Spectrophotometric Determination Of Copper (Ii) In Soil From Ahero Rice Irrigation Schemes Using Hydroxytriazene.

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Abstract

A simple, rapid, sensitive and cost effective spectrophotometric technique for the determination of copper (II) using 3-hydroxy-3-m-tolyl-1-O-carboxyphenyltriazene has been reported. The complex formed was greenish-yellow having stoichiometry of 1:1 (Cu (II): hydroxytriazene) at a pH range of 6.0-6.6. The measurements were carried out at a working wavelength of 405nm with a molar absorptivity of $2.8 \times 10^4 Lmol^{-1}cm^{-1}$ and sandell's sensitivity of $2.26ng/cm^2$. Lambert –Beer's law is obeyed over a range of 1.27-7.63mg/L of copper. The correlation coefficient of the standard calibration curve was 0.997. The complex formed between copper (II) and hydroxytriazene was greenish –yellow with composition of 1:1(Cu (II): hydroxytriazene). The relative standard deviation (n=10) for standard dilution of $635.46 mgL^{-1}$ copper (II) was 0.92%. The change in Gibb's free energy at $27^0 C$ was determined using Purohit's, Harvey and Manning's method and found to be respectively. The study showed that, the following cations and anions do not interfere when present at equivalent amount of copper: Cl^- ,Br^-, CH_3COO^-, CO_3^{2-}, PO_4^{3-},SO_4^{2-}, $C_2O_4^{2-}$, I^-, $S_2O_3^{2-}$, NO^-, SO_3^{2-},S^{2-},HPO_4^{2-}, F^-, NO_3^- , WO_4^{2-}, $MO_7O_{24}^{6-}$, NH_4^+, Na^+, k^+, Ba^{2+} , Cd^{2+}, Mg^{2+}, Ca^{2+}. However, UO^{2+}, Mn^{2+}, Pb^{2+}, Hg^{2+}, Sn^{2+}, Th^{4+},ZrO^{2+},Co^{2+}, Zn^{2+}, Ni^{2+} interferes with copper (II) determination at equivalent amount, hence the need for the masking agent. The present method was applied to the determination of copper (II) in agricultural soil samples.

Keywords: *spectrophotometric, copper (II), Cu (II) – Hydroxytriazene complex, molar absorptivity, Gibb's free energy, interference studies.*

I. Introduction

Copper is one of the essential micronutrient for plants growth and development [1]. It participates in many physiological processes in plants due to its existence in multiple oxidation states [2].It acts as a structural element in regulatory proteins and it's also involved in photosynthetic electron transport, cell wall metabolism, hormone signaling, oxidative stress responses, mitochondrial respiration, ethylene perception, response to pathogens and molybdenum cofactor biosynthesis [3]. However, copper can have adverse effect to plant development due to direct or indirect interference with a number of physiological processes. Hence minimal amount of copper is recommended for ensuring cellular functions because of the sensitivity of plants to copper toxicity which may attribute to metabolic disturbances and growth inhibition when the levels of copper in the tissue is slightly higher or low than the normal [4].

The plants in aqueous media containing copper are generally exposed to copper toxicity. Copper can accumulate in the root tissue with little upward movement towards the shoots; hence it hinders root elongation and growth. The symptoms associated with this includes chlorosis, necrosis and leaf discolouration. The excess copper in plant tissue might attach themselves to the sulfhydryl group of cell membrane or induce lipid peroxidation which ends up damaging membrane and producing free radicals in different plant organelles and other parts. The toxicity of copper due to redox process cycling between Cu^+ and Cu^{2+} triggers the formation of reactive oxygen species such as singlet and hydroxyl radicals, leading to injuries to macromolecules [5, 6]. The environmental contamination might arise from anthropogenic, domestic and agricultural use of metals and copper containing compounds [7, 8, 9]. The contamination can also occur through copper corrosion, atmospheric deposition, soil erosion of copper ions and leaching of copper sediment re-suspension and copper evaporation from water resources to soil and ground water, waste dumps, combustion of fossil fuels and wastes, wood production, natural phenomena(weathering and volcanic eruptions, wind-blown dust from native soils, decaying vegetation, forest fires and sea spray),industrial sources(copper processing) in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, textiles, microelectronics, plastics, wood preservation and paper processing plants [10,11,12,13].

The deficiency of copper in plants contribute to reduced growth rate, chlorosis especially in young leaves, curling of leaf margins, defects in cell wall formation, damage at the apical meristem, lignification which contributes to insufficient water transport, limited fruit formation, defective pollen development and viability

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and diminished seed production and viability [14]. The deficiency of copper may be caused by copper binding strongly to organic and inorganic colloids in soil and sediments contributing to reduction in mobility of copper inland plants [15]. The mobility of copper is also related to the chemical properties of soil. Some types of soil which consist of carbonate contribute to copper being effectively immobilized by providing an adsorbing surface or by buffering the pH. The plants grown in calcareous or alkaline soils in arid and semi-arid environments are likely to contain low concentration of copper in edible plant tissue [16].

Copper plays a vital role in biological processes like metabolism, nerve function, bone development and hemoglobin synthesis [17]. However, excess of copper in the human body causes many diseases like heart disease, anemia and liver damage. Heavy metals like copper disrupt metabolic functions by accumulating and thereby disrupt function in vital organs and glands like heart, brains, kidney, bone, liver, etc. and also by displacing the vital nutritional minerals from their original place, hence hindering their biological function [18]. Copper may enter the body through consumption of foods, beverages, skin exposure and inhaled air. This has raised an increasingly ecological and global public health concern about environmental contamination by heavy metals such as copper as a result of human activities which have drastically altered copper geochemical cycles and biochemical balance [19]. Therefore, the copper levels in environmental samples should be monitored continuously using simple, rapid, selective and cost effective analytical technique and thereafter, appropriate cost effective remediation techniques can then be applied to reduce copper levels in the environmental samples to be within the permissible limits. A survey of literature reveals that, a number of analytical techniques have been used for copper (II) determination which includes ICP-AES, ICP-OES, ICP-MS, AAS, GF-AAS, microwave Plasma-AES, potentiometry, voltammetry, spectrofluorimetry, photoluminescence spectroscopy, dynamic light scattering [20, 21,22]. However, some of these methods have drawbacks such as time-consuming, labour intensive, the demand of large volume of organic solvents that may increase pollution, the use of hazard solvents, highly costly and well controlled experimental conditions [23]. UV-visible spectrophotometric technique is widely used in most of quality control laboratories for copper (II) ions determination in aqueous media because of its inherent simplicity, low cost, availability and provides a high accuracy and reproducibility from a quite small number of samples [24]. The various spectrophotometric reagents for the determination of copper (II) ions containing a proper functional analytical groups that enables its interaction with copper (II) ions and subsequently resulting to its analytical signals have been reported in literature. Most of these reagents for copper (II) determinations have limitation such as heating of the solution, narrow Lambert-Beer's range, more time for colour development, the period for the stability of colour developing reagent and the absorbing product takes a short time, interferences from many foreign ions, which implies that, they can be rendered selective through the introduction of masking agents, control of pH, solvent extraction, adjustment of the oxidation state or prior removal of foreign ions and this increases the cost of the method and time for analysis [25, 26]. Hydroxytriazenes have different applications as chemical and biological reagents for the metal determination as well as intermediate compounds in organic synthesis. They have been used in the study of supramolecular arrangements in metallic complexes and also as antibacterial, antileukemic, antitripanosomal and anticancer activities. Hydroxytriazenes being Azo compounds are expected to act as fluorescence sensors with biological and environmental functions, hence can be used as chemical sensors [27]. A survey of the literature has revealed that, a number of hydroxytriazenes have been used as analytical reagent for the spectrophotometric determination of transition and non-transition metal ions. The study has shown that 6.35ppm of Cu (II) can be determined with 3-hydroxy-3-propyl-1-p-tolyltriazene as a spectrophotometric reagent in the presence of ten cations and anions. However, the presence of 10ppm of Mn^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Cr^{3+}, Hg^{2+}, F^-, I^-, molybdate and oxalate interferes with the determination. Another study has also revealed that, 6.35ppm of copper can be determined in the presence of twenty two cations and anions with only Cd^{2+}, Zn^{2+}, Mg^{2+}, Hg^{2+}, Co^{2+}, Ni^{2+} and CO_3^{2-} interferes at 10ppm [28]. These studies show that different hydroxytriazenes have different selectivity. Therefore, there is a need to focus in finding a new spectrophotometric reagent from the class of hydroxytriazenes which can attribute to simpler, rapid, accurate and selective spectrophotometric method for determination of copper.

II. Materials and Methods

Equipment/apparatus.

Shimadzu uv-1800 uv-spectrophotometers, PG-990 Atomic Absorption spectrophotometer equipped with deuterium lamp, electronic analytical balance (mettle Teledo), Ethos easy advanced microwave digestion, pH meter (Hanna instrument), ultra-pure water equipment (model milli-Q equipped with Q-pod), Mrc-MPA 12 melting point apparatus, vario E2 III-Elementar, glassware and polyethylene containers.

Reagents and solutions

All the working solutions were prepared with ultra-pure water. The analytical grade reagents were used throughout. Glassware and polyethylene containers were washed with a neutral detergent and thereafter soaked overnight in 10% (V/V) HNO_3 solutions. This was followed by ultrasonification for 1hr and finally rinsed several times with ultra-pure water. The standard series of copper solutions for calibration curve of AAS were prepared by dilution of 1000mg/L of stock solutions of copper. Analytical reagent grade of 70% HNO_3, 37% and 30% H_2O_2 were used.

Synthesis of hydroxytriazenes
Step 1: preparation of aryl hydroxylamine
0.25 moles of 3-nitrotoluene, 30gms of NH_4Cl and 250ml of ultra-pure water were mixed in 1000ml beaker and stirred mechanically at 40^0C and then 40gms of zinc dust was added slowly in small amounts in such a way that, when the temperature of reaction mixture reached 58^0C, half of the zinc dust had already been added. The temperature of reaction mixture was then maintained between 58^0C-60^0C by occasionally adding zinc dust. The mixture was further stirred for 15 minutes after all the zinc dust had been added. The product obtained was filtered under vacuum, washed with ice cold ultra-pure water and the filtrate obtained was kept in refrigerator.

Step 2: preparation of aryldiazonium salt
0.25 moles of 2-aminobenzoic acid was dissolved in 100ml of 50% HCl in a 250ml beaker. 0.25 moles of sodium nitrite was dissolved in minimum quantity of ultrapure water in a 100ml beaker. These solutions were kept in refrigerator at about 0^0C for 2hrs. Thereafter the solution of sodium nitrite was added drop wise to the solution of arylamine hydrochloride which was maintained at the temperature between 0-5^0C while stirring.

Step 3: coupling
The temperature for both arylhydroxylamine (step 1) and aryldiazonium salts (step 2) were maintained between 0-5^0C. The aryldiazonium salts (step 2) was added dropwise to the solution of arylhydroxylamine (step 1) while maintaining the pH between 5-6 using sodium acetate buffer. The resultant product was filtered under vacuum, washed with ice cold ultra-pure water and dried. The presence of hydroxytriazene in the crude compound synthesized was detected using four spot tests namely naphthylamine test, picric test, N, N- Dimethylaniline test and sulphuric acid test. Both tests gave positive tests; an indication of hydroxytriazene [29].The crude compound was subjected to purification and recrystallization using ethanol. The purity of hydroxytriazene was checked by physical characteristics and the composition of the synthesized hydroxytriazene was verified using elemental analysis.

Preparation of Reagents and standard solutions.
EDTA solution.
0.37224g of Disodium dihyrogenethylene diaminetetra-acetate of analytical reagent quality (99%) which was dried at 80^0C was accurately weighed. It was then transferred into 100ml volumetric flask and diluted to the mark using ultra-pure water.
Stock solution of copper (II) solution
0.06355g AR crystalline copper sulphate ($CuSO_4.5H2O$) was dissolved in 100ml of ultrapure water. A few drops of concentrated sulphuric acid was added in order to prevent hydrolysis. A liquot of this stock solution was standardized by EDTA solution using murexide as an indicator. A series of solution of lower concentrations were prepared by appropriate dilution of aliquots from the stock solution with ultra-pure water.

MUREXIDE (AMMONIUM PURPURATE) INDICATOR SOLUTION.
0.15g of the dye was dissolved in 100g of absolute ethylene glycol.
Solution of pH adjustments.
1.0% tris buffer solution was prepared by dissolving 1.0gm of tris- buffer in minimum quantity of water and then diluted to 100ml with ethanol. 1.0% Perchloric acid solution was prepared by diluting 1.0ml of Perchloric acid with ethanol to 100ml.
HYDROXYTRIAZENE SOLUTION
The required quantity of hydroxytriazene was dissolved in ethanol. The solutions of lower concentrations were prepared by proper dilution of stock solution of hydroxytriazene using ethanol. Fresh solutions of hydroxytriazene were prepared when required because hydroxytriazene deteriorate in solution when left to stay for a long time.
Soil sample collection
A total of six samples were collected using soil auger at 0-20cm depth from the six different agricultural areas that are used for cultivation of rice in Ahero irrigation schemes in Kenya. Randomly sampling technique was

used to collect, a total of six samples from each agricultural area and then pooled together to form six composite samples.

Samples preparation

The six composite samples were air dried for 14days and then placed in electric oven at a temperature of 40^{0}C for 2hrs. The samples were then removed from oven and cooled to a room temperature. Thereafter, they were ground, mixed using conning and quartering and then sieved through 0.5mm mesh sieve. The dried samples of constant weight which had already been reduced, sieved and mixed were transferred into six separate clean, labeled polythene bag for digestion.

Digestion of soil samples

Microwave digestion Ethos Easy EPA 3051(alternative) method was used in the present study. 0.5gms of dried, ground, sieved and mixed soil samples were transferred into Teflon-PFA microwave digestion vessel where 9ml of 70% high purity HNO_3 and 3ml of 37% HCl were added in each Teflon tube containing samples. The tubes were transferred into microwave digesters and then digested for 30 minutes. The digested sample solutions were removed from the digester after 24hrs. The solutions were then transferred into six of 50ml volumetric flask and each solution was diluted to the mark using ultra-pure water.

Absorption spectra

The spectrum of complex formed between copper (II) and hydroxytriazene at pH 6.0 was taken in the wavelength region 370nm to 500nm against reagent blank. The spectrum of hydroxytriazene against ethanol was also taken in the same wavelength region. The wavelength where there was maximum difference between the absorption due to complex and the hydroxytriazene was taken as a working wavelength.

Effect of pH

The effect of pH on the formation of the copper complex has been studied by preparing a series of solutions containing copper (II) and hydroxytriazene. The concentration of Cu(II) and hydroxytriazene in each of the solutions were kept constant but having different pH. 1.0% tris buffer and 1.0% Perchloric acid were used for pH adjustment. Absorbance of these solutions was measured at 405nm against reagent blank. The pH range (6.0-6.6) where the absorbance was maximum and remained constant was noted and used throughout the study. The greenish-yellow colour which developed at this pH range was instantaneous, stable for about 24hrs.

MOLAR COMPOSITION OF COPPER (II) COMPLEX WITH HYDROXYTRIAZENE

The composition of the complex formed was studied using Harvey and Manning's method and Purohit's method under optimum condition of pH, solvent and copper(II) to hydroxytriazene following the procedure described by [30].

THE VALIDITY OF THE PROPOSED METHOD

The validity of the proposed method was carried out by investigating the applicability of Lamberts-Beer's law. This was due to the fact that, the validity of any method for determining the concentration of a coloured substance by measuring its absorption of monochromatic light depends upon the ability of that substance to obey certain physical laws such as Lambert- Beer's law [31].

Precision studies

The precision of the proposed method was evaluated by measuring the absorbance of ten solutions having the same concentration of copper (II) and the hydroxytriazene.

Interference studies

The effect of foreign ions on the determination of copper (II) ions using hydroxytriazene as a spectrophotometric reagent under the optimum conditions has been investigated. This involved determination of copper (II) spectrophotometrically using hydroxytriazene in the presence of equivalent amounts, fivefold excess and tenfold excess. Sodium, potassium and ammonium were used for preparation of solution of anions whereas chloride and nitrate were used for the preparation of cations solution.

APPLICATION

The applicability of the proposed method was evaluated by determining six soil samples from agricultural areas using both proposed method and flame AAS (a standard method). The results from the two methods were compared using F-test and T-test in order to establish whether the two methods have comparable standard deviations and whether there is statistical difference in the results of the proposed method and standard reference method.

III. Results and Discussions

The physical characteristics and CHN values for the 3- hydroxy-3-m-tolyl-1-O-carboxyphenyltriazene were determined in order to check the purity of hydroxytriazene synthesized. The results obtained are summarized in table 1.

Table 1: physical characteristics and CHN values for the hydroxytriazene.

Colour and shape of crystals	Solvent used	Elemental analysis			M.P ^{0}C
		%C	%H	%N	
Light yellow shinning needles	Ethanol	TH. 61.96 EXP. 61.61	4.83 4.78	15.49 15.23	178
Deviation		-0.35	-0.05	-0.26	

3-hydroxy-3-m-tolyl-1-o-carboxyphenyltriazene is a compound, consisting of light yellow shining needles with melting point of 178^{0}C. The reagent is stable and can be stored for a long time. The purity of compound was confirmed by carrying out crystallization repeatedly until a constant of small range of melting point was achieved and also by determining the contents of carbon, hydrogen and nitrogen using elemental analyzer. The results showed no significant difference between experimental and theoretical values. The deviation of experimental values was within 0.4% of the theoretical values and this indicated that, the compound was in pure form.

Absorption spectra and optimum condition for complexation.

3-hydroxy-3-m-tolyl-1-o-carboxyphenyltriazene reacts with Cu (II) ions to form a stable greenish- yellow colour complex in ethanol. The absorption spectrum of the hydroxytriazene solution and the complex solutions was studied over the Wavelength range of 3700nm to 500nmagainst reagent and ethanol, respectively. The Cu (II) - hydroxytriazene spectra shows an absorption maximum at 396nm, while the hydroxytriazene had appreciable absorbance at this wavelength at pH 6.0. Hence at 405nm where there was maximum difference between the absorption due to complex and the reagent was chosen as the working wavelength for subsequent studies.

The optimum pH range required for the colour development of constant maximum absorbance of Cu (II)- Hydroxytriazene complex solutions were studied in the pH range 3.0-9.0. The results revealed that the absorbance of maximum and constant occurs in the pH range of 6.0-6.6. It was further observed that, the colour developed was greenish-yellow, which was stable for atleast 24hours and the colour development was instantaneous. Hence the pH range of 6.0-6.6 was selected as the optimal condition for subsequent studies and all the measurements were made at room temperature.

The absorbance of Cu (II) - hydroxytriazene complex solutions at 405nm were taken at various concentrations of hydroxytriazene solution while keeping Cu (II) concentration constant at the optimal condition of pH. The results showed that, a fivefold molar excess of hydroxytriazene was required for the maximum development of Cu (II) - hydroxytriazene complex and the excess of hydroxytriazene had no effect on the absorbance of the Cu (II)-hydroxytriazene solution and therefore, further studies were carried out at six fold molar excess of hydroxytriazene. The data of λ-max, working wavelength and optimum conditions have been incorporated in table 2.

Table 2: determination of λmax, working wavelength and optimum conditions

Colour of complex	λmax nm	Working wavelength(nm)	Optimum pH range	[Cu(II)]: [R]
Greenish-yellow	396	405	6.0-6.6	1:6

Applicability of Lambert- Beer's law, molar absorptivity and sandell's sensitivity.
The values of Lambert-Beer's law range, molar absorptivity, sandell's sensitivity, standard deviation and percent are given in table 3.

Table 3: Determination of Lambert-Beer's law range, molar absorptivity, sandell's sensitivity of Cu (II) - hydroxytriazene complex.

Lambert-Beer's law range ×10^{-5} M	Molar absorptivity Lmol^{-1}cm^{-1}	Sandell's sensitivity ng/cm	Cu(II) taken ppm	Standard deviation ppm	% error
0.2-1.2	28,000	2.269	0.635	0.006	0.92

The verification of Lambert-Beer's law of Cu (II) - hydroxytriazene complex was carried out in order to explore the possibility of trace determination by utilizing the colour reaction due to Cu (II) ions and 3-hydroxy-3-m-tolyl-1-o-carboxyphenyltriazene. It was observed that, Lambert-Beer's law was obeyed in the

concentration range of 0.2×10^{-5}M to 1.2×10^{-5}M and the molar absorptivity and sandell's sensitivity were found to be 28,000Lmol^{-1}cm^{-1} and 2.269 ng/cm respectively. The values of molar absorptivity and sandell's sensitivity showed that, the method is fairly sensitive and can be used in trace amount determination of copper. The analyses of ten replicate of 0.635ppm Cu (II) ions solution showed that accurate and consistently reproducible results are obtainable with error of 0.92% and standard deviation of 0.006.

Composition of the complex and stability constant.
The composition and stability constant of Cu (II)-hydroxytriazene complex are given in table 4.

Table 4: Determination of the composition and stability constant of the complex.

Harvey and Manning's method	Composition		
	1:1	6.1245	-8.408
Purohit's method	1:1	5.9186	-8.122

The composition of Cu (II) - hydroxytriazene was determined using two methods, namely: Harvey and Manning's method and Purohit's method. Both methods confirmed that, 3-hydroxy-3-m-tolyl-1-O-carboxyphenyltriazene which acts as bidentate ligand forms 1:1 Cu (II) complex. This might suggest that, only one ligand is coordinated to the Cu (II) ion and the other coordination positions are occupied by water molecules. Hence a five- coordinated in a distorted square-pyramidal geometry in which the nitrogen and oxygen atoms of bidentate ligand as well as water molecules form irregular pyramidal structure. This has been supported by previous studies of Cu (II)- hydroxytriazene and hydroxytriazene ligand [32, 33,34] which have showed that, the shifting of the band at 1474-1489cm^{-1} due to –N=N- to 1513-1515cm^{-1} during complexing of Cu(II) ions and hydroxytriazene was attributed to the coordination of Cu(II) ion with nitrogen atom of diazo group while the disappearance of the band at 3201-3248cm^{-1} due to O-H stretching and the band at 3298- 3510cm^{-1} due to N-H stretching frequency of tautomeric hydroxytriazene supported the formation of Cu- hydroxytriazene complex. The bands which appeared at 415-417cm^{-1} and 491-624cm^{-1} which were not present in the spectrum of free hydroxytriazene were attributed to Cu-O and Cu-N vibrations respectively. This further supported the involvement of nitrogen and oxygen atoms in complexation with Cu (II). The medium stretch bands at 840-850cm^{-1} confirmed the presence of coordinated water molecules.

The conditional stability constant and Gibb's free energy were determined using two methods, namely; Harvey and Manning's method and Purohit's method. The stability or formation or binding constant was determined in order to establish the strength of interaction between the hydroxytriazene and Cu (II) ions that are involved in complex formation in the solution. The results in table 4 showed that, log value obtained using the two methods were fairly in good agreement which was attributed to simultaneous complex formation. The conditional stability constant obtained was higher than that of Cu (II)-chlorosubstituted pyrazoles [35] which was attributed to bulkiness/steric hindrances which contributed to preventing the dissociation of the Cu (II) - hydroxytriazene complex in solution [36,37]. The value of thermodynamic parameter in table 4 was determined using Harvey and Manning's method and Purohit's method. The results obtained by these two methods were in good agreement. The values had negative values which showed spontaneity of the reaction process. The spontaneousy of the reaction revealed that, entropy change was positive which might have attributed to the release of bound water molecules from the Cu (II)aq during formation of Cu(II)- hydroxytriazene complex. This could have resulted to the primary hydration sphere of the Cu (II) ion being displaced by the hydroxytriazene ligand, resulting to increase in the number of particles in the system (randomness of the system increases, Cu^{2+}aq +hydroxytriazene aq $\rightarrow$ Cu (II)-Hydroxytriazene aq +nH2O). The water molecules which bounded to the Cu (II) ions was expected to be highly distorted and oriented which may have contributed to low value of entropy, although high value is generally expected when positively cations and negatively charged ligands combined which involves the displacement of water molecules. The stability constant of Cu (II) –hydroxytriazene in the present study was fairly high and this implied that Cu (II) –hydroxytriazene bond was fairly strong and the change in enthalpy could have been exothermic hence the formation of Cu (II) –hydroxytriazene was favourable [38].

Interference studies
The interference study for Cu (II) ions determination in the presence of different cations and anions was investigated. The tolerance limit was taken to be concentration of foreign ion which caused a relative error of in the determination of Cu (II) ions. The results showed that, the following ions did not interfere even at tenfold excess: Cl^-, Br^-, CH_3COO^-, CO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, F^-, NO_3^-, NH_4^+, Na^+, K^+, Ba^{2+} whereas I^-, SO_3^{2-}, S^{2-}, HPO_4^{2-}, $Mo_7O_{24}^{6-}$, Cd^{2+}, Mg^{2+} could be tolerated up to fivefold excess and Ca^{2+}, WO_4^{2-}, NO_2^-, $C_2O_4^{2-}$, PO_4^{3-} up to equivalent amount. UO_2^{2+}, Mn^{2+}, Pb^{2+}, Hg^{2+}, Sn^{2+}, Th^{4+}, ZrO_2^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+} interfered at equivalent amount with the Cu

(II) ions determination. However, the effect of foreign ions at lower than equivalent amount and higher than tenfold excess was not studied. However, some of these interfering ions can be removed by using a suitable separating technique such as masking and demasking agent [39].

Analytical applications

The spectrophotometric technique using 3-hydroxy-3-m-tolyl-1-o-carboxyphenyltriazene as spectrophotometric reagent was used to determine the concentration of copper (II) ions in agricultural soil and the results were compared with those of flame Atomic absorption spectrophotometric method (standard reference method). The results from these two techniques are incorporated in table 5.

Table 5: Determination of Cu (II) in agricultural soil samples using the proposed method and flame AAS technique.

Sample No.	1	2	3	4	5	6	Mean, coefficient of variation and relative standard deviation	95% confidence level
Proposed method (ppm)	34.6	57.8	63.1	37.9	60.7	51.8	= 50.98 CV=23.67% S(mean)rel.=9.66	Fcalculated=1.0967 Fcalculated=5.05
Reference method (ppm)	24.1	45.7	55.4	32.1	49.2	43.1	= 43.1 CV=19.48 S(mean)rel.=7.95	Tcalculated=11.797 Ttabulated=2.228

=mean, CV=coefficient of variation, S (mean) rel. =relative standard error

The F-test and T-test were used in comparing the results from proposed technique and flame-AAS. The results of F-test at the 0.05 level showed that, the two methods have comparable standard deviations. Furthermore, the difference between coefficients of variation of the two methods was only which further supported the conclusion reached based on f-test. However, the t-test results at the 0.05 level indicated that, there is statistical difference in the results of the proposed method and flame-AAS method given that. The relative standard error of the proposed method was found to be 9.66 while that of flame-AAS was 7.95 which gave a difference of and this cannot be ignored. The difference in results of the two techniques has been attributed to the use of inorganic fertilizers like urea, calcium superphosphate, iron sulphate and also pesticides such as herbicides, fungicides by rice farmers to obtain maximum yields and this might have attributed to accumulation of heavy metals like Zn, Pb, Cd, As, Co, Cu, Ni, Fe, Mn as impurities in the soil [40, 41]. The present investigation have revealed that, heavy metals like Mn, Pb, Co, Ni, Zn etc. do interfere positively with the determination of copper when using 3-hydroxy-3-m-tolyl-1-o-carboxyphenyltriazene as a spectrophotometric reagent and when such interference species are present, appropriate separation techniques must be employed. Slightly high value of Cu (II) obtained in the present investigation using proposed method compared to flame-AAS might be due to the presence of such heavy metals in the soil sample.

IV. Conclusion

Hydroxytriazene have been synthesized, characterized using physical characteristics and elemental analysis. The detection of hydroxytriazene was achieved by employing the four spot tests. The hydroxytriazene synthesized is a bidentate N,O donor, which usually coordinates with Cu (II) ions in 1:1 ratio. The results obtained showed that, the proposed method in which the reaction involves hydroxytriazene and Cu (II) ions can be used for estimation of Cu (II) in the environmental samples and the results can be used in making the decision whether the soil remediation is required or not. The proposed method is precise, simple, rapid, low cost and fairly sensitive method for the determination of trace amounts of copper in the environmental sample. This spectrophotometric reagent can operate at fairly a wide range of concentrations and might be utilized as a sensitive chemo-sensor of copper monitoring in the environmental samples given that, the colour change due to reaction of hydroxytriazene with Cu (II) is instantaneous and stable for atleast 24hrs.

CONFLICT OF INTEREST
The author would like to take this opportunity to declare that, the conflict of interest with respect to the publication of this paper does not exist.

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