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Application of Stripping Voltammetry for the Determination of Cadmium, Lead, Copper and Zinc in Yemeni soils and Vegetables

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تحديد العناصر الزهيدة من الكادميوم والرصاص والنحاس والخارصين في أراضي وخضراوات محافظة إب بالجمهورية اليمنية بطريقة الخلع المصعدي الجهدي

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يقدم هذا البحث النتائج العملية لتحديد عناصر الكادميوم، الرصاص، النحاس، والزنك الموجود في مستويات منخفضة جداً في الأراضي والخضراوات اليمنية بإستخدام طريقة الخلع المصعدي الجهدي.
أوضحت النتائج أن المتوسط الهندسي للعناصر الأربعة في التربة الجافة هو ٠,٥٦ ، ٩,٤ ، ٣٥ ، ٥٨ ملغم/كغم على التوالي وهذا يدل على إن العينات المدروسة خالية من التلوث بهذه العناصر وفقاً للقيم المنشورة، أما تحليل هذه العناصر في الخضراوات فقد أظهر بأن أعلى وأدنى قيمة للكاديموم هي في الجرجير والخيار (٣٧,٦ و ٢,٦ ميكروغم/كغم على التوالي) وللرصاص في الجرجير والطماطم (٣٠٦,٨ و ٣٠,٢ ميكروغم/كغم على التوالي) وللنحاس في الملوخية والملفوف (٤٧٣٤ و ٥٢١ ميكروغم/كغم على التوالي) وللزنك في الملوخية والطماطم (٥٥٦١ و ٥٥٩ ميكروغم/كغم على التوالي)، إن هذه التراكيز لا تشكل أي خطورة على المستهلك اليمني وفقاً لمعايير الصحة العالمية.

Keywords: *Anodic Stripping Voltammetry, Cadmium, Lead, Copper, Zinc, Edible vegetables, Yemeni soils.*

ABSTRACT

Heavy metals contents in the solutions from acid digestion of Yemeni soils and vegetables were determined by differential pulsed anodic stripping voltammetry. The geometric mean for Cd, Pb, Cu and Zn concentrations for the soils (to a depth of 40 cm) were 0.56, 9.4, 35 and 58 mg kg^{-1} dry weight respectively. These levels are closely related to the literature values for uncontaminated soils. Highest and lowest concentrations of Cd were in watercress and cucumber (37.6 and 2.6µg kg^{-1}, resp.); Pb in watercress and tomato (306.8 and 30.2µg kg^{-1}, resp.); Cu in Jew's marrow and cabbage (4734 and 521µg kg^{-1}, resp.); and Zn in Jew's marrow and tomato (5561 and 559µg kg^{-1}, resp.). These values do not pose a health risk for consumers, according to the levels proposed by FAO / WHO.

Introduction

Food, fiber, and many products essential to sustain human life come directly or indirectly from plants, which are supported by soils. Through food grown on soils, humans obtain energy and essential elements. Also, through food grown on soils, humans may be exposed to harmful substances that are absorbed by plants.

In developing countries like Yemen industry is not too intensive to cause substantial pollution. The major source of contamination comes from agricultural activities, such as the application of fertilizers, pesticides, and the use of sewage sludge coming from biological wastewater treatment plants.

To some fertilizers have been added elements such as copper, zinc, and may contain cadmium and lead as impurity [1]. Thus the monitoring of heavy metals in the soil and plants becomes a task of a high priority and significance in environmental research and protection.

Several techniques have been used in trace metal analysis with varying degrees of success and convenience [2]. Among the various techniques, stripping analysis offers the advantages of high accuracy with extraordinary detection sensitivity, good precision and the ability to determine simultaneously several heavy metals over a large concentration range [3]. Moreover, voltammetry requires considerable lower costs for instrumentation and its operation and maintenance.

In the present study a convenient analytical procedure has been developed for the simultaneous determination of Cd, Pb, Cu and Zn in Yemeni soils and vegetables. Yemeni soils and vegetables may be considered as a typical representative for a media in which the major source of contamination comes mainly from agricultural activities.

Exposure to toxic metals is often assessed by measurements in air, food, water, soil and other materials. This is valuable in identification of sources of exposure and checking of effects and measures to reduce them [4]. In this study the contribution of vegetables to the daily intake of the trace elements under study and repercussions with respect to the recommended directives is discussed briefly next.

In the Middle Eastern diet, vegetables account for 14.7% of the total daily food consumption (193 grams person kg^{-1} day^{-1}). This figure is very close to the average vegetable consumption recommended by the food and Agricultural Organization (FAO) to be used in order to estimate human exposure to pollutants [5]. Based on this figure, the lowest and highest average daily intake of Cd and Pb by Yemeni consumer are estimated to be 0.5-7.3 and 5.8-59.2μg/day respectively.

The FAO/WHO Joint Expert Committee on Food Additives recommended a provisional maximum tolerable daily intake of Cd and Pb from all sources (food, air and water) of 1 - 1.2 and 3.5 - 4 g kg^{-1} body mass respectively [6]. These values correspond to a provisional daily intake of 60 - 72 and 210 - 240 μg (assuming an average Yemeni weight of 60 kg). According to these directives the daily intake of Cd and Pb by Yemeni consumers from vegetables alone is far below the FAO/WHO Provisional Tolerable Daily Intakes.

The lowest and highest daily intake of Zn by vegetable consumer was estimated to be 107.9 - 1073.3 μg. Obviously these values do not pose any health risk according to the 1989 Recommended Daily Allowance (RDA) levels (15 mg/day for males and 12mg/day for females) [7]. Similarly, the average daily intake of copper from vegetables is ranging from 106.9-913.7μg (Table 3). This level is also below the current estimated safe and adequate daily intake (ESADDI) range from 2 to 3 mg/day [7]. The amount of copper and zinc ingested in food through uncontaminated soils are almost never harmful to humans because they are relatively low and because most mammals and vertebrate have the ability to maintain homeostasis by a combination of decreased absorption and enhanced excretion. Toxic amounts are encountered only in unusual circumstances such as the use of uncontrolled amounts of copper and zinc salts in agricultural products.

Materials and Methods

Sampling and Sample Pretreatment

Cadmium, lead, copper and zinc were measured in a total of 100 samples of Yemeni soils and 600 samples of edible vegetables (from eighteen different species) commonly grown and eaten in Yemen. The samples are chosen from various agricultural locations in Ibb province, which represent the most fertilized lands in Yemen.

Each soil is homogenized in the laboratory using a mortar and pestle and air-dried for 24 h before analysis. An aliquot of 300 mg of finely ground soil is digested under pressure with a 3:1 of HNO_3/HCl at 170°C [8,9]. The entire digestion takes about 3 h. The analyte resulting from the digestion is made up to a volume of 250 ml and the pH is adjusted to 4 for the simultaneous determination of Cd, Pb, Cu and Zn.

Different procedures given in the literature were examined to digest the vegetables. Based on the experience with these methods the following recipe was adopted [10]. Each vegetable was taken into a pre-cleaned polyethylene bag, washed thoroughly with distilled water and air dried before analysis. One gram of each sample is wet digested with 1 ml of 70% nitric acid (Electronic grade), 0.5 ml of 96% sulphuric acid and 1 ml of 70% perchloric acid at 170°C until a clear digest was obtained (approximately 3 h). The digested sample was evaporated to nearly dryness. The residue was taken in 10 ml of 0.25% nitric acid (pH = 2), which was directly transferred to the voltammetric cell for lead, cadmium and copper estimation. For zinc estimation 5 ml of sample solution in 0.25% nitric acid was buffered with equal volume of 0.3 M ammonium acetate and then transferred to voltammetric cell. Optimum quantities of acids were used so that the wet oxidation of the sample was complete and at the same time the reagent blank values for Cd, Pb, Cu and Zn were sufficiently low.

Apparatus

Stripping voltammetric experiments were carried out with a Metrohm (Herisau, Switzerland) 746 VA Trace Analyzer connected to a Metrohm 747 VA multimode electrode used in the hanging mercury drop electrode (HMDE) mode. A platinum rod and a saturated Ag /AgCl electrode were used as auxiliary and reference electrodes, respectively [11]. pH was measured with a digital pH-meter JENWAY, Model 3310. Dissolved oxygen was removed from the samples by purging with purified nitrogen (99.99%) through the measuring vessel for 5 min. During the experiments, nitrogen was passed over the solution to prevent oxygen interference.

The optimum experimental conditions were established as follows: the potential was swept using differential-pulse modulation (DPASV) with a pulse rate of 3.33 s^{-1}, a scan rate of 6 mV s^{-1} and a pulse amplitude of 50 mV. The standard additions technique was used to give the concentrations of cadmium, lead, copper and zinc simultaneously when a sweep potential was applied between -1.150 V and 200 mV (for zinc -1.150 V to -800 mV, for cadmium -800 mV to -450 mV, for lead -500 mV to -200 mV and for copper -200 mV to 200 mV). All quoted potentials are referred to the Ag/AgCl electrode.

Chemicals

All chemicals were of analytical-reagent grade. Deionized water was used to prepare all solutions. The standard metal solutions were prepared as follows: cadmium, copper and lead stock solutions were prepared by dissolving the corresponding nitrates and a zinc stock solution by dissolving the sulphate in deaerated 2% (v/v) HNO_3. The working standard solutions were prepared daily by suitable dilution of this stock solution in the matrix required. All glassware were stored in 8 M nitric acid for 1 week and rinsed thoroughly with deionized water.

Statistical analysis

Quantification of metal concentrations in the samples was carried out by use of the standard addition method. This is the preferred method as the sensitivity of the stripping voltammetric analysis may vary between samples of different ionic strength. The best fitting line through the data pairs was calculated by linear least-squares regression analysis. The concentration of each element in the sample is equal to the quotient of the intercept and the regression coefficient.

The scatter of the results was examined visually to assess its closeness to a normal distribution. All data relating to Cd, Pb, Cu and Zn in soils and vegetables were skewed severely and results were subjected to logarithmetic transformation to achieve approximate normality. The results are expressed as means and standard deviations.

Results

An anodic stripping voltammogram of a digested soil sample is shown in Figure 1. Well-defined peaks for cadmium, lead, copper and zinc were observed, indicating that the digestion of the sample was relatively complete. The small overlap between Pb and Cu peaks is successfully resolved (after smoothing and removing background) using the model presented by Huang W et al [12]. The peak height of the voltammetric signal increases linearly with the deposition time in the range of 30-180s for all four metals studied allowing thus the adaptation of the deposition time to the level of the metal.

The calibration curves were linear in the concentration range of 0-300 ppb with a correlation coefficient lies between 0.9950 and 0.9957 for the four elements. Based on the calibration curve, the limits of detection were also determined. The limit of detection is the analyte concentration giving a signal equal to the blank signal, plus three standard deviations [13]. The limits of detection were 0.15 $\mu g\ l^{-1}$ Cd, 0.30 $\mu g\ l^{-1}$ Pb, 2.80 $\mu g\ l^{-1}$ Cu and 1.20 $\mu g\ l^{-1}$ Zn.

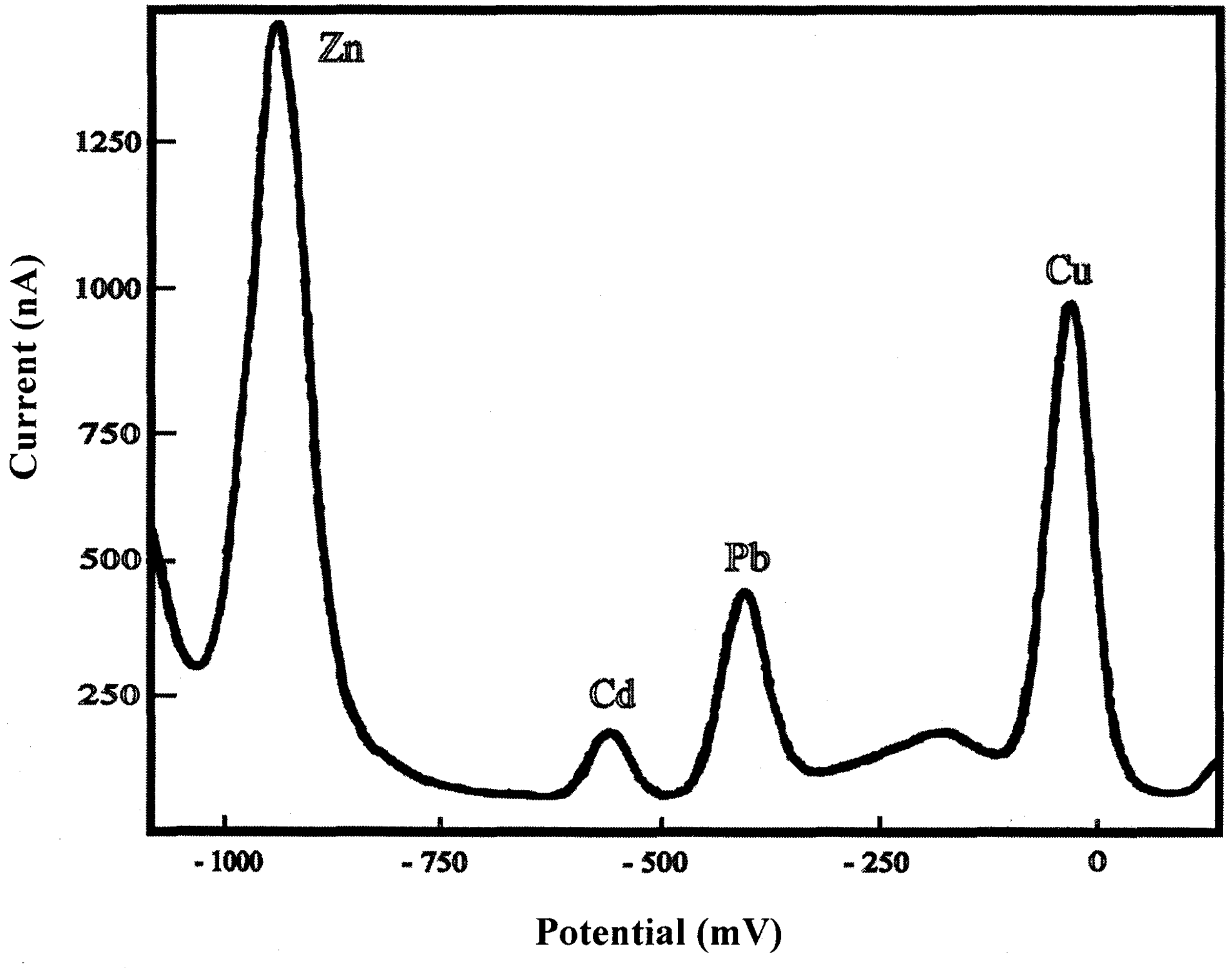

Figure 1: Typical voltammogram of wet digested khat (300 mg) after dilution to 250ml. Deposition potential -1100 mV; deposition time 120s for Cd, Pb and 30s for Cu. Zn; scan rate 6 mV/s; pulse amplitude 50 mV.

The precision and accuracy of the proposed method were checked with Orchard leaves (NBS Standard Reference Material 1571) and with river sediment (NBS Standard Reference material 1645) after digestion to provide aqueous solutions whose final concentrations were within the range of the metal contents expected in soil and vegetables. Our mean results agree to 94% with the certified values (Table 1).

Table 1: Trace metal determination in Standard Reference Materials (values in mg kg^{-1} dry weight).

Standard Reference Material	Cd	Pb	Cu	Zn
NBS 1571 (Orchard leaves)				
Certified value	116 ± 13*	48 ± 5	11.6 ± 0.4	27.3 ± 4.2
Own value	110 ± 10	45 ± 3	12.0 ± 1.0	25.0 ± 3.0
NBS 1645 (River sediment)				
Certified value	10.2 ± 1.5	714 ± 28	109 ± 19	1720 ± 169
Own value	9.6 ± 2.1	698 ± 51	96 ± 15	1642 ± 280
* Standard deviation for 6 determinations.				

Recoveries were done by five replicate voltammetric determinations of the metals under consideration in five samples. The average recoveries were 99% (range 90-108%).

Tables 2,3 and 4 summarize the results of our studies using the wet digestion procedure.

Table 2: Concentrations of cadmium, lead, copper and zinc in soil samples from unpolluted regions in Ibb-Yemen (values in mg kg^{-1} dry weight).

Element	Range of Values	Geometric Mean	Standard Deviation
Cadmium	0.05 - 2.47	0.56	0.79
Lead	0.2 - 46.3	9.4	6.8
Copper	6 - 117	35	27
Zinc	14 - 231	58	40

Table 3: Concentrations of cadmium, lead, copper and zinc in soil samples from relatively polluted regions in Ibb - Yemen (values in mg kg^{-1} dry weight).

Sampling location	Cd	Pb	Cu	Zn
Jibla	0.19	33.0	45	119
Dhahoob	0.46	20.4	83	103

Table 4: Concentrations of cadmium, lead, copper and zinc elements of edible vegetables grown on uncontaminated Yemeni soils (fresh weight basis).

Sample**	**Cd /µg kg^{-1}**	**Pb /µg kg^{-1}**	**Cu /µg kg^{-1}**	**Zn /µg kg^{-1}**	**Plant Part*** Examined**
Lettuce	19.9 ± 11.4	222.3 ± 148.3	1793 ± 859	2116 ± 1126	Leaf
Cabbage	3.6 ± 1.4	84.8 ± 58.6	521 ± 182	1023 ± 280	Leaf
Parsley	9.8 ± 4.2	212.0 ± 70.1	3218 ± 1634	3741 ± 1596	Leaf
Leek	8.5 ± 3.9	169.5 ± 99.4	1968 ± 1233	1559 ± 658	Leaf
Radish	14.0 ± 9.3	269.3 ± 175.7	1406 ± 1092	2903 ± 1120	Leaf
Watercress	37.6 ± 17.4	306.8 ± 140.3	2089 ± 979	3645 ± 1419	Leaf
Mint	9.6 ± 7.6	292.8 ± 123.3	3948 ± 2044	4852 ± 1460	Leaf
Jew's marrow	34.4 ± 18.4	164.2 ± 95.4	4734 ± 1585	5561 ± 1667	Leaf
Coriander	11.8 ± 7.0	175.3 ±74.1	3207 ± 1188	4546 ± 2067	Leaf
Cucumber	2.6 ± 1.7	30.4 ± 19.4	554 ± 296	859 ± 363	Immature fruit
Carrot	3.0 ± 1.2	44.5 ± 22.3	873 ± 504	1215 ± 541	Root
Egg-Plant	8.1 ± 3.9	36.9 ± 20.9	1311 ± 346	1277 ± 360	Immature fruit
Potato	7.5 ± 5.0	34.0 ± 21.1	1504 ± 554	1957 ± 768	Tuber
Zucchini	3.5 ± 2.6	39.9 ± 34.5	928 ± 218	1969 ± 546	Young fruit
Onion	5.8 ± 3.8	57.9 ± 48.8	1057 ± 420	1276 ± 783	Bulb
Okra	9.0 ± 4.4	58.6 ± 24.2	2145 ± 770	3791 ± 1636	Immature fruit
Tomato	4.4 ± 2.2	30.2 ± 15.6	1410 ± 704	559 ± 305	Ripe fruit
Pepper, sweet	6.3 ± 3.6	63.5 ± 26.2	1279 ± 365	1350 ± 718	Mature fruit

* Each value is the mean ± SD

** Number of samples: n = 30

*** Classified according to ref. [14]

Discussion

Yemeni soils

Metal concentrations of soils around the world have been extensively reported and they vary considerably from location to location, reflecting differences in the soil genesis processes. Nearly all metals produce toxic effects when their exposures are excessive, and a reduction of such exposures should be a prudent practice wherever possible. In general the following average levels are accepted for unpolluted soil sample: Cd = 0.35 mg kg^{-1}, Pb = 19 mg kg^{-1} [5], Cu = 20 mg kg^{-1} and Zn = 50 mg kg^{-1} [15]. Taking these values as representative data for unpolluted soils the metal levels in Ibb province (to a depth of 40 cm) (Table 2) are very close to the literature values, which indicate that Ibb is almost free of toxic metal pollution in the soil. This is well understandable as most agricultural fields in Ibb are far away from industrial areas and polluted irrigation sources. The elevated Cd values are the consequence of the fact that Cd is present in all Zn ores as a minor element.

Among ten sites only two (Jibla and Dhahoob) are relatively polluted (Table 3). The average content of lead in Jibla (22.6mg kg^{-1}) is much higher than the average value of all the investigated sites (9.4 mg kg^{-1}). This may reflect the natural geochemical background in this area. Recently the Geological Survey & Mineral Research Board in Yemen has reported that in addition to zinc and copper occurrences lead is widespread in Jibla [16]. The other field (Dhahoob) is slightly enriched with lead, copper and zinc (20.4, 83 and 103 mg kg^{-1} resp.) relative to the average values of other sites (9.4, 35 and 58 mg kg^{-1} resp.). This is probably due to the long period irrigation of these lands with municipal wastewater. The use of Pb-elevated sewage sludge as a soil amendment will lead to an increase in dietary Pb and cannot be recommended. However, the average concentration of elements in these two sites is still not critical in comparison of other soils in the world [15,17].

Yemeni Vegetables

Trace elements indigenous to the soils are rarely bioaccumulated in the plant tissue as their solubilities are low and the kinetics of their solubilization from the solid phase is low. With growing industrial and agricultural activities world-wide, trace metal concentrations of the soils have been steadily increasing. The plant uptake of potentially toxic trace elements from contaminated soils, however, would be significantly higher than those grown in the uncontaminated sites. Our data indicate that Yemeni soils are almost free of toxic metal pollution; thence we expect that Yemeni plant exposure to trace elements be significantly below the traditional levels in other areas. Indeed, table 4 indicates that the highest and lowest concentrations of Cd were in watercress and cucumber (37.6 and 2.6μg kg^{-1}, resp.); Pb in watercress and tomato (306.8 and 30.2μg kg^{-1}, resp.); Cu in Jew's marrow and cabbage (4734 and 521μg kg^{-1}, resp.); and Zn in Jew's marrow and tomato (5561 and 559μg kg^{-1}, resp.). Compared to literature data these levels proved to be in the normal range for uncontaminated areas (a fresh weight to dry weight conversion factor of ~ 0.08 was applied whenever required) [10,18].

An examination of Table 4 reveals two interesting features. Firstly, the highest accumulations of all elements were observed for leafy vegetables followed by tuber and bulb vegetables and finally immature fruits. Green cabbage leaves generally assimilated the lowest levels of heavy metals and is thus potentially suitable for the estimation of soil heavy metal hazard potential. These results agree well with those reported by other workers [19,20]. Secondly, it is evident that watercress leaves exhibited conspicuously and exceptionally, high Cd levels, whereas Jew's marrow had significantly higher concentrations of copper and zinc than did other vegetables. The relatively higher values of elements in watercress and Jew's marrow cannot be related to the concentration of Cd, Cu and Zn in the soil only since all the investigated vegetables came from the same unexposed agricultural areas. It seems that in addition to the soil chemical properties, the geometry and absorbing capacity of root systems, solute diffusion, and tortuosity of the growing media all affect the rates and amounts of trace metal ions absorbed by plants [5,21].

Nowadays increasing attention is being paid to the relation between nutrition and health, particularly regarding the possible linkage between nutrition and the development of chronic disease. Zinc and copper are two trace minerals essential for important biochemical functions and necessary for maintaining health throughout life. Zinc deficiency results in a variety of immunologic defects whereas copper deficiency is characterized by anemia, neutropenia and skeletal abnormalities [22,23]. In many metals, such as Cu and Zn, the range of desirable to toxic levels may be less than one order of magnitude. It is therefore appropriate that we put increased emphasis on the relation between nutrition and health in our societies.

Yemen is a developing nation where life expectancy is lower than in developed societies. Their intakes of meat products, which are good sources of trace elements, are limited. Therefore, increasing the amount of vegetables (such as parsley, mint, coriander and Jew's marrow) in Yemeni diets would be helpful to maintain sufficient amounts of zinc and copper in the body for good health.

REFERENCES

[1] **Kjuus, B. E., 1985.** Determination of Cadmium, Copper, Lead and Zinc in Inorganic Fertilizers by Anodic Stripping Voltammetry, Fresenius Z. Anal Chem 322: 577 - 580.

[2] **Wang J., 1996,** in Laboratory Techniques in Electroanalytical Chemistry: Kissinger P. T. and Heineman W. R. (Eds), Marcel Dekker, New York, 719 pp.

[3] **Staden J. F. and M. C. Matoetoe, 2000.** Simultaneous Determination of Copper, Lead, Cadmium and Zinc Using Differential Anodic Voltammetry in a Flow System, Analytica Chimica Acta, 411: 201-207.

[4] **Matloob, M. H.,** Simultaneous Determination of Cadmium, Lead, Copper and Zinc in Urine of Heavy Khat-Chewers Using Anodic Stripping Voltammetry, Submitted to Chemia Analityczna (Warsaw) for publication.

[5] **Chang, L.W., 1996.** Toxicology of Metals, CRC Press, Boca Raton, FL, 3pp.

[6] **Marquardt, H., S. G. Schafer, R McClellan and F. Welsch, 1999.** Toxicology, Academic Press, California, 766 pp.

[7] **Recommended Dietary Allowance, 1989.** 10th ed., NRC-National Academy of Science, 284pp.

[8] **Crompton, T. R. 1996.** Analysis of Solids in Natural Waters. Springer-Verlag Berlin Heidelberg, 32 pp.

[9] **Hall, G. E., 1997,** in Manual of Physico-Chemical Analysis of Aquatic Sediments: Mudroch, A., J. M. Azcue and P. Mudroch, (Eds) Lewis: Boca Raton, FL, 85 pp.

[10] **Yaman, M. and S. Gucer, 1995.** Determination of Cadmium and Lead in Vegetables After Activated-carbon Enrichment by Atomic Absorption Spectroscopy, Analyst, 120: 101 - 105.

[11] **Matloob, M. H., A. M. Al - Joufi and M. H. A. Saleh, 2001.** The Assessment of the Quality of Drinking Water in Ibb City, Yemen, University Researcher, 3: 107-130 (in Arabic); see also: O Metrohm, 746 VA Trace Analyzer/747 VA Stand, 1997. Instructions for Use, CH-9 101 Herisau-Switzerland, 7 pp.

[12] **Huang, W., T. L. E. Henderson, A. M. Bond and K. B. Oldham, 1995.** Curve Fitting to Resolve Overlapping Voltammetric Peaks: Model and Examples, Analytica Chimica Acta, 304: 1-15.

[13] **Xiaomel, Y., Y. Haodan, G. Tedeusz and P. Januusz, 1999.** Determination of Lead in Blood and Urine by SPME/GC, Analytical Chemistry, 7: 2998.

[14] **Maynard, D. N. and G. J. Hochmuth, 1997.** Knott's Handbook for Vegetable Growers. 4th ed., John Wiley & Sons, New York, 2 pp.

[15] **Reddy, S. J., P. Valenta and H. W. Nurnberg , 1982.** Simultaneous Determination of the Toxic Trace Metals Cd, Cu, Pb and Zn in Soils by Differential Pulse Anodic Stripping Voltammetry, Fresenius Z. Anal Chem 313: 390 - 394.

[16] **Ministry of Oil and Mineral Resources, Republic of Yemen, 2001.** Geology and Mineral Resources of Yemen, 2nd ed. 108 pp.

[17] **Moir, A. M. and Thronton, I., 1989.** Lead and Cadmium in Urban Allotment and Garden Soils and Vegetables in the United Kigdom, Environ. Geochem. Health, 11(3-4): 113-19.

[18] **Kozak, L., M. Bubicz, M. Mikos-Bielak and Z. Warda, 1994.** Lead, Cadmium, nickel, Zinc, Copper, Manganese and Iron Content of Fruits Available in the Region of Lublin, Bromatol. Chem. Toksycol., 27(2): 123-7.

[19] **Bossque, M. A., M. Schuhmacher, J. L. Domingo and J. M. Llobet, 1990.** Concentration of lead and Cadmium in Edible Vegetables from Terragona Province, Spain, Sci. Total Environ, 95: 61-7.

[20] **Schoenhard, G. and C. Von Lear, 1992.** Heavy Metal Loads of Plants Grown in Horti- and Agriculturally Utilized Areas of the Conurbation of Berlin (West), Gesunde Pflanz, 44(1): 21-30 (Ger).

[21] **Trueby, P. and Angelica Raba, 1990.** Heavy Metal Uptake by Vegetables from Field Irrigated with Wastewater near Freiburg, Agribiological Research, 43(2): 139 - 46.

[22] **Lonnerdal, B. and R. Uauy, 1998.** Genetic and Environmental Determinants of Copper Metabolism, American Journal of Clinical Nutrition, 67(supl): 951S.

[23] **Prentice, A., 1993.** Does Mild Zinc Deficiency Contribute to Poor Growth Performance, Nutrition Review, 5: 268.