DETERMINATION OF SOME ESSENTIAL TRACE ELEMENTS (CHROMIUM, ZINC AND SELENIUM) IN SELECTED EGYPTIAN SEEDS AND MEDICINAL PLANTS

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ABSTRACT

There is a strong relationship between Cr^{3+} , Zn^{2+} and Se^{4+} and insulin. These essential trace elements is well known to take part in the synthesis, secretion and activity of insulin. Some Egyptian aromatic plants are utilized as tisanes by diabetic people as medicinal plants. Their active principle is not yet known, and the importance of their Cr^{3+} , Zn^{2+} and Se^{4+} contents in the claimed therapeutic properties should not be discarded.

The purpose of this study was to determine Cr^{3+} , Zn^{2+} and Se^{4+} in some plants and herbals. Cr^{3+} was determined using Graphite Atomic Absorption, Se estimation was performed using Hydrid Generation atomic, Men and Zn determination was made by Flame Atomic Absorption. Most of analyzed plants contain Cr^{3+} at the normal level for this element (1.173 – 4.632 $\mu g/g$: n=16) but some plants used to prepare tisanes to alleviate diabetic conditions contained higher levels (8.64 -18.115 $\mu g/g$, n=4) than others (0.280 – 0.479 $\mu g/g$, n=2). Zinc was found at the normal levels (36.825 – 17.30, n=9), but some plants had higher levels (142.50 – 55.27 $\mu g/g$, n=15) than others (10.55 – 9.152 $\mu g/g$, n=2). On the other hand, Se was present at a high level in most plants (1832.25-73.50, n=13 $\mu g/kg$), but in a modest level in some plants (21.75-40.05,n=8 $\mu g/kg$) and low in others (1.75-14.75 g/kg,n=6).

Key words: chromium, medicinal plants, seeds, selenium, zinc.

1. INTRODUCTION

Chromium is an essential trace element needed in very small quantities (50-1,000 micrograms daily, Anderson 1998) for normal health in humans.

Chromium is a vital nutrient and there is accumulating evidence linking low levels of chromium with heart disease, diabetes and possibly cancer. In 1959, trivalent chromium was identified as the active component of the "glucose tolerance factor", which alleviated impaired glucose tolerance in rats fed diet inadequate in chromium. In 1960, reports indicated that Cr³⁺ could help improve blood sugar control in diabetics.

Cr³⁺ enhances insulin action and thus influences carbohydrate, lipid, and protein metabolism.(Althuis *et al.*, 2002; Berrio *et al.*, 1995; Abraham *et al.* 1992; Anderson *et al.*, 1991; Anderson 1993; Anderson *et al.*, 1997; and Anderson 1998).

The recommended dietary allowance (RDA) for Cr³⁺ is 50 µg daily. The anti-aging dosage of Cr³⁺ for older people is recommended at 200 µg daily (National Research Council, 1989).

There is a strong relationship between Zinc and insulin. This essential trace element is well known to take part in the synthesis, secretion and activity of insulin (Faure et al., 1992). Furthermore, zinc also mimics some actions of insulin (Shisheva et al., 1992). Fredric et al. (1995) suggested that zinc improves glucose assimilation, as evidenced by increase in weight, and improvement results mainly from an increase in glucose effectiveness (Insulin-like effect) rather than an action on insulin response or insulin sensitivity.

Chen et al. (1998) reported that zinc supplementation alleviated the hyperglycemia of obese and obmice, which may be related to its effect on the enhancement of insulin activity.

Administration of oral and /or injectable trace elements and some antioxidants have been proposed as a therapeutic (Coppey et al., 2001). Ezaki (1990) first showed the effect of sodium selenate as a potential insulinlike agent. Other studies performed by both sodium

selenate 2.5- as an insulin mimetic and as an antioxidant in vivo, depending 4.5 mg/d) and sodium selenite 0.5 mg/d) showed that selenium also acted on its concentration and tissue (McNeill et al., 1991; Becker et al., 1996).

The RDA of Se for men and women is 70 and 55 μ g/d, respectively (National research council,1989)..

In Egypt, some medicinal seeds are consumed as tisanes by diabetic people to alleviate their health problems. In popular medicine, these plants are well known in relation to that therapeutic property, but the involved active principles are unknown. Perhaps compounds containing trace elements as Cr, Zn and Se could be responsible for their pharmacological effect. For this purpose, some Egyptian plants commonly employed to reduce blood sugar were analyzed for these elements using atomic absorption spectrometry.

The aim of this study was to make an available database for Cr, Zn and Se in some Egyptian seeds and medicinal plants.

2.MATERIALS AND METHODS

2.1.Sample preparation

Some plants shown in Tables 2,3 and 4, were purchased from herb stores that sell them as having antidiabetic properties, but others were obtained from North Sinai. Only the parts claimed to be active were used.

The first step in sample preparation was air drying at room temperature (25°C), followed by grinding in a porcelain vessel, avoiding contact with stainless steel to prevent contamination. The obtained powder was further dried at 105°C for 3 hrs. After cooling, 1.00 g of the material was incinerated at 500°C for 3 hrs (in triplicate) in porcelain crucible. To the resulting ash, 10 ml of superpure HCl: HNO₃: H₂O (1:1:8) were added, and after boiling for 2 mins, the cooled solution was quantitatively transferred to a 25 ml volumetric flask to be used without further dilution. This procedure was performed according to Curtius and Campos (1982).

2.2.Instrumentation and analysis conditions

2.2.1.graphite furnace

A Perkin Elmer Atomic Absorption Spectrophotometer . model 3300 AAS equipped with Perkin Elmer graphite furnace, model HGA, a deuterium lamp background corrector, and an auto sampling system model were utilized in all determinations. As a light source, a hollow cathode lamp was utilized, and pyro/plat form tubes were used as atomizers.

The conditions for analysis of Cr were based on the instruction presented by the instrument manual. Samples 10 μ L were carried to the graphite tube by the autosampling system and submitted to furnace established conditions (Table 1).

Table(1):Recommended HGA analytical conditions for chromium.

Wave length	: 357.9	Pretreatment (°C)	:1650
Slit	:0.7	Atomization temp(°C)	:2500
Tube site	:pyro/platform		
Matrix modifi Mg(NO ₃)	ier : 0.05 mg	· · · · · · · · · · · · · · · · · · ·	
Characteristic	mass (pg/0.004 As)	: 3.0	

Comments: Diluted used to obtain data 0.2% ANO₃.

2.3. Chromium determination

Direct determination of the chromium concentration in the prepared samples was performed by comparison with aqueous standard chromium (Merck). A stock solution (100ppm) was prepared and stored in a polyethylene container. Working aqueous standards were made fresh daily by volumetric dilution of the stock solution to the desired concentration with deionized water. A calibrated curve automatically prepared between 10 and 40 ppb of Cr³⁺ was obtained (r2=0.9998). Cr³⁺ measurements were closer to the mean value obtained with the standard calibration curve method. (Perkin-Elmer, 1982).

2.4.Zinc determination

Samples for zinc concentrations were determined by the method of Flame Atomic Absorption Spectrophotometry, wavelength 213.9, slit 0.7 air-acetylene oxidizing. (Perkin-Elmer, 1982).

2.5. Selenium determination

The Se determination by Hydride Generation Atomic Absorption Spectrophotometry (HGAAS) was performed according to (Thnat and Miller 1977) for plants. Samples to be analyzed for Se should prereduced prior to analysis by adding 1:1 diluted HCl to the sample or standard solution followed by heating at 20°C for 20 mins. After prereduction , the solution may be diluted without the risk of back oxidation from Se⁴⁺ to Se⁶⁺.

The element Se⁴⁺ was also measured by using the same Atomic Absorption and with the Hydride technique involving the reaction of acidified aqueous samples with a reducing agent of sodium borohydride. The reaction was transported to a quartz cell by means of an argon carrier gas in which it was converted to gaseous metal atoms. The amount of light absorbed was then measured by using the lamps as a source of light. The conditions used for measuring Se element were as follows:

Reagents:

Carrier solution:

10% (v/v) HCI

Reducing agent:

0.2% NaBH₄ in 0.05% NaOH

Sample solution: Se^{4+} in 10%(v/v) HCl.

3.RESULTS AND DISCUSSION

In spite of the fact that insulin has become one of the most important therapeutic agents known to medicine, research workers again have been making efforts to find insulin substitutes from synthesis or plant sources for the treatment of diabetes. Many of them have remained as an alternative to conventional therapy in areas where insulin is not readily available (Sanchez et al., 1994). Cr, Zn and Se in some plants with antidiabetic properties were measured according to the standard calibration curve methods.

Tables(2, 3 and4) present the results for Cr and Zn contents in $\mu g/g$ dry wt and Se in $\mu g/kg$, some of which are used in the herbal treatments of diabetes. The plants claimed to have hypoglycemic activity have a higher concentration (1.1-3.8 $\mu g/g$ dry wt) (Valdemar, 1998). Lupinus termis has a high value of Cr (12.83 $\mu g/g$ dry wt). Glombitza et al., 1994 studied the use of many plants for the treatment of diabetes mellitus e.g. Bryonia cretica, Salex species, Lupinus termis, Fenugreek and Allium species.

Chromium and zinc contents in sage were 2.615 and 20.357 $\mu g/g$ (dry wt), respectively while it was $13\mu g/kg$ in the case of selenium.

Sage may be of value to people with diabetes. Laboratory studies indicated that sage may boost insulin's action, since sage was found to boost insulin activity two-to five folds or more in patients with Type II (non-insulin dependent) (Tyagi and Delanty, 2003).

In the Egyptian folk medicine, many plants were used in the treatment of diabetes mellitus e.g., Bryonia cretica, Salix species, Lupinus termise, Fenugreek and Allium species. The highest value of Cr was found in Nigella sativa (18.918 µg/g dry wt), the zinc and selenium in the same plant were concentrations of 84.57µg/g and 10.5 µg/kg, respectively. Salem et al. (2004) reported that the ratio of total cholesterol/HDL-cholestrol highly significantly decreased in hypercholestrolemic rats by giving Nigella sativa diets, which proved the beneficial effect of these hypercholestrolemic agents. In addition, Salem et al., (2004) indicated that hypercholestrolemic diet induced significant increase in liver enzymes activities(ALT, AST, GCT, LDH and alkaline phosphates AIP). Nigella sativa at different levels significantly (p<0.01) decreased ALT. AST GCT, LDH and the levels of alkaline phosphatase activity. Shils and Shike(1994) found that Cr³⁺ is either involved with binding of insulin to its receptors or with chromium facilitates insulin binding and subsequent uptake of glucose into the cell. Supplemented Cr³⁺ has been shown to decrease fasting glucose level, improve glucose tolerance, and decrease total cholesterol and triglycerides while increasing HDL-cholesterol in normal, elderly and type2 diabetic subject (Mooradian et al., 1994 and Baker 1996).

Table(2): Chromium concentration in the studied samples.

Соттол пате	Arabic(local)name	Latin name	Cr (μg/g) ± SD(n=3)
Lavender cotton(flower)	El-Qaissom	Achillea fragrant	18.061±0.158
vender cotton(whole seed)	El-Qaissom	Achillea fragrant	2.94±0.032
Thymifolius	Qussaib El-raal	Fumana thymifolia	1.473±0.023
Rose of Jericho	Kaff Mariam	Anastatica hierochuntica	1.865± 0.055
and wormwood (flower)	Al-Ader	Artemisia monosperma	1.606±0.007
and wormwood .(whole seed)	Al-Ader	Artemisia monosperma	2.33 ±0.011
Juniper	Al-Arar	Juniperus phoenicea	2.26±0.026
Rocket(seeds)	Gargeer	Eruca vesicaria sativa	3.123±0.38
Harmeli	Harmal	Pegnum harmola	0.280±0.141
Wild thyme	Zaatar Barri	Thymus syriacum	1.768±0.078
Black cumin	Habbet Al-Barakah	Nigella sativa	18.918±0.098
Nettle	Aras	Urtica dioica	4.632±0.228
Lupine	Termis	Lupinus digitatus	12.83±0.365
ajoram or sweet majoram	Bardakosh	Majoran hortenis	8.699±0.309
ankincense or Olibanum	Leban dakar	Boswelia Carterii	0.479±0.028
Fenugreek	Helba	Trigonella foenum	2.497±0.188
Wormwood	Damseesah	Artemisia anomala	2.134±0.119
Aloes	Sabbar	Aloe spp.	13.949±0.118
Myrrh	Могт	Commiphora myrrha	2.615±0.110
Morning-glory	Hantit	Ipomoea spp.	1.165±0.095
Saga or garden sage	Marmariah	Salvia officinalis	1.353±0.013
Turmeric	Korkom	Curcuma domestica	1.1655±0.095
Chick pea(cooking)	Hommos Tabkh	Cicer arietinum, spp asiaticum	2.94±0.032
Chick pca(sweets)	Hommos Halawiat	Cicer arietimum, spp arientale	18.155±0,095
Barley	Shaeir	Hordeum vulgare	4.555±0.058

Table(3): Selenium concentration in the studied samples.

Arabic(local)name				
Common пате		Latin name	Se ($\mu g/kg$) \pm SD(n=3)	
Lavender cotton(flower)	El-Qaissom	Achillea fragrant	40.50±.0114	
Lavender cotton(whole seed)	El-Qaissom	Achillea fragrant	39.75±0.122	
Thymifolius	Qussaib El-raal	Fumana thymifolia	106.00±0.58	
Rose of Jericho	Kaff Mariam	Anastatica hierochuntica	215.00±0.114	
Sand wormwood (flower)	Al-Ader	Artemisia monosperma	31.5±0.029	
Sand wormwood .(whole seed)	Al-Ader	Artemisia monosperma	84.50±0.154	
Juniper	Al-Arar	Juniperus phoenicea	24.25±.0.26	
Rocket(seeds)	Gargeer	Eruca vesicaria sativa	1832.25±0.151	
Harmeli	Harmal	Pegnum harmola	115.625±0.024	
Wild thyme	Zaatar Barri	Thymus syriacum	77.75±0.068	
Black cumin	Habbet Al-Barakah	Nigella sativa	10.5±0.045	
Nettle	Aras	Urtica dioica	73.50±0.138	
Lupine	Termis	Lupinus digitatus	200.25±0.119	
Majoram or sweet majoram	Bardakosh	Majoran hortenis	28.75±0.045	
Frankincense or Olibanum	Leban dakar	Boswelia Carterii	14.47±0.026	
Fenugreek	Helba	Trigonella foenum	24.25±0.34	
Wormwood	Damseesah	Artemisia anomala	231.255±0.013	
Aloes	Sabbar	Aloe spp.	1.75±0.19	
Myrrh	Morr	Commiphora myrrha	9.50±0.016	
Morning-glory	Hantit	Ipomoea spp.	219.25±0.0106	
Saga or garden sage	Marmariah	Salvia officinalis	13.00±0.026	
Turmeric	Korkom	Curcuma domestica	34.75±0.064	
Chick pea(cooking)	Hommos Tabkh	Cicer arietinum, spp asiaticum	465.25±0.101	
Chick pea(sweets)	Hommos Halawiat	Cicer arietinum, spp arientale	14.25±0.013	
Barley	Shaeir	Hordeum vulgare	211.75±0.632	
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Table(4): Zinc concentration in the studied plants.

Common name	Arabic(local)name	he studied plants. Latin name	Zn(μg/g) ±
Lavender cotton(flower)	El-Qaissom	Achillea fragrant	SD(n=3) 77.007±0.032
Lavender cotton(whole	El-Qaissom		77.007±0.032
seed)	`	Achillea fragrant	33.57±0.008
Thymifolus	Qussaib El-raal	Fumana thymifolia	55.257±0.0585
Rose of Jericho	Kaff Mariam	Anastatica hierochuntica	21.525±0.0112
Sand wormwood (flower)	Al-Ader	Artemisia monosperma	124.50±0.0031
Sand wormwood (whole seed)	Al-Ader	Artemisia monosperma	33.225±0.0026
Juniper	Al-Arar	Juniperus phoenicea	20.650±0.058
Rocket(seeds)	Gargeer	Eruca vesicaria sativa	88.00±0,0028
Harmeli	Harmal	Pegnum harmola	40.525±0.057
Wild thyme	Zaatar Barri	Thymus syriacum	35.15±0.0116
Black cumin	Habbet Al-Barakah	Nigella sativa	84.75±0.0016
Nettle	Aras	Urtica dioica	66.25±0.292
Lupine	Termis	Lupinus digitatus	57.800±0.0425
Majoram or sweet majoram	Bardakosh	Majoran hortenis	105.357±0.0077
Frankincense or Olibanum	Leban dakar	Boswelia Carterii	10.55±0.0064
Fenugreek	Helba	Trigonella foemum	65.35±0.0403
Wormwood	Damseesah	Artemisia anomala	. 74.20±0.0661
Aloes	Sabbar	Aloe spp.	26.125±0.004
Myrrh	Morr	Commiphora myrrha	9.125±0.041
Morning-glory	Hantit	lpomoea spp.	17.30±0.034
Saga or garden sage	Marmariah	Salvia officinalis	20.357±0.0083
Turmeric	Korkom	Curcuma domestica	17.30±0.344
Chick pea(cooking)	Hommos Tabkh	Cicer arietinum , spp asiaticum	60.625±0.045
Chick pea(sweets)	Hommos Halawiat	Cicer arietinum , spp arientale	56.20±0.0809
Barley	Shaeir	Hordeum vulgare	36.825±0.0101

Experts recommended a higher dosage (400-1000 $\mu g/day$) for men and for people desiring to reduce high cholesterol and triglycerides, improve glucose tolerance, boost immune system functioning, thwart heart disease and cancer(Vincent,2000). It is virtually impossible to get adequate daily Cr3+ from the average normal diet (Vincent, 2000).

Chromium has a very low toxicity level without side effects at normal doses (Sutherland et al., 2000). According to experts (Vincent, 2000), a person could even take three hundred times the ecommended dose of 2000 micrograms without "getting in trouble". However, excessively high doses of chromium do bio-accumulate in liver and kidneys over time but high doses are unnecessary. The anti-aging dose of 200 µg daily is considered to be perfectly safe and effective according to experts(Anderson, 1998). The two stable valence states of Cr found in biological systems, hexavalent Cr⁶⁺ is much more toxic than trivalent Cr³⁺ (Sutherland et al., 2000).

From Tables (2,3 and 4), it could be noted that Cr^{3+} in chick pea (cooking type) (18.155 $\mu g/g$) is higher than chick pea (sweet type)(1.353 $\mu g/g$). That was on contrast to the concentrations of Se in the two plants since it was higher 33 folds in the case of chick pea (cooking type) when compared to chick pea (sweets type). Zinc in both types of chick pea was almost of the same concentrations.

Abdel-Rahim et al. (2004) suggested that supplementation with raw chick pea seed meal (var G35), raw or soaked, decreased either glucose or cholesterol in plasma. Also, the authors concluded that chick pea could be used at about 100g/day to regulate the blood glucose of diabetic patients.

The values of Cr ,Zn and Se for turmeric were 1.165,17.30 µg/g. and 34.075µg/kg. Lucy Dey et al.(2002) found that Cr content of turmeric species had a beneficial effects to decrease fasting glucose levels, improved glucose tolerance and lowered insulin levels. Shallan et al.(2004) concluded that biscuits made using the powder of turmeric exhibited significant hypoglycemia as serum glucose or hypocholestrolemic activity agent. Some plants are used in the herbal treatment of diabetes. The values of Cr in herb Juniperus phoenicea is 2.26 µg/g. Asad et al. (2002) suggested that the best result in blood glucose, AST, ALT and lipids pattern were shown in

5% level of *Juniperus phoenicea* dry plant supplementation when compared with diabetic control. The value of Cr in Fumana thymifolia is (1.47 µg/g). Regarding the Recommended Dietary Allowances (RDA), zinc and selenium contents in Fumana thymifolia were found to be of sufficient levels. The results obtained by Asad et al.,(2001) showed that the optimum level of Fumana thymifolia plant that can be used was 6% supplementation significantly decrease the weight gain ,blood glucose, lipid pattern and transaminase activities compared to correspond control. Artimisia monosperma Cr values was (1.606 µg/g). Asad et al., (2003) showed that the optimum level of the dried plant can be used at 7.5%. This level decreased the weight gain, blood glucose, urea serum, uric acid, total lipids, total cholesterol, triglycerides. ALT and AST. Also barley contains high values of Cr, Zn and Selenium. In Iraq, for example, bread from barley flour is traditionally used in the management of diabetes mellitus, and the flour was postulated to explain its beneficial effect (Mahdi and Naismith, 1991).

The Cr and Zinc contents of Anastacia hierochuntica plant were of adequate levels but it was higher in the case of selenium. The highest level of Anastacia hierochuntica (dry plant) that can be used was 8% concerning consumer acceptability. Also improved increase the weight gain while the blood glucose, GOT,GPT and lipids pattern were decreased when compared with positive control. (Asad et al., 1999).

Some plants not claimed to have medicinal properties, which have lower values of the metal, may also be active if Cr is also involved with adequate ligands to be well absorbed and used by the organism.

Failla and Gardll (1985) and Lau and Failla (1984) findings support the notion that in the diabetic state, the alterations in trace metal metabolism and concentration resulted mainly from the lack of insulin. Endocrine imbalance, mainly hypoinsulinemia causing an elevation of metallotheioneion, is thought to be a major contributor to the alterations of trace metal metabolism and concentration in chemical induced diabetic rats.

Additionally, Tables (3) and (4) reveal the concentration of Zn and Se.

Brun et al. (1995) suggested that 20 mg zinc gluconat as zinc load, at physiological doses, improve glucose-induced glucose disposal, and thus improves intravenous glucose tolerance. This effect colud be related to the insulinl-ike effects of this metal, which have been reported at higher superphysiological doses in animals.

Chen et al. (1998) concluded that Zn supplementation alleviated the hyperglycemia of ob/ob mice, which may be related to its effect on the enhancement of insulin activity. Craft and Failla (1983) reported that the metabolism of trace elements e.g., Zn and Cr in diabetic rats suggested that similar abnormalities also exist for Zn. Additionally, it was shown that the increased urinary Zn excretion in the insulin-dependant rats could be reversed by the administration of insulin (Failla &Gardll, 1985 and Lau & Failla (1984).

Selenium contents of individual foods vary widely and are dependant on the introduction of that metal in the growing, transport, processing, and fortification of the food. Berkenboom *et al.* (1997) have shown that oral administration of sodium selenite is able to prevent cyclosporine-A-induced vascular toxicity. Ayaz *et al.* (2002) suggested that sodium selenite treatment may alleviate late diabetic complications when it is used under controlled conditions.

In conclusion, these results suggested that the Egyptian plants used as active agents to alleviate diabetes may have such activity owing to their high levels of Cr, Zn and Se. which should be in complex form with organic compounds to have the best therapeutic effect.

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تقدير بعض العناصر الضنيلة الأساسية (الكروميوم و الزنك و الساينيوم) في بعض البذور و النباتات الطبية المصرية

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ملخص

توجد علاقة قوية بين الكروميسوم و الزنسك و السلينيوم و هرمسون الانسولين ، حيث وجد ان هذه العناصر لها دور في عمليسات تخليسق و إفراز وكذلك نشاط هرمون الأنسولين. يستخدم الكثير من مرضى السكر بعسض هذه النباتات والبذور كنباتات طبية. و على الرغم من ان الدور الحيوى لهذه النباتات ليس معروفا بعد على وجه الدقة إلا ان محتوى هذه النباتات من الكروم و الزنك و السلينيوم هو من الموضوعات الجديرة بالدراسة.

تهدف هذه الدراسة لتقدير محتوى بعض النباتات و الأعشاب المصريـــة من هذه العناصر الثلاثة حيث تم تقدير هذه العناصر باستخدام جهاز الامتصاص الذرى.

أوضحت النتائج ان معظم النباتات و الأعشاب المختبرة احتوت على مستويات مناسبة من عنصر الكروميوم ((7,10-777.3) ميكروجرام/جم) بينما احتوت باقى النباتات المستخدمة فى علاج مرضى السكر على نسب أعلى من هذا العنصر ((7,7.8-7.4) ميكروجرام/جم) مقارنة بغيرها ((7,7.8-7.4) ميكروجرام/جم).

و بالنسبة للزنك فقد وجد في مستويات كافية في معظم النباتات و لكنه وجد ايضا ان بعض النباتات تحتوى على نسب أعلى منه (١٤٢,٥٠-٥٥,٢٧ ميكروجرام/جم. (أما ميكروجرام/جم) مقارنة بالبعض الآخر (١٩٠١- ١٠,٥٥ ميكروجرام/جم. (أما عنصر السلينيوم فقد وجد بنسب عالية في معظم النباتات تستراوح بين عالم ١٤,٧٥ ميكروجرام/كجم.

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