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NON-EXTRACTIVE SECOND DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF CD(II) IN ENVIRONMENTAL, SOIL, BIOLOGICAL, MEDICINAL AND GREEN LEAFY VEGETABLE SAMPLES USING 2- BENZOYLPYRIDINE 4-METHYL-3-THIOSEMICARBAZONE (BPMT)

[1]*V. Saleem Basha and [2]K. Hussain Reddy

[1]*Department of Chemistry, Government College, (Autonomous), Anantapur -515 001, A.P, India.

[2]Department of Chemistry, Sri. Krishnadevaraya University, Anantapur -515 001, A.P, India.

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***Corresponding Author**
V. Saleem Basha
Department of Chemistry, Government College, (Autonomous), Anantapur - 515 001, A.P, India.

ABSTRACT

2-benzoylpyridine 4-methyl 3- thiosemicarbazone (BPMT) is proposed as a new sensitive, selective, direct and non-extractive chromogenic reagent for extractive spectrophotometric determination of trace amounts of cadmium (II) in aqueous dimethyl formamide (DMF). BPMT reacts with cadmium (II) to give a greenish yellow colored complex 1:1 complex in ammonium chloride-ammonium hydroxide buffer medium of pH 8.0 at λ_{max} 400 nm. The color reaction has been investigated in detail. The molar absorptivity and Sandell's sensitivity are found to be 1.6 x 10^4 $Lmol^{-1}$ cm^{-1}, and 0.00702 μgcm^{-2} respectively. The interference of various diverse ions has also been studied. The method has been successfully applied for the non-extractive second derivative spectrophotometric determination of trace amounts of Cd (II) in several standard reference materials such as Environmental, soil, Biological Samples, Medicinal, and green leafy vegetable samples.

KEYWORDS: Cadmium, Spectrophotometry, Environmental, leafy vegetables, Biological, medicinal Samples.

INTRODUCTION

Among the toxic heavy metals cadmium is of great environmental concern because of its higher bio availability. It is a lustrous, silver-white, ductile and highly malleable metal. It is soluble in acids but not in alkalis. About 75% of cadmium is used in Ni-Cd batteries and the remaining is used mainly for pigments, coating and plating and as stabilizers for plastics.[1, 2] Cadmium has been used particularly to electroplate steel and as a barrier to control nuclear fission. Naturally a very large amount of cadmium is released into the environment, through weathering of rock, through forest fires and volcanoes. The rest of the cadmium is released through human activities, such as manufacturing processes, e-waste materials, and through sewage-sludge and fertilizers[3-5] (due to enrichment of heavy metals in soil which impacts human health) etc., It is easily absorbed by plants, through their root system and by leave.[6] Acid reaction of soil increases its mobility and availability. It remains in tissues for a relatively long time and is accumulated in vital organs especially in kidney and liver.[7]

Human intake of cadmium takes place mainly through diet like liver, mushrooms, shellfish, mussels, cocoa powder and dried seaweed.[8] One of the main reasons for cadmium accumulation in the body is the extensive use of tobacco. Growing plants acquire and concentrate Cd (II) with in the same biochemical set up, because of its higher bioavailability in soil-plant relationship and toxicity to human and livestock by readily getting in to food chain.[9]

The outbreak of cadmium (II) poisoning occurred in Japan in the form of itai itai or ouch ouch disease. Symptoms include fragileness of bones. At high levels cadmium causes kidney problems anamemia and bone marrow disorders.[10] Trace amounts of cadmium are important in industry[11], as a toxicant[12], and biological non-essential[13], as an environmental pollutant[14], and an occupational hazard.[15] It is an extremely toxic metal, and the effects of acute cadmium poisoning are manifested in a variety of different symptoms including high blood pressure, kidney damage and destruction of red blood cells.[16]

Substituted - thiosemicarbazones are important organic reagents and good chelating agents and have potential sites for the formation of complexes with various metal ions by bonding through azomethine *nitrogen* atom and thioketo sulphur atom.[17,18] The metal chelates of these sulfur and nitrogen containing reagents find wide range of applications in medicine and agriculture.[19]

This paper describes the non-extractive spectrophotometric determination of cadmium as its BPMT complex in aqueous medium. A survey of literature reveals that 2-benzoylpyridine 4-methyl 3- thiosemicarbazone (BPMT) have not been employed for direct, non- extractive spectrophotometric determination of Cd (II), so the authors have introduced it a new analytical reagent for the first time, for the spectrophotometric determination of trace amounts of cadmium (II). The present method does not require a solvent extraction step, hence the use of carbon tetra chloride or chloroform as solvents is avoided which are reported as toxic, environmental pollutants and carcinogens. Compared to recently published methods, the present method here offers several distinct advantages.

MATERIALS AND METHODS

Preparation of BPMT: The reagent was prepared by simple condensation of 2-benzoylpyridine (0.0155 mol, 2.5 ml) dissolved in 10 ml of methanol and 4-methyl, 3-thiosemicarbazide (0.0155 mol, 1.63 gram) dissolved in10 ml of hot distilled water. Suitable quantity (~2 ml) of glacial acetic acid was added and heated under reflux with stirring for 5 hours. Shiny deep yellow crystals were separated out on cooling the reaction mixture. It was collected by filtration and washed with hot water and with 1: 1 cold methanol. The compound was recrystallized from methanol and dried in vacuum. Yield is 92 percent, M.P. 145– 147^0 C.

The reagent solution (0.01M) was prepared by dissolving 67 mg of the compound in 25 ml of dimethylformamide (DMF) and it is stable for more than 12 hours.

Hydrochloric acid (1M)-sodium acetate (1M) (pH0.5-3.5); 0.2M sodium acetate – 0.2M acetic acid (pH 4.0-6.5) and 0.2M ammonium chloride-0.2M ammonium solutions (7.5-10.5) were used in the present study.

A known amount of cadmium acetate is dissolved in water and then diluted to 100 ml with distilled water. The stock solution is standardized by EDTA titration[20] using xylenol orange as an indicator. Further, required dilute solutions are prepared by diluting the stock solution suitably with distilled water.

Recommended procedure

A known aliquot of the Cd (II) solution was taken in a 25- ml standard flask containing 10 ml of buffer solution of pH 8.0 and 1.5ml 1 x 10^{-2}M reagent solution. The contents are made up

to the mark with distilled water. The absorbance of the solution was measured at 400 nm against the reagent (BPMT) blank. The absorbance values were referred to the predetermined calibration plot and computed the amount of cadmium in sample solutions.

Shimadzu 160A UV visible spectrophotometer equipped with 1.0 cm (path length) quartz cells, A Hitachi model 170-30 atomic absorption spectrophotometer and Elico Model LI-120 digital pH meter were used in present study. A Hitachi model 170-30 atomic absorption spectrophotometer is used for comparison of results.

Characterization of BPMT

The compound was chararacterized by IR, NMR, Mass and UV spectral analysis.

The reagent, 2- benzoylpyridine 4-methyl, 3-thiosemicarbazon (BPMT) was easily prepared. The compound was characterized by IR, NMR and mass spectral data. Infrared spectrum of BPMT shows bands (cm^1) at 3296(s), 3056(m), 2936(m), 1585(s), and 1045(s) respectively assigned to υ(NH) secondary, υ(C-H) aromatic stretch (sp^2 C–H), υ (C-H) aliphatic stretching, υ(C=N) Schiff's base and υ(C=S) vibrations respectively. ^{1}H-NMR spectrum of BPMT ($CDCl_3$+DMSO-d_6) showed signals (δ ppm) at 3.23 (3H, s), 8.90 (1H, s), 13.72 (1H, s) and 7.3-7.8 (9H, m) due to -CH_3, -NH, >NH– (imino) and aromatic/ and pyridine protons of BPMT respectively. Mass spectrum shows molecular ion peak at m/z 271. Other peaks due to loss of methyl radical and NH-CH_3 radical are also observed in mass spectrum. Based on above spectral data, the structure of the reagent is given in Fig. 1.

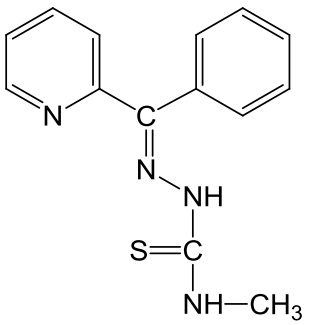
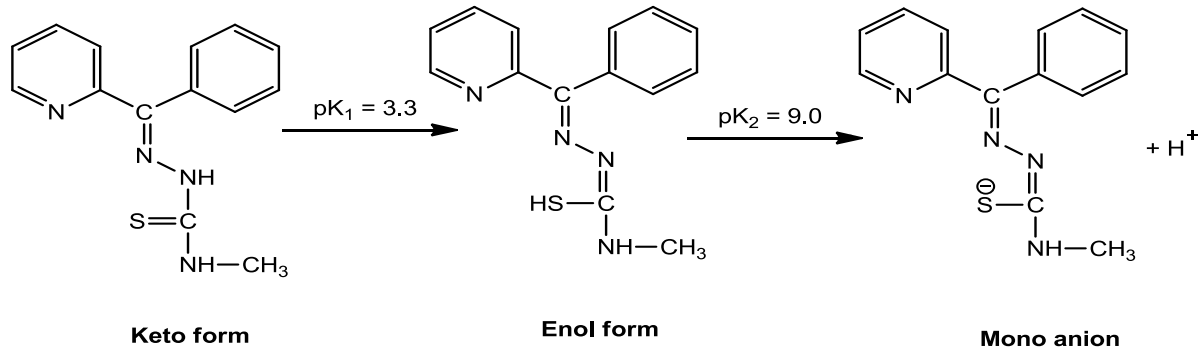


Fig. 1: Structure of 2- benzoylpyridine 4-methyl, 3-thiosemicarbazon (BPMT).

Absorption spectra of 2 × 10^{-5}M solution of BPMT at different pH values were recorded and pKa values were determined spectrophotometrically using Phillip and Merritt method.[21] The bathochromic shift from 280 to 350nm indicates that in solution on increasing pH the C=S

group of reagent (BPMT) is enolised and dissociated (Scheme.1). The values of deprotonation constants of BPMT are 3.3 (pK_1) and 9.0 (pK_2) respectively. The pK_1 and pK_2 values are presumably due to *thione-thiol tautomerism* and *deprotonation of thiol* (SH) group respectively.



Scheme. 1.

A 0.01M solution of reagent is stable for 12 h. The colour reactions of metal ions with the reagent are summarized in Table 1. At higher pHs (>4) the *ligand presumably exists in enolic* form.

Chromogenic Characteristics of BPMT

Metal ion	λ_{max}	$\varepsilon \times 10^4$ L.mol^{-1}cm^{-1}	pH range	Colour of the complex
Cu(II)	410	1.45	5.0 – 8.0	Yellow
Hg(II)	410	4.7	5.0 – 8.0	Greenish Yellow
Co(II)	380	1.04	5.0 – 8.0	Orange yellow
Ni(II)	390	2.0	6.0 – 8.0	Yellow
Zn(II)	390	3.02	5.0 – 8.0	Pale yellow
Cd(II)	400	1.6	7.0 – 10.0	Greenish Yellow
Pb(II)	390	2.9	5.0 – 7.0	Pale yellow

Determination of Cadmium in various Environmental, soil, biological, medicinal and leafy vegetable samples

The present methods are applied for the determination of Cd (II) in Environmental, and green leafy vegetable and biological samples.

Water samples

Each filtered (with whattman No. 40) water sample[22,26] (250 ml) was mixed with 10 ml of concentrated nitric acid in a 500 ml distillation flask. The sample was digested in the presence of an excess potassium permanganate solution according to the method

recommended by Fifield *et al.*,[23] the solution was cooled and neutralized with dilute NH_4OH solution. The digest was transferred into a 25-ml calibrated flask and diluted upto the mark with deionized water. The results were given in Table 2.

Soils samples

A 2 g weight of soil, 5 – 7 ml of concentrated H_2SO_4 and an excess of $KMnO_4$ are mixed in a conical flask equipped with a reflux condenser.[24,26] The crystals of $KMnO_4$ are added slowly in small portions, while stirring. It is heated until vapours of SO_3 are evolved. After cooling down, 10 ml of distilled water are added. The excess of $KMnO_4$ and manganese oxides are eliminated by adding H_2O_2. Iron is isolated by precipitation as hydroxide. After filtration, the solution is transferred into 25-ml standard flask and the volume is brought to the mark with distilled water. Aliquots of this solution were taken for analysis by following procedure given above. The results were given in Table 2.

Leafy Vegetable and biological samples

Dry ashing method was used in the analysis of organic samples. The leafy vegetables and medicinal leaves analyzed are procured from the city grocery stores. The samples[25,26] are cleaned and dried in open air, protecting them from the mineral contamination. The dried samples are pulverized to finely powdered particles in a mortar for the analysis of Cd (II).

A 10 g of dried leafy vegetable sample or liver sample was taken in a silica dish. The sample was heated over a low burner until the material chars. The charred mass was moistened with 1: 1 HNO_3. Occasionally a 20 percent solution of magnesium nitrate was used for this purpose, particularly if the ash content is very low. Again evaporated to dryness, and transferred to a muffle furnace. The temperature to about 500^0C was reached in the course of about 3 hours. Heating was continued until the ash becomes white. The dish was cooled and the ash was dissolved in a 5 ml portion of 1: 1 HCl. Distilled water was added amounting to about twice the volume of acid added. The solution was filtered to remove any insoluble residue and washed with 1: 4 HCl. The solution was diluted to 50-ml in a standard flask.[27] Aliquots of this sample were taken for the determination of Cadmium by following procedure given above. The results were given in Table 2.

Recommended procedure

A known aliquot of the sample solution was taken in a 25 ml standard flask containing buffer solution of pH 8.0, and reagent [BPMT; 1.0ml 1 x 10^{-2}M; Stock solutions] solution and made

up to the mark with distilled water. Absorbance of the solution was measured at λ_{max} 400 against the reagent blank. The absorbance values were referred to the predetermined calibration plot to compute the amount of Cadmium.

RESULTS AND DISCUSSIONS

The colour reaction of reagent with cadmium (II) is instantaneous at room temperature. The order of addition of reagent, metal ion, and buffer has no effect on the absorbance of the complex. Various physico-chemical and analytical characteristics of Cd-BPMT complex are summarized in Table.1. Stoichiometry of the complex (M: L=1:1) was determined by job's continuous variation method and molar ratio method (Fig.5). Ammonium chloride-ammonium hydroxide buffer (pH8.0, μ=0.2 and T=300K) and equimolar solution of cadmium (II) and reagent were used in these studies. The dissociation constant (α) and concentration (c) of the reagent at intersecting point (Fig.5) are used in the calculation of stability constant ($\beta = \frac{1-\alpha}{\alpha^2 C}$) of the complex. The stability constant of the complex is found to be 1.0×10^7

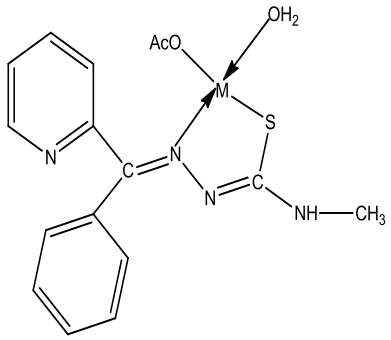


Fig. 2: The Structure of Cd-BPMT Complex.

M = Hg, Cu, Co, Cd

Optimum reaction conditions for the quantitative determination of the metal-ligand complex are established through a number of preliminary studies, such as the effect of the pH, choice of the solvent, reagent concentration and diverse ions, in order to develop a rapid, selective and sensitive non-extractive spectrophotometric method for the determination of cadmium (II) at micro gram levels.

Absorption Spectra of the Reagent and Cd (II) – BPMT Complex

The absorption spectra of Cd(II)- BPMT complex and the reagent show maximum absorbance at 400 nm Figure 3. The reagent showed a minimum absorbance at the wavelength of maximum absorbance of the complex. Hence, all the spectral measurements of the complex have been carried out at 400 nm.

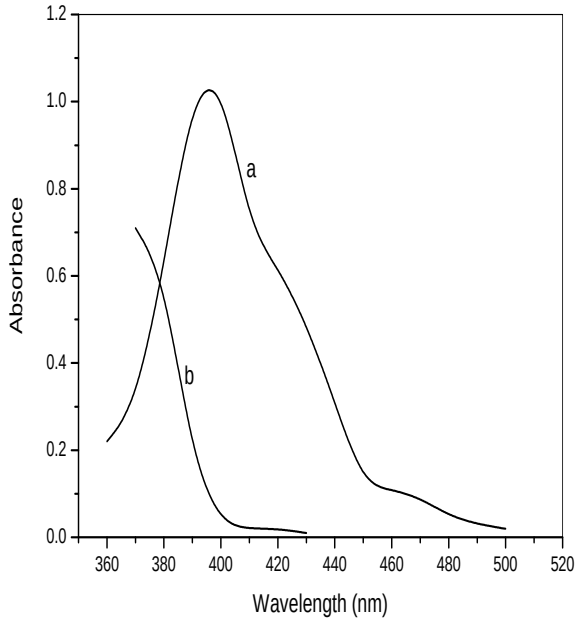


Fig. 3: Absorption spectra of

a) BPMT vs Water blank b) Cd(II) BPMT complex vs BPMT solution

[Cd(II)] = 4×10^{-5}M; [BPMT] = 4×10^{-4}M; pH =8.0

The study of the effect of pH on the color intensity of the reaction mixture showed that constant and maximum color is obtained in the pH range 7.0-10.0. The complex has maximum absorbance in buffer solution of pH (8.0). The analytical studies were therefore, carried out at pH 8.0.

Different molar excesses of BPMT are added to fixed metal ion concentrations and the absorbances were measured adopting the standard procedure. It is observed that a 10 fold molar excess of reagent with respect to metal is necessary to get maximum absorbance. Hence, a 10 fold molar excess of the reagent was used in all experimental studies.

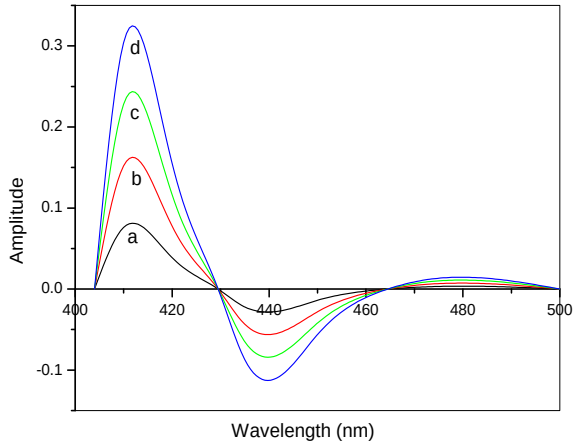


Fig. 4: Second derivative spectra of Cd(II) – BPMT *Vs* Reagent blank.

Cd(II) µg/ml a) 0.8086; b) 1.6047; c) 2.4070; d) 3.2093

[BPMT] = 4 x 10^{-4}M; pH = 8.0

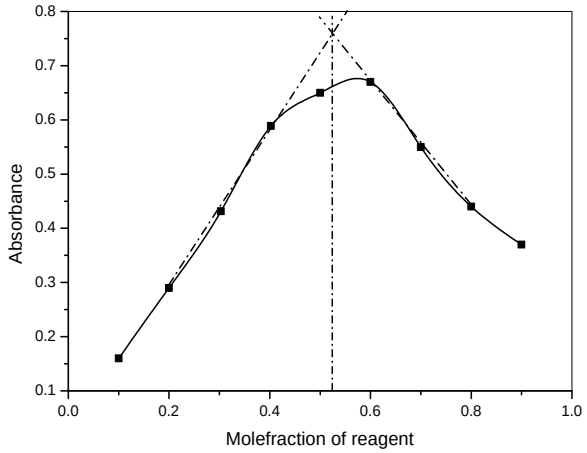


Fig. 5: Job's method of continuous variation.

Cd (II) – BPMT = 5 × 10^{-4}M (stock solution)

Wavelength = 400 nm; pH 8.0

The absorbance of the solution was measured at different time intervals to ascertain the time stability of the color complex. It is observed that the color development was instantaneous and remained constant for more than 12 hours. Physicochemical and analytical properties of cadmium (II) complex of BPMT are summarized in Table 1.

Table 1: Physico – chemical and analytical characteristics of Cd(II) – BPMT complex.

S. No.	Characteristics	Results
1	λ_{max} (nm)	400
2	Mean absorbance	0.232 ± 0.0006
3	pH range (optimum)	7.0 – 10.0
4	Mole of reagent required for mole of metal ion for full colour development	10 fold
5	Time stability of the complex (in hours)	12
6	Beer's law validity range (µg/ml)	1.798 – 17.98
7	Molar absorptivity (lit $mol^{-1}cm^{-1}$)	1.6×10^4
8	Specific absorptivity ml $g^{-1}cm^{-1}$	0.145
9	Sandell's sensitivity µg of Hg(II) cm^{-2}	0.00702
10	Composition of the complex as obtained in Job's and molar ratio methods	1 : 1
11	Stability constant of the complex	1.0×10^7
12	Standard deviation(s) in the determination of 1.798 µg/ml of Cd (II) for ten determinations	0.0024
13	Relative Standard deviation (RSD), or coefficient of variation	0.21%

Adherence of the Cd (II) - BPMT Complex System to Beers law:

For the possible determination of cadmium (II) at micro levels, the absorbance of the solution containing different amounts of the metal ion is measured at 400 nm. The linear plot between the absorbance and the amount of cadmium (II) is drawn and the straight line obtained fits the equation A_{400}= 0.2189C - 0.0077. Further Beers law is obeyed in the range of 1.79 – 17.98 $\mu g ml^{-1}$. The molar absorptivity and Sandell's sensitivity are found to be $1.6x10^4$ $L.mol^{-1}cm^{-1}$ and 0.00702 $\mu g cm^{-2}$ respectively. The standard deviation of the method for ten determinations of 1.798 µg ml^{-1} is ± 0.0024.

To assess the precision and accuracy of the method, estimations were carried out for a set of five determinations of cadmium (II), under optimum conditions. The results show that standard deviation of the method is not more than 0.0024 and relative standard deviation is less than 0.21%. These results indicate that this method has good precision, besides being accurate.

Second order derivative Spectrophotometry:

Second order derivative spectra were recorded for the above solutions in Figure.4 with a scan speed of fast (nearly 2400 nm min^{-1}) slit width of 1 nm with nine degrees of freedom in the wavelength range 400-500 nm. The derivative amplitude was measured at wavelengths 411 nm and 440 nm and plotted against amount of Cd(II) to obtain the calibration plots.

The calibration graph follows the straight line equation Y = a. C + b; where the C is Concentration of the solution, Y is measured absorbance or peak or valley height and a & b are constants. By substituting the corresponding experimental data substituted in the above equation, the calibration equations were calculated as A_{400} = 0.2189C-0.0077 for zero order method, A_{411} = 0.0097C -0.0005 and A_{440} = 0.0028C - 0.001 for second derivative method.

Effect of foreign ions on the extraction of the Cd (II) - BPMT complex

The effect of various diverse ions which often accompany cadmium has been studied by measuring the absorbance of the reaction mixture containing 1.798 μgml^{-1} of cadmium (II) in the presence of different amounts of foreign ions. An error of ± 2 percent in the absorbance reading is considered tolerable. The colour developed as described in the recommended procedure. However, in the presence of 300 µg of iodide, Hg (II) does not interfere even in 100 fold excess. Co (II) is tolerable up to 90 fold excess in the presence 200 µg of Thiocyanate.

Applications

The present method was applied for the determination of cadmium (II) when present alone and present in various water, soil, biological and leafy vegetable samples and the results were comparable with those obtained by atomic absorption spectrometric method. The data obtained in the analysis are given in Table.2.

Table 2: Determination of Trace amounts Cadmium (II) in environmental, soil, Biological, medicinal and green leafy vegetable samples.

Normal range of Cd in soil (ppm) (Source: Kabata and Pendians (1984)	Cd - 0.01-7.0
Critical levels (mg L^{-1}) of Cd in Irrigation water (Source: FAO (1979)	Cd - 0.01

Name of the samples	**Amount of cadmium[a] found (µg/L) or (µg/Kg)**	
	BPMT method	**AAS method**
Effluent water[b]	4.82	4.76
Waste water[c]	2.81	2.76
River Water[d]	2.00	1.94
Fish liver	1.45	1.23
Sheep liver	1.04	0.98
Urban soile[e]	1.49	1.47
Road side soil[e]	2.34	2.33
Radish (Raphanus setaivus)	1.75	1.79

Vepaku (Azadirachta indica)	0.352	0.338
Tutikura (Ipomoea Reptams	0.342	0.330
Chukkaku (Rumex vesicarius)	0.998	1.012
Thotakura(Amaranths Gangeticus)	0.125	0.128
Cauliflower green (Brassica Deraceavar, botntis)	0.183	0.180
Bangi (Tagetes erecta willd)	0.121	0.122
Cigarette Tobacco (Nicotiana Tabacum)	19.68	19.92

[a] Average of three determinations.

[b] Laboratory effluent water, (Dept. of Chemistry, S.K.U)

[c] waste water,(Anantpur Town drain water)

[d] River Water,(Tungabhadra river water, Kurnool)

[e] Urban Soil, (Anantapur Town)

[f] Road side soil (Anantapur town)

[g]Vegetables grown at waste water/laboratory effluent area, SKU, Anantapur and rural areas)

CONCLUSION

The literature available indicates that a few thiosemicarbozones have been used for the direct spectrophotometric determination of metal ions (Hg, Cd etc.,) but substituted thiosemicarbazones (especially BPMT) have not been exploited in the spectrophotometric determination of metal ions. The cadmium content can be determined in ppm level in the samples using the present method.

Green leafy vegetables in general and Crops members of the family *Solanaceae*, tomato (*Licopersicon esculentum* L.), eggplant (*Solanum melongena* L.) and pepper (*Capsicum annum L.*), in particular, have got accumulated the cadmium in roots, stems, leaves, seeds, flowers etc., when grown in industrially polluted region/drain water/laboratory waste water. So care must be taken. Smoking leads to highest amount of Cd accumulation in the body. All these findings may play a vital role in providing the awareness among the public.

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