Iranian Journal of Analytical Chemistry

Cloud Point Extraction and Spectrophotometric Determination of Cadmium in Some Vegetables

Foroozan Hasanpour[*], Masoumeh Taei, Zahra Alimirzaei, Hamed Karimi, R. Samimi

Chemistry Department, Payame Noor University, 19395-4697, Tehran, Iran.
[*]Email: F.hasanpour@pnu.ac.ir

ABSTRACT
Cloud point extraction and spectrophotometric determination of trace quantities of cadmium in some vegetables have been developed. The research area for vegetables analysis was near to the Zarin Shahr where the soil received heavy metals from the Esfahan Steel Company. Cloud point extraction used dithizone as complexing agent and non ionic surfactant Triton-X114 as micellar phase. Several important variables affecting the separation and determination process were optimized. Under optimized conditions calibration graph was linear in the range of 1.0-180 ng mL^{-1} with detection limit of 0.2 ng mL^{-1}. Preconcentration of 10 mL of samples gave an enhancement factor of 50. The proposed method was applied for determination of Cd(II) in vegetable samples with satisfactory results.

KEYWORDS: Cadmium; Cloud point extraction; Dithizone; Spectrophotometric determination.

1. INTRODUCTION

Cadmium is known as prevalent toxic metal which has no necessity function in human body and can damage liver and lungs even at low concentration level [1, 2]. One of the pathways that cadmium enters in human body is through daily intake of water and food. Cadmium is highly labile in the soil-plant system; its relative availability to plants is much greater than that of other potentially harmful heavy metal contaminants, such as lead [3]. The metal content of garden-type vegetables is important because some species, such as lettuces, accumulate relatively high concentrations of cadmium [4]. In general, leafy vegetables tend to accumulate higher concentrations of cadmium than root, grain or fruit crops [5]. The sources of low-level cadmium contamination of agricultural soils are usually phosphatic fertilizers, atmospheric deposition and soil contamination from industrial sources such as Esfahan Steel Company. The Word Health Organization reported tolerably weekly intakes of Cd(II) as 0.007 mg/kg body weight for all human groups [6]. Thus monitoring of trace amount of cadmium in environmental samples is important in the field of environmental analysis. Several methods have been reported for cadmium determination such as flame atomic absorption [7,8], inductively coupled plasma atomic emission [9] and electrothermal atomic absorption spectroscopy [10]. Because of low concentration of cadmium in food samples, an efficient preconcentration step is necessary prior to aforementioned techniques. Cloud point extraction (CPE) is based on micelle-mediated phase separation. It uses non ionic surfactant in aqueous solution to form micelle when they heated above cloud point temperature. After the formation of sparingly water soluble complex it bound to micelles and extracted to the surfactant rich phase. Compared to solid phase extraction, liquid-liquid extraction and ion exchange, CPE is considered as the most simple, low cost, high extraction efficiency, easy disposal of surfactant and low toxicity [11]. Although flame atomic absorption and inductively coupled plasma are frequently used for determination of trace metal, spectrophotometry is still popular because of common availability of instrumentation, simplicity, speed, precision and accuracy of technique [12]. In this work we proposed a CPE method in Triton X-114 micelle media for preconcentration of Cd(II) prior to spectrophotometric determination. The aim is quantification of accumulated cadmium in two vegetable crops that grown near the Esfahan Steel Company and investigation the impact of factory pollution on the two leafy vegetables cultivated near there. The method was successfully applied to determination of cadmium in vegetables such as cabbage and lettuce. The response characteristics of the proposed method are compared with recently reported cloud point extraction methods for determination of cadmium with various detection systems [13-22] and the results are given in Table 1. As data show, the proposed method has rather wide dynamic range and good enhancement factor.

Table 1. Comparison of recently CPE with various detection methods and the proposed method for determination of cadmium.

Method	Linear range($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	EF	Ref.
UV-vis spectrophotometry	10-500	3.8	24	13
[a]FAAS	10-250	2.9	35	14
FAAS	2-200	0.95	10.5	15
[b]ETAAS	0.02-0.4	0.007	22	16
FAAS	1-100	0.37	52	17
FAAS	0.9-70	0.33	157	18
[c]GFAAS	0.1-20.0	0.0059	50	19
[d]TS-FF-AAS	0.1-5.0	0.0178	55.5	20
UV-vis spectrophotometry	0.32-7.50	0.19	84	21
ICP	20-2000	1	97.9	22
Proposed method	1.0-180	0.2	50	-

[a] *Flame atomic absorption spectrometry*

[b] *Electrothermal atomic absorption spectrometry*

[c] *Graphite furnace atomic absorption spectrometry*

[d] *Thermospray flame furnace atomic absorption spectrometry*

2. EXPERIMENTAL

2.1. Apparatus

T80 Double Beam UV/Vis Spectrophotometers with fixed bandwidth from 190 to 1100 nm (Canadawide Scientific Ltd) and 1.0×0.5 cm glass cell were used. The temperature of cell compartment was controlled with water bath model LO-TEMPTROL 154. A centrifuge with 15 mL calibrated centrifuge tubes (Superior, Germany) were used to accelerate the phase separation process. pH adjustment was performed using a Metrohm digital pH meter (model 780).

2.2. Chemicals and solutions

All reagents were of analytical grade. Doubly distilled water was used throughout. Stock solution of cadmium (1000 ng mL^{-1}) was prepared and working standard solutions were obtained by appropriate dilution of the stock standard solution. The non ionic surfactant Triton X-114 (Merck) stock solution (4%) was prepared in hot water. Acetic acid-acetate buffer solution (0.1 mol L^{-1}) was used to adjust the pH. Dithizone standard solution (1×10^{-2} mol L^{-1}) was prepared daily by dissolving the reagent in ethanol.

2.3. Procedure

For the cloud point extraction an aliquot of 10 mL of a solution containing 100 ng m L^{-1} cadmium, Triton X-114, 0.6% (v/v), 3×10^{-5} mol L^{-1} of dithizone and 2 mL of acetate buffer (pH=5), were kept for 15 min in water bath at 65 °C. Separation of two phases was achieved by centrifuging for 10 min at 5000 rpm. Then centrifuge tubes containing two phases were cooled to 0 °C at an ice bath in order to surfactant-rich phase became viscous and was accumulated at the bottom of the tubes. The aqueous phase was then decanted by inverting. Finally surfactant rich-phase was diluted up to 1.0 mL by adding methanol. The absorbance of cadmium-dithizone complex was measured at the λ_{max} of 500 nm.

2.4. Procedure for vegetables analysis

The analyzed vegetables included cabbages and lettuces. These were received a small application of nitrogen-phosphorus-potassium (NPK) fertilizer. After harvest, the leaves of plants were thoroughly scrubbed and washed in distilled water to remove any soil, a bout 2.0±0.001g dry sample was oven-dried at 100°C and milled ready for analysis [23]. The vegetable samples were refluxed in concentrated nitric acid, taken to dryness and the residue dissolved in hydrochloric acid (0.001 mol L^{-1}). This solution was then filtered and made up to a known volume in distilled water. The final solution was determined by recommended procedure.

3. RESULT AND DISCUSSION

The coordination of dithizone (diphenyl thiocarbazone) with several metal ions is well known [8]. Fig. 1 shows Cd(II)–dithizone complex. The complex is water insoluble thus the solvent extraction step required before complex determination [24]. The cadmium ions, like many other metals form an intense red complex with dithizone. This nonspecific character of dithizone reaction is one of its major disadvantages as a complexing agent for trace determination of metals [25].
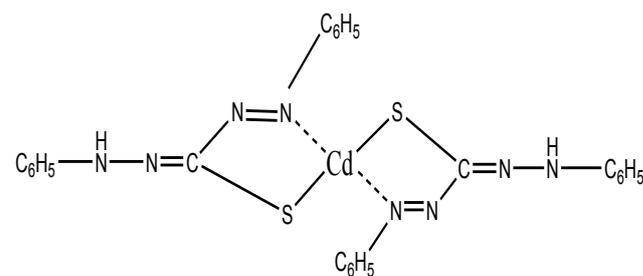
Fig. 1. The structure of Cd(II)-dithizone complex.

However, dithizone can be used as rather selective ligand for extracting a large number of metals by a careful control of the pH and proper choice of surfactant. The absorption spectrum of Cd–dithizone was determined in the range from 300 to 700 nm. The absorption spectrum of the ligand and complex shown in Fig. 2. As seen the absorbance maxima of the ligand is between 450 nm and 590 nm. The longer wavelength corresponding to thione form and the shorter to thiol form [26]. The maximum absorption wavelength for the Cd(II)-dithizone complex is at 500 nm. The effect of pH, amount of Triton X-114, concentration of dithizone, incubation time and equilibrium temperature were optimized.
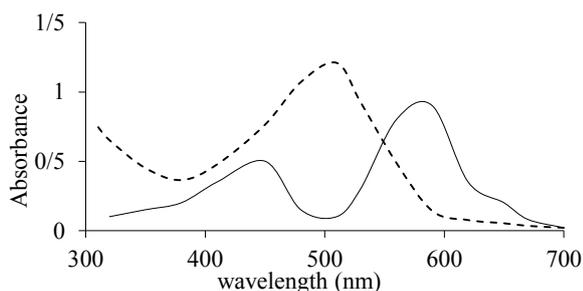

Fig. 2. Absorption spectra of dithizone (solid line) and dithizone-Cd(II) complex (dash line), pH=5, $[Cd^{2+}]$=80 ng mL^{-1}, [dithizine] = 3.0×10^{-5} mol L^{-11} and 0.6% (v/v) of triton X-114.

3.1. Effect of pH

The solution pH plays a significant role in the formation of metal complexes with a chelating agent. Dithizone selectivity decreases with increase of pH, probably due to formation of insoluble hydroxide complexes with other metals [8]. The effect of pH in the range of 2-8 was investigated in phosphate buffer. On increasing pH the λ_{max} has a little bathochromic shift. At pH 5 maximum sensitivity was achieved. The Influence of the buffer type (at pH=5) on the absorbance was also studied. It is realized that the buffer composition (phosphate, acetate or ammonium) had little influence on absorbance response. However, with acetate buffer the reproducibility is somewhat better. The subsequent studies were, therefore, made at pH 5 acetate –acetic acid buffer (Fig.3).
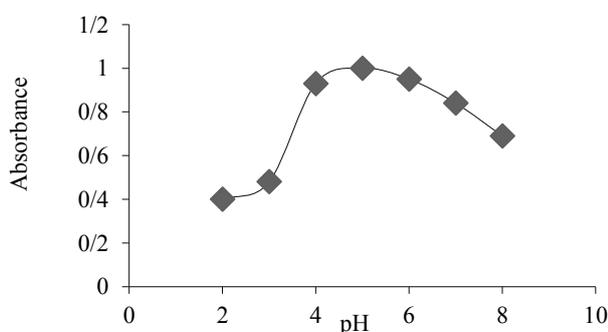

Fig. 3. Effect of pH on the absorbances of the complexes, $[Cd^{2+}]$=80 ng mL^{-1}, [dithizine]= 3.0×10^{-5} mol L^{-1} and 0.6% (v/v) of triton X-114.

3.2. Effect of Dithizone concentration

The effect of dithizone concentration on complex absorbance at λ_{max} was studied in the range of 1×10^{-6} - 1×10^{-4} mol L^{-1}. As can be seen (Fig.4) the signal increases up to 1.5×10^{-5} mol L^{-1} and then reaching plateau. Dithizone concentration of 3.0×10^{-5} mol L^{-1} was chosen as optimum for lowering the interference effect of some other metals in vegetable samples that may extract with Cd(II).
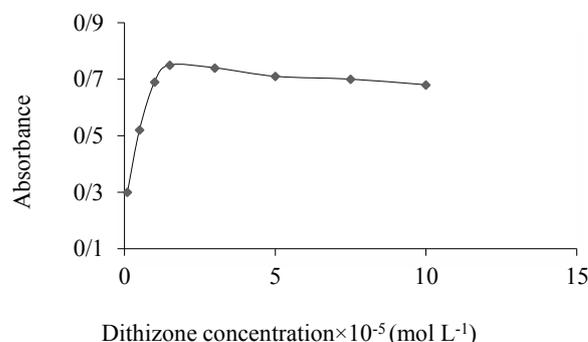

Fig. 4. The effect of dithizone concentration on complex absorbance, $[Cd^{2+}]$=80 ng mL^{-1} and 0.6% (v/v) of triton X-114.

3.3. Effect of Triton X-114

The concentration of surfactant used in CPE is a critical factor. The non-ionic surfactant Triton X-114 was chosen because of its low toxicological properties and high density of surfactant-rich phase [27]. The effect of surfactant concentration was investigated in the range of 0.04-0.9% (v/v). The absorbance of the complex in rich phase at 500 nm increased by increasing the triton X-114 concentration up to %0.5 (v/v) and remained constant at 0.6%-0.9% which is considered as complete extraction. Therefore 0.6% Triton X-114 was used as optimum concentration.

3.4. Effect of equilibration temperature and incubation time

It is desirable to achieve the shortest incubation time and the lowest equilibrium temperature in CPE. The equilibrium temperatures were selected above the cloud point temperature of the surfactant. The dependence of extraction recovery on equilibrium temperature and incubation time was studied over the ranges of 25-70 °C and 5-25 min, respectively. The result shows that an equilibrium temperature of 65 °C and incubation time of 15 min were adequate to achieve the best extraction recovery. The enhancement factor (EF) of 50 calculated as the ratio of absorbance of preconcentrated sample to that obtained without preconcentration.

3.5. Interferences

As one of the versatile chelating agents, dithizone could form complex with other heavy metal ions besides cadmium. More selective extraction of cadmium could be accomplished by controlling the pH, masking agent and varying the extracting solvents [28]. After digestion of the sample with HNO$_3$-HCl to decompose organic matter, any large amount of silver was precipi-

tated as AgCl [29]. Copper and mercury together with remaining silver are then removed by extraction with dithizone in chloroform at pH 2. The solution was adjusted to pH 9 and nickel was removed by adding of dimethylglyoxime (0.001mol L^{-1}) and extraction with chloroform. Another attention must be focused on interference from zinc. The zinc-dithizone complex had approximately the similar color and maximum wavelength as Cd(II)-dithizone. By adding of phosphate (0.001mol L^{-1}) the formation of phosphate zinc complex (above pH=10) makes extraction of cadmium selective [30]. The effect of foreign ions at different concentrations on the absorbance of solution containing 80 ng mL^{-1} of Cd(II) was investigated. The tolerance limit of the coexisting ions, defined as the largest amount of interference produced a variation in the absorbance of the cadmium complex more than 5% given in Table 2.

Table 2. Maximum tolerable concentration of interfering species for a cadmium (II) concentration of 80 ng mL^{-1}.

Foreign ions	Foreign ion to Cd(II) ratio
K$^+$, Ca^{2+}, Mg^{2+}	1000
Fe^{3+}, Al^{3+}, Mn^{2+},	800
SCN$^-$, HCO$_3^-$	800
SO$_4^2$	500
Zn^{2+}	10
+Hg2	5
Cu^{2+}, Ag$^+$, Ni^{2+}	1

3.6. Analytical parameters

Under the optimized condition, a calibration graph (Fig.5) was linear in the range of 1.0-180.0 ng mL^{-1} with equation of A=(57.54 ×10^{-4} X+33.24×10^{-2}) where A is absorbance of complex at 500 nm and C is the concentration of Cd(II) in ng mL^{-1}. The limit of detection (LOD) defined as 3S$_B$/m (where S$_B$ was standard deviation of blank and m was slope of calibration graph) obtained 0.2 ng mL^{-1}.
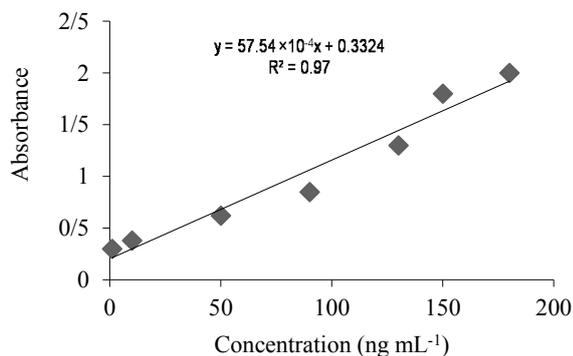

Fig. 5. Calibration curve of complex, for [Cd(II)]=1, 10,50, 90,130, 150,180 ng mL^{-1}, [dithizine]= 3.0 ×10^{-5} mol L^{-1} and 0.6% (v/v) of triton X-114.

The preconcentration factor F$_c$ defined as the ratios of the Cd(II) concentration in surfactant-rich phase to that in bulk phase initially, will remain constant in the same surfactant solutions [31].

$$F_c = C_s/C_0$$

Where C$_s$ is the Cd(II) concentration (ng mL^{-1}) in surfactant rich phase after phase separation and C$_0$ is initial concentration of Cd(II). The preconcentration factor obtained 50 ±0.8 (n=3). To evaluate the precision of the method a series of standard samples was used. The result shows that RSDs for determination were 2.2% and 3.8% for Cd(II) concentration of 1 and 40 ng mL^{-1}.

3.7. Application

The applicability of the proposed method was evaluated by determining of Cd(II) concentration in lettuce and cabbage samples. These vegetables were cultivated near the Esfahan Steel Company. The results were presented in Table 3. The accuracy of the method was examined by obtaining recovery of experiment that varying between from 96.5% to 104.2%. This proved that this procedure was not affected by the sample matrix. Comparison of the results in Table 3 with those found in literature [32] show that the effect of Esfahan Steel Company pollution on two leafy vegetables cultivated near the factory is noticeable.

Table 3. Cadmium content in two cabbages and lettuces.

Sample	Cd (II) added (μg L-1)	Cd (II) found (μg L-1)	(%) Recovery
Lettuce 1	0	78.5±1.2	-
	20.0	102.6±1.4	104.2
Lettuce 2	0	81.2±0.9	-
	40	117.0±1.5	96.5
Cabbage 1	-	50.2±0.08	-
	20	68.4±0.9	97.4
Cabbage 2	0	58.3±1.1	-
	40	95.7±1.4	97.3

± shows the standard deviation with three replicates determination

4. CONCLUSIONS

The proposed method offers several advantages including low cost, safety and good extraction efficiency. The method also gives low limit of detection and low RSD values. The results of this study show noticeable effect of Esfahan Steel Company pollution on two leafy vegetables cultivated near the factory.

ACKNOWLEDGEMENTS

5. REFRENCES

[1] A.C. Davis, P. Wu, X.F. Zhang, X.D. Hou and B.T. Jones, Determination of cadmium in biological samples, *Appl. Spectrosc. Rev.* 41 (2006) 35-75.

[2] X.D. Wen, P. Wu, L. Chen and X.D. Hou, Determination of cadmium in rice and water by tungsten coil electrothermal vaporization-atomic fluorescence spectrometry and tungsten coil electrothermal atomic absorption spectrometry after cloud point extraction, *Anal. Chim. Acta* 650 (2009) 33–38.

[3] B.J. Alloway and H. Morgan, *The behaviour and availability of Cd, Ni and Pb in polluted soils,* Kluwer Academic Press, Dordrecht. (1986) pp. 101-113.

[4] P.J. Peterson and B.J. Alloway, *Cadmium in soils and vegetation. Elsevier,* North Holland and Biomedical Press, Amsterdam (1979) pp. 45-92.

[5] B.J. Alloway, A.P. Jackson and H. Morgan, The accumulation of cadmium by vegetables grown on soils contaminated from a variety of sources, *Sci. Total. Environ.* 91 (1990) 223-236.

[6] H. Tavallali and F. Kazempourfard, Determination of cadmium ions by designing an optode based on immobilization of dithizone on a triacetylecelluose membrane in polluted soil and water samples, *J. Korean Chem. Soc.* 53 (2009) 144-151.

[7] E. Carasek, J.W. Tonjes and M. Scharf, A new method of microvolume back-extraction procedure for enrichment of Pb and Cd and determination by flame atomic absorption spectrometry, *Talanta* 56 (2002) 185–191.

[8] F.A.C. Amorim and S.L.C. Sergio, Determination of cadmium and lead in table salt by sequential multi-element flame atomic absorption spectrometry, *Talanta*, 65 (2005) 960-964.

[9] H. Wang, F. Liu, C. Wu, G. Wang, Y. Xu, W. Zhang and J. Zhou, Determination of trace cadmium in steel by inductively coupled plasma-atomic emission spectrometry after removal of iron matrix with an adsorption column, *Analytical Methods* 6 (2014) 1936-1940.

[10] I. Lipez-Garcıa, Y. Vicente-Martınez and M. Hernandez-Cordoba, Determination of lead and cadmium using an ionic liquid and dispersive liquid–liquid microextraction followed by electrothermal atomic absorption spectrometry, *Talanta* 110 (2013) 46-52.

[11] P. Liang and J. Yang, The composition of camel milk: A meta-analysis of the literature data, *J. Food Compost. Anal.* 23 (2010) 95-101.

[12] A. Safavi, H. Abdollahi, M.R. Hormozi Nezhad and R. Kamali, Cloud point extraction, preconcentration and simultaneous spectrophotometric determination of nickel and cobalt in water samples, *Spectrochim. Acta, Part A.* 60 (2004) 2897-2901.

[13] R. Gurkan and N. Altunay, Determination of trace cadmium in nonalcoholic beverages by coupling cloud point extraction with spectrophotometry, *Food Nutr. Sci.* 63 (2013) 253-260.

[14] M. Chamsaz, A. Atarodi and M. Eftekhari, Vortex-assisted ionic liquid microextraction coupled to flame atomic absorption spectrometry for determination of trace levels of cadmium in real samples, *J. Adv. Res.* 4 (2013) 45-31.

[15] C. Bosch Ojeda, F. Sánchez Rojas and J.M. Cano Pavón, Preconcentration of cadmium in environmental samples by cloud point extraction and determination by FAAS, *Am. J. Anal. Chem.* 1 (2010) 127-134.

[16] H. Filik, F. Dondurmacıoglu and R. Apak, Micelle mediated extraction of cadmium from water and tobacco samples with glyoxal-bis (2-hydroxyanil) and determination by electrothermal atomic absorption spectrometry, *Intern. J. Environ. Anal. Chem.* 88 (2008) 637–648.

[17] J.L. Manzoori and Gh. Karim-Nezhad, Development of a cloud point extraction and preconcentration method for Cd and Ni prior to flame atomic absorption spectrometric determination, *Anal. Chim. Acta* 521 (2004) 173–177.

[18] M. Arvand, A. Pourhabib, A. Afshari, M. Bagherinia, N. Ghodsi and R. Shemshadi, Determination of cadmium and zinc in water samples by flame atomic absorption spectrometry after cloud-point extraction, *J. Anal. Chem.* 63 (2008) 954–959.

[19] X. Zhu, X. Zhu and B. Wang, Determination of trace cadmium in water samples by graphite furnace atomic absorption spectrometry after cloud point extraction, *Microchim. Acta* 154, 95–100 (2006).

[20] H.C. Rezend, C.C. Nascentes and N.M.M. Coelho, Cloud point extraction for determination of cadmium in soft drinks by thermospray flame furnace atomic absorption spectrometry, *Microchem. J.* 97 (2011) 118–121.

[21] Z.A. A Khammas, A.A. Ghali and K.H. Kadhim, combined cloud point extraction spectrophotometric extraction and spectrometric detection of lead and cadmium in honey samples using a new ligand, *Int. J. Chem. Sci.* 10 (2012) 1185-1204.

[22] E.L. Silva, P.D.S. Roldan and M.F. Giné, Simultaneous preconcentration of copper, zinc, cadmium, and nickel in water samples by cloud point extraction using 4-(2- pyridylazo)-resorcinol and their determination by inductively coupled plasma optic emission spectrometry, *J. Hazard. Mater.* 171 (2009) 1133–1138.

[23] Z.Y. Hseu, Evaluating heavy metal contents in nine composts using four digestion methods, *Bioresour. Technol*. 95 (2004) 53–59.

[24] E.B. Sandell and H. Onishi, *Photometric Determination of traces of Metals*, Willey, Newyork (1978) pp.579-647.

[25] G.A. Shar and M.I. Bhanger, Spectrophotometric determination of zinc with dithizone in anionic micellar media of dodecyl sulphate salt, *J. Chem. Soc. Pak*. 23 (2001) 74-79.

[26] M.H. Memon and P.J. Worsfold, Analytical applications of microemulsions Spectrophotometric determination of zinc using dithizone, *Analyst* 113 (1988) 769-771.

[27] H. Tavallali, E. Asrari, A.M. Attaran and M. Tabandeh, Sensitive determination of lead in soil and water samples by cloud point extraction-flame atomic absorption spectrometry method, *Inter. J. Chem. Tech. Res*. 2 (2010) 1731-1737.

[28] M. Stoeppler, *Hazardous Metals in the Environment: Techniques and Instrumentation in Analytical Chemistry*, Elsevier, Amsterdam (1992) pp: 177-219.

[29] C.H. Williams, D.J. David and O. Iisma, The determination of cadmium in soils, plants and fertilizers by dithizone extraction and atomic absorption spectroscopy, *Commun. Soil Sci. Plant Anal*. 3 (1972) 399-407.

[30] O. Klinghoffer, J. Ruzincka and E.H. Hansen, Flow-injection analysis of traces of lead and cadmium by solvent extraction with dithizone, *Talanta* 27 (1980) 169-175.

[31] L. Machackovaa and M. Zemberyova, Cloud point extraction for preconcentration of trace beryllium and chromium in water samples prior to electrothermal atomic absorption spectrometry, *Anal. Methods* 4 (2012) 4042-4048.

[32] F. Itanna, metals in vegetables grown in addis ababa and toxicological implications, *Ethiop. I. Health. Dev*. 16 (2002) 295-302.