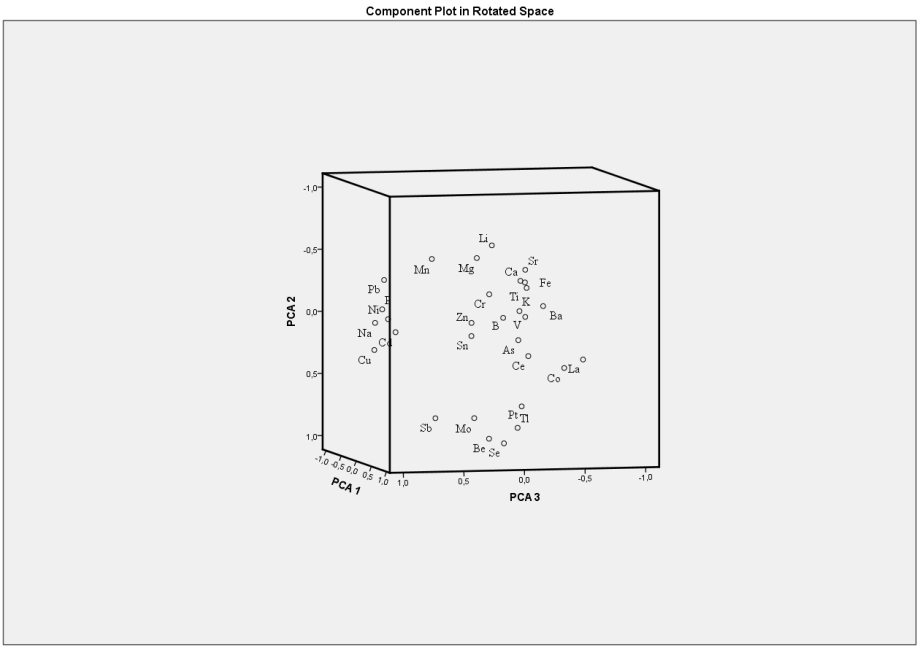
**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

The relationship between the elements in the Rhubarb plants has also been applied to the multi-component analysis principal component analysis (PCA), which is a stronger chemo metric technique.

### 4.5. Principal component analysis for the element concentrations in rhubarb

By using the PCA subroutine of the IBM SPSS statistic V22 package (Release 22.0.0.0, 1989–2003) and Minitab 17 (Licensing: 17.1.0.0, 2013), principal component analysis (PCA) was subsequently carried out on the correlation matrix to identify the underlying pattern of metals and to help the data interpretation. The PCA was applied to the data matrix of total metal concentrations (30 × 7 cases) of the Rhubarb samples. The results show that five Eigen values explain about 93.45% of the total variance and the fifth Eigen value explains about 12.07 % of the variance. Principal component loading for Rhubarb samples extracted five components in which it explains about 93.45% of the total variance with the contribution of each factor being 22.17%, 21.60%, 20.67%, 16.95% and 12.07%, respectively. For this reason, the first five Eigen values were selected for further analysis. The five factor loadings extracted after performing the maximum rotation and communalities are given in Table 6. The factor 1 has high loadings for As, Ce, V, Fe, K, Co, Ti, Li, Mg and Ba, explains about 22.17 of the total variance. The factor 2 is loaded Se, Be, Tl, Mo, Sb and Pt explains 21.60% of the total variance. Factor 3 is loaded by Na, Cu, Pb, Ni, P, Cd, Sn and Mn, explains 20.67% of the total variance. The loading were for B, Ba, Sr, Ca and Mn on the fourth component and for Zn and Cr on the fifth component. A 3-D plot of the PCA loadings is illustrated in Figure 1-3and the relationships among




the elements are readily seen.

**Figure 4.37.** The space plot of loading of the first three principle components.

**Table 4.11.**The loading and the scores of the first three rotated principal components.

| | The loading | | | | | The score | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Element** | PC1 | PC2 | PC 3 | PC 4 | PC 5 | Vocational | PC1 | PC2 | PC 3 | PC 4 | PC 5 |
| As | **0.965** | 0.079 | 0.015 | 0.126 | -0.212 | Pervari | -0.578 | -0.091 | -1.495 | -1.162 | -0.634 |
| Ce | **0.915** | 0.213 | -0.081 | 0.004 | 0.130 | Şirvan | 0.246 | 2.020 | -0.097 | 0.170 | -0.472 |
| V | **0.910** | -0.102 | -0.056 | 0.349 | -0.127 | Sor | -0.749 | 0.143 | 1.810 | -0.220 | -0.346 |
| Fe | **0.889** | -0.378 | -0.059 | 0.169 | -0.042 | Rash | 0.790 | -1.026 | 0.528 | -1.157 | -0.246 |
| K | **0.745** | -0.135 | -0.050 | 0.538 | 0.361 | Karajar | -0.870 | -0.892 | -0.353 | 1.616 | -0.706 |
| Co | **0.682** | 0.336 | -0.435 | 0.090 | 0.286 | Gara | 1.803 | -0.207 | -0.189 | 0.788 | 0.252 |
| Ti | **0.682** | -0.314 | -0.124 | 0.604 | -0.085 | Qalandar | -0.642 | 0.053 | -0.204 | -0.035 | 2.152 |
| Se | 0.055 | **0.989** | -0.094 | -0.067 | -0.066 | | | | | | |
| Be | 0.017 | **0.953** | 0.020 | 0.038 | 0.291 | | | | | | |
| Tl | 0.040 | **0.868** | -0.211 | -0.203 | -0.315 | | | | | | |
| Mo | -0.045 | **0.790** | 0.126 | -0.222 | -0.510 | | | | | | |
| Sb | 0.027 | **0.777** | 0.465 | -0.293 | -0.039 | | | | | | |
| Pt | -0.141 | **0.712** | -0.289 | -0.406 | -0.471 | | | | | | |
| Li | **0.468** | -0.644 | 0.109 | 0.261 | -0.330 | | | | | | |
| Mg | **0.541** | -0.551 | 0.251 | 0.362 | 0.120 | | | | | | |
| Na | 0.001 | 0.002 | **0.956** | -0.188 | -0.218 | | | | | | |
| Cu | -0.250 | 0.242 | **0.900** | -0.190 | 0.134 | | | | | | |
| Pb | 0.034 | -0.345 | **0.889** | -0.292 | -0.056 | | | | | | |
| Ni | 0.055 | -0.031 | **0.861** | -0.003 | 0.497 | | | | | | |
| P | -0.218 | -0.086 | **0.842** | -0.028 | 0.395 | | | | | | |
| Cd | 0.020 | 0.079 | **0.792** | 0.015 | -0.149 | | | | | | |
| La | **0.520** | 0.286 | -0.631 | 0.015 | -0.108 | | | | | | |
| B | 0.365 | -0.049 | -0.010 | **0.891** | 0.001 | | | | | | |
| Ba | 0.150 | -0.117 | -0.394 | **0.870** | -0.172 | | | | | | |
| Sn | -0.054 | 0.130 | **0.147** | -0.809 | -0.382 | | | | | | |
| Sr | 0.261 | -0.423 | -0.218 | **0.783** | -0.263 | | | | | | |
| Ca | 0.632 | -0.368 | -0.086 | **0.639** | -0.067 | | | | | | |
| Mn | 0.253 | -0.525 | **0.550** | **0.567** | 0.176 | | | | | | |
| Zn | -0.322 | 0.048 | 0.080 | -0.049 | **0.938** | | | | | | |
| Cr | 0.244 | -0.230 | 0.075 | -0.006 | **0.928** | | | | | | |
| Eigenvalue | 6.651 | 6.479 | 6.200 | 5.084 | 3.621 | | | | | | |
| **Variance (%)** | 22.170 | 21.596 | 20.667 | 16.945 | 12.070 | | | | | | |
| **Cumulative (%)** | 22.170 | 43.766 | 64.433 | 81.378 | 93.448 | | | | | | |

The two way loadings and score plots are shown in Figure 2 PC1 component was plotted against to every principle component to show high percentage of the total variance (43.77–34.24).

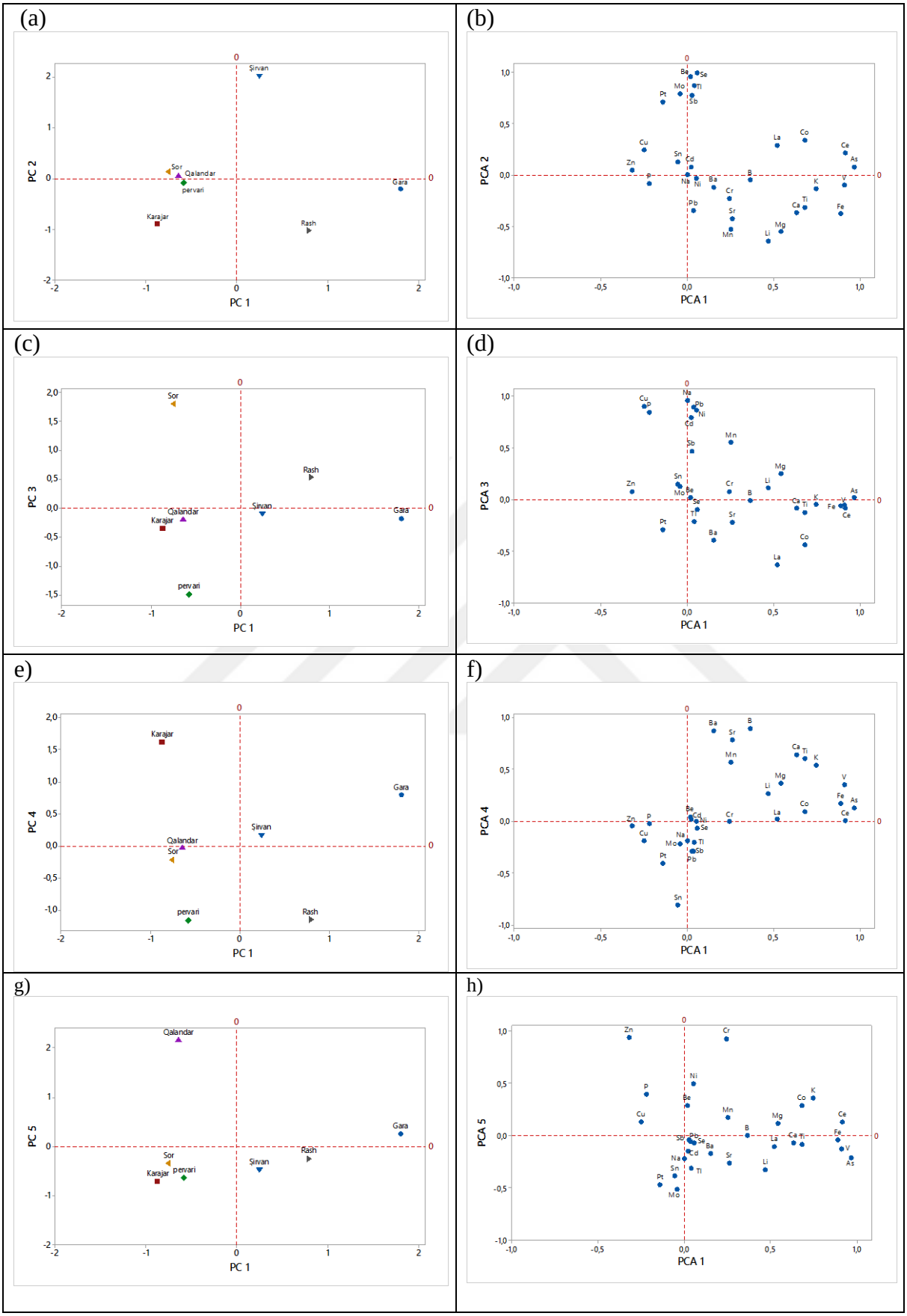



**Figure 4.38.** The score and loading plots (a, c, e and g are the score plots and b, d, f and h are the loading plots).

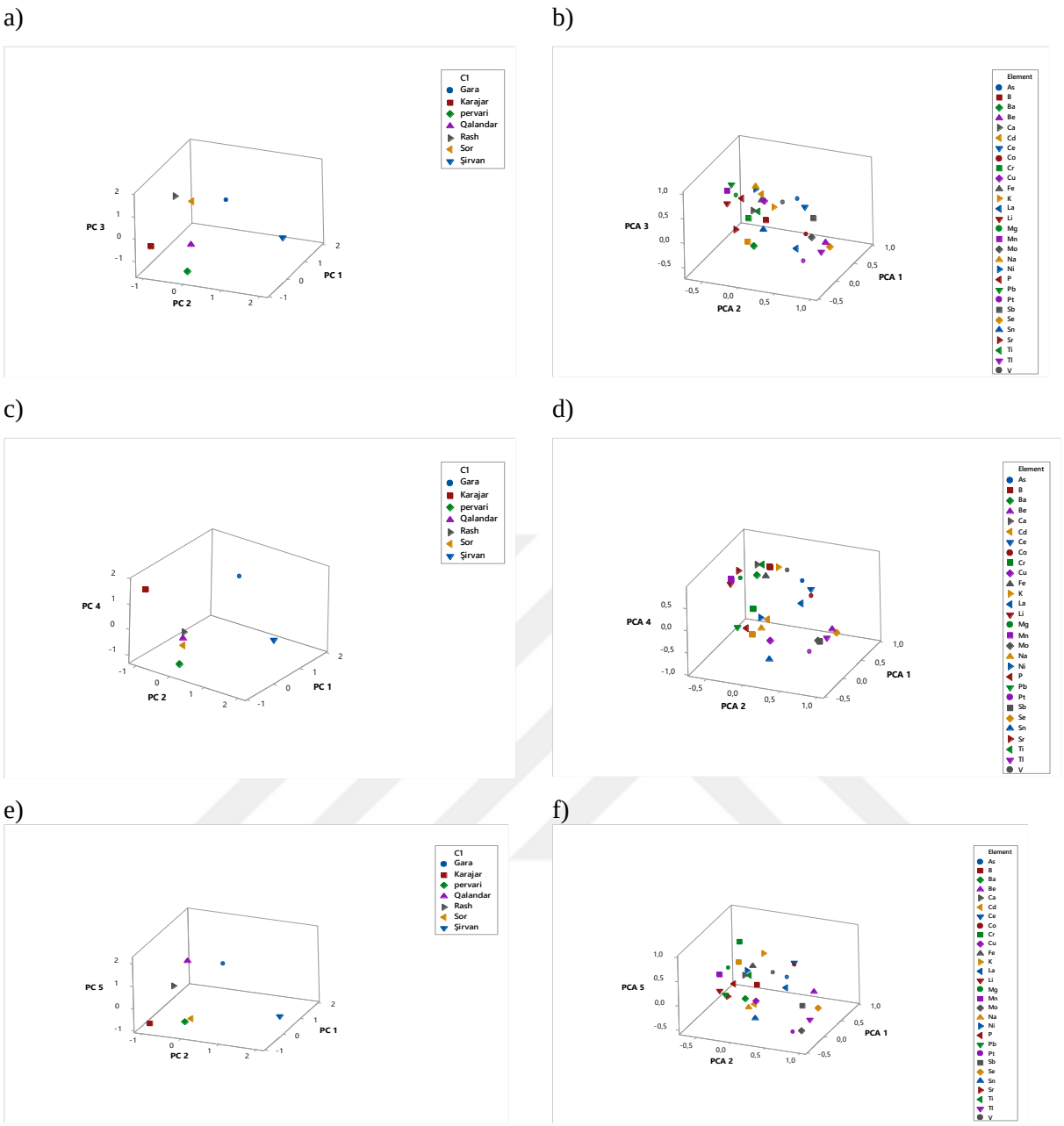



**Figure 4.39.** Three way PCA score and loading plots (a, c and e are the score plots and b, d and f are the loading plots).

As seen Figure 4.39, the classification of the species of metal contents in Rhubarb can be made by using three way PC score graphs.

The percentage of total variance of about 43.77 was observed with PC 1–2 while PC 1–3 score graph shows about 42.84 % of total variance. It can be seen from the PC 1–2, PC 1–3 graphs (Figure 2), Table 2 and Figure 3 that the thermal waters of spas can be classified into three groups. These groups were:

Group 1: Pervari, Qalandar and Şirvan

Group 2: Sor and Rash

Group 3: Karajar and Gara

### 4.6. Cluster analysis for the element concentrations in rhubarb

The cluster analysis technique can be used for classification process that involves a measurement of the similarity between objects to be clustered (Brereton, 2003; Kara, 2009). Rhubarb samples will be grouped in clusters in terms of their similarity or similarity. The measurement of the similarity is based, among others, on the Squared Euclidean distance. The clustering method used was the Ward's method. The cluster analysis was applied using the Statistical package Minitab 17 (Licensing: 17.1.0.0, 2013). The results obtained in the cluster analysis are presented as a dendrogram given in Figure 4 displays mainly three clusters: These clusters represent the similarity on the origins of elements for Rhubarb samples. Similar results to cluster analysis were also obtained after the application of PCA. For Rhubarb samples:

Group 1: Pervari, Qalandar and Şirvan

Group 2: Sor and Rash

Group 3: Karajar and Gara

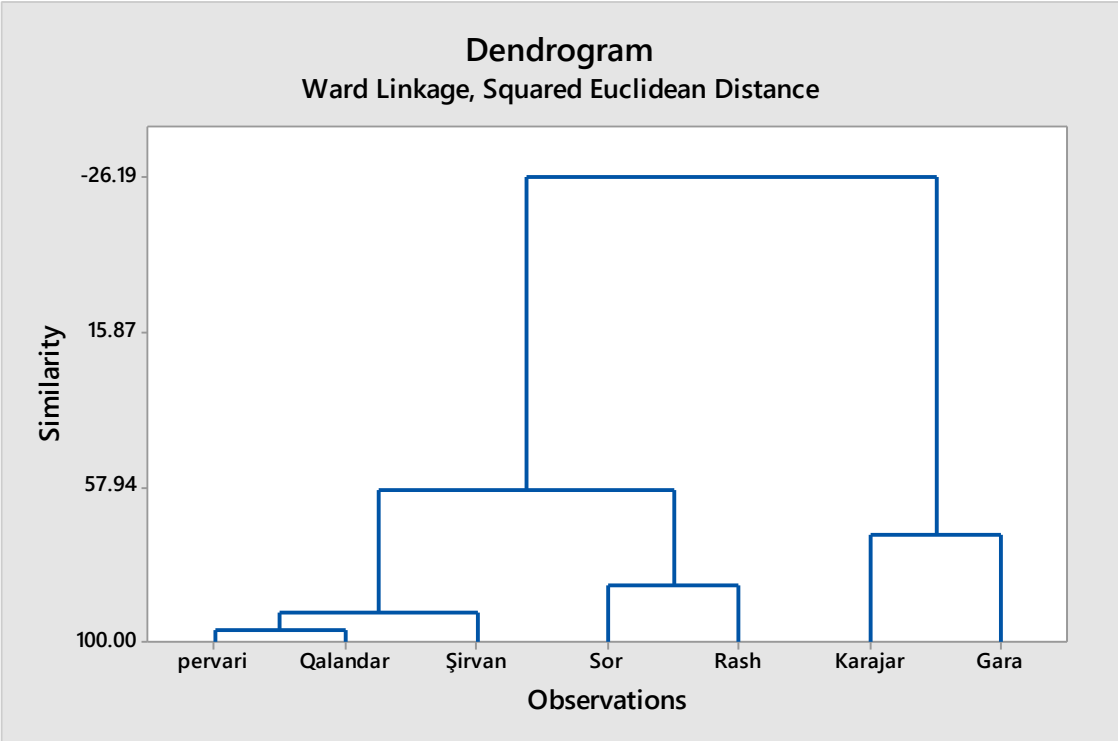



**Figure 4.40.** Dendrogram of cluster analysis for Rhubarb samples

Similarly, as shown in figure 5, classification can also be made between elements. As can be seen in Figure 5, five groups can be separated. This grouping can be shown as follows.

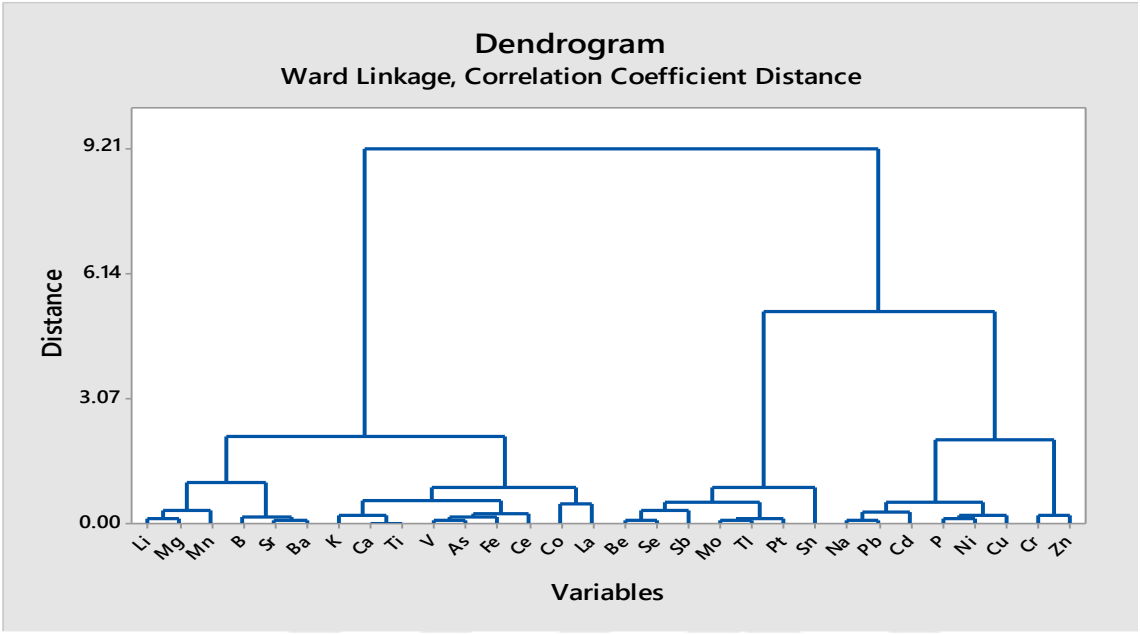



**Figure 4.41.** Dendrogram of cluster analysis for metal concentrations

For elements:

Group 1 had Li, Ba, Mn, B, Sr and Ba, Group 2 had K, Ca, Ti, V, As, Fe, Co and La, Group 3 had Be, Se, Sb, Mo, Tl, Pt and Sn, Group 4 had Na, Pb, Cd, P, Ni and Cu, Group 5 had Cr and Zn.

## 5. CONCLUSION

Of the 7 samples of Rhubarb studied, the 30 type of heavy metals Mg, K and P were found to be present in higher concentrations than the other metals. The metals Pt, Ti, Be, and As were present in much lower concentrations. The concentration range of the metals was similar to that found in most East Asian Rhubarb. No clear trend was apparent in the concentration range of metals from the various parts of herbal plants. A few samples were found to contain relatively higher concentrations of the metals cd, Pb, and Hg. The toxic metals were probably from contamination during the drying and preservation steps. However, at the level present in the herbal plants, they are unlikely to cause any adverse health effects unless consumed in large quantity continuously.

This work provides a rapid, sensitive and accurate LC-MS / MS method for the simultaneous determination of 37 types of phenolic compound in rhubarb. The phenolic compounds content varied significantly within the same species. The relationship of the anthraquinone glycoside content with plant species, geographic distribution and altitude were studied using correlation analysis, principal component analysis and spatial autocorrelation analysis. Plant species and geographic distribution were found not to affect the content of the phenolic compound in rhubarb. The variations in the phenolic compounds content were primarily due to the different altitude where the plant was grown.

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**APPENDICES**

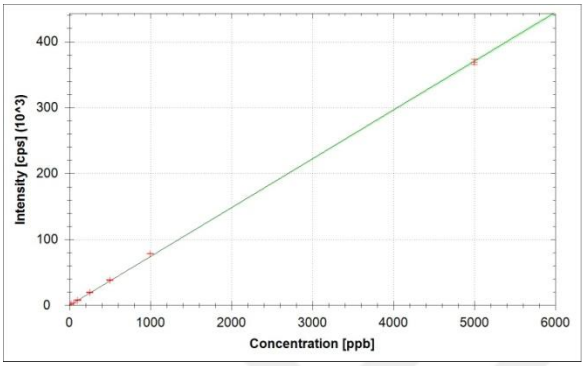
**Appendix 1. Calibration charts of metal analysis**

## Calibration Curves:

Instrument Nam: iCAP Q

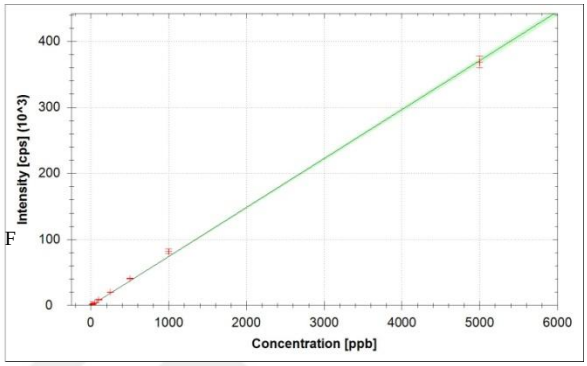
Serial Number: Undefined

$_{7}$Li




f(x) = 74.0467*x
$R_2$ = 0.9998
BEC = 0.000 ppb
LOD = 0.0000 ppb

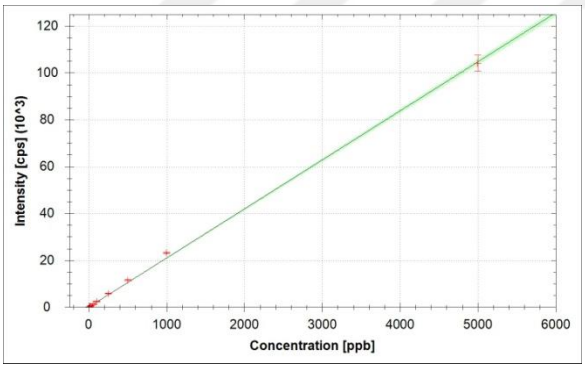
$_{9}$Be




F

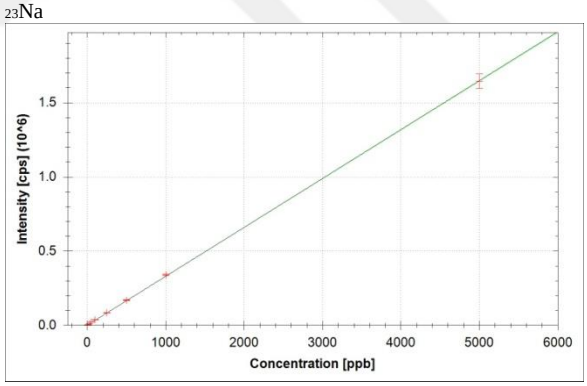
(x) = 74.1049*x + 3.3333
$R_2$ = 0.9993
BEC = 0.045 ppb
LOD = 0.2337 ppb

$_{11}$B



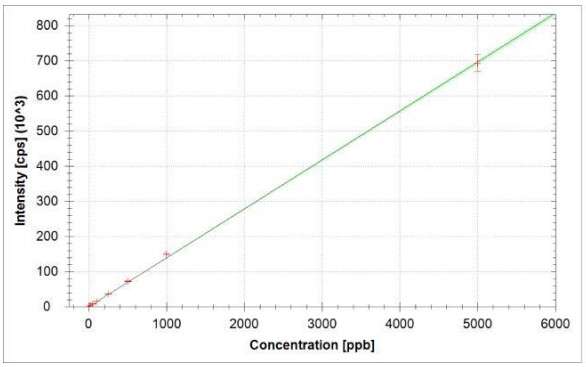

f(x) = 20.9200*x + 103.3341
$R_2$ = 0.9993
BEC = 4.939 ppb
LOD = 3.6089 ppb

$_{23}$Na



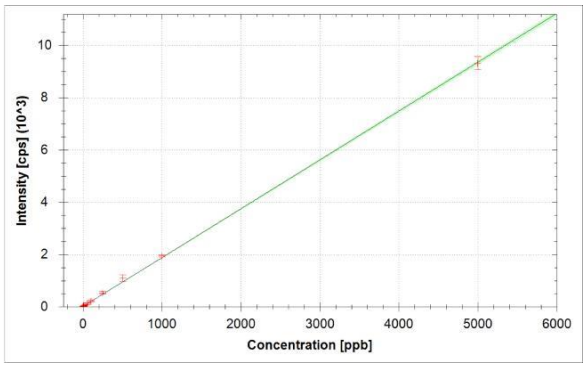

f(x) = 329.3396*x + 580.0151
$R_2$ = 0.9999
BEC = 1.761 ppb

$_{24}$Mg



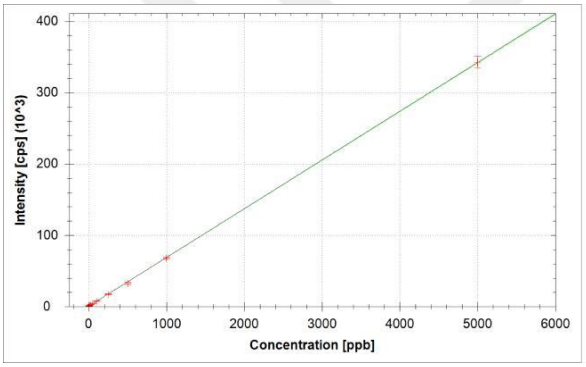

f(x) = 139.2022*x + 6.6667
$R^2$ = 0.9996
BEC = 0.048 ppb
LOD = 0.2489 ppb

$_{31}$P



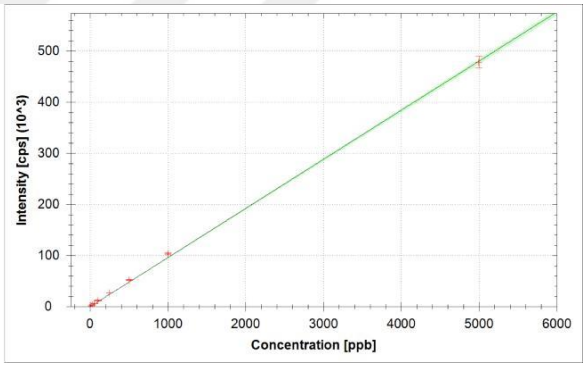

f(x) = 1.8676*x + 13.3334
$R^2$ = 0.9996
BEC = 7.139 ppb
LOD = 9.2745 ppb

$_{39}$K



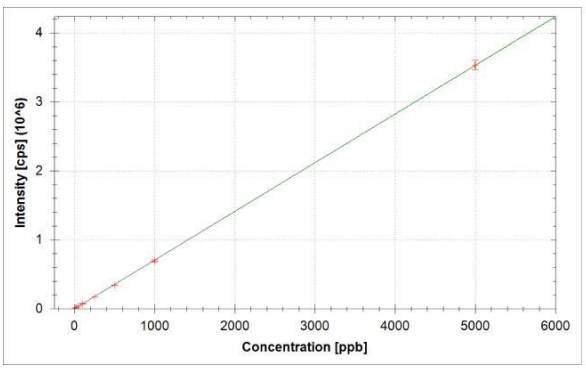

f(x) = 68.2591*x + 753.3588
$R^2$ = 0.9999
BEC = 11.037 ppb
LOD = 4.2690 ppb

$_{44}$Ca



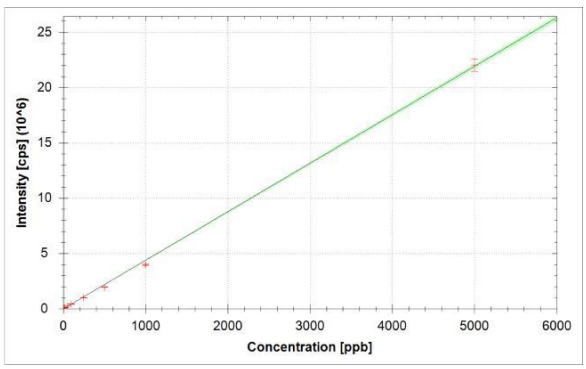

f(x) = 95.9947*x + 26.6669
$R^2$ = 0.9995
BEC = 0.278 ppb
LOD = 0.3609 ppb

$_{48}$Ti



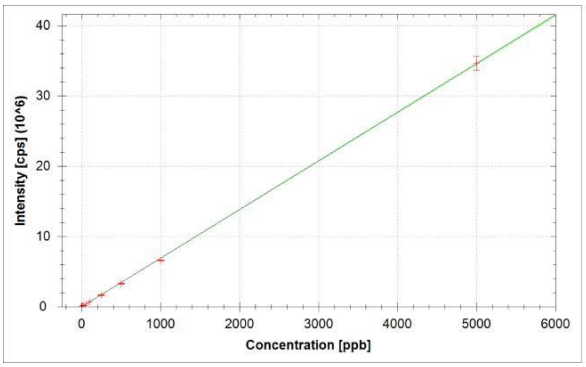

f(x) = 705.8034*x + 3.3333
$R^2$ = 1.0000
BEC = 0.005 ppb
LOD = 0.0245 ppb

$_{51}$V



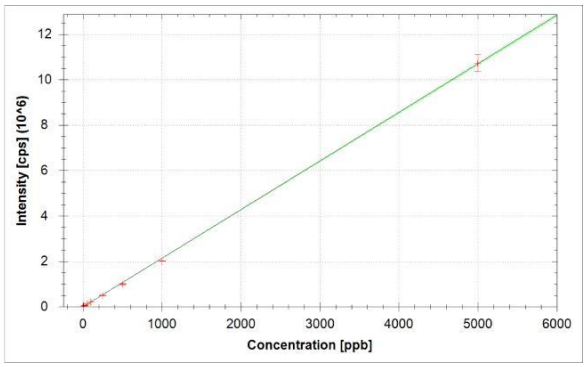

f(x) = 4383.3481*x
$R^2$ = 0.9994
BEC = 0.000 ppb
LOD = 0.0000 ppb

$^{52}$Cr



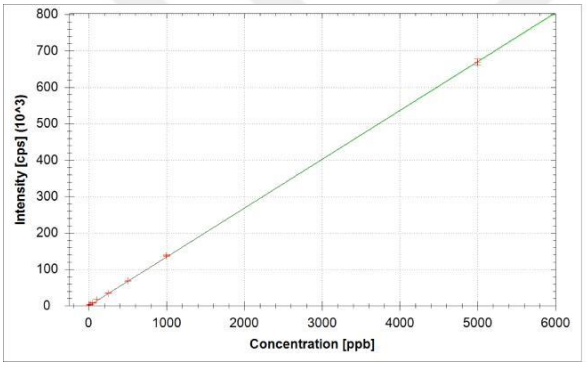

f(x) = 6917.2912*x + 26.6668
$R^2$ = 0.9998
BEC = 0.004 ppb
LOD = 0.0025 ppb

$^{55}$Mn



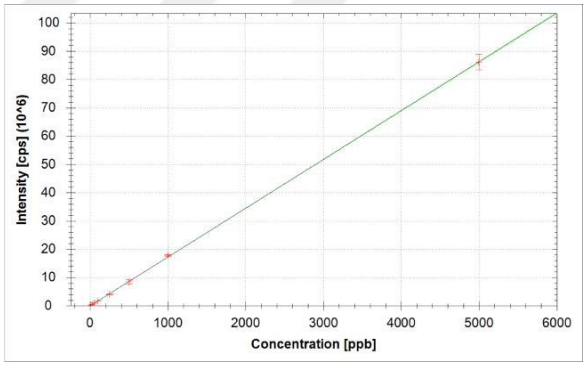

f(x) = 2139.0694*x + 33.3335
$R^2$ = 0.9997
BEC = 0.016 ppb
LOD = 0.0081 ppb

$^{57}$Fe



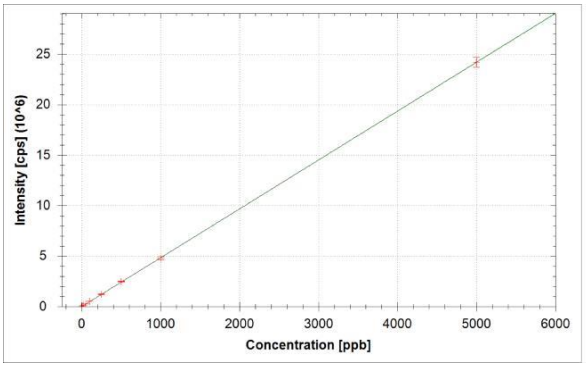

f(x) = 134.0377*x + 10.0000
$R^2$ = 0.9999
BEC = 0.075 ppb
LOD = 0.0000 ppb

$^{59}$Co



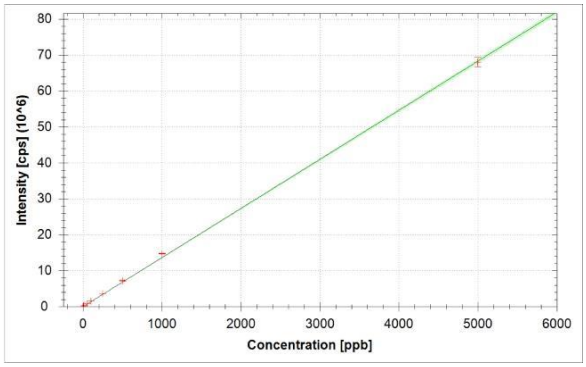

f(x) = 17246.2538*x + 113.3344
$R^2$ = 0.9999
BEC = 0.007 ppb
LOD = 0.0089 ppb

$^{60}$Ni



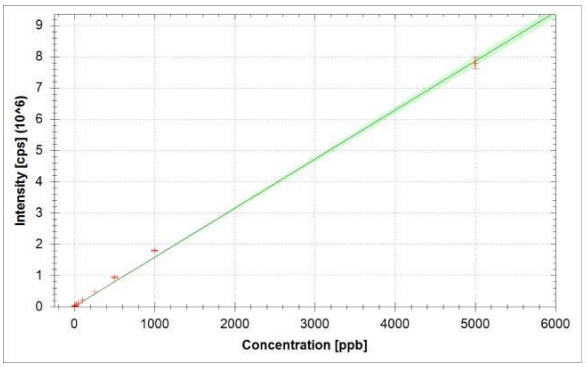

f(x) = 4838.3663*x + 20.0001
$R^2$ = 1.0000
BEC = 0.004 ppb
LOD = 0.0062 ppb

$^{63}$Cu



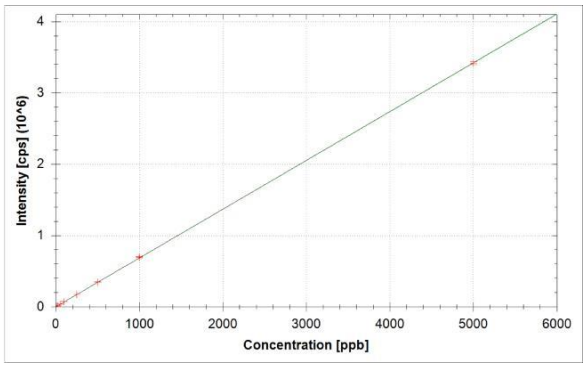

f(x) = 13652.8900*x + 116.6677
$R^2$ = 0.9996
BEC = 0.009 ppb
LOD = 0.0063 ppb

$_{66}$Zn



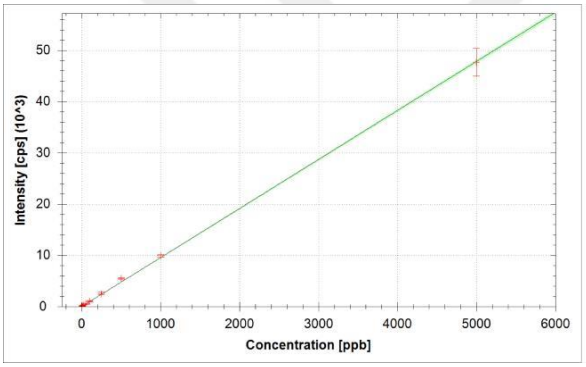

f(x) = 1568.0454*x + 15209.3923
$R^2$ = 0.9985
BEC = 9.700 ppb
LOD = 2.1074 ppb

$_{75}$As



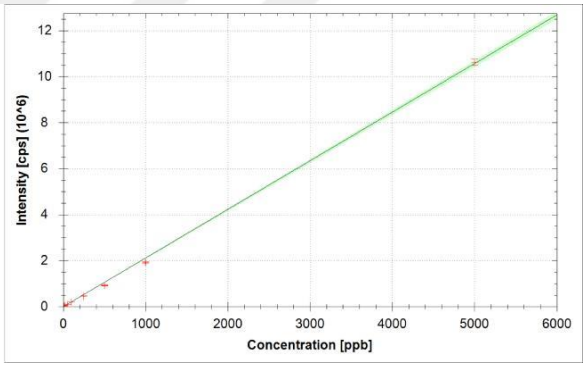

f(x) = 683.9898*x
$R^2$ = 1.0000
BEC = 0.000 ppb
LOD = 0.0000 ppb

$_{77}$Se



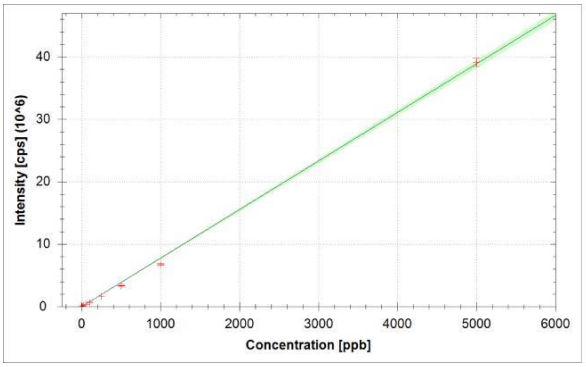

f(x) = 9.5640*x + 3.3333
$R^2$ = 0.9996
BEC = 0.349 ppb
LOD = 1.8110 ppb

$_{88}$Sr



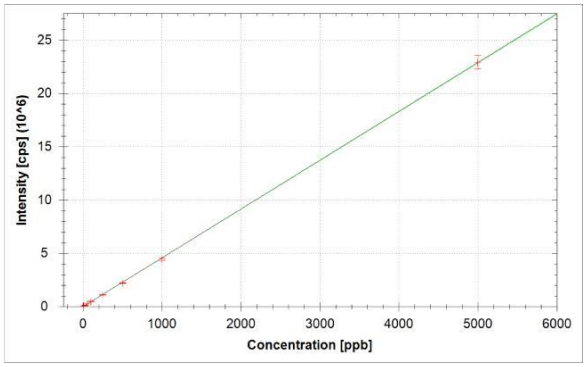

f(x) = 2112.9353*x
$R^2$ = 0.9993
BEC = 0.000 ppb
LOD = 0.0000 ppb

$_{95}$Mo



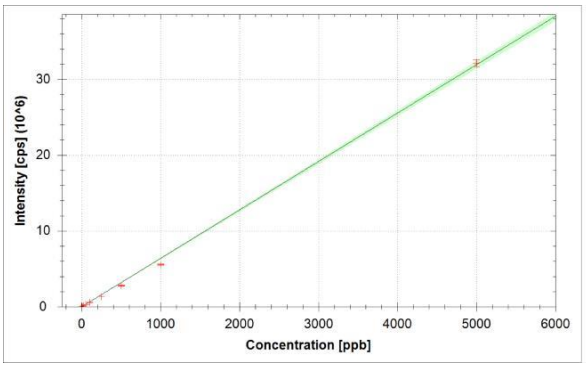

f(x) = 7768.8252*x + 80.0006
$R^2$ = 0.9988
BEC = 0.010 ppb
LOD = 0.0077 ppb

$_{111}$Cd



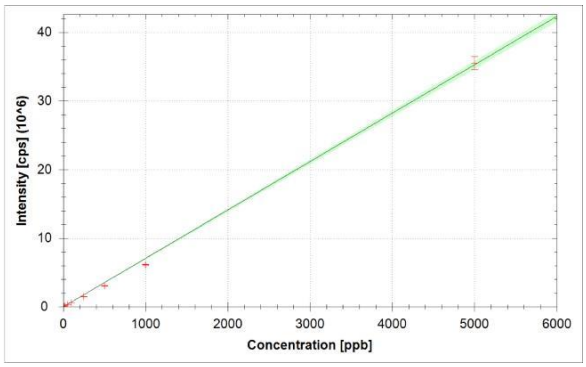

f(x) = 4577.4282*x + 33.3335
$R^2$ = 0.9999
BEC = 0.007 ppb
LOD = 0.0136 ppb

$^{118}$Sn



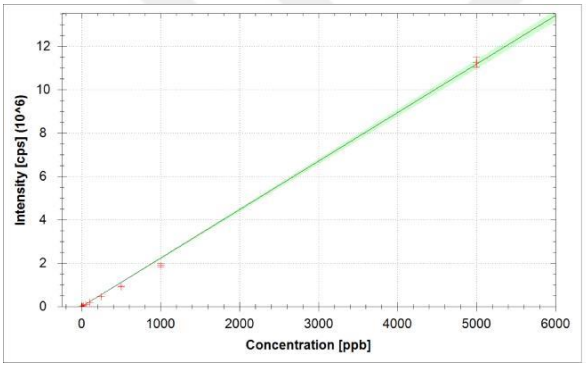

f(x) = 6383.6315*x + 76.6672
$R^2$ = 0.9989
BEC = 0.012 ppb
LOD = 0.0072 ppb

$^{121}$Sb



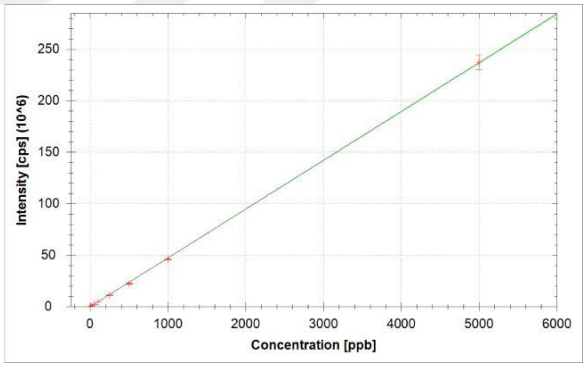

f(x) = 7055.1055*x
$R^2$ = 0.9988
BEC = 0.000 ppb
LOD = 0.0000 ppb

$^{137}$Ba



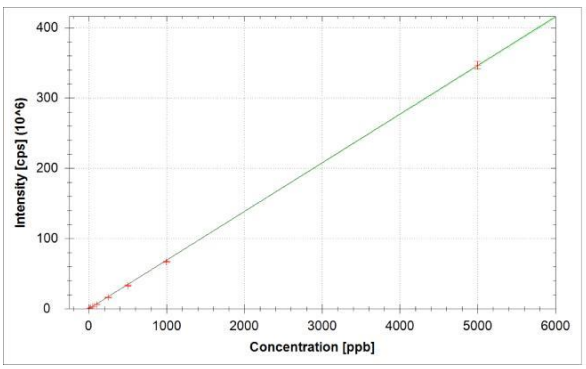

f(x) = 2235.0224*x + 13.3334
$R^2$ = 0.9985
BEC = 0.006 ppb
LOD = 0.0155 ppb

$^{139}$La



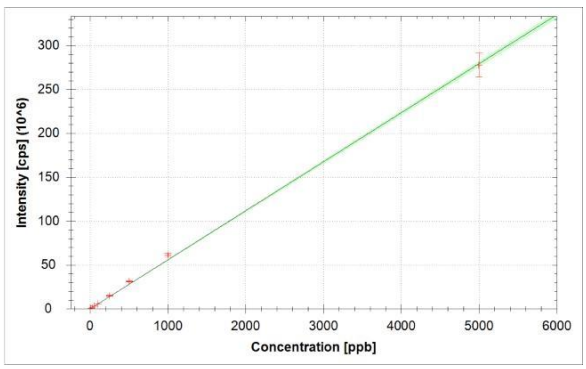

f(x) = 47360.5541*x + 10.0000
$R^2$ = 0.9999
BEC = 0.000 ppb
LOD = 0.0006 ppb

$^{140}$Ce



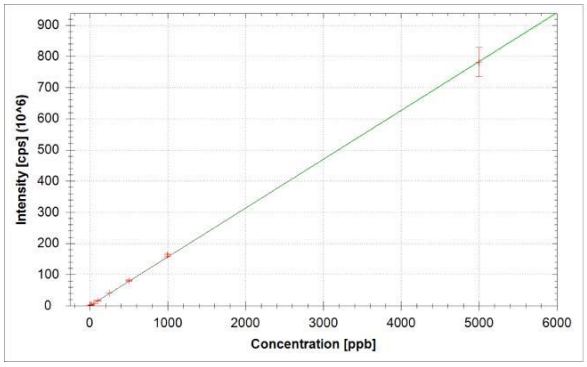

f(x) = 69223.9711*x + 13.3334
$R^2$ = 0.9999
BEC = 0.000 ppb
LOD = 0.0007 ppb

$^{195}$Pt



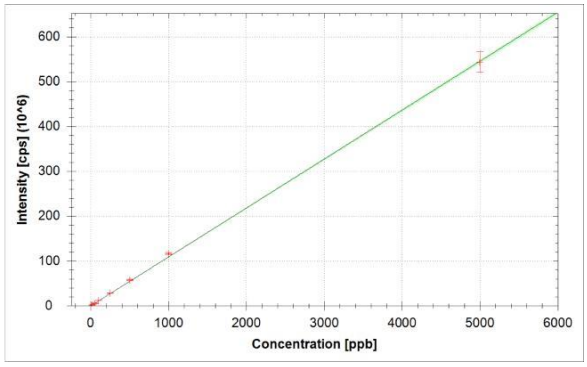

f(x) = 55859.6529*x + 40.0002
$R^2$ = 0.9993
BEC = 0.001 ppb
LOD = 0.0005 ppb

$_{205}$Tl



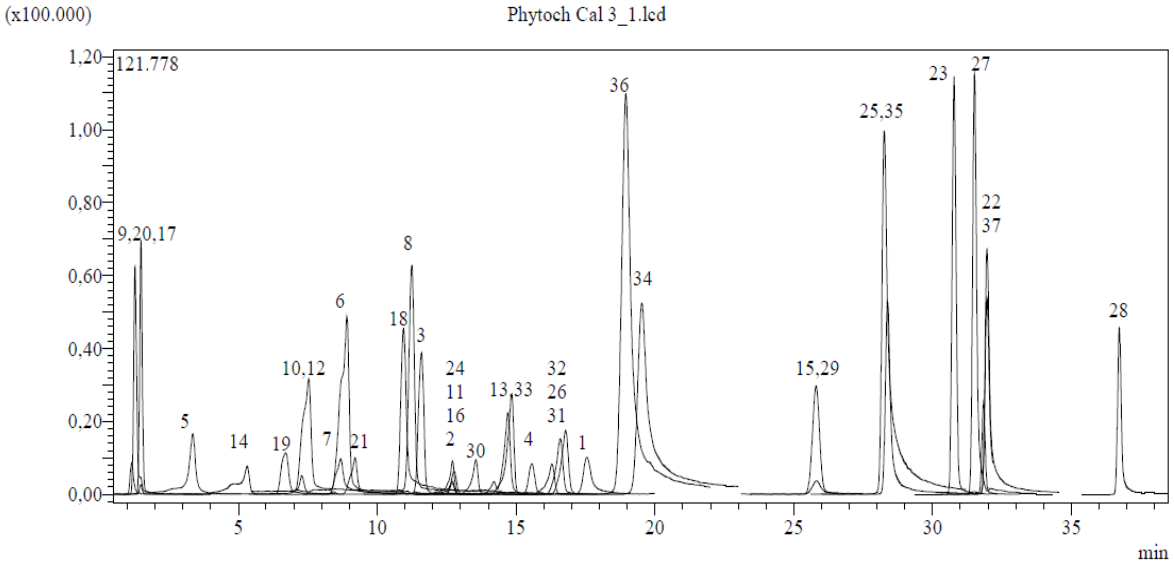

f(x) = 156622.0831*x + 20.0002
$R_2$ = 0.9999
BEC = 0.000 ppb
LOD = 0.0004 ppb

$_{208}$Pb

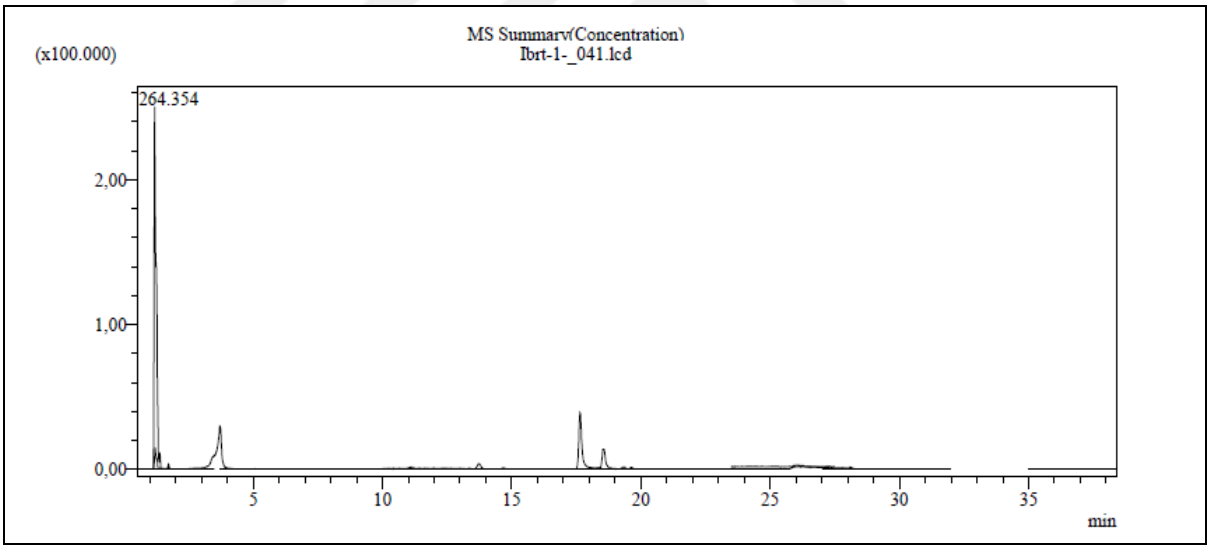



f(x) = 109032.9730*x + 2336.8958
$R_2$ = 0.9997
BEC = 0.021 ppb
LOD = 0.0055 ppb

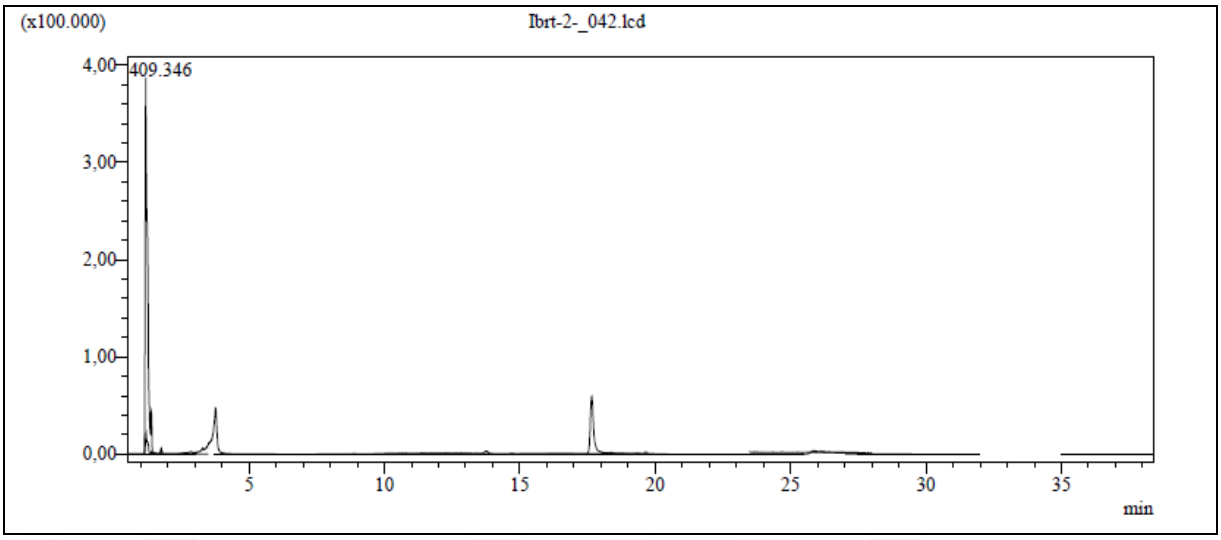
## Appendix 2. Phenolic compound picks (1-8)



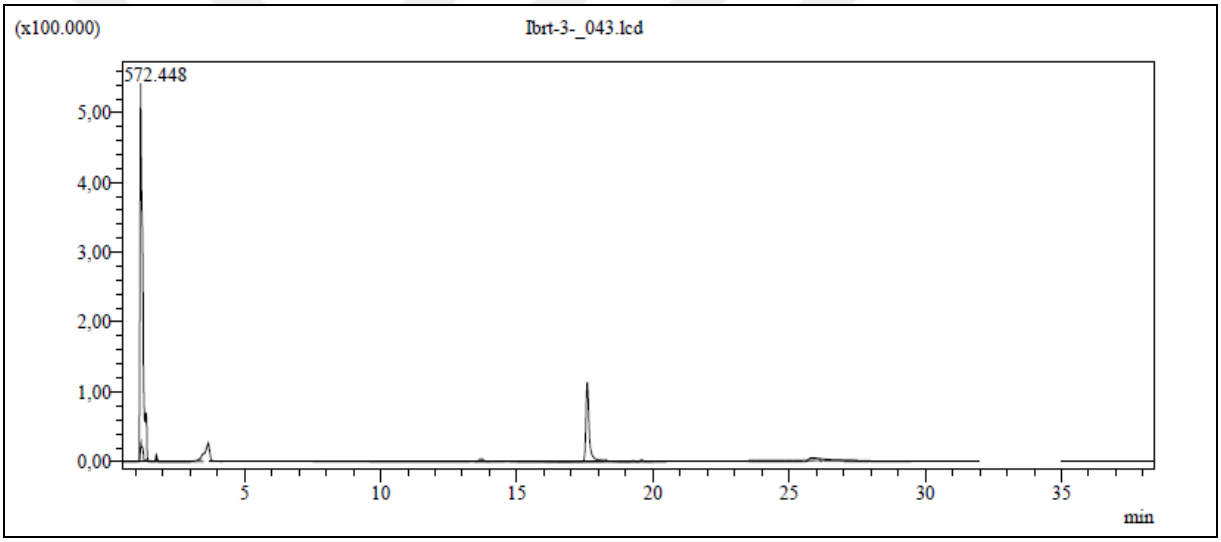

**App. 2.1.** The sdandart chromatograms of phenolic compound



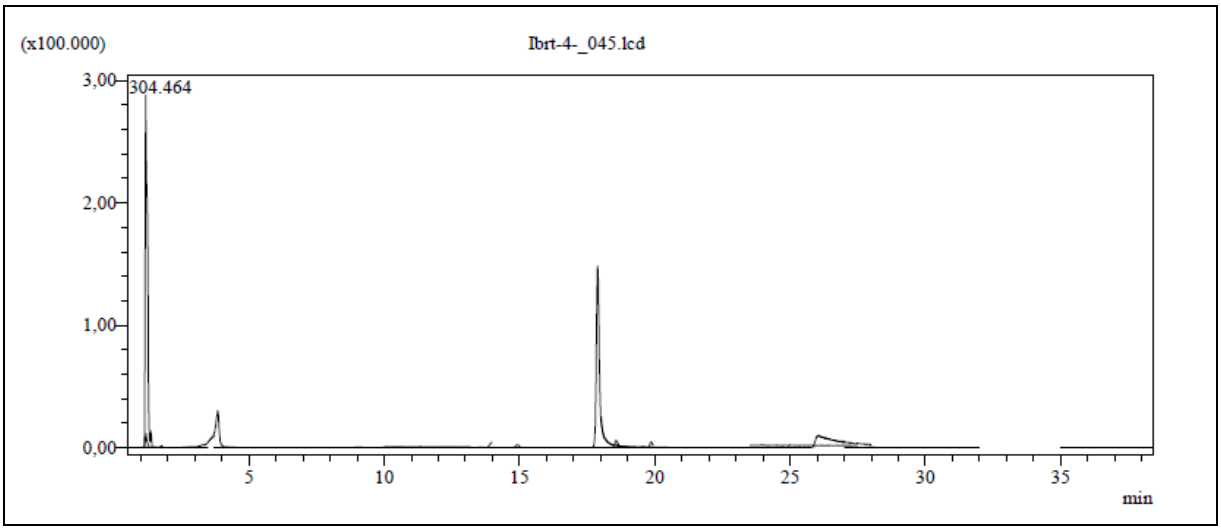

**App. 2.2.** The chromatograms of Gara



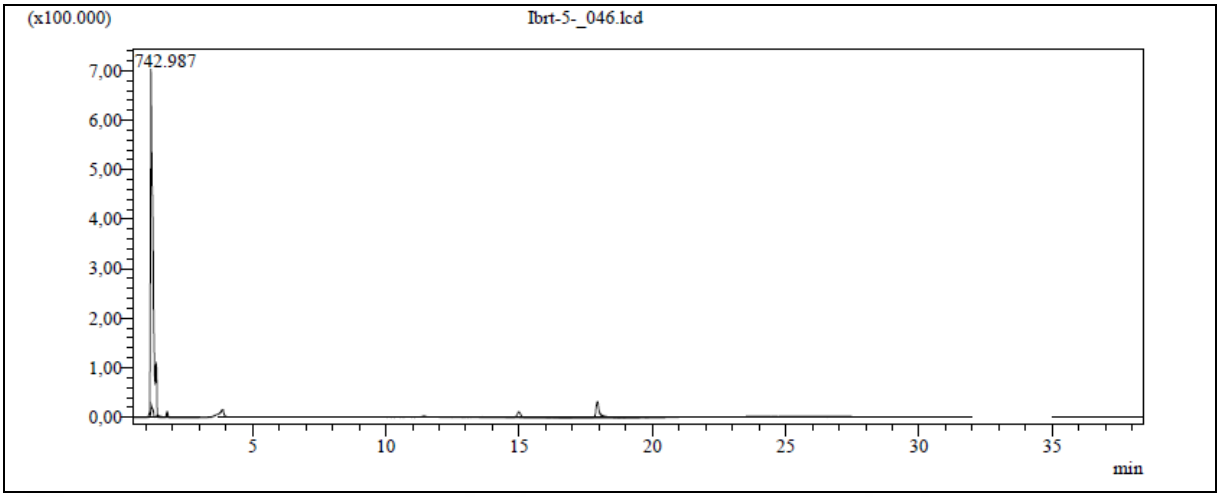

**App. 2.3.** The chromatograms of Korajar



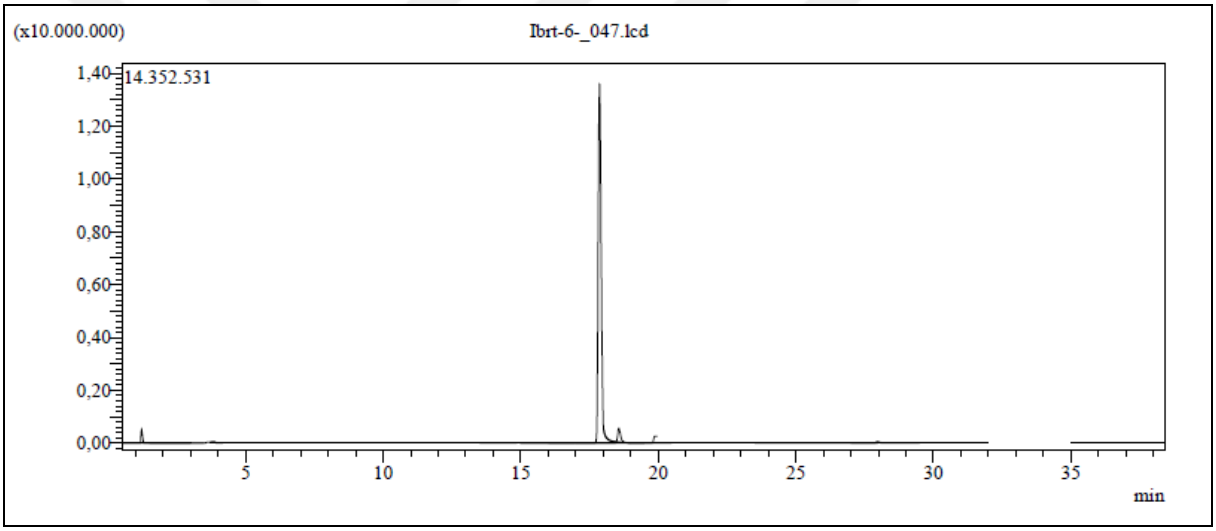

**App. 2.4.** The chromatograms of Sor



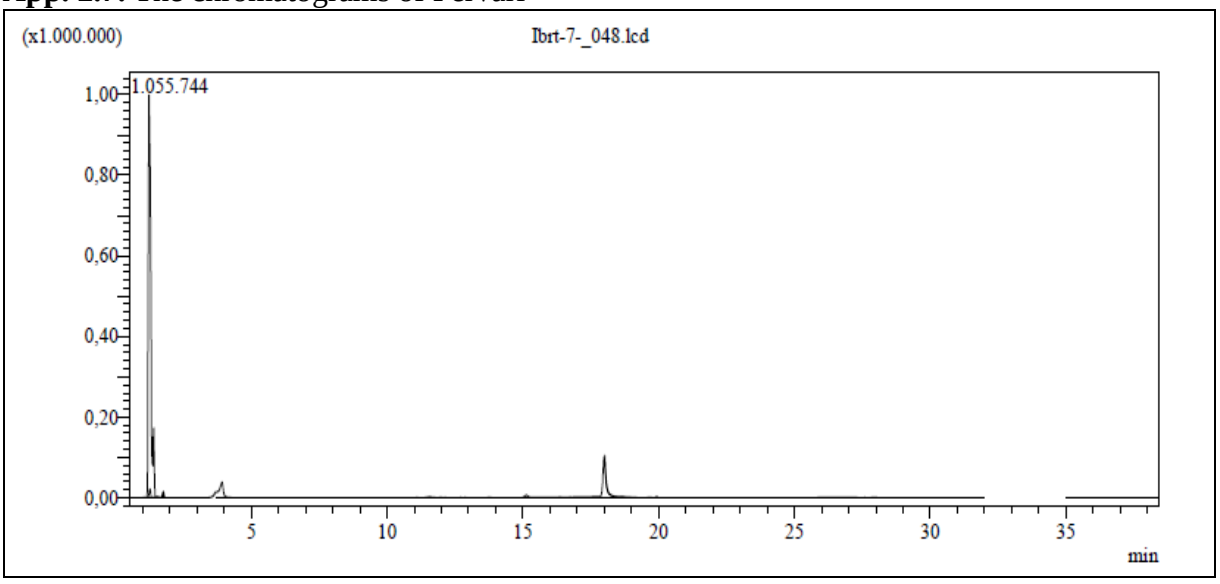

**App. 2.5.** The chromatograms of Rash




**App. 2.6.** The chromatograms of Qalandar




**App. 2.7.** The chromatograms of Pervari




**App. 2.8.** The chromatograms of Pervari

**CURRICULUM VITAE**

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| | |
|---|---|
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| High school | : | Robad , Duhok, Iraq | **2002** |
| Üniversite | : | Duhok University college of Education | **2006** |

**WORK EXPERIENCES**

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|---|---|---|
| 2006 | High school | Teacher |
| 2012 | The independent high Electoral commission | employee |
| 2014 | English language (ILES) | Student |
| 2015 | Information of computer (ICDL) program | Student |

**SPECIALTY AREA: -**

**FOREIGN LANGUAGES:** Turkish, Persian, English, Kurdish

**OTHER FEATURES YOU WANT TO SPECIFY:**