International Journal of Modern Chemistry, 2014, 6(1): 1-17

Modern Scientific Press

International Journal of Modern Chemistry **ISSN: 2165-0128**

Journal homepage: www.ModernScientificPress.com/Journals/IJMChem.aspx *Florida, USA*

Article

Spectrophotometric Determination of Traces of Lead (II) in Spinach Samples Marketed in Chuka, Kenya

O. Ombaka[1,] *, A. Ndanu[1], D. I. Kibaara[2], A. O. Njiri[2] and K. W. Kosgei[3]

[1]*Department of Physical Sciences, Chuka University, Kenya*

[2]*Department of Biological Sciences Chuka University, Kenya*

[3]*Department of Pure and Applied Sciences Murang'a University College, Kenya*

* Author to whom correspondence should be addressed; E-Mail: ombakaochieng@gmail.com

Article history: Received 14 December 2013, Received in revised form 6 January 2014, Accepted 8 January 2014, Published 10 January 2014.

Abstract: Three hydroxytriazenes were synthesized and studied for the spectrophotometric determination of Lead (II) in spinach samples. Lead (II) reacts with hydroxytriazenes to form a 1:2 yellow complex, having a sensitive absorption peak at 403, 403 and 413 nm for reagent (i), (ii) and (iii) respectively. The Lead (II) complexes were detected at PH range of 7.4 – 7.85, 7.20 – 7.50, 7.0 – 7.5 for reagent (i), (ii) and (iii) respectively. Under the optimum conditions Beer-Lambert's law was obeyed in the range of 0.8 – 4.8 x 10^{-5}, 0.8 – 4.8 x 10^{-5}, 1.0 – 6.0 x 10^{-5}M for reagent (i), (ii) and (iii) respectively. The molar absorptivity ($moL^{-1}dm^3cm^{-1}$), detection limit (mg/L), Sandell's sensitivity (ng/cm^3), stability constant and Free energy of Formation (kcal/moL) ranged between 3900 to 6125, 5.75 to 11.45, 33.829 to 53.129, 0.282 to 2.12 x 10^{10} and -12.671 to -13.522. Except Zn, Co, Ni and Cu, all foreign ions studied do not interfere with the determination. The method has high precision and accuracy in the determination of Lead (II) in spinach sample.

Keywords: Lead, Spinach, Graphite Furnace, Atomic Absorption, Hydroxytriazenes, Spectrophotometry, Interferences.

1. Introduction

Lead is a common environmental pollutant. Most of this metal's high levels found throughout the environment come from human activities [1-3]. Lead can enter the environment through releases from mining that make or use Lead, Lead alloys, or Lead compounds. Lead is released into the air during burning of coal, oil or waste [4]. People living near hazardous waste sites may be exposed to Lead and chemicals that contain Lead by breathing air, drinking water, eating foods, or swallowing dust containing Lead [5-8]. Leafy fresh vegetables grown in Lead containing soils may have Lead-containing dust on them, the amount of which depends on the species, the chemical composition of the soil, the amount of Lead in the soil and soil temperature [9-12].

The main target of Lead toxicity is the nervous system, both in adults and children. Long term exposure of adults to Lead can result in decreased performance in some tests that measure functions of the nervous system. Lead exposure may cause weakness in fingers, wrists and ankles. It may also cause anemia and small increases in blood pressure, especially in middle aged and older people. At high levels of exposure, Lead can severely damage the brain and kidney in both adults and children, ultimately causing death. In pregnant women, exposure to high levels of Lead may cause miscarriage. High-level exposure in men can damage the organs responsible for sperm production [13-16]. Children are more vulnerable to Lead poisoning than adults. They can be exposed to Lead in the womb, when they breastfeed, from eating other foods, drinking water, swallowing and breathing dirt or dust [17].

In view of the above, there is a need to be concerned about elevated Lead in the environment. This involves environmental monitoring of Lead levels using accurate and reliable analytical methods possessing high sensitivity and selectivity, coupled with convenience and economy, applicable to real world samples.

To determine trace amount of Lead, some analytical instrument with high sensitivity detection capabilities are available, including inductively coupled plasma mass spectroscopy (ICP-MS) and graphite furnace [18]. Although their sensitivities make ICP-MS and GFAAS the methods of choice for Lead analysis, these instruments are very expensive and their daily operational costs are high. In addition, because only a small amount of solution can be introduced into the instrument, they require a very clean working environment, which adds to the maintenance cost of operating the instrument.

A large number of spectrometric methods for determination of Lead are reported to face interference due to the presence of several matrices in the samples [19]. For this reason there is an ongoing search for new organic reagents for direct and rapid spectrophotometric determination of trace amounts of Lead in environmental samples. Based on these observations, this paper reports three new spectrophotometric methods for the determination of trace levels of Lead (II) in spinach samples.

2. Materials and Methods

2.1. Apparatus

Systronics double beam UV-vis recording spectrophotometer 108 (Systronics Limited, India) and a systronics PH meter 324, with a combination of electrode were used for the measurements of absorbance and PH, respectively. PG 990 graphite (platform coated) furnace atomic absorption spectrometer (GF-AAS) was used for comparing the results. The instrumental parameters used are shown in Table 1. The melting point and composition verification of hydroxytriazenes were carried out using melting point apparatus (model Kruess M500) and elemental analyzer (Perkin Elmer 2400 series II CHN Analyzer), respectively.

Table 1: GF-AAS Parameters used in the determination of Lead

Analytical line	283.3 nm
Bandwidth	0.4 nm
Filter Factor	0.1
Lamp Current	3.0 mA
Integration Time	3.0 sec
Background	None
Sample Size	10 µL
Acidity	0.1% Nitric
Sensitivity	5.18 pg/mL
Detection Limit	3.88 pg/mL
Working Range	1.0 - 100 ng/mL

2.2. Reagents and Solution

All chemicals used were of high purity reagent grade obtained from Merck, Germany. Throughout all analytical work, deionized double distilled water, which is non-absorbent under visible radiation, was used. The standard solution of 0.01M Pb^{+} was prepared by dissolving the appropriate amount of $Pb(NO_3)_2$ in water. The required volumes of this solution were used to prepare the working solutions. 1.0% hexamine solution (1.0gm of hexamine was dissolved in minimum quantity of water and then diluted with acetone to 100mL) and 1.0% perchloric acid solution (1.0 mL of perchloric acid was diluted to 100mL with acetone) were used to adjust the desired PH. Standard solutions for Lead determination using GF-AAS were prepared from 1000ppm spectro econ stock. Hydroxytriazenes were synthesized according to the recommended method [20-21].

2.3. Quality Control of Analytical Procedure

a) Accuracy of GF-AAS method

The method was validated by analyzing samples of three certified reference materials (citrus leaves [NIST 1572], orchard leaves [NIST 1571] and olive leaves [CRM 62]).

b) Precision of Spectrophotometric method

This was carried out by measuring the absorbance of ten solutions. Each solution contained equal concentration of Lead (II) and the reagent. The absorbance was measured against reagent blank under optimum conditions of PH, solvent and Lead (II) to reagent ratio. On the basis of their absorbance values, standard deviation was calculated.

c) Repeatability and Reliability of data generated

All the tests were carried out in five replicates to establish confidence in the repeatability of data generated.

2.4. Sampling Techniques

100 spinach samples were collected from each of the five markets selected randomly within Chuka area. The sample from each market was rinsed quickly using 2% non-phosphate detergent solution followed by two double distilled-deionized water rinses. The washing was done quickly to minimize the leaching of certain elements from the leaves. The sample was then blotted dry with absorbent paper and placed in a large envelope to ensure integrity of the sample. After this it was transported to the laboratory and dried at 60ºC in ventilated oven until a dry constant weight was obtained. It was then ground with a mortar and pestle and sieved through a mesh of 1mm diameter. Mixing using conning and quartering was done to obtain analytical sample.

2.3. Methods of Digestion

It is common practice to perform an oxidative digestion of the plant material before analysis to destroy the organic material and take the Lead into solution. Dry ashing procedures are less suitable, as Lead is a volatile metal, and can easily be lost during the ashing process. Wet oxidative digestion in open vessels using concentrated acid mixtures can be successful, but require careful control to ensure complete destruction of the organic material and to avoid contamination. Digestion in sealed vessels reduces the likelihood of contamination and allows higher temperatures and pressures to be obtained, aiding the destruction of the organic material.

2.3.1. Digestion in a sealed vessel

2gm of the dried, mixed vegetable material sample was weighed in a metal weighing funnel and transferred to the flat-bottomed 100mL flask of the digestion apparatus. 20mL of the digestion mixture (40 mL of 14.4M HNO_3 + 4 mL of 11.6M $HclO_4$ + 1.0 mL of 18.0M H_2SO_4) and 4 carborandum beads were added followed by swirling the flask to wet the vegetable material. The lower cone of the condenser was moistened with water and placed on the flask. The upper cone of the glass tube was also moistened with water and placed on top of the condenser. The stopcock was closed and thereafter the whole digestion apparatus was fixed upright. It was then left to stand overnight at room temperature to prevent excessive foaming. Nitric acid was used in order to destroy all the easily oxidizable material and this prevented any explosions which could have occurred due to rapid reaction by perchloric acid; sulphuric acid was used to dilute the perchloric acid hence preventing any explosions which could have occurred at final stage.

The mixture was moderately heated (about 170ºC) for at least 40 min until most of the nitric acid had been distilled off. The temperature was then raised stepwise and this allowed the nitric acid that had remained together with some water to be distilled over. During this process the contents of the flask gradually turned black. The increase in temperature continued until azeotropic boiling point of perchloric acid (205ºC) was reached. At this temperature perchloric acid attacked remaining organic material with a violent oxidation reaction resulting in white dense fumes. The heating supply was adjusted in such a way that the perchloric acid vapors condensed halfway the side arm. The digestion continued for one more hour after the digest had slightly turned color. The resulting mixture was cooled a little, and then 20mL water and 2mL of sodium nitrite solution was added. After this the boiling was done for 10 minutes followed by cooling. The contents of the condenser were discarded, and the side arm of the condenser was rinsed with water and all rinsings were collected in the flat bottomed digestion flask. The contents of the flask were then transferred to a 100 mL volumetric flask. The digestion flask was rinsed with water and the rinsings were collected in the same volumetric flask. The solution was made up to the mark followed by mixing. The solution was filtered through course paper into a 100 mL Erlenmeyer flask. Sodium nitrite was used to reduce insoluble higher oxides of manganese which may have been formed. Boiling was done in order to dissolve salts after the digestion. Blank digestion was also prepared following same procedure but minus the samples. Azeotropic mixture solution was not evaporated to dryness because of the presence of salts of metals which could cause explosions.

2.4. Determination of Working Wavelength

The spectrum of the complex formed with each reagent ([M]:[R]) was obtained in wavelength region 389 nm to 500 nm against reagent blank. Further, spectrum of reagent was also measured in the same wavelength region against acetone. The working wavelength was chosen such that there was maximum difference between the absorbance of the complex and the reagent.

2.5. Determination of PH Range

The absorbance of a series of solutions containing Lead (II) and reagent in the molar ratio 1:5 ([M]:[R]) was measured against reagent blank at corresponding working wavelength at various PH values to determine PH range of constant and maximum absorbance. The color that developed in PH range of constant absorbance was yellow for each reagent. The color development in each case was instantaneous and stable for about 24 hours. All the measurements were made at room temperature in all the three cases.

2.6. Determination of Molar Composition of Lead (II) Complex

Three different methods, namely; Job's method, Mole ratio method and Slope ratio method of Harvey and Manning were used for the determination of molar composition of metal: reagent [22].

2.7. Conformity with Beer-Lambert's Law

Under optimum conditions of PH, solvent and Lead (II) to reagent ratio, the validity of Beer-Lambert's law was studied. The results of absorbance obtained were plotted against the corresponding concentration of Lead (II). The calibration curve obtained was used to determine the concentration of Lead in sample and digestion blank. The difference between these absorbance was taken as the absorbance corresponding to the sample.

2.8. The Stability Constant

The apparent stability constant of the complex was calculated using Harvey and Manning's method and Purohit's method [23].

2.9. Effect of Foreign Ions

The influence of other ions such as alkali, alkaline earth and transition metals on the determination of Lead (II) using hydroxytriazenes was studied. The tolerance limit was fixed at the maximum amount of an ion causing a relative error not greater than 5% in the absorbance.

2.10. Comparison with GF-AAS Results

Analytical results obtained with the UV-Visible method were validated by comparison with those acquired with a well-established GF-AAS method.

2.11. Recovery of the Results

Certified reference material No.23 Tea leaves II with non-detectable Lead was selected for Lead recovery testing. 0.1 g CRM Tea leaves sample was spiked with 1.2 mL of 1000ppm Lead (II) and digested in the same manner as the spinach sample. The solution was diluted to 100 mL. Five analyses were performed, each using both hydroxytriazene and GF-AAS. The results obtained are summarized in Table 7. The recovery results was set at 90- 150 % of the spiked Lead.

3. Results and Discussion

The results from the validation experiment are shown in Table 2. The calculated t-value were found as 1.916, 1.100 and 1.212 for NIST 1572 citrus leaves, NIST 1571 orchard leaves and CRM 62 olive leaves respectively while critical value of t_3 is 3.18 at 95% (p= 0.05). From these values, it can be seen that observed t-values were less than the tabulated t-values; hence there is no significant difference between the certified values and those obtained using GF-AAS.

Table 2: Results for certified Reference materials

Sample	**Analyte Certified value**	**Lead (II) found in mg/Kg**	**t-values calculated at 95% (p= 0.05)**	**t-tabulated (critical value) p= 0.05**
NIST 1572 citrus leaves	13.3 ± 2.4	12.67 ± 0.52	1.1916	3.18
NIST 1571 orchard leaves	45 ± 3	45.16 ± 0.23	1.100	3.18
CRM 62 olive leaves	25 ± 1.5	24.78 ± 0.31	1.212	3.18

The results of the physical characteristics and elemental analysis are shown in Table 3. The color and shape of reagents (i), (ii) and (iii) were light yellow needles, orange shining needles and olive-green needles respectively. The melting points of these hydroxytriazenes ranged from 86ºC to 140ºC. Crystallization was carried out using acetone. The results of elemental analysis indicate that theoretical values of C, H and N are identical to the experimental values.

Table 3: Physical characteristics and Elemental analysis of hydroxytriazenes

Reagent no.	Synthesized hydroxytriazenes	Physical Characteristics			Elemental Analysis						Molecular formula
					C%		H%		N%		
		Color and Shape of crystals	Crystallized media	M.P (ºC)	TH	EXP	TH	EXP	TH	EXP	
(i)	3-hydroxy-3-p-tolyl-1-o-chlorophenyltriezene	Light yellow needles	Acetone	86	59.64	58.86	4.62	4.72	16.06	16.42	$C_{13}H_{12}N_3OCl$
(ii)	3-hydroxy-3-p-tolyl-1-m-chlorophenyltriezene	Orange shining needles	Acetone	140	59.64	59.03	4.62	4.47	16.06	16.67	$C_{13}H_{12}N_3OCl$
(iii)	3-hydroxy-3-p-tolyl-1-p-chlorophenyltriezene	Olive-green needles	Acetone	146	59.64	59.35	4.62	4.40	16.06	16.72	$C_{13}H_{12}N_3OCl$

The spectrum of the complex formed between Lead (II) and hydroxytriazenes was recorded in the range of 389 to 500 nm by plotting the absorbance against the wavelength. The wavelength where maximum absorption occurred was very close to that of working wavelength. This ensured high sensitivity and constant molar absorptivity, hence Beer-Lambert's law was obeyed. The results of ƛmax and working wavelength have been incorporated in Table 4.

The effect of reagent concentration was examined by measuring the absorbance of the solution containing a known concentration of Pb(II) and different amounts of organic reagent. The absorbance was also measured at a constant concentration of organic reagent and varying concentration of Lead (II) as well as Job's method. These methods were employed in order to establish the stoichiometry of the complex. The results indicated the formation of a complex Pb(II):Hydroxytriazene = 1:2. The effect of PH on the absorption efficiency was studied. The results indicated that , maximum and constant absorbance of the complex occurred in the range of 7.4-7.85, 7.20-7.50 and 7.0-7.50 for reagents (i), (ii) and (iii) respectively. Under the optimum conditions, Beer-Lambert's law was followed for Pb(II) concentration in the range of (0.8 - 4.5) x 10^{-5}, (0.8 - 4.8) x 10^{-5} and (1.0 – 6.0) x 10^{-5} for reagents (i), (ii) and (iii) respectively. The correlation coefficient for reagents (i), (ii) and (iii), were 0.987, 0.973 and 0.988 respectively, showing an acceptable linearity of the calibration curve. The calculated t-values for reagents (i), (ii) and (iii) were 10.64, 7.30 and 11.08 respectively while the

tabulated t-value for n-2= 3 at 95% (p= 0.05) confidence limit is 3.18. This revealed a significant correlation between absorbance and concentration. From t-values it was deduced that a straight line was stronger when reagent (iii) was used in comparison with the other reagents.

Table 4: Spectrophotometric determination of Lead (II) with hydroxytriazenes

Reagent No.		(i)	(ii)	(iii)
Concentration of the complex Pb:R		1:2	1:2	1:2
Color of the complex		Yellow	Yellow	Yellow
ƛmax in nm		399	396	395
Working wavelength in nm		403	403	413
Optimum PH range		7.4-7.85	7.2-7.5	7.0-7.5
Beer-Lambert's range x10^{-5} M		0.8-4.8	0.8-4.8	1.0-6.0
Molar absorptivity in mol^{-1} dm^3 cm^{-1}		6125	4875	3900
Sandell's sensitivity in ng/cm^2		33.829	42.503	53.128
Stability constant for complex by Harvey Manning's x10^{10}		1.216	0.4801	1.089
Stability constant for complex by Purohit's method x10^{10}		1.201	0.2819	2.212
Free energy of formation of the complex at 25ºC using Harvey		-13.522	-12.981	-13.458
Free energy of formation of the complex at 25ºC using Purohit's method Kcal/mol		-13.514	-12.671	-13.444
Precision	**Pb taken in ppm**	8.288	10.201	12.538
	Standard deviation in ppm for ten determinations	0.134	0.177	0.300

The limit of detection signal for reagents (i), (ii) and (iii) were calculated by taking the mean of blank signals plus three times the standard deviation of the blank signals; they were found to be 0.193, 0.143, 0.215 mg/L for Pb respectively. These limits were used in the regression equation which gave a detection limit for reagents (i), (ii) and (iii) as 5.75, 5.82 and 11.45 mg/L respectively. These results

indicated that the lowest concentration of Pb (II) which the instrument signal significantly differed from that of the blank was observed with the reagent (i), hence this reagent is more applicable for trace determination in comparison with the other two reagents.

The values of molar absorptivity and Sandell's sensitivity revealed that reagent (i) was the most sensitive and reagent (iii) was the least sensitive amongst the hydroxytriazenes used in the present studies. Moreover, reagent (i) is more sensitive than hexamethyleneiminedithiocarbonmate (HMICDT) in chlorotoluene. However, its sensitivity is slightly lower than all the reagents which have been incorporated in Table 5. The stability constant data revealed that, the complex formed by reagent (iii) was more stable in comparison with those formed by the other two reagents. The high amount of the free energy of formation of the complex was observed when reagent (i) and (iii) were employed.

Table 5: Comparison of analytical parameters for the spectrophotometric determination of Lead

Reagent	**λ (nm)**	**$\sum\lambda \times 10^{-14}$**	**Remark**
1-(2-Thiazoylazo)-2-naphthol	575	3.6	Light-sensitive Co, Zn, Fe, Mn, EDTA interfere
Porphyrin compounds	479	2.2	Cu, Ni, Mn, Cd, Ca, Mg, Zn, Fe interfere
Xylenol orange	580	2.3	Light-sensitive Hg, Zn, Al, Bi, Ni, Re EDTA interfere
2-(2-Thiazoylazo)-p-cresol	650	2.1	Ni, Co, Zn, Fe, Cd interfere
4-(2-Pyridylazo)-2-resorcinol	520	4.3	Cd, Ni, Cu, Co, Ag, Hg, Zn, Fe interfere
Arsenazo III	660	3.0	Al, Cu, Th, Ti, U, Fe interfere
Benzoic Acid Azo phenyl cal-ix[4]arene (BAPC)	440	1.2	Cu, Ca, Fe, Cr, Co, Ni interfere
Dibromo-p-methyl-carboxysulfonazo (DBMCSA)	648	10.4	Ca, Ba
3-hydroxy-3-p-tolyl-1-o-chlorophenyltrazene	403	0.6125	Zn, Co, Ni, Cu interfere
3-hydroxy-3-p-tolyl-1-m-chlorophenyltrazene	403	0.4875	Zn, Co, Ni, Cu interfere
3-hydroxy-3-p-tolyl-1-p-chlorophenyltrazene	413	0.3900	Zn, Co, Ni, Cu interfere

The precision studies were carried out in order to check the reliability of the results of Lead (II) determination with each of the hydroxytriazenes and the results showed a fair replication of the results with the reagents studied.

Table 6 consists of the percentage recoveries. Examination of this table revealed that the percentage recoveries using each hydroxytriazenes ranged between 157.25 to 177.58% and was out of the acceptable range. This was contributed by the presence of matrices in the Tea leaves samples. These recovery test results showed that the method is fairly effective and reliable for estimation of the Lead content in a given environmental sample.

Table 6: Lead Recovery test results

Reagent no.	**Lead (II)/ mg/L**		**Recovery ±SD %**
	Added	**Found**	
(i)	12	19.83	165.25 ± 2.752
(ii)	12	21.31	177.58 ± 3.18
(iii)	12	18.87	157.25 ± 2.911
GF-AAS	12	12.32	102.67 ± 0.213

Possible interference from various ions which may be found in the environmental samples were examined by introducing various amounts of diverse ions to a solution containing 8.29, 10.2 and 12.54 ppm of Pb (II) for the case of reagents (i), (ii) and (iii) respectively. The results have been incorporated in Table 7-9. The tolerance limit was fixed at the maximum amount of an ion causing an error not greater than 5% in the absorbance of the solution. Almost all of the cations and anions were tolerated except $Co^{2+,}$ Ni^{2+}, Cu^{2+} and Zn^{2+} which interfered causing a positive error. The positive error could be due to the formation of the complex between these interfering species and hydroxytriazenes which absorbs electromagnetic radiation at the working wavelength hence contributing to high absorbance. A close look at the results in Table 5 reveals that hydroxytriazenes are more selective than all the reagents in this table except for dibromo-p-methyl-carboxysulfonazo (DBMCSA). However in the presence of Ca^{2+} and Ba^{+} in the sample, hydroxytriazenes are more reliable than DBMCSA.

The concentration levels of Lead (II) in spinach samples obtained from five markets within Chuka have been shown in table 10. The results show that the samples from Marima market had the highest concentration of Lead (II) {average of 0.103 ± 0.0029, 0.097 ± 0.0027, 0.121 ± 0.0019, 0.084 ± 0.0082 using reagents (i), (ii), (iii) and GF-AAS respectively}, while samples from Cheera had the least {0.062 ± 0.0068, 0.055 ± 0.0047, 0.059 ± 0.0018, 0.047 ± 0.0016 for reagents (i), (ii), (iii) and GF-AAS respectively}. According to the guideline by EU commission regulation 2011, concentration

of Lead should not exceed 0.1-0.2 mg/Kg depending on the kind of vegetables. The results obtained showed that the levels of Lead (II) in the samples collected from markets were less than the maximum allowable limits hence consumers of this commodity are safe from effects of Lead (II). The results obtained using three hydroxytriazenes were compared with those of GF-AAS. The observed values of t in each case was less than critical value (tabulated value; t_8= 2.31) hence the null hypothesis was retained. This means that there is no significant difference between the two methods. The results reveal that, the spinach samples did not have matrices which do interfere with Lead (II) determination.

Table 7: Determination of Lead (II) in the presence of diverse ions using 3-Hydroxy-3-p-Tolyl-1-o-chlorophenyltriazene

Diverse ions	**Diverse ion taken (ppm)**	**Pb (II) found* (ppm)**	**Relative error**	**Standard deviation**	**Diverse ion**	**Diverse ion taken (ppm)**	**Pb (II) found* (ppm)**	**Relative error**	**Standard deviation**
Cl^-	1.42	8.02	-3.26	0.07	NH_4^+	0.72	8.06	-2.77	0.11
Br^-	3.20	8.45	+1.93	0.13	Na^+	0.92	8.08	-2.53	0.07
CH_3COO^-	2.36	8.01	-3.38	0.12	K^+	1.56	8.33	+0.48	0.10
CO_3^{2-}	2.40	8.21	-0.97	0.04	UO^{22+}	10.80	8.48	+2.29	0.11
PO_4^{3-}	3.80	8.00	-3.50	0.07	Mn^{2+}	2.20	7.99	-3.62	0.13
SO_4^{2-}	3.84	8.31	+0.24	0.15	Ba^{2+}	5.49	8.30	+0.12	0.14
C_2O^{2-}	3.92	8.03	-3.14	0.09	Zn^{2+}	2.62	8.79	+6.03	0.14
I^-	5.08	8.15	-1.69	0.13	Hg^{2+}	8.02	8.18	-1.34	0.12
$S_2O_3^{2-}$	4.49	8.39	+1.21	0.14	Sn^{2+}	4.75	7.99	-3.62	0.15
NO_2^-	1.84	8.39	+1.21	0.03	Th^{4+}	9.28	8.12	+0.02	0.12
SO_3^{2-}	3.20	7.88	-4.95	0.13	Cd^{2+}	4.50	8.02	-3.26	0.15
S^{2-}	1.28	8.32	+0.36	0.05	Mg^{2+}	0.97	7.91	-4.58	0.13
HPO_4^{2-}	3.84	7.98	-3.74	0.08	Ca^{2+}	1.60	8.09	-2.44	0.12
F^-	0.76	8.02	-3.26	0.10	Co^{2+}	2.36	18.53	+123.52	0.13
NO_3^-	2.48	8.12	-2.05	0.12	Ni^{2+}	2.35	21.48	+159.11	0.13
WO_4^{2-}	9.91	8.15	-1.69	0.13	Cu^{2+}	2.54	14.31	+72.62	0.11
$Mo_7O_{24}^{6-}$	42.22	7.95	-4.10	0.12	ZrO^{2+}	4.29	8.03	-3.14	0.13

*Average of the five determinations

Table 8: Determination of Lead (II) in the presence of Diverse ions using 3-Hydroxy-3-P-Tolyl-1-m-chlorophenyltriazene. Pb(II) taken = 10.2 ppm

Diverse ions	**Diverse ion taken (ppm)**	**Pb(II) found* (ppm)**	**Relative error**	**Standard deviation**	**Diverse ions**	**Diverse ion taken (ppm)**	**Pb(II) found* (ppm)**	**Relative error**	**Standard deviation**
Cl^-	1.73	9.86	-3.33	0.12	$NH4^+$	0.90	9.92	-2.75	0.11
Br^-	4.0	10.16	-0.39	0.15	Na^+	1.15	10.23	+0.29	0.23
CH_3COO^-	2.95	10.43	+2.25	0.12	K+	1.95	10.08	-1.18	0.08
CO_3^{2-}	3.00	10.54	+3.33	0.07	UO_2^{2+}	13.50	10.05	-1.47	0.17
PO_4^{3-}	4.75	10.70	+4.90	0.12	Mn^{2+}	2.75	10.16	-0.39	0.16
SO_4^{2-}	7.80	10.14	-0.59	0.12	Ba^{2+}	6.87	9.94	-2.55	0.20
$C_2O_4^{2-}$	4.90	9.73	-4.61	0.06	Zn^{2+}	3.27	13.58	+33.14	0.14
I^-	6.35	10.55	+3.43	0.12	Hg^{2+}	10.03	9.95	-2.45	0.12
$S_2O_3^{2-}$	5.62	9.82	-3.73	0.16	Sn^{2+}	5.94	10.50	+2.94	0.15
NO_2^-	2.30	10.18	-0.20	0.15	Th^{4+}	11.60	10.53	+3.23	0.15
SO_3^{2-}	4.00	10.09	-1.08	0.07	Cd^{2+}	5.62	10.39	+1.86	0.14
S^{2-}	16.03	9.91	-2.84	0.14	Mg^{2+}	1.22	10.41	+2.06	0.14
HPO_4^{2-}	4.80	10.70	+4.90	0.12	Ca^{2+}	2.00	10.03	-1.67	0.13
F^-	0.95	10.54	+3.33	0.17	Co^{2+}	2.95	25.94	+154.31	0.13
NO_3^-	3.10	10.23	+0.29	0.19	Ni^{2+}	2.93	30.72	+201.18	0.15
WO_4^{2-}	12.39	10.11	-0.88	0.08	Cu^{2+}	3.18	21.45	+110.29	0.11
$Mo_7O_{24}^{6-}$	52.78	10.13	-0.69	0.15	Zro^{2+}	5.36	9.74	-4.51	0.17

*Average of the five determinations

Table 9: Determination of Lead (II) in the presence of Diverse ions using 3-Hydroxy-3-P-Tolyl-1-P-chlorophenyltriazene. (Pb(II) in ppm taken = 12.54

Diverse ions	**Diverse ion taken (ppm)**	**Pb(II) found* (ppm)**	**Relative error**	**Standard deviation**	**Diverse ions**	**Diverse ion taken (ppm)**	**Pb(II) found* (ppm)**	**Relative error**	**Standard deviation**
Cl^-	2.13	12.13	-4.02	0.20	$NH4^+$	1.08	12.53	-0.08	0.20
Br^-	4.79	12.22	-2.55	0.19	Na^+	1.38	12.71	-2.95	0.15
CH_3COO^-	3.54	12.59	+0.40	0.21	K+	2.35	12.84	+2.39	0.08
CO_3^{2-}	3.60	12.70	+1.28	0.14	UO_2^{2+}	16.20	12.78	+1.91	0.18
PO_4^{3-}	5.70	12.34	-1.59	0.07	Mn^{2+}	3.30	12.56	+0.16	0.10
SO_4^{2-}	5.76	12.29	-2.45	0.06	Ba^{2+}	8.24	12.60	+0.48	0.19
$C_2O_4^{2-}$	5.88	12.05	-3.91	0.09	Zn^{2+}	3.92	13.24	+5.58	0.10
I^-	7.61	12.39	-1.20	0.24	Hg^{2+}	12.04	12.58	+0.32	0.17
$S_2O_3^{2-}$	6.74	12.28	-2.07	0.22	Sn^{2+}	7.12	12.68	+1.12	0.18
NO_2^-	2.76	12.43	-0.88	0.22	Th^{4+}	13.92	13.03	+3.91	0.20
SO_3^{2-}	4.80	12.86	+2.55	0.29	Cd^{2+}	6.74	12.94	+3.19	0.21
S^{2-}	1.92	12.28	-2.07	0.23	Mg^{2+}	1.46	12.07	-3.75	0.15
HPO_4^{2-}	5.76	12.27	-2.15	0.24	Ca^{2+}	2.40	12.80	+2.07	0.17
F^-	1.14	12.48	-0.48	0.14	Co^{2+}	3.54	16.26	+29.67	0.17
NO_3^-	3.72	12.24	-2.39	0.34	Ni^{2+}	3.52	22.83	+82.06	0.23
WO_4^{2-}	14.87	12.64	+0.80	0.08	Cu^{2+}	3.81	14.82	+18.18	0.273
$Mo_7O_{24}^{6-}$	63.33	12.54	0.00	0.16	Zro^{2+}	6.43	12.53	-0.08	0.18

*Average of the five determinations

Table 10: Lead content in spinach sample (in mg/ Kg of dry vegetable sample)

Reagent no.	**Sampling Points/ concentration (mg/Kg)**					**t-values**
	Chuka	**Ndagani**	**Kaanwa**	**Cheera**	**Marima**	
(i)	0.077 ±0.0097	0.080 ±0.0017	0.067 ±0.0033	0.062 ±0.0068	0.093 ±0.0029	1.651
(ii)	0.089 ±0.0051	0.076 ±0.0022	0.059 ±0.0011	0.055 ±0.0047	0.097 ±0.0027	1.305
(iii)	0.081 ±0.0023	0.079 ±0.0041	0.063 ±0.0073	0.059 ±0.0018	0.091 ±0.0019	0.976
GF-AAS	0.069 ±0.0011	0.067 ±0.0008	0.052 ±0.0013	0.047 ±0.0016	0.084 ±0.0082	

Tabulated t^+ value = 2.31

4. Conclusion

Fairly sensitive and reasonably selective spectrophotometric reagents for the determination of trace amount of Lead (II) in spinach samples have been introduced. The results obtained using hydroxytriazenes are comparable with those of GF-AAS. The concentration of Lead (II) in spinach samples from selected markets varied from 0.047 to 0.84 with mean ± SD value of 0.0008 ± 0.0082 mg/Kg. On the basis of the results obtained in this study, it can be postulated that the spinach leaf from Chuka, Ndagani, Kaanwa, Cheera and Marima markets within Chuka are not contaminated with Lead and so are safe for human consumption as the Lead levels are far below the tolerated limit of the EU commission.

Acknowledgement

The authors are especially indebted to the administration of Chuka University for providing the laboratory facilities.

References

[1] Anil, K. S. and Harshall, S. K., Novel method for micro determination of Lead in water samples. *Sci. Revs. Commun.*, **2013**, *3*(1): 43-53

[2] Adelene, B.; Ipsita, M. and Krishnajyoti, G., Concenrtrations of Lead in selected vegetables grown and marketed along major highway in Kolkata (India). *IIOAB Journal*, **2013**, *4*(2): 32-35

[3] Qin, F. and Chen, W., Lead and Copper levels in tea samples marketed in Beijing (China). Bull. *Environ. Contam. Toxicol*. **2010**, *78*: 128-131

[4] Sezgin, B. and Mehmet, Y., Determination of Lead, Cadmium and Copper in roadside soil and plants in Elazig, Turkey. *Environ. Monit. Assess.* **2008**, *136*: 401-410

[5] Blake, C. and Bourqui, B., Determination of Lead and Cadmium in food products by Graphite Furnace Atomic Absorption Spectroscopy. *Atom. Spectr*. **1988**, *19*(6): 207-213

[6] Shahida, B. N.; Arshad, A. G. and Younas, M. Khokhar. Flame Atomic Absorption Spectrophotometric determination of Lead in root vegetables after activated Carbon enrichment. Jour. Chem. Soc. Pak. **1999**, *4*: 386-391

[7] Sezgin, B.; Tolga, Y.; Nihan, T.; Mehmeet, D.; Kemal, A. F.; Dnur, M. and Abdullah, K., Determination of As, Cd and Pb in tap water and bottled water samples by using optimized GFAAS system with Pd-Mg and Ni as matrix modifiers. *Jour. Spectr*. **2013**, ID *824817*:1-7

[8] Rakesh, K. M.; Tejinder, P.; Singh, W. S. and Guru, N., Stripping Voltammetric determination of Zinc, Cadmium, Lead and Copper in blood samples of children aged between 3 months and 6 years. *J. has*. **2005**, *4*(1): 1-8

[9] Nan, D.; Qian, C.; Hong, Z.; Yimin, Y.; Lixizeng, Y. H.; Kaixiang, X. and Guangwei, W. Colorimetric Assay for determination of Lead (II) based on its incorporation into Gold nano particles during their synthesis. *Sensors*. **2010**, *10*: 11144-11155

[10] Opajobi, A. O.; Esume, C. O.; Osasuyi, A. and Okehie, C. C., Determination of the Lead content of pumpkin leaf *Telfairia occidentalis* in selected towns of Delta state covering the three senatorial districts of the state. *Curr. Worl. Environ. 2011*, *6*(1): 39-44

[11] Sitki, B.; Abdurrahim, K. and Ali, R. T., Determination of Lead, Iron and Nickel in water and vegetable samples after preconcentration with *Aspergillus niger* loaded on Silica gel. *Clean*, **2007**, *35*(6): 607-611

[12] Maria, D. M.; Monika, R. and Stefan, S. S. Concentration of Lead and Cadmium in selected vegetables grown in the region of Warmia and Mazury. *Pol. J. Food Nutri. Sci*. **2007**, *57*(4): 119-121

[13] Opaluwa, O. D.; Aremu, M. O.; Ogbo, L. O.; Abiola, K. A.; Odiba, I. E.; Abubakar, M. M. and Nweze, N. O. Heavy metal concentrations in soils, plant leaves and crops grown around dump sites in Lafia Metropolis, Nasarawa State, Nigeria. *Adv. Appl. Sci. Res*. **2012**, *3*(2): 780-784

[14] Alizera, A. Simultaneous determination of trace amounts of Lead and Zinc by Adsorptive Cathodic Stripping Voltammetry. Malay. *Jour. Analy. Sc*. **2008**, *12*(2): 410-418

[15] Le, V. T. and Nguyen, T. N. L. Spectrophotometric determination of Lead in environment samples by Benzoic Acid Azo Phenylcalix [4] arene (BAPC). *Intern. Jour. Chem*. **2010**, *2*(2): 86-90

[16] Guozhen, F.; Yongwen, L.; Shuangming, M. and Yong, G. Spectrophotometric Determination of Lead in Vegetables with Bromo-P-methyl-carboxysulfonazo. *Talanta*. **2002**, *57*(6):1155-1160

[17] Kailaasa, S. K.; Lingaladinne, K.; Sunnapu, H.B.; Kanchi, S.; Lokanath, B. S. and Pattium, Chiranjeevi. Spectrophotometric determination with a chromogenic reagent of Lead in vegetables. *Chem. & Biodiv*. **2005**, *2*(3): 386-391

[18] Sabah, S.; Ali, D.; Ali H.; Abdolhossein, P.; Babok, G. and Sina, S. Determination of trace amounts of Lead using the Floatation Spectrophotometric method. *Analy. Chem. Insigh*. **2011**, *6*: 15-20

[19] Guo, Y.; Liu, Y. C. X. and Meng, S. Determination of Lead using a new chromogenic reagent 2-(2-Sulfo-4-acetylphenyl-azo)-7-(2,4,6-trichlorophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid. *Microchim. Acta*. **2004**, *144*: 257-261

[20] Purohit, D. N.; Kumar, S. and Goswami, A. K., A review of Hydroxytriazenes. Rev. Analy. Chem. (Israel). **2003**, *22*(1):73-80

[21] Sharma, J. C.; Upadhyav, M.; Jain, M. C. and Dashora, R. Complexometric determination of Copper in pharmaceutical sample using Hydroxytriazenes. *Asian J. Chem.* **2006**, *18*(4): 3144-3146

[22] Ombaka, O.; Musundi, S. W.; Gitonga, L. K.; Kibaara, D. and Olivia, A. N., Rapid economical and selective complexometric determination of Iron (III) in its synthetic alloys using 3-hydroxy-3-phenyl-1-(2,4,6-tribromophenyl)-triazene. *Intern. Jour. Sci & Eng. Res*. **2013**, *4*(9):140-150

[23] Ombaka, O. and Gichumbi, J. M., Spectrophotometric determination of Cobalt (II) in low concentrations using hydroxytriazenes as selective chelating agent. *Afr. Jour. Pur. And Appl. Chem.* **2011**, *5*(15): 494-502