# A Highly Selective and Sensitive Spectrophotometric Method for the Determination of Lead at Ultra-trace Levels in some Real, Environmental, Biological, Food and Soil Samples Using 5,7-Dibromo-8-Hydroxyquinoline

**M. Jamaluddin Ahmed[1*], M. Tazul Islam[2] and Sumaira Aziz[3]**
***Laboratory of Analytical Chemistry***
***Department of Chemistry, University of Chittagong, Chittagong-4331, Bangladesh.***
***E-mail: pmjahmed55@gmail.com***
tazul78j@gmail.com

UNDER PEER REVIEW

**Abstract**

A very simple, ultra-sensitive and highly selective non-extractive spectrophotometric method for the determination of trace amounts of lead using 5,7-dibromo-8-hydroxyquinoline (DBHQ) has been developed. DBHQ reacts in a slightly acidic (0.0006-0.0025 M HCl) aqueous solution with lead (II) in 30% ethanolic media to produce highly absorbent a greenish-yellow chelate which has an absorption maximum at 390 nm. The reaction is instantaneous and the absorbance remains stable for over 24 h. The average molar absorption co-efficient and Sandal's sensitivity were found to be 6.16 x $10^5$ L $mol^{-1}cm^{-1}$ and 5 ng $cm^{-2}$ of lead (II), respectively. Linear calibration graphs were obtained for 0.01- 60.0 mg $L^{-1}$ of lead (II) having detection limit of 1.0 µg $L^{-1}$ and RSD 0-2%. The stoichiometric composition of the chelate is 1:2 (Pb : DBHQ). A large excess of over 60 cations, anions and complexing agents (like, chloride, phosphate, azide, tartrate, oxalate, $SCN^-$ etc.) do not interfere in the determination. The developed method was successfully used in the determination of lead levels in several Standard Reference Materials (alloys , steels, natural water, bovine liver, human urine and hair) as well as in some environmental waters (potable and polluted), biological samples (human blood, urine and hair), soil samples, food samples (vegetables, rice, wheat) solutions containing both lead (II) and lead (IV) and complex synthetic mixtures. The results of biological and food analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS.

**Keywords:** *Spectrophotometry; lead determination; 5,7-dibromo-8-hydroxyquinoline; alloys, steels, environmental, biological, soil, food samples;*

## Introduction

Lead in trace amounts is important industrially[1], as a: toxicant[2], biological nutrient[3], environmental pollutant[4] and occupational hazards[5]. Lead[6] is a cumulative body poison[7] that enters the body from lead water pipes, lead-based paints and leaded petrol. Presence of even traces of Pb(II) in environmental samples leads to environmental pollution and many fatal diseases including dysfunction of renal blood and neurological systems. Pb(II) easily deposits in blood, kidney, reproductive system, nervous system and brain, and acute lead poisoning can result in colic shock, severe anemia and irreversible brain damage. Lead compounds is used as anti-knocking agents in automobile fuels cause air pollution. The toxicity[8] of lead has been studied extensively[9]. Inorganic lead ($Pb^{2+}$) binds itself with the -SH group in enzymes or proteins and acts as an enzyme inhibitor. Lead interferes with the calcium metabolism and gets deposited in the bones. Organic lead compounds, such as tetramethyl lead, are highly poisonous because they are absorbed readily by the body through skin and mucus membranes. Acute lead poisoning in humans causes severe damage in the kidneys, liver, brain, reproductive system, and central nervous system, and sometimes causes death. Mild lead poisoning causes anemia, headache, and sore muscles and the victim may feel fatigued and irritable. All these findings cause great concern regarding public health, demanding accurate determination of this metal ion at trace and ultra-trace levels.

Lead[10] in environmental and biological samples has been determined by NAA[11], inductively coupled plasma – atomic[12] emission spectrometry(ICP-AES)[13], inductively coupled plasma - mass spectrometry(ICP-MS)[14], anodic stripping voltammetry[15] and reversed – phase high performance liquid chromatography coupled with UV-Vis or fluorescence detection[16], AAS[17] and spectrophotometry[18-65]. The first four methods have disadvantages[66] in terms of cost and instrument used in routine analysis. AAS is often lacking in sensitivity and affected by matrix condition of samples such as salinity. Spectrophotometry[67] is a relatively sensitive method for lead (II) as it is of low cost, simple and within the reach of even ordinary laboratories, which is based on reaction between lead and chromogenic reagents. For this reason there is an ongoing search for new chromogenic reagents for direct and rapid spectrophotometric estimation lead at trace levels, especially in aqueous solution. Spectrophotometry[68] is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis.

The aim[69] of this study is to develop a simpler direct spectrophotometric method for ultra-

UNDER PEER REVIEW

trace determination of lead. 5, 7-dibromo-8-hydroxyquinoline (DBHQ) has been reported as a spectrophotometric reagents and forms colored water – soluble complexes with vanadium[70] and cadmium[71] and has not previously been used for spectrophotometric determination of lead. This paper reports its use in a very sensitive, highly specific spectrophotometric method for the ultra-trace determination of lead. The method possesses distinct advantages over existing methods[18–65]with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal stability, accuracy, precision, and ease of operation. The method is based on the reaction of non-absorbent DBHQ in slightly acidic solution (0.0006-0.0025 M HCl) with lead(II) to produce a highly absorbent greenish-yellow chelate product, followed by direct measurement of the absorbance in aqueous solution. With suitable masking, the reaction can be made highly selective and the reagent blank solutions do not show any absorbance.

## Experimental Section

### *Apparatus*

A Shimadzu (Kyoto, Japan) (Model-1800) double-beam UV/VIS spectrophotometer and a Jenway (England, UK) (Model-3010) pH meter with combination of electrodes were used[72] for measurements of the absorbance and pH, respectively. The calibration and linearity of the instrument were frequently checked with standard quinine sulphate (100-$\mu gL^{-1}$). A Thermo Fisher[73] Scientific (Model-iCE 3000, origin USA) atomic absorption spectrophotometer equipped with a microcomputer-controlled nitrous oxide-acetylene flame was used to compare of the results[74]. Infrared spectrum was recorded with FTIR Spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 $cm^{-1}$.

### *Reagent and solutions*

All of the chemicals used were of analytical reagent grade or the highest purity available. Doubly distilled de-ionized water, HPLC-grade ethanol which is non-absorbent under ultraviolet radiation, were used throughout. High- purity water was obtained by passing tap water through cellulose absorbent and to mixed bed ion exchange columns, followed by distillation in a corning AG-II unite. Glass vessels were cleaned by soaking in acidified solution of $KMnO_4$ or $K_2Cr_2O_7$ followed by washing with concentrated $HNO_3$ and rinsed several times with deionized water. Stock solutions and environmental water samples (1000-mL each) were kept in polypropylene bottles containing 1-mL of concentrated $HNO_3$. More

UNDER PEER REVIEW

rigorous contamination control was used when the lead levels in the specimens were low.

***DBHQ solution ( 3.3 ×10$^{-3}$ M)***

A 25-mL amount of stock solution was prepared by dissolving the requisite amount (0.025g) of DBHQ (Merck, Darmstadt, Germany, proanalysis grade 99%) in a known volume solution of distilled ethanol. More dilute solutions of the reagent were prepared as requited as when required. A freshly prepared reagent[75] (DBHQ) solution ($10^{-4}$M) was used whenever as required.

***Purity Test***

The purity of DBHQ was tested by taking[76] the melting point, an FTIR spectrum and thermogravimetric analysis. The melting of the reagent (DBHQ) was (200± 2)ºC ( Lit. 198º-200ºC)[77]. The FTIR spectrum of the reagent (DBHQ) shows a peak at 1581.70 cm$^{-1}$ due the characteristic C=N double bond peak (C=N,1580-1660 cm$^{-1}$)[78] ,the peak at 1607.74 cm$^{-1}$ due to the characteristics C=O double bond peak (C=O, 1600-1735 cm$^{-1}$), the presence of a peak at 1568.19 cm$^{-1}$ due to the characteristic C==C double bond peak (C=C,1560-1620)[78] of DBHQ.

Both melting point and FTIR spectral analysis[79] data indicated the purity of the reagent DBHQ. The steadiness of the thermogravimetric curve obtained for about 1g of the reagent at 80-90ºC, which indicates that the reagent did not contain any moisture.

***Lead (II) standard[8] solution (4.83×10$^{-3}$ M)***

A 100-mL amount of stock solution (1mg mL$^{-1}$) of divalent lead was prepared by dissolving 159.9 mg of lead nitrate {$Pb(NO_3)_2$}(Merck, proanalysis grade, 99.8%) in doubly distilled de-ionized water. One ml of dilute nitric acid was added to the stock solution to prevent hydrolysis. Aliquots of this solution were standardized with EDTA titration using xylenol orange as indicator[80]. Working standard solution was prepared by suitable dilutions of the stock solution as when required.

***Lead (IV) standard solution (4.83×10$^{-3}$ M)***

A 100-mL volume of lead(IV) stock solution (1mg mL$^{-1}$) was prepared by dissolving 110.3 mg of purified grade lead(II) oxide (The British Drag House Ltd. England) in de-ionized water containing 1-2-mL of hydrochloric acid (1+1). The working standard was prepared by approximate dilution of this stock solution.

UNDER PEER REVIEW

*EDTA solution*

A 100-mL stock solution of EDTA (0.01%) was prepared by dissolving 10 mg of A.C.S. grade (≥90%) ethylenediaminetetraacetic acid, disodium salt dehydrate in (100-mL) de-ionized water.

***Tartrate solution***

A 100-mL stock solution of tartrate (0.01%) was prepared by dissolving 10 mg of A.C.S. grade (99%) potassium sodium tartrate tetrahydrate in (100-mL) deionized water.

***Dilute ammonium hydroxide solution***

A 100-mL solution of dilute ammonium hydroxide was prepared by diluting 10-mL concentrated $NH_4OH$ (28-30% A.C.S. grade) to 100-mL with de-ionized water. The solution was stored in a polypropylene bottle.

***Other solutions***

Solutions of a large number of inorganic ions and complexing agents were prepared from their Analytical grade or equivalent grade water soluble salts (or the oxides and carbonates in hydrochloric acid); those of niobium, tantalum, titanium, zirconium and hafnium were specially prepared from their corresponding oxides (Specupure, Johnson Matthey) according to the recommended procedures of Mukharji [81]. In the case of insoluble substances, special dissolution methods were adopted[82].

***General Procedure***

A volume of 0.1-1.0-mL of neutral aqueous solution containing 0.1-600 µg of lead in a 10-mL volumetric flask was mixed with a 1:50 to 1:200 fold molar excess (preferably 1-mL of 3.3 $x10^{-3}$ M) of 5,7-dibromo-8-hydroxyquinoline (DBHQ) reagent solution followed by the addition of 0.05 – 0.70-mL (preferably 0.5-mL) of 0.001M hydrochloric acid. The solution was mixed well. After 1 minute 3-mL of ethanol was added. The mixture was diluted up to the mark with de-ionized water. The absorbance was measured at 390 nm against a corresponding reagent blank. The lead content in an unknown sample was determined using a concurrently prepared calibration graph.

***Sample collection and preservation***

**Water:** Water samples were collected in polythene bottles from shallow tube-wells, tap-wells, river, sea and drain of different places of Chittagong region, Bangladesh. After collection, $HNO_3$ (1 mL $L^{-1}$) was added as preservative.

UNDER PEER REVIEW

**Blood and Urine:** Blood and urine samples were collected in polypropylene bottles from effected persons of CSCR Hospital & Chittagong Medical College Hospital, Bangladesh. Immediately after collection they were stored in a salt-ice mixture and latter, at the laboratory, were kept at-20$^0$C.

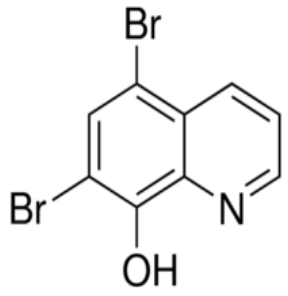
**Soil:** Soil[73] (surface) samples were collected from different locations in Chittagong region, Bangladesh. Samples were dried in air and homogenized with a mortar.

**Food samples:** Food samples (rice, wheat, and vegetables) were collected from local market of Chittagong. After collection the samples (vegetables) were stored in refrigerator for preservation. Samples (rice, wheat,) were used as dry condition and homogenized with a mortar.

## Results and Discussion

### *Factors Affecting the Absorbance*

*Absorption spectra:* The absorption spectra of a lead-DBHQ system in aqueous medium in presence of 1-mL 0.001M hydrochloric acid[74] solution, was recorded using the spectrophotometer. The absorption spectra of the lead- DBHQ is a asymmetric curve with maximum absorbance at 390 nm and an average molar absorptivity of 6.16 x $10^5$ L $mol^{-1}cm^{-1}$ (**Fig. 2**). The reagent blank exhibited negligible absorbance despite having wavelength at 390 nm. The reaction mechanism of the present method is as reported earlier[83]. The structure of the reagent (DBHQ) is shown **Scheme 1.**




**Scheme-1**. The Structure of 5, 7-Dibromo-8-hydroxyquinoline ( DBHQ)

UNDER PEER REVIEW

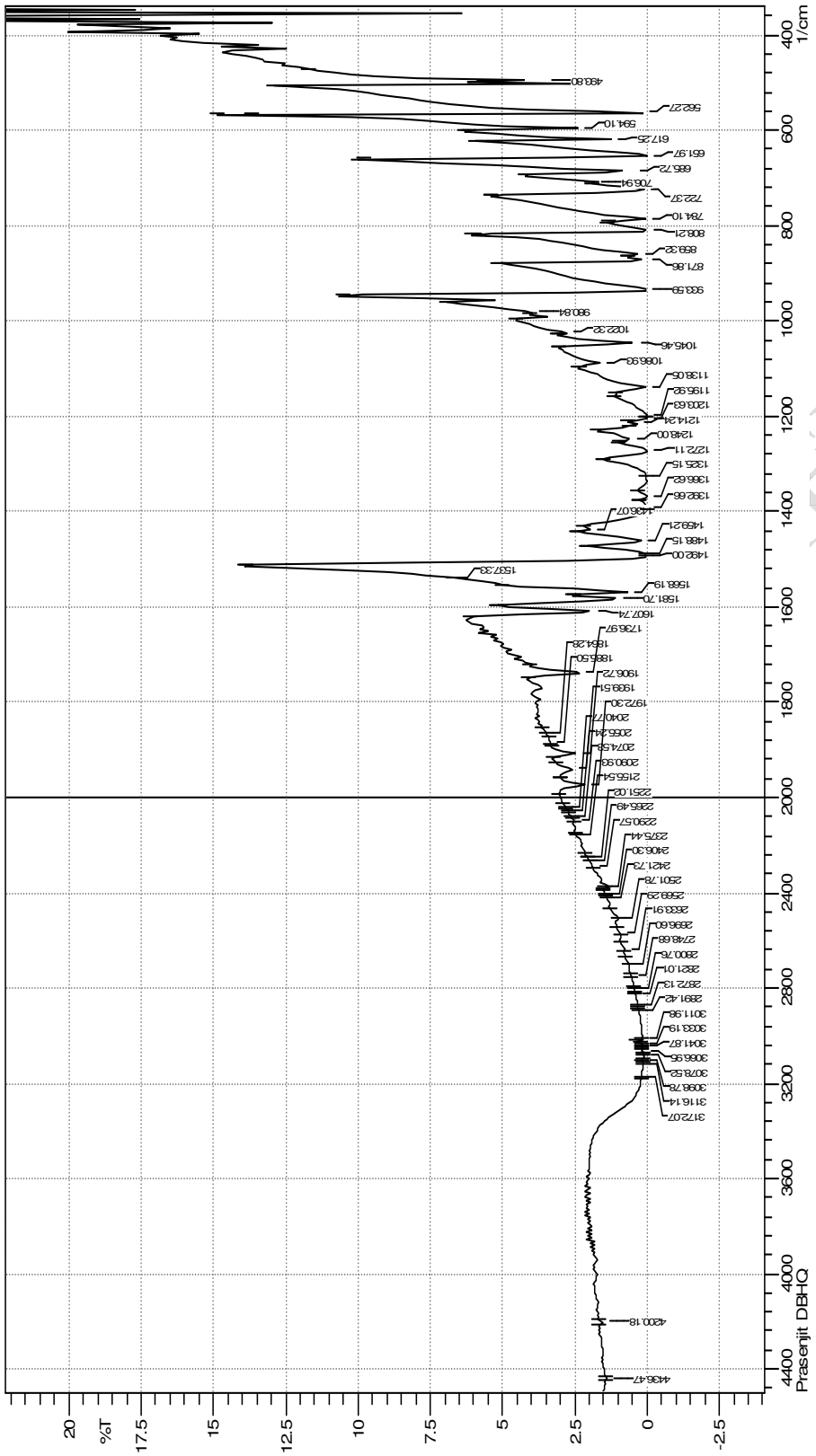



**Fig. 1.** FTIR Spectrum of 5,7-dibromo-8-hydroxyquinoline (DBHQ)

**Live subject statement**. We were not aiming to carry out detailed human studies but some samples from individuals were used in our study and as such we abided by all the necessary procedures and regulations and our University gave consent. University of Chittagong, Bangladesh is committed to the protection and safety of human subjects involved in research.

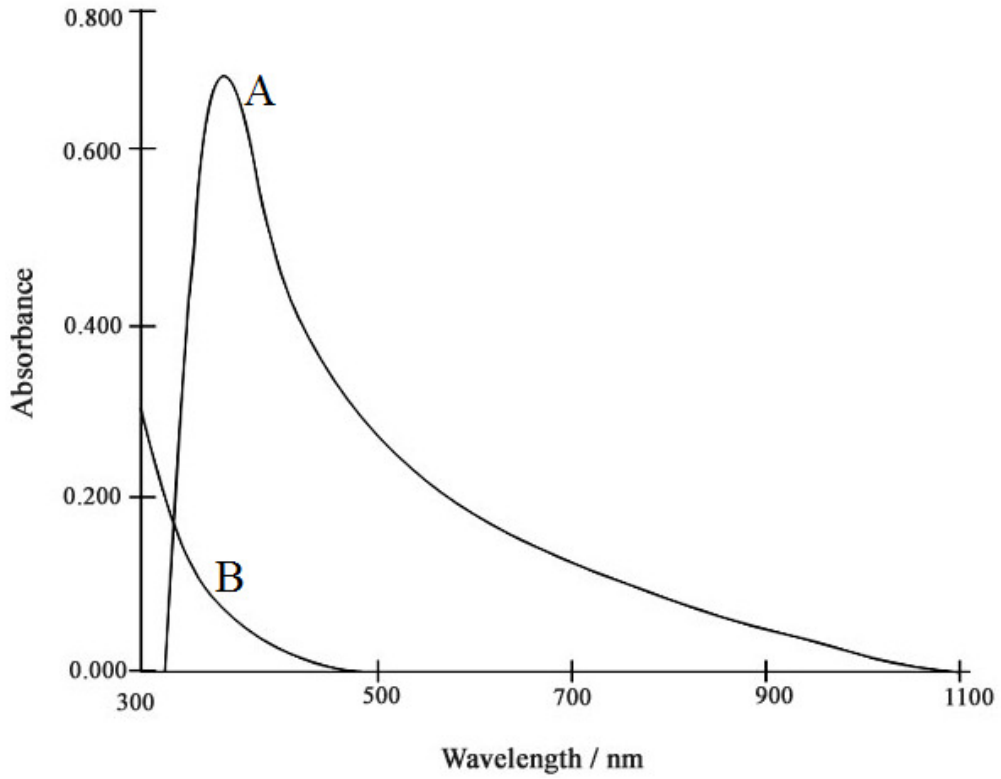



**Fig. 2.** A and B absorption spectra of $Pb^{II}$-DBHQ system and the reagent blank ( $\lambda_{max}$ =390 nm) in aqueous solutions, respectively.

***Optimization of some Parameters on the Absorbance***

***Effect of solvent***

Because DBHQ is partially soluble in water, an organic solvent was used for the system, consideration of cost, availability , toxicity and volatility of the solvent etc. Of the various solvents (acetone, benzene, carbon tetrachloride, chloroform, ethanol, 1-butanol, isobutyl methyl ketone, N.N-dimethyl formamide (DMF), methanol[74] and 1,4-dioxane) studied, ethanol was found to be the best solvent for the system. Different volumes (0-7mL) of ethanol were added to fixed metal ion concentration and the absorbance were measured according to the general procedure. Maximum absorbance was observed in (30 ± 2%) (v/v) ethanol/water medium, hence, a 30% ethanol solution was used in the determination

procedure. It was observed that 20-70% (2-7mL) ethanol produced a constant absorbance of the Pb-chelate (**Fig. 3**). For all subsequent measurements, 30% (3-mL) of ethanol was added.

***Effect of acidity***

Of the various acids[74] (nitric, sulfuric, hydrochloric, percloric and phosphoric) studied, hydrochloric acid was found to be the best acid for the system. The variation of the absorbance was noted after the addition of 0.05-2.0-mL of 0.001 M hydrochloric acid to every 10-mL of test solution. The maximum and constant absorbance was obtained in the presence of 0.1-1.0-mL of 0.001M sulfuric acid at room temperature $(25\pm5)^0$C. Outside this range of acidity, the absorbance decreased (**Fig.4**). For all subsequent measurements 0.5-mL of 0.001 M hydrochloric acid was added.

***Effect of time***

The reaction[73] is instantaneous. The lead(II)-DBHQ system attained maximum and constant absorbance immediately(within 1 min) just after dilution of the solution to final volume and remained strictly constant for over 24 h.

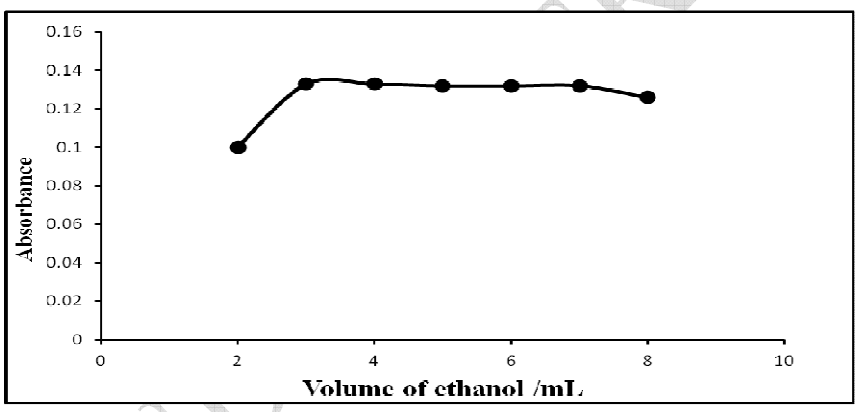



**Fig.3.** Effect of solvent (ethanol) on the absorbance of $Pb^{II}$-DBHQ system

UNDER REVIEW

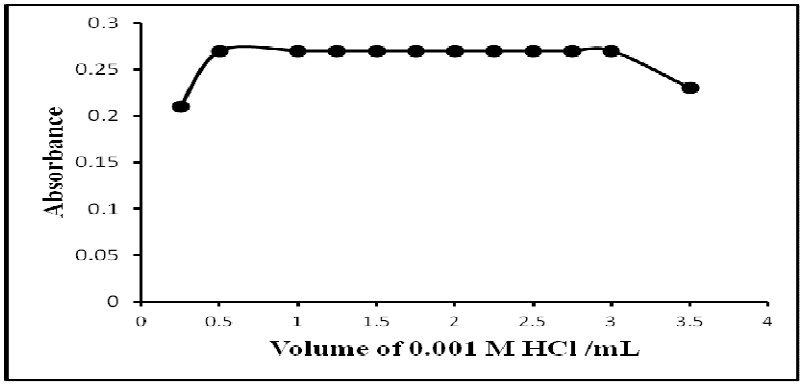



**Fig. 4.** Effect of acidity on the absorbance of $Pb^{II}$-DBHQ system

***Effect of temperature***

The Pb - DBHQ system attained maximum and constant absorbance at room temperature $(25\pm5)^0C$ . Outside this range of temperature, the absorbance decreased. All subsequent measurements were done at room temperature $(25\pm5)^0C$.

***Effect of reagent concentration***

Different molar excesses of DBHQ were added to a fixed metal ion concentration and the absorbance was measured according to the general procedure. It was observed that 1.0-$mgL^{-1}$ lead metal, the reagent molar ratios of 1:50 to 1:200 produced a constant absorbance of Pb-chelate (**Fig. 5**). For different (0.5 and 10.0 $mgL^{-1}$) lead concentrations an identical effect of varying the reagent concentration was noticed. A greater excess were not studied. For all subsequent measurements, 1 mL of $3.30 \times 10^{-3}$ M DBHQ reagent was added.

***Calibration graph (Beer's law and sensitivity)***

The well known equation for a spectrophotometric analysis in a very dilute solution was derived from Beer's law. The effect of the metal concentration was studied over 0.01-100 mg $L^{-1}$ distributed in four different sets (0.01 -0.1, 0.1-1.0, 1.0-10 and 10.0-100.0 mg $L^{-1}$) for convenience of the measurement. The absorbance was linear for 0.01-60.0 mg $L^{-1}$ at 390 nm. Of four calibration graphs, the one showing the limit of the linearity range **Fig.6;**the next three were straight-line graphs passing through the origin($R^2$=0.9986). The molar absorption co-efficient and the Sandal's sensitivity[84] were found to be 6.16 x $10^5$ L $mol^{-1}$ $cm^{-1}$ and 5 ng $cm^{-2}$ of lead, respectively. The selected analytical parameters obtained with the optimization experiments are summarized in **Table 1**.

UNDER PEER REVIEW

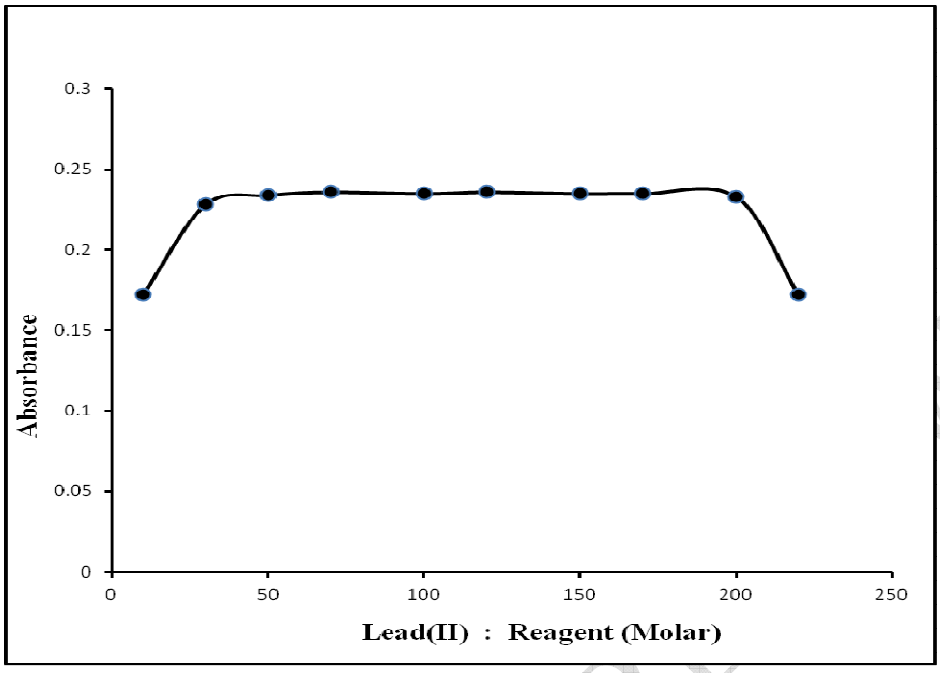



**Fig.5 .** Effect of reagent on the absorbance of $Pb^{II}$-DBHQ system

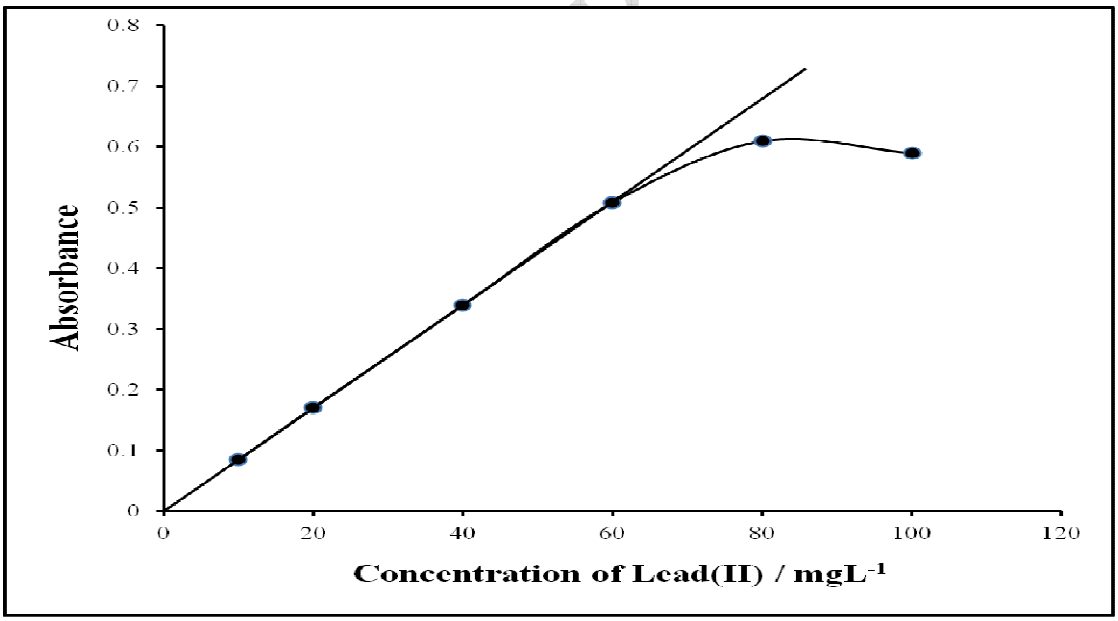



**Fig. 6.** Calibration graph : 10 – 60 $mgL^{-1}$ of lead(II)

Table 1. Selected analytical parameters obtained with the optimization experiments[85]

| Parameters | Studied range | Selected value |
|---|---|---|
| Wavelength / $\lambda_{max}$ (nm) | 200-800 | 390 |
| Solvent / % (Ethanol) | 10-80 | 20-70 (Preferably 30) |
| Acidity / M HCl | 0.0001-0.05 | 0.0006-0.0025 (Preferably 0.001) |
| pH | 4-1.30 | 3.10-2.48 (Preferably 3) |
| Time / h | 0-72 | 1min-24 h (Preferably 1 min) |
| Temperature / °C | 10-80 | 20-70 (Preferably 25±5) |
| Reagent (fold molar excess, M:R) | 1:1 - 1:500 | 1:50 - 1:200 (Preferably 1: 100) |
| Linear range/mg $L^{-1}$ | 0.001-100 | 0.01-60.0 |
| Molar absorption coefficient / L $mol^{-1}$ $cm^{-1}$ | $5.0 \times 10^5$ –$7.32 \times 10^5$ | $6.16 \times 10^5$ |
| Sandell's sensitivity/ng $cm^{-2}$ | 1-100 | 5.0 |
| Detection limit/ µg $L^{-1}$ | 0.01-10 | 1.0 |
| Reproducibility (% RSD) | 0-10 | 0-2 |
| Regression Co-efficient ($R^2$) | 0.9989-0.9998 | 0.9995 |
| Molar Ratio ( Pb : DBHQ) | 1 : 9 – 9 : 1 | 1 : 2 |

UNDER PEER REVIEW

*Effect of foreign ions*

The effect of over 60 anions, cations and complexing agents on the determination of only 1 mg $L^{-1}$ of lead was studied. The criterion for an interference[86] was an absorbance value varying by more than 5% from the expected value for lead alone. The results are summarized in **Table 2**. As can be seen, a large number of ions have no significant effect on the determination of lead. The interference were from V(V), Mo(VI) and Cd(II) ions. Interference from these ions is probably due to complex formation with DBHQ. The greater tolerance limits for these ions can be achieved by using several masking methods. In order to eliminate interference of V(V), Mo(VI) and Cd(II); EDTA and tartarate used as masking agent, respectively. During the interference studies, if a precipitate was formed, it was removed by centrifugation. The strong reducing agents such as tin(II), chloride, iron(II), sulfate, hydroxylamine hydrochloride and sodium azide, which would otherwise reduce lead(IV) had no reducing effect on lead(II). The amount mentioned is not the tolerance limit but the actual amount studied. However, for those ions whose tolerance limit has been studied, their tolerance ratios are mentioned in **Table 2**.

UNDER PEER REVIEW

**Table 2.** Table of tolerance limits of foreign ions[a], tolerance ratio [species(x) / Pb(II)(w/w)]

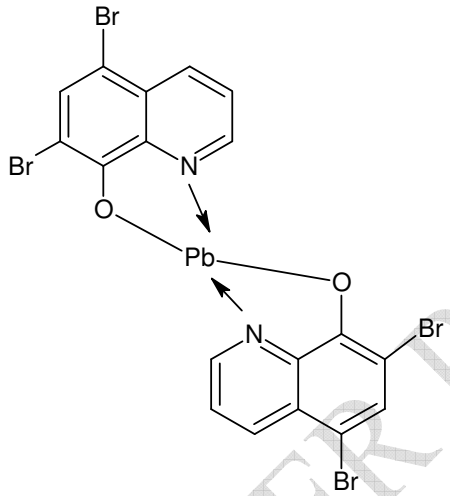
| Species x | Tolerance ratio $x/Pb^{II}$(w/w) | Species x | Tolerance ratio x/Pb (w/w) |
|---|---|---|---|
| Ammonium (I) | 50 | Lithium | 50[b] |
| Arsenic (III) | 50 | Lead (II) | 100 |
| Arsenic (V) | 50 | Magnesium | 100 |
| Aluminium | 100 | Manganese (II) | 100 |
| Azide | 100 | Manganese (VII) | 100 |
| Ascorbic acid | 100 | Mercury (II) | 100 |
| Antimony | 100 | Molybdenum (VI) | 50[b] |
| Barium | 100 | Nitrate | 100 |
| Bromide | 100 | Nickel | 50 |
| Bismuth (III) | 100 | Oxalate | 1000 |
| Beryllium (II) | 100 | Potassium | 50 |
| Calcium | 50 | Phosphate | 100 |
| Chloride | 100 | Selenium (VI) | 50 |
| Cobalt (II) | 100 | Selenium (IV) | 50 |
| Cobalt (III) | 100 | Silver | 100 |
| Chromium (III) | 100 | Sodium | 100 |
| Chromium (VI) | 50 | Strontium | 50 |
| Cadmium | 50[c] | Sulfate | 100 |
| Carbonate | 1000 | Titanium (IV) | 50 |
| Cerium(III) | 100 | Tellurium (IV) | 100 |
| Cerium(IV) | 100 | Tellurium (VI) | 100 |
| Cesium | 50 | Tartrate | 1000 |
| Citrate | 100 | Thiocyanate | 1000 |
| Copper (II) | 50 | Thiourea | 1000 |
| Cyanide | 100 | Tungsten (VI) | 100 |
| EDTA | 1000 | Tin (II) | 100 |
| Fluoride | 100 | Tin (IV) | 100 |
| Iodide | 100 | Vanadium (V) | 50[c] |
| Iron (II) | 100 | Zinc | 50[c] |
| Iron (III) | 100 | | |

[a] Tolerance limit was defined as ratio that causes less than ± 5 percent interference.
[b] with 10 mg $L^{-1}$ tartrate.
[c] with 10 mg $L^{-1}$ EDTA

UNDER PEER REVIEW

*Composition of the absorbent complex*

Job's method[87] of continuous variation method was applied to ascertain the stoichiometric composition of the complex under the optimum conditions (**Table 1**). A Pb - DBHQ (1:2) complex was indicated by this method. The molar- ratio method[88] was also applied to ascertain the stoichiometric composition of the complex. A Pb - DBHQ complex was indicated by both methods and the stoichiometry was also found to be 1:2 (Metal : Ligand).




**Scheme 2 .** Structure of [Pb(DBHQ)$_2$] complex

UNDER PEER REVIEW

***Precision and accuracy***

The precision of the present method was evaluated by determining different concentrations of lead (each analyzed at least five times). The relative standard deviation (n = 5) was 2-0% for 0.1-60 µg of lead in10-mL, indicating that this method is highly precise and reproducible. The detection limit (The detection limit (3s/S 's' is the standard deviation of the blank & 'S' is slope)) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for lead were found to be 1 µg $L^{-1}$ and 5 ng $cm^{-2}$, respectively. The method was also tested by analyzing several synthetic mixtures containing lead and diverse ions (**Table 3**).The results for total lead were in good agreement with certified values (**Table 4**). The reliability of our Pb-chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition of lead spike to some environmental water samples was quantitative as shown in (**Table 5**.) The results of biological and food analyses by the spectrophotometric method

were in excellent agreement with those obtained by AAS (**Table 6 & 8** ). The results of speciation of lead(II) and lead(IV) in mixtures are shown in **Table 9.** Hence, the precision and accuracy of the method were excellent. With suitable masking, the reaction can be made highly selective.

## *APPLICATIONS*

The proposed method[89] was successfully applied to the determination of lead in a series of synthetic mixtures of various compositions (**Table 3**) and also in a number of real samples e.g. several Certified Reference Materials (CRMs) (**Table 4**). The method was also extended to the determination of lead in a number of environmental, biological, food, vegetable and soil samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such samples were analyzed for lead content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in good agreement (**Table 5**). The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (**Table 6**). The results of soil samples analyzed by the spectrophotometric method are shown in **Table7**. The results of vegetable and food analyses by the spectrophotometric method were[90] found to be in excellent agreement with those obtained by AAS (**Table 8**). The results of speciation of lead(II) and lead(IV) in mixtures are shown in **Table 9**. The precision and accuracy of the method were excellent.

***Determination of lead in synthetic mixtures***

To test the validity of the proposed method several synthetic mixtures[91] of varying compositions containing lead and diverse ions of known concentrations were determined by the present method using tartrate or EDTA as masking agent and the results were found to be highly reproducible. The results are shown in **Table 3**. Accurate recoveries were achieved in all solutions.

**Table 3.** Determination of lead in some synthetic mixtures

| Sample | Composition of mixtures ($mg\ L^{-1}$) | Lead (II) / $mg\ L^{-1}$ | | |
|---|---|---|---|---|
| | | Added | Found[a] (n=5) | Recovery ± SD[b] (%) |

| | | | | |
|---|---|---|---|---|
| A | $Pb^{II}$ | 0.50<br>1.00 | 0.49<br>1.00 | 98 ± 0.3<br>100 ± 0.0 |
| B | As in A + Ag (25) + Cd (25) + K(25) + $Co^{2+}$ (25) | 0.50<br>1.00 | 0.50<br>1.02 | 100 ± 0.0<br>102 ± 0.5 |
| C | As in B + Na (25) + $Te^{IV}$ (25) + $Sn^{2+}$(25) + Mg(25) | 0.50<br>1.00 | 0.49<br>0.99 | 98 ± 0.6<br>99 ± 0.2 |
| D | As in C + Ca (25) + $Cr^{III}$ (25) + Ba(25) + $Hg^{2+}$ (25) + EDTA(50) | 0.50<br>1.00 | 0.52<br>1.04 | 104 ± 1.3<br>104 ± 1.5 |
| E | As in D + Sr (25)+ $Ce^{IV}$ (25) + $Mn^{2+}$ (25) + Sb(25) | 0.50<br>1.00 | 0.54<br>1.07 | 108 ± 1.6<br>107 ± 1.8 |

[a] Average of five analyses of each sample.
[b] The measure of precision is the standard deviation (s).

***Determination of lead in some Certified Reference Materials***

Certified Reference Materials, alloys, steels, brass and some synthetic compounds were analyzed to evaluate the validation of the method. A 0.1g amount of an alloy or steel or brass containing 0.23 – 2.25% of lead was accurately weighed and placed in a 50-mL Erlenmeyer flask in the presence excess reducing agent to reduce Pb(IV) to Pb(II), following a method recommended by Mitra[92]. To it, 10-mL of concentrated $HNO_3$ and 2-mL of concentrated $H_2SO_4$ were carefully added. The solution was heated and simmered gently after the addition of another 10-mL of concentrated $HNO_3$ until all carbides were decomposed. The solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen and then cooled to room temperature $(25\pm5)^0$C. After suitable dilution with de-ionized water, the contents of the Erlenmeyer flask were warmed to dissolve the soluble salts. The solution was then cooled and neutralized with a dilute $NH_4OH$ solution in the presence of 1-2-mL of 0.01%(w/v) tartrate solution. The resulting solution filtered, if necessary, through Whatman no. 40 filter paper into a 100-mL calibrated flask. The residue (silica and tungstic acid) was

UNDER PEER REVIEW

washed with a small volume of hot (1+99) $H_2SO_4$, followed by water; the volume was made up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the above solution was taken into a 10-mL calibrated flask and the lead content was determined as described under general Procedure using EDTA or tartrate as masking agent. The proposed method for the spectrophotometric determination of lead was applied to the analysis of natural water(NIST-SRM-1640a), bovine liver(NIST-SRM-1677c), human urine(NIST- SRM-2670a) and human hair(CRM-397) obtained from National Research Council of Canada using EDTA or tartrate as a masking agent, following a method recommended by Sun et al.[93]. Based on five replicate analyses, the average lead concentrations determined by spectrophotometric method were found to be in good agreement with the certified values. The results are shown in **Table 4.**

**Table 4.** Determination of lead in some Certified Reference Materials

| Sample | Certified reference materials (Composition, %) | Lead(%) | | |
|---|---|---|---|---|
| | | In C.R.M sample | Found (n=5) | RSD[a] (%) |
| 1 | BAS-CRM -5g, Brass (Cu=67.4, Sn=1.09, Pb=2.23, Zn=28.6, Ni=0.33, P=0.01) | 2.23 | 2.25 | 1.5 |
| 2 | BAS-CRM-10g, High-tensile Brass: (Cu=60.8, Fe=1.56, Pb=0.23, Ni=0.16, Sn=0.21, Zn=32.0,Al=3.34 Mn=0.12, ) | 0.23 | 0.22 | 1.0 |
| 3 | BAS-Brass-5f: (Cu=70.8, Fe=0.31, Pb=2.52, Ni=0.17, Sn=1.84, Zn=24.2, Mn=0.12, P=0.06) | 2.25 | 2.30 | 1.8 |
| 4 | NIST-SRM-$1640_a$ :Natural water[b] | 12.101±0.05 | 12.05±0.06 | 2.0 |
| 5 | NIST-SRM[R] -$1677_c$: Bovine liver[b] | 62.8±1.0 | 62.5±1.2 | 2.5 |

UNDER PEER REVIEW

| | | | | |
|---|---|---|---|---|
| 6 | NIST-SRM$^{R}$ -2670$_{a}$ :Urine(Freez-dried)$^{c}$ | 125.0±4.3 | 123.0±3.5 | 2.8 |
| 7 | CRM-397 :Human hair $^{d}$ | 33.0±1.2 | 32.8±1.5 | 2.6 |

[a] The measure of precision is the relative standard deviation (RSD).
[b] Values in µg $kg^{-1}$
[c] Values in µg $L^{-1}$
[d] Values in µg $g^{-1}$

***Determination of lead in some environmental water samples***

Each filtered (with Whatman No. 40) environmental water sample (1000 mL) was evaporated nearly to dryness with a mixture of 3-mL concentrated $H_2SO_4$ and 10-mL of concentrated $HNO_3$ in a fume cupboard in presence fresh excess sodium azide solution(2.5%), following a method recommended by Greenberg *et al.*[94] and was cooled to room temperature. The residue was heated with 10-mL of de-ionized water in order to dissolves the salts. The solution was then cooled and neutralized with dilute $NH_4OH$ solution in the presence of a 1–2-mL of 0.01 % (w/v) tartrate or EDTA solution. The resulting solution was then filtered (if necessary) and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water.

An aliquot (1-2-mL) of this preconcentrated water sample was pipetted into a 10-mL calibrated flask and the lead content was determined as described under the Procedure, using tartrate or EDTA as a masking agent. The analyses of environmental water samples for lead from various sources are shown in **Table 5**.

Most spectrophotometric methods for the determination of lead in natural and sea-water require preconcentration of lead[94]. The concentration of lead in natural and sea-water is a few µg$L^{-1}$ in Japan[6]. The mean concentration of lead in US drinking water is 5 µg$L^{-1}$ [94].

**Table 5. Determination of lead in some environmental water samples**

| Sample | Lead (II) / µg $L^{-1}$ | Recovery ± s (%) | $s_r^b$ (%) |
|---|---|---|---|

UNDER PEER REVIEW

| | | Added | Found [a] (n=5) | | |
|---|---|---|---|---|---|
| **Tap water** | | 0<br>100<br>500 | 25.0<br>119.0<br>525.0 | <br>99±0.5<br>100±0.0 | <br>0.35<br>0.00 |
| **Well water** | | 0<br>100<br>500 | 16.0<br>115.0<br>517.0 | <br>99.5±0.4<br>100.0±0.3 | <br>0.27<br>0.17 |
| **Rain water** | | 0<br>100<br>500 | 10.0<br>108.0<br>510.0 | <br>98.18±1.0<br>100.0±0.0 | <br>0.24<br>0.00 |
| **River water** | Karnaphully (upper) | 0<br>100<br>500 | 25.0<br>123.0<br>524.0 | <br>98.4±1.0<br>99.8±0.5 | <br>0.25<br>0.23 |
| | Karnaphully (lower) | 0<br>100<br>500 | 30.0<br>131.0<br>530.0 | <br>100.0±0.03<br>100.0±0.0 | <br>0.15<br>0.00 |
| | Halda (upper) | 0<br>100<br>500 | 20.0<br>121.0<br>519.0 | <br>100.8±0.5<br>99.8±0.7 | <br>0.26<br>0.27 |
| | Halda (lower) | 0<br>100<br>500 | 22.0<br>120.0<br>524.0 | <br>98.0±1.0<br>104.0±0.6 | <br>0.25<br>0.23 |
| **Sea water** | Bay of Bengal (upper) | 0<br>100<br>500 | 15.0<br>116.0<br>514.0 | <br>100.8±1.0<br>99.8±0.7 | <br>0.42<br>0.26 |
| | Bay of Bengal (lower) | 0<br>100<br>500 | 13.0<br>114.0<br>512.0 | <br>100.8±0.8<br>99.8±0.5 | <br>0.09<br>0.10 |
| **Drain water** | T. S. P. Complex [c] | 0<br>100<br>500 | 65.0<br>165.0<br>568.0 | <br>100.0±0.0<br>99.5±0.7 | <br>0.00<br>0.21 |
| | PHP[d] | 0<br>100<br>500 | 125.0<br>230.0<br>635.0 | <br>102.0±0.8<br>101.6±0.9 | <br>0.28<br>0.26 |
| | BSRM [e] | 0<br>100<br>500 | 95.0<br>198.0<br>590.0 | <br>98.4±0.5<br>101.0±0.4 | <br>0.21<br>0.29 |
| | K.P.M Water[f] | 0<br>100<br>500 | 120.0<br>225.0<br>620.0 | <br>97.8±0.9<br>100.0±0.0 | <br>0.37<br>0.00 |
| | Eastern Refinery [g] | 0<br>100<br>500 | 150.0<br>255.0<br>660.0 | <br>98.0±0.5<br>98.5±0.8 | <br>0.35<br>0.48 |

[a] Average of five replicate determinations of each sample .
[b] The measure precision is the relative standard deviation($s_r$).
[c] T. S. P. Complex Ltd., Patenga, Chittagong.
[d] PHP glass factory, Kumira, Chittagong.
[e] Bangladesh Steel Re-rolling Mills Ltd.(BSRM) , Baizid Bosthami, Chittagong.
[f] Karnaphuly Paper Mills, Chandraghona, Chittagong.
[g] Eastern Refinery, North Patenga, Chittagong.

***Determination of lead in some biological samples***

UNDER PEER REVIEW

Human blood (2-5-mL) or urine (20-50-mL) was collected in polyethane bottles from the affected persons. Immediately after collection, they were stored in a salt-ice mixture and later, at the laboratory, were kept at -20°C. The samples were taken into a 100-mL micro-Kjeldahl flask. A glass bead and 10-mL of concentrated nitric acid were added, and the flask was placed on the digester under gentle heating. The sample was digested in presence of excess reducing agent according to the method recommended by Stahr[95]**.** When the initial brisk reaction was over, the solution was removed and cooled at room temperature. 2-mL volume of concentrated sulfuric acid was added carefully, followed by the addition of 2-mL of concentrated HF and heating was continued to dense white fumes, repeating nitric acid addition if necessary. Heating was continued for at least ½ hr and then cooled. The content of the flask was filtered then neutralized with dilute $NH_4OH$ solution in the presence of 1-2-mL of a 0.01 % (w/v) tartrate or EDTA solution. The resultant solution was then transferred quantitatively into a 10-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1-2-mL) of the final solution was pipetted into a 10-mL calibrated flask and the lead content was determined as described under the Procedure using tartrate or EDTA as masking agent. The results of biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The results are shown in **Table 6**.

The abnormally high values for the lung cancer and convulsion patients are probably due to the involvement of high lead concentration with As and Zn. The occurrence of such high lead content are also reported in lung cancer and convulsion patients from developed countries[96]**.** The abnormally high value for the lung cancer patient is also probably due to high lead concentrations in air due to use of tetramethyl lead in gassoline[97].

UNDER PEER REVIEW

Table 6. Determination results of lead for human fluids and hair samples

| **Serial No.** | **Sample** | **Lead / µg $L^{-1}$** | | | | **Sample Source[a]** |
|---|---|---|---|---|---|---|
| | | **AAS (n=5)** | | **Proposed method (n = 5)** | | |
| | | **Found** | **RSD[b] (%)** | **Found** | **RSD[b] (%)** | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | Blood<br>Urine | 252.0<br>72.8 | 2.0<br>1.2 | 258.0<br>75.5 | 2.0<br>1.1 | Convulsion patient(Male) |
| 2 | Blood<br>Urine | 210.5<br>55.6 | 2.3<br>1.3 | 215.8<br>58.5 | 1.8<br>1.2 | Hemolytic anemia patient(Female) |
| 3 | Blood<br>Urine | 373.5<br>94.8 | 2.2<br>1.0 | 381.6<br>95.7 | 2.5<br>1.3 | Lung cancer patient (Male) |
| 4 | Blood<br>Urine | 85.0<br>21.5 | 1.5<br>0.8 | 91.0<br>23.8 | 1.7<br>1.2 | Asthma patient (Male) |
| 5 | Blood<br>Urine | 33.5<br>8.6 | 1.8<br>0.8 | 35.8<br>8.8 | 1.9<br>1.0 | Normal Adult(Female) |
| 6 | Hair[b] | 45.5 | 1.3 | 48.6 | 1.5 | Normal human hair (Female) |

[a]Samples were from Chittagong Medical College Hospital.
[b]Values in $\mu g\ g^{-1}$.

***Determination of lead in some surface soil samples***

An air dried homogenized soil sample (100 g) was weighed accurately and placed in a 100-mL micro-Kjeldahl flask. The sample was digested in presence of excess reducing agent (1-mL of 2.5%(w/v) freshly[98] prepared sodium azide solution), following the method recommended by Jackson[99]. The contents of the flask was filtered through a Whatman No. 40 filter paper into a 25-mL calibrated flask and neutralized with dilute $NH_4OH$ solution in the presence of 1-2-mL of a 0.01% (w/v) tartrate or EDTA solution. Then the solution of the flask was made up to the mark with de-ionized water.

Suitable aliquots (1-2-mL) were transferred into a 10-mL calibrated flask and a calculated amount of 0.001 M HCl needed to give a final acidity of 0.0006-0.0025 M HCl was added followed by 1-2-mL of 0.01% (w/v) tartrate or EDTA solution as masking agent. The lead content was then determined by the above Procedure and quantified from a calibration graph prepared concurrently. The results are shown in **Table 7.** The average value of different surface soil samples of Bangladesh containing 78.47 mg $kg^{-1}$ of Pb.

**Table 7. Determination of lead in some surface soil samples**

UNDER PEER REVIEW

| Serial No. | Lead ($mg\ kg^{-1}$) [a] (n=5) | RSD (%) | Sample Source[b] |
|---|---|---|---|
| $S_1$[b] | 10.5 | 1.0 | Agriculture soil (Chittagong University Campus) |
| $S_2$ | 6.55 | 1.5 | Marine soil (Bay of Bengal) |
| $S_3$ | 175.0 | 1.3 | Traffic soil (Kadamtali Bus Terminal) |
| $S_4$ | 76.5 | 2.0 | Industrial soil (Estern Cables) |
| $S_5$ | 85.6 | 2.1 | Industrial soil (T.S.P. Complex, Chittagong) |
| $S_6$ | 104.5 | 2.5 | Industrial soil (Bangladesh Steel Re-rolling Mills Ltd., Chittagong, Bangladesh) |
| $S_7$ | 78.6 | 2.0 | Road side soil (Dhaka-Chittagong Highway) |
| $S_8$ | 125.0 | 2.3 | Paint soil (Elite Paint, Chittagong) |
| $S_9$ | 56.0 | 1.6 | River soil (River Halda, Chittagong) |
| $S_{10}$ | 67.0 | 1.8 | River soil (River Karnaphully, Chittagong) |

[a]Average of five analyses of each sample
[b]The measure precision is the relative standard deviation(RSD).
[c]Composition of the soil samples: C,N, P, K, Na, Ca, Mg, Cu, Mo, Fe, Pb,Zn,V,Mn,Co,$NO_3$,$SO_4$

***Determination of lead in some vegetable and food samples***

UNDER PEER REVIEW

The vegetable and food samples collected prior to the determination were pretreated in the following way. Edible portion of samples was first washed clean with tap water followed by rewashing with de-ionized water. After removing de-ionized water from the surface of vegetables and fruits, the samples were cut into small pieces and dried at 65º C in oven. An air dried vegetables and fruits samples (10gm) were taken in a 100-mL micro-Kjeldahl flask in presence of reducing agent and digested following a method recommended by Stahr[95]. A glass bead and 10-mL of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled at room temperature. 1-mL volume of concentrated sulfuric acid was added carefully, followed by the addition of 2-mL of concentrated HF, and heating was continued for at least ½ hr and then cooled. The content of the flask was reduced from lead(IV) to lead(II) by using freshly prepared sodium azide solution (2.5% w/v) and excess of azide was removed by boiling and then filtered. The solution of flask then neutralized with dilute ammonia in the presence of 1-2-mL of a 0.01 % (w/v) tartrate or EDTA solution. The resultant solution was then transferred quantitatively into a 25-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1-2-mL) of the final solution was pipetted into a 10-mL calibrated flask and the lead content was determined as described under the Procedure using tartrate as masking agent. High value of lead for *Brassica oleracea cupitata* (White Cabbage) is probably due to the involvement of high lead concentration in the soil[100]. The results are shown in **Table 8**.

UNDER PEER REVIEW

**Table 8. Determination of lead in some food and vegetable samples**

| Serial No. | Sample | Lead / mg kg$^{-1}$ Found[a] ± s (n=5) | | | | Sample source |
|---|---|---|---|---|---|---|
| | | AAS (n=5) | | Proposed method (n = 5) | | |
| | | Found | RSD[b] (%) | Found | RSD[b] (%) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | White Cabbage *(Brassica oleracea cupitata)* | 48.0±1.0 | 1.8 | 50.0±1.5 | 2.0 | Local Market, Chittagong |
| 2 | Spinach *(Spinacia oleracea)* | 15.0±0.8 | 1.5 | `17.0±1.0 | 2.3 | Local Market, Chittagong |
| 3 | Tomato Leaves *(Lycopersicon esculentum)* | 21.0±1.0 | 2.0 | 20.0±0.9 | 1.8 | Local Market, Chittagong |
| 4 | Lettuce Leaves *(Lactuca sativa)* | 35.0±1.2 | 2.1 | 36.5±1.3 | 2.5 | Local Market, Chittagong |
| 5 | Cauliflower Leaves *(Brassica oleracea)* | 34.0±1.0 | 1.8 | 35.5±0.8 | 2.0 | Local Market, Chittagong |
| 6 | Almonds *(Prunus dulcis)* | 22.0±0.6 | 1.5 | 25.0±1.0 | 2.2 | Local Market, Chittagong |
| 7 | Rice *(Oryza sativa)* | 0.20±0.5 | 1.8 | 0.30±0.08 | 1.6 | Local Market, Chittagong |
| 8 | Wheat powder *(Tritricum aestivum)* | 0.50±1.0 | 1.6 | 0.6±0.08 | 1.8 | Local Market, Chittagong |
| 9 | *Cacao (Theobroma cacao)* | 15.0±0.5 | 1.8 | 16.0±0.8 | 1.7 | Local Market, Chittagong |
| 10 | Cashews *(Anacardium occidentale)* | 17.0±1.0 | 1.8 | 16.0±1.5 | 2.0 | Local Market, Chittagong |
| 11 | Peanuts *(Arachis hypogaea)* | 21.0±1.5 | 2.0 | 23.0±1.6 | 2.1 | Local Market, Chittagong |

[a] Average of five replicate analyses of each sample.
[b]The measure precision is the relative standard deviation(RSD).

UNDER PEER REVIEW

***Determination of Lead(II) and Lead(IV) Speciation in Mixtures***

Suitable aliquots (1-2 mL) of lead (II + IV) mixtures (preferably 1: 1, 1: 5, 1:10) were taken in a 25-mL conical flask. A few drops (2-3drops) of 4 M $H_2SO_4$, 3-4 mL of a freshly prepared sodium azide solution (2.5% w/v) was added to reduce the tetravalent lead to divalent lead and heated gently with the further addition of 5 mL of water, if necessary, for 5 minutes to drive off the excess azide cooled to room temperature. The reaction mixtures was

neutralized with dilute $NH_4OH$ and transferred quantitatively into a 10-mL volumetric flask. 1-mL of $3.3 \times 10^{-3}$M DBHQ reagent solution was added followed by the addition of 0.5-mL of 0.001 M HCl and 3-mL ethanol. It was made up to the mark with de-ionized water. The absorbance was measured after 1 min at 390 nm against a reagent blank. The total lead content was calculated with the help of a calibration graph prepared concurrently.

An equal aliquot (1-2-mL) of the above lead (II+ IV) mixture was taken into a 25-mL beaker. Neutralize the solution with dilute $NH_4OH$ in presence of 1-2 mL of 0.01% (w/v) tartrate solution. After, the content of the beaker was transferred quantitatively into a 10-mL volumetric flask; 1-mL of $3.3\times10^{-3}$ M DBHQ reagent solution was added, followed by the addition of 0.5-mL of 0.001 M HCl and 3-mL ethanol. It was made up to the mark with de-ionized water. After 1 min the absorbance was measured at 390 nm against a reagent blank, as before. The lead concentration was calculated in mg $L^{-1}$ or µg $L^{-1}$ with the aid of a calibration graph. This gives a measure of lead (II) originally present in the mixture. This value was subtracted from that of the total lead to get the lead(IV) present in the mixture. The results were found to be highly reproducible. The occurrence of such reproducible results is also reported for different oxidation states of lead[101]. The results of a set of determination are given in **Table 9**.

UNDER PEER REVIEW

**Table 9. Determination of Pb(II) and Pb(IV) speciation in mixtures**

| Serial No. | Pb(II) : Pb(VII) | Pb, taken (mg $L^{-1}$) | | Pb, found (mg $L^{-1}$) | | Error (mg $L^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | | Pb(II) | Pb(IV) | Pb(II) | Pb(IV) | Pb(II) | Pb(IV) |
| 1 | 1: 1 | 1.00 | 1.00 | 0.99 | 0.98 | 0.01 | 0.02 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 1: 1 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 | 0.00 |
| 1 | 1: 1 | 1.00 | 1.00 | 0.98 | 0.99 | 0.02 | 0.01 |
| Mean error : Pb(II) = ± 0.017; Pb(IV) = ± 0.017<br>Standard deviation : Pb(II) = ± 0.05; Pb(IV) = ± 0.006 | | | | | | | |
| 1 | 1: 5 | 1.00 | 5.00 | 0.98 | 4.99 | 0.01 | 0.01 |
| 1 | 1: 5 | 1.00 | 5.00 | 0.98 | 4.98 | 0.02 | 0.02 |
| 1 | 1: 5 | 1.00 | 5.00 | 0.99 | 4.98 | 0.01 | 0.02 |
| Mean error : Pb(II) = ± 0.013; Pb(IV) = ± 0.016<br>Standard deviation : Pb(II) = ± 0.0058; Pb(IV) = ± 0.006 | | | | | | | |
| 1 | 1:10 | 1.00 | 10.00 | 0.98 | 9.98 | 0.02 | 0.02 |
| 1 | 1:10 | 1.00 | 10.00 | 0.98 | 9.99 | 0.02 | 0.01 |
| 1 | 1:10 | 1.00 | 10.00 | 0.99 | 9.98 | 0.01 | 0.02 |
| Mean error : Pb(II)= ± 0.016; Pb(IV) = ± 0.017<br>Standard deviation : Pb(II) = ± 0.0058; Pb(IV) = ± 0.006 | | | | | | | |

**Conclusions**

A new simple, sensitive, and inexpensive method with the lead(II)-DBHQ complex was developed for the determination of lead in some real, environmental, biological, soil, food, and vegetable samples, for continuous monitoring to establish the trace levels of lead in several difficult samples matrices. Compared with other methods , the proposed method has several remarkable analytical characteristics. Firstly, the proposed method is highly sensitive with molar absorptivity of the complex of $6.16\times10^{5}$ L $mol^{-1}$ $cm^{-1}$. Thus, amount of ng $g^{-1}$ of lead can be determined without preconcentration. Secondly, the proposed method is very simple, rapid, and stable. The reaction of lead(II) with DBHQ is completed rapidly in 1 min at room temperature($25\pm5^{0}$C) so it does not involve any stringent reaction conditions and offer the advantages of high complex stability (24h). Thirdly, the method has added the advantage of determining individual amounts of Pb(II) and Pb(IV). With suitable masking agents, the reaction can be made highly selective. The proposed method using DBHQ in aqueous solutions not only is one of the most sensitive methods for the determination of lead but also is excellent in terms of selectivity and simplicity. Therefore, this method will be successfully applied to the monitoring of trace amounts of lead in real, environmental, biological, soil, food and vegetable samples.

UNDER PEER REVIEW

## Consent

We were not aiming to carry out detailed human studies but some samples from individuals were used in our study and as such we abided by all the necessary procedures and regulations and our University gave consent. University of Chittagong, Bangladesh is committed to the protection and safety of human subjects involved in research.

## References


1. *Patty's Industrial Hygiene and Toxicology,* G. D. Clayton, and F. E. Clayton (eds.) Wiely, New York, 3rd edn. Vol. 2A, **,** p.1725(1981)
2. R. A. Goyer and T.W. Clarkson, In: C. D. Klaassen, M. O. Amdur, J. Doull (Eds.), "*Casarett and Doull's Toxicology: The Basic Science of Poisons",* 6th edn., MacMillan Publishing Company, New York, p.828 (2001).
3. J. Guarterman,Trace Elements in Human and Animal Nutrition, vol. 2, 5th edn., Academic Press, New York(1986).
4. J. L. Stauber and T. M. Florence, *Sci. Total Environ*. **74,** 235(1988)
5. *Occupational Diseases – A guide to Their Recognition*, ed. M. M. Key, A. F. Henschel, J. Butter, R. N. Ligo, I. R. Tabershad, U.S. Department of Health, Education and Welfare, US Government Printing, Washington DC(1977).
6. Ccsenet.org (2017)
7. A. K. De, Environmental Chemistry, 3rd ed., New Age International (P) Limited, New Delhi, , p. 263(1996).
8. M. J. Ahmed, *Talanta*,55,43 (2001)
9. B. Venugopal and T. D. Lukey, "*Metal Toxicity in Mammals",* vol. 2, Plenucm Press, New York, p. 235 (1979).
10. K. Humaira, M. Jamaluddin Ahmed, and M. Iqbal Bhanger, *Analytical Sciences,***23,** 193 (2007)
11. Determination of Metals in Ambient Particulate Matter Using Neutron Activation Analysis (NAA) Gamma Spectrometry,Center for Enviromental Protection, U.S. Enviromental Protection Agency(1999) **.**
12. www.mdpi.com (2018)
13. S. Recknagel, P. Bratter, A. Tomiak and U. Rosick, *Fresnius J. Anal. Chem.,* **346***,* 833(1993).
14. D. R. Lide, CRC Handbook of Chemistry and Physics, 86th edn, Boca Raton (FL): CRC Press (2005).
15. J. Zhao, S. Meng and D. Li, *Solvent Extr. Ion. Exch.*, **22**, 429(2004).
16. L. F. Arenas, C. Ponce de León, and F. C. Walsh, *Electrochimica Acta*, **205,** 226(2016).
17. A. Safavi and M. Mirzaee*, Talanta*, , **51,** 225(2000).


UNDER PEER REVIEW

18. S. Mar´ıa, Di Nezio, E. M. Palomeque, S. Beatriz and F. Band, *Talanta* , , **63,** 405 (2004).

19. M. Jamaluddin Ahmed and Mosaddeque-Al Mamun, *Talanta,* **55**, 43(2001).

20. Le Van Tan, *International Journal of Chemistry,* **2** , 86 (2010).

21. M. T, Nazimtiaz, R. A. Khan and J. Iqbal, *J.Chem.Soc.Pak.*, , **34,** 1111(2012).

22. G. Fang, Y. Liu, S. Meng and Y. Guo, *Talanta*, **57,** 1155 (2002).

23. A. P. Argekar and A. K. Shetty, *Talanta* , , **45,** 909 (1998).

24. B. Jankiewicz, B. Ptaszyński and M. Wieczorek, *Polish Journal of Environmental Studies,* **2 ,** 123(2001),.

25. J. Aznarez, F. Palacios, J. Carlos Vidal and J. Galban,*Analyst*,, , **109,** 713 (1983).

26. O. Cankur, D. Korkmaz and O. Y. Ataman, *Talanta*, 2005, **66,789** ( 2005).

27. N. Chakrabarti and S. K. Roy,*Transactions of the Indian Ceramic Society*, , **51** , 73(1992).

28. R. M. Dangali, T. S. West and P. Young, *Talanta*, , **12,** 583(1965).

29. H. T. Delves, *Analyst,* **96,** 431(1970).

30. B. Du , J. Yang , Q. Wei and G. Chang, *Analytical Letters*, **35 ,** 895(2002).

31. S. Conradi, L. O. Ronnevi and O. Vesterberg, *Journal of Neurology, Neurosurgery, and Psychiatry*, , **41,** 389(1978).

32. L. Ebdon and A. Lechotycki, *Microchemical Journal*, **34,** 340(1986).

33. G. Fang, S. Meng , G. Zhang and J. Pan, *Talanta*, **54**, 585 (2001).

34. *S.* L. C. Ferreira, M. G. M,Andrade, I.P. Lobo and A. C. *S.* Costa, *Analytical Letters*, , **24,** 1675(1991).

35. M. Kuramochi, K. Tomioka, M. Fujinami and K. Oguma *Talanta,* **68,** 287( 2005).

36. Z. Li, Z. Zhua, T. Jana and J. Pan, *Analyst*, , **124,** 1227(1999).

37. Z. Li , J. Tang and J. Pan, *Food Control*, **15,** 565 ( 2004).

38. B. Raquel, R. Mesquita, M. Sılvia, V. Fernandes and A. Rangel, *Talanta*, **62,** 395(2004*)*.

39. S. Ninan, A. Varadarajan, S. B. Jadhav, A. J. Kulkarni and S. P. Malve, *Spectrochimica Acta Part A*, , **55**, 825(1999).

40. E. A. Novicov, L. K. Shpigun and Y. A. Zolotov, *Analytca Chemica Acta*, , **230,** 157(1990).

41. A. A. Ramirez, D. G. Bzquez, I. M. de la Rosa and F. Moreno, *Analytical Letters*, **27** 1595(1994).

42. T. Prasada Rao and T. V. Ramakrishna, *Talanta*, **, 27**, 439(1979).

43. J. G. T. Regan and J. Warren, *Analyst*, , **103,** 447(1978).

UNDER PEER REVIEW

44. J. A. Schneider" and J. F. Hornig , *Analyst*, , **118**, 933(1993).

45. S. Selander and K. Cramer, *Brit. J. industr. Med*, , **25,** 209(I968).

46. M. Stoeppler and K. Brandt and T. C. Rains, *Analyst*, **103,** 714(1978).

47. M. Thakur and M. K. Deb, *Analyst*, **124,** 1331(1999).

48. M. Yan, S. Liu and X. Chen and B. Du, *Analytical Letters,* **35,** 5 (2002)

49. L. Zaijun ,Y. Yuling , T. Jian and P. Jiaomai, *Talanta*, , **60,**123(2003).

50. M. A. Taher, *Bull. Chem. Soc. Ethiop.*, **17,** 129(2003).

51. M. Novakova and V. Kuban, *Chem. Papers*, **42,** 183(1988).

52. M. J. Ahmed, H. Khan and M. Iqbal, *Spectroscopy*, , **20,** 285(2006).

53. M. P. Bettenshaw" and D. Gelsthorpe, *Analyst* , **106,** 23(1981).

54. D. W. Yeager, J. Cholakl, and E. W. Henderson, *Environmental Science & Technology*, , **5,** 1020 (1971).

55. S. Shiri, A. Delpisheh, A. Haeri, A. Poornajaf, B. Golzadeh and Sina Shiri, *Analytical Chemistry Insights*, , **6,** 15 (2011).

56. B. Saritha, A. Giri and T. Sreenivasulu Reddy, *Journal of Chemical and Pharmaceutical Research*, **6** , 1571 (2014).

57. D. M. Hubbard, *Industrial and Engineering Chemistry*, **9 ,** 493 (1937,).

58. Dr. M. Renuka , Dr. Naga Kavitha, V.Venkateswarulu and K. Hussain Reddy, *International Journal of Scientific Engineering and Applied Science (IJSEAS)*, 2015, **1**, 414(1937).

59. N. Parveen and Y. Rohan, *Journal of Environmental Research And Development*, , **6** , 57(2011).

60. A. K. Shrivastava and H. S. Khaladkar, *Sci. Revs. Chem. Commun.* **3 ,** 43 (2015).

61. S. Skurnik-Sarig and M. Zidon, *Israel Journal of Chemistry* , **8,** 545 (1970).

62. K. Deepa, Y. Paul raj and Y. Lingappa , *Scholars Research Library Der Pharmacia Lettre*, **6**, 380 (2014).

63. J. Klamtet, S. Sanguthai and S. Sriprang, *NU Science Journal*, , **4** , 122 (2007).

64. J. Sun, *Advanced Materials Research*, **301.** 1030 ( 2014) .

65. Q. Z. Zhai, J. M. Li and J. P. Zhang, *Asian Journal of Chemistry*, **25 ,** 538 ( 2013).

66. www.csass.org (2015)

67. Jocpr.com (2015)

68. www.ics-ir.org (2013)

69. M. J. Ahmed and Enamul Haque, *Analytical Sciences*,**18**,433 (2002)

70. M. J. Ahmed and A. K. Banerjee, *Analyst,* ,**120,** 2019 (1995).

71. M. J. Ahmed and M. T. Islam Chowdhury, *Analytical Sciences,* **, 20,** 987 (2004).

UNDER PEER REVIEW

72. H. W. Darwish, A. S. Abdelhameed, A. H. Bakheit and A. M. Alanazi, *RSC Advances,* **51,**40455 (2015)

73. www.sdiarticle 1.org (2012)

74. Ceacsu.edu.pk(2008)

75. B. K. Pal, M. J. Ahmed and A. K. Chakrabarti, *Analyst,***15**,439 (1990)

76. M. J. Ahmed, M. Nasir Uddin and T. Zannat and S. Sultana, *Analytical Methods,* **6**, 2282 (2014)

77. Sigma-Aldrch, *"5.7-Dibromo-8-hydroxyquinoline, CAS No. 551-74-4, Registry No. 53624"*, (2010)

78. K. Bahagat and A. G. Righeb, *Central European Journal of Chemistry*, **5,** 201 (2007).

79. www.ceacsu.edu.pk(2010)

80. A. I. Vogel, *'Vogel's Quantitative Chemical Analysis',* London, 6th edn., p. 185 (2002).

81. A. K. Mukharjee, "*Analytical Chemistry of Zirconium and Hafnium*", 1st ed., Pergamon Press, New York, p. 12 (1970).

82. B. K. Pal, B. Chowdhury, *Mikrochim. Acta*, , **11,** 121 (1984).

83. A. I. Busev, V. G. Tiptsova, V. M. Lvanov (eds.), *"Analytical Chemistry of Rare Elements"*, Mir Publisher, Moscow, p. 389 (1981)

84. E. B. Sandell, "*Colorimetric Determination of Traces of Metals*", 3rd ed., Interscience, New York, , p. 269 (1965).

85. M. J. Ahmed, .M. Tazul Islam and M. J. Nime, *Analytical Methods*,**7,** 7811(2015)

86. C. Bosch Ojeda, A. Garcia de Torres, F. Sanchez Rojas and J. M. Cano Pavon, *Analyst*, **112,** 1499 (1987).

87. P. Job., *Ann. Chim.* (Paris), **, 9,** 113(1928).

88. J. A. Yoe and A. L. Jones, *Ind. Eng. Chem. Anal. Ed*., **16,** 11 (1944).

89. www.eurjchem.com (2018)

90. Humaira Khan, *Spectroscopy*, **20,**285 (2006)

91. www.eurasianjournals.com (2011)

92. S. Mitra (ed.), *Sample Preparation Techniques in Analytical Chemistry*, Wiley-Interscience, New Jersey, **,** p. 28(2003).

93. C. Sun, J. Y. Yang and S. R. Tzeng, *Analyst,* **124**, 421(1999).

94. E.A. Greenberg, S.L. Clesceri, D.A. Eaton (Eds.), *Standard Methods for the Examination of Water and Wastewater*, 18th ed., American Public Health Association, Washington, DC, p. 3-254 (1992).

95. H. M. Stahr, "*Analytical Methods in Toxicology*", 3rd ed., John Wiley & Sons, New York, p. 75(1991).

96. R. L. Boecks, *Anal. Chem.,* **58,** 275 (1986).

97. C. Baird, N. Cann, Environmental Chemistry (5th edn.), (2012).

UNDER PEER REVIEW

98. Journals.tubitak.gov.org(2016)

99. M. L. Jackson, Soil Chemical Analysis, Prentice Hall, Englewood Cliffs, NJ, p.336(1965).

100. A. L. Page, A. C. Chang and M. El-Amany, *In: Lead, Mercury, Selenium, Cadmium, Manganese and Arsenic in the Environment*, T.C. Hutchinsm and K. M. Meema, John Wiley and Sons Ltd., New York, , p. 115(1987).

101. *Hazardous Metals in Human Toxicology,* ed., A. Vercruysse, Elsevier, Amsterdam, p. 275 (1984).

UNDER PEER REVIEW