Determination of cadmium, lead, copper and zinc in Yemeni khat by anodic stripping voltammetry

M.H. Matloob[1]

تحديد مقادير الكادميوم، والرصاص، والنحاس، والزنك في القات اليمني بواسطة قياس الفولت بالتجريد المصعدي

محمد هاشم مطلوب

الخلاصـة: لقد تم التحرّي عن تركيز العناصر الزهيدة في القات لأنها قد تحدث اضطراباً في مستويات العناصر الزهيدة في جسم الإنسان. فقد تم تحديد مستويات الكادميوم والرصاص والنحاس والزنك في القات وفي 6 من الخضراوات الورقية الشائعة الاستهلاك في الجمهورية اليمنية، وذلك بواسطة قياس الفولت النبضي بالتجريد المصعدي عقب الهضم الرطب للمادة العضوية. وقد تبيّن أن القات يحتوي على تركيزات عالية من الزنك والنحاس أكثر من الخضراوات الورقية، وإن كان يحتوي على نفس النسبة من الرصاص والكادميوم. ويقدَّر متوسط الجرعة اليومية التي يتحصَّل عليها مستهلكو القات من الكادميوم ما بين 2.0 و10.2 مكروغرام/يوم؛ ومن الرصاص ما بين 23.6 و118.0 مكروغرام/يوم؛ ومن النحاس ما بين 530 و2654 مكروغرام/يوم، ومن الزنك ما بين 662 و3311 مكروغرام/يوم. وبالرغم من ارتفاع هذه المستويات إلا أنها في الحدود المسموح بها التي حدَّدتها منظمة الأغذية والزراعة ومنظمة الصحة العالمية.

ABSTRACT Trace element concentrations in khat were investigated as they can disturb trace element levels in the body. Cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) levels in khat and 6 leafy vegetables commonly consumed in the Republic of Yemen were determined by differential pulse anodic stripping voltammetry after wet digestion of the organic matter. Khat had significantly higher concentrations of Cu and Zn than did the leafy vegetables, but similar amounts of Cd and Pb. The average daily intake of khat consumers of Cd, Pb, Cu and Zn from khat only was estimated to be 2.0–10.2 μg/day, 23.6–118.0 μg/day, 530–2654 μg/day and 662–3311 μg/day respectively. Although high, these values were within Food and Agriculture Organization/World Health Organization tolerance limits.

Détermination du cadmium, du plomb, du cuivre et du zinc dans le khat yéménite par voltamétrie à redissolution anodique

RESUME On a examiné les concentrations d'oligo-éléments dans le khat étant donné qu'elles peuvent influencer les taux d'oligo-éléments dans l'organisme. Les concentrations de cadmium, de plomb, de cuivre et de zinc dans le khat et six légumes-feuilles couramment consommés en République du Yémen ont été déterminées par voltamétrie à redissolution anodique en tension différentielle surimposée après digestion par voie humide de la matière organique. Le khat avait des concentrations significativement plus élevées de cuivre et de zinc que les légumes-feuilles mais les quantités de cadmium et de plomb étaient similaires. On estime que l'absorption quotidienne moyenne de cadmium, de plomb, de cuivre et de zinc chez les consommateurs de khat s'élève respectivement à 2,0-10,2 μg/jour, 23,6-118,0 μg/jour, 530-2654 μg/jour et 662-3311 μg/jour. Bien qu'élevées, ces valeurs se situent dans les limites de tolérance fixées par l'Organisation des Nations Unies pour l'alimentation et l'agriculture/l'Organisation mondiale de la Santé.

[1]*Department of Chemistry, College of Science for Girls, University of Babylon, Babylon, Iraq.*
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Introduction

Khat (*Catha edulis Forsk*) is a plant whose leaves and stem tips are chewed for their stimulating effect. The plant grows wild in certain areas of East Africa and the Arabian Peninsula. Among the various compounds in the plant, two phenylalkylamines, namely cathine and cathinone, seem to account for most of the stimulant effect. When ingested, users get a feeling of well-being, mental alertness and excitement. The after-effects are usually insomnia, numbness and lack of concentration [*1,2*].

In 1973, the World Health Organization reported that 80% of men and between 7% and 10% of women in Yemen were khat users [*3,4*]. More women and an increasing number of juveniles now chew khat regularly [*5*]. Financially, khat sellers achieve an above-average long-term return compared with other investment activities. Wide areas of fertile lands and most water sources are used now to grow khat. Moreover, Yemeni farmers are using excessive amounts of fertilizers and insecticides to improve and protect their crops.

During the last two decades, important progress has been made in understanding the pharmacological and social effects of khat, but less attention has been paid to the concentration of trace elements in khat and their role in disturbing trace element levels in human tissues and body fluids [*6–9*].

Differential pulse anodic stripping voltammetric (DPASV) procedures for the direct determination of low levels of cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) in Yemeni khat were used in our study. Stripping electrochemical techniques combine high detection sensitivity, accuracy and precision with sufficiently high determination rates, convenience in application and moderate costs for instrumentation [*10–12*].

Since values for khat previously had not been reported, we compared mineral levels of khat with mineral levels of six leafy vegetables commonly grown and freshly eaten in the Republic of Yemen. For our investigation, leaves were used as they generally accumulate greater amounts of elements than do other parts of plants.

Methods

Cd, Pb, Cu and Zn were measured in 100 samples of Yemeni khat and 300 samples of 6 different species of leafy vegetables commonly grown and eaten in the country (lettuce, parsley, cabbage, leek, radish and watercress). The samples were chosen from various agricultural locations in Sana'a, Ibb and Taiz, which were unexposed to industrial activities and not irrigated by municipal wastewater. Each sample was collected in a pre-cleaned polyethylene bag, washed thoroughly with distilled water and air dried before analysis. For approximately 3 hours, 1 g of each sample was wet digested with 1 mL of 70% nitric acid (electronic grade), 0.5 mL of 96% sulfuric acid and 1 mL of 70% perchloric acid at 170 °C until a clear digest was obtained. The digested sample was evaporated until nearly dry. The residue was collected in 10 mL of 0.25% nitric acid (pH = 2) and transferred to the voltammetric cell for Cd, Pb and Cu estimation. For Zn estimation, 5 mL of sample solution in 0.25% nitric acid was buffered with equal volume of 0.3 mol/L ammonium acetate and then transferred to the voltammetric cell. Optimum quantities of acids were used so that the wet oxidation of the sample was complete and so that the reagent blank values for Cd, Pb, Cu and Zn were sufficiently low.

Stripping voltammetric experiments were done with a Metrohm 746 VA Trace

Analyser connected to a Metrohm 747 VA multimode electrode (Metrohm Limited, Herisau, Switzerland), 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 [*13*]. A digital pH-meter (Jenway/Barloworld Scientific, Model 3310, Essex, England) was used to measure pH. Dissolved oxygen was removed from the samples by purging with purified nitrogen (99.999%) through the measuring vessel for 5 minutes. During the experiments, nitrogen was passed over the solution to prevent oxygen interference.

Optimum experimental conditions were established as follows: the potential was swept using DPASV with a pulse rate of 3.33 s^{-1}, a scan rate of 10 mV/s and a pulse amplitude of 50 mV. The standard additions technique was used to give the concentrations of Zn, Cd, Pb and Cu simultaneously when a sweep potential was applied between –1.150 V and 200 mV (–1.150 V to –800 mV for Zn, –800 mV to –450 mV for Cd, –500 mV to –200 mV for Pb and –200 mV to 200 mV for Cu). All quoted potentials refer to the Ag/AgCl electrode.

All chemicals were analytical reagent grade. Deionized water was used to prepare all solutions. Cd, Cu and Pb stock solutions were prepared by dissolving the corresponding nitrates. A Zn stock solution was prepared by dissolving the sulfate in deaerated 2% (v/v) nitric acid. The working standard solutions were prepared daily by dilution of the stock solution in the required matrix. All glassware was stored in 8 mol/L nitric acid for 1 week and rinsed thoroughly with deionized water [*14*].

The standard addition method was used to quantify metal concentrations in the samples. This method was preferred as the sensitivity of the stripping voltammetric analysis could vary between samples of different ionic strengths. The best fit line through data pairs was calculated by linear least-squares regression analysis. The concentration of each element in the sample was equal to the quotient of the intercept and the regression coefficient.

The scatter of the results was examined visually to assess its fit to a normal distribution. All data relating to Cd and Pb were approximately normally distributed. Those for Cu and Zn were skewed severely and results were logarithmically transformed to achieve approximate normality. The results were expressed as mean and standard deviation.

Results

Figure 1 shows an anodic stripping voltammogram of a digested khat sample. Well-defined peaks for Cd, Pb, Zn and Cu were observed, indicating that the digestion of the sample was relatively complete. As the concentrations of the four trace elements varied by more than an order of magnitude from each other, the current sensitivity was altered two times as indicated by the sudden changes of the voltammetric signal between the Zn and Cd peaks and the Pb and Cu peaks. The small overlap between Pb and Cu peaks was successfully resolved after smoothing and removing background as per the Huang et al. model [*15*]. The peak height of the voltammetric signal increased linearly with deposition time in the range of 30–180 s for all four metals, thus allowing for the adaptation of deposition time to the metal.

The calibration graph for the four elements was determined in the concentration range of 0–300 ppb (Figure 2). The calibration curves were linear over the entire range with a correlation coefficient between 0.9950 and 0.9957 for all four ele-

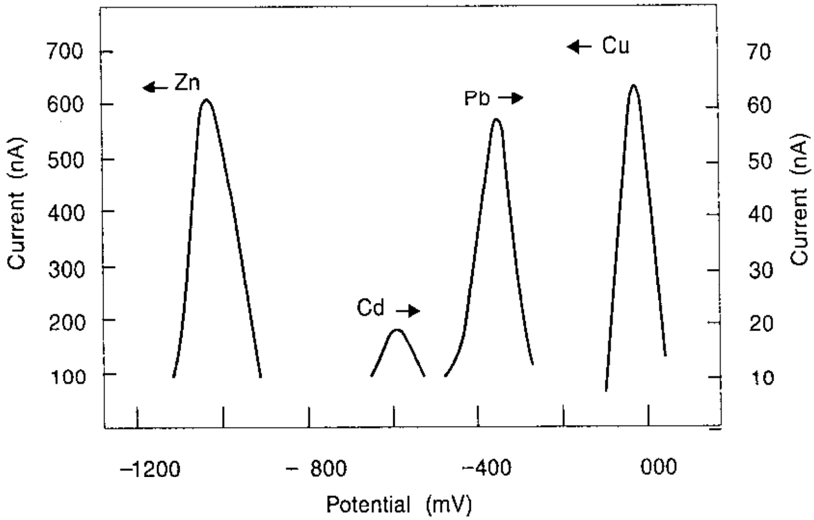

Figure 1 **Typical voltammogram of 1 g wet digested khat after dilution to 20 mL. The peaks represent 2 ppb Cd, 19 ppb Pb, 296 ppb Cu and 174 ppb Zn. Deposition potential was –1100 mV; deposition times were 120 s for Cd and Pb and 30 s for Cu and Zn; scan rate was 10 mV/s; pulse amplitude was 50 mV.**

ments. Based on the calibration curve, the limits of detection were also determined. The limit of detection was the analyte concentration that gave a signal equal to the blank signal plus three standard deviations [*16*]. The limits of detection were 0.15 µg/kg Cd, 0.30 µg/kg Pb, 2.80 µg/kg Cu and 1.20 µg/kg Zn.

The precision and the accuracy of the proposed method were checked with orchard leaves (National Bureau of Standards, Standard Reference Material 1571, Washington DC) after dilution to 1000 mL. Table 1 shows the analytical data obtained by DPASV, indicating that this method was reliable for analysis.

Recovery tests were as follows: half of a batch of 10 samples from the khat mixture were spiked with aliquots of each analyte prior to analysis. The whole batch was then subjected to the digestion/analysis procedure (Table 2). Recovery was satisfactory for all elements.

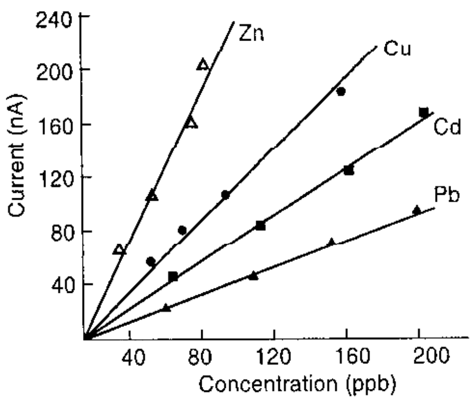

Figure 2 **Calibration graph for Cd, Pb, Cu and Zn by DPASV. Deposition time = 60 s. Deposition potential = –1100 V. Scan rate = 10 mV/s. pH = 2 for Cd, Pb and Cu; pH = 4 for Zn.**

Table 1 **Determination of cadmium, lead, copper and zinc in orchard leaves (National Bureau of Standards, Standard Reference Material 1571, Washington DC)**

Value[a]	Cd (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Measured	116 ± 13	48 ± 5	11.6 ± 0.4	27.3 ± 4.2
Certified	110 ± 10	45 ± 3	12.0 ± 1.0	25.0 ± 3.0

[a]*Each value is the mean ± standard deviation of 5 runs.*

Digestion of khat was also attempted by dry ashing at 480 °C for 14 h. The ashed samples were dissolved in nitric acid–perchloric acid mixture (1:1) and diluted to 20 mL with distilled water before the analysis. The recovery levels for Cd, Pb, Cu and Zn with dry ashing were 10%–20% lower than with the wet ashing procedure. It appeared that volatilization of the elements occurred, as volatile salts were the prime source of low recoveries.

Table 3 shows the results of the wet ashing procedure. On a fresh weight basis (µg/kg) the results indicate that khat had significantly higher concentrations of Cu and Zn than did the leafy vegetables, but almost the same amounts of Cd and Pb.

Discussion

The pollution of air, soil, food and water is widely anticipated and reported in industrialized and densely populated areas. In countries like the Republic of Yemen, there is not enough industry to cause substantial pollution. The major source of contamination comes from agricultural activities, such as the application of fertilizers and pesticides and the use of sewage sludge from biological wastewater treatment plants. National and international regulations on food quality have lowered the maximum permissible levels of toxic metals in human food; hence, an increasingly important aspect of food quality should be to control the concentrations of trace metals in food.

The mean Cd and Pb content of khat, lettuce, parsley, cabbage, leek, radish and watercress ranged from 3.6 to 37.6 µg/kg and 84.8 to 306.8 µg/kg fresh weight respectively (Table 3). Compared with the literature, these levels were normal for uncontaminated areas (a fresh weight to

Table 2 **Recovery for 10 samples of khat (1 g each) after wet digestion and dilution to 30 mL**

Metal	Mean level in khat[a]	Aliquot spike[a]	Total[a] (%)	Found[a]	Recovery
Cd	11.2	50.0	61.2	66.1	108
Pb	224.6	600	824.6	857.6	104
Cu	8540	5000	13540	14491	107
Zn	11230	5000	16230	17100	105

[a]*Concentrations are given as µg/kg fresh weight.*

Table 3 **Metal concentrations in khat and selected Yemeni vegetables determined by differential pulse anodic stripping voltammetry (fresh weight basis)**

Sample	*n*	Cd (μg/kg)	Pb (μg/kg)	Cu (μg/kg)	Zn (μg/kg)
Khat	100	20.3 ± 10.3 (6.3–52.3)	233.6 ± 141.5 (66.4–700.0)	5308 ± 1888 (2649–10454)	6622 ± 1822 (3520–11961)
Lettuce	50	19.9 ± 11.4 (3.5–36.9)	222.3 ± 148.3 (20.2–738.1)	1793 ± 859 (607–3385)	2116 ± 1126 (800–5722)
Cabbage	50	3.6 ± 1.4 (1.5–5.3)	84.8 ± 58.6 (19.3–209.6)	521 ± 182 (321–814)	1023 ± 280 (773–1519)
Parsley	50	9.8 ± 4.2 (3.1–15.6)	212.0 ± 70.1 (50.4–294.8)	3218 ± 1634 (780–6473)	3741 ± 1596 (1744–8371)
Leek	50	8.5 ± 3.9 (3.5–11.2)	169.5 ± 99.4 (40.9–387.8)	1968 ± 1233 (519–5261)	1559 ± 658 (475–3034)
Radish	50	14.0 ± 9.3 (1.9–47.4)	269.3 ± 175.7 (33.9–877)	1406 ± 1092 (373–5598)	2903 ± 1120 (1408–5905)
Watercress	50	37.6 ± 18.4 (10.1–76.0)	306.8 ± 140.3 (95.2–658.2)	2089 ± 979 (898–4247)	3645 ± 1419 (1293–6284)

Values are mean ± standard deviation with range shown in brackets.

dry weight conversion factor of approximately 0.08 was applied when necessary) [*17–19*]. Surprisingly, watercress leaves, which were collected from the same locations as the other produce, had exceptionally high Cd levels. Green cabbage leaves generally had the lowest levels of heavy metals and thus were potentially useful for the estimation of soil heavy metal hazard.

The average quantity of khat chewed by Yemenis ranges from 100 g to 500 g daily. Thus the consumption of khat contributes 2.0 μg to 10.2 μg of Cd and 23.6 μg to 118.0 μg of Pb daily. The Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives has recommended a provisional maximum tolerable daily intake of Cd and Pb from all sources (food, air and water) of 1.0 to 1.2 μg/kg body mass and 3.5 to 4.0 μg/kg body mass respectively [*20*]. These values correspond to a provisional daily intake of 60 μg to 72 μg and 210 μg to 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 khat alone is below the FAO/WHO provisional tolerable daily intakes. However, if other Pb and Cd sources are included and if khat is digested without washing (as mostly the case in the Republic of Yemen), the daily intake may exceed the recommended levels. Indeed we observed that substantial amounts of heavy metals were removed by washing the samples with distilled water.

Continuous exposure to Cd and Pb results in their gradual accumulation in human vital organs, which may cause profound biochemical and neurological changes in the body. According to the 1994 Piomelli study, lead poisoning is the most important environmental health problem for young children [*21*].

The geometric mean concentration levels of Cu and Zn in fresh khat samples were

5308 μg/kg and 6622 μg/kg respectively (Table 3). Compared with the other 6 leafy vegetables, Yemeni khat contained significant amounts of Cu and Zn, although the samples came from the same unexposed agricultural areas and had similar Pb and Cd levels. Many researchers have reported the same phenomena. Chang reviewed approximately 300 references and concluded that the plant tissue concentrations of chromium, copper, nickel and zinc were not related to their respective amounts in the soil, even if soil pH and soil texture were considered [*22*]. The differences in Cu and Zn contents of khat and other plants have been reported to be heavily dependent upon plant growth properties and species [*23*].

Zn and Cu are two trace minerals essential for important biochemical functions and necessary for maintaining health throughout life. Zn deficiency results in a variety of immunological defects whereas Cu deficiency is characterized by anaemia, neutropenia and skeletal abnormalities [*24–26*].

The mean daily intake of Zn by khat consumers was estimated to be 0.662 mg to 3.311 mg. These values do not pose a health risk according to the 1989 recommended daily allowance (RDA) levels (15 mg/day for males and 12 mg/day for females) [*27*]. Continued supplementation with Zn in excess of the RDA, however, interferes with Cu absorption [*28*]. Recent observations of 100 healthy volunteers indicated that after heavy khat digestion 24-hour urinary Zn and Cu excretion was substantially elevated over pre-khat digestion values [*29*].

The average daily intake of Cu by khat chewers ranged from 0.531 mg to 2.654 mg (Table 3). The current estimated safe and adequate daily intake range of Cu is 2–3 mg/day [*27*]. If other Cu sources were included, the daily intake would substantially exceed the recommended level [*30*]. Negative consequences of excessive Cu intake have been extensively documented [*26*]. Liver cirrhosis typically develops from toxic intake and abnormalities in red blood cell formation also occur.

In summary, based on our analysis:

- The khat load of metals in the Republic of Yemen is generally not critical, although the continuous exposure to heavy metals does require attention.
- Daily metal intake can be decreased by washing khat properly and reducing the amount of khat digested.

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Substance abuse disorders

There are a number of disorders resulting from the use of psychoactive substances including alcohol, opioids such as opium or heroin, cannabinoids such as marijuana, sedatives and hypnotics, cocaine, other stimulants, hallucinogens, tobacco and volatile solvents. The conditions include acute intoxication, harmful use, dependence and psychotic disorders. Tobacco and alcohol are the substances which are used most widely across the globe and which pose the most serious public health consequences.

Goals of therapy: To reduce illness, disability and death due to the use of psychoactive substances and to help patients lead a drug-free life. Strategies include:

- Early diagnosis;
- Identification and management of risk of infectious diseases as well as other medical and social problems;
- Counselling and access to services and opportunity to achieve social integration;
- Medical detoxification is only the first stage of dependence treatment — it must be accompanied by long-term care to de crease rates of relapse.

Source: WHO Fact sheet No. 265