SPECTOPHOTOMETRIC DETERMINATION OF Cu, Ni, AND Fe IN TWO ENVIRONMENTAL SAMPLES USING PARA-PHENYLENEDIAMINE EXTRACTED FROM A NATURAL MINERAL DYE

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ABSTRACT

A simple, sensitive and novel spectrophotometric method has been developed for the determination of trace elements (Cu, Ni and Fe) using para-phenylenediamine extracted from a mineral dye with diethyl ether. The method was applied to the determination of these metals in algae and cattle manure samples obtained from a polluted river and cattle house within Ilorin Metropolis. The concentration of the metals in algae sample were 30.15±4.1 51.9±9.4 and 1055.25±8.8 ppm for Cu, Ni, and Fe respectively while cattle manure were found to be 15.5±2.1, 25.15±5.5 and 429.15±17.2, ppm for Cu, Ni and Fe, respectively. Two standard methods of determination of heavy metals, AAS and XRF were also used to determine the same metals in the environmental samples. The results obtained were found to be of no siginificant difference ($p < 0.05$) from the result of developed method.

Keywords: Spectrophotometric, mineral dye, para-phenylenediamine, copper, nickel, iron, algae and cattle manure

INTRODUCTION

Heavy metals refer to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include Cu, Mn, Zn, Cr, Ar, Fe, Ni and Pb. They cannot be degraded or destroyed but tend to bioaccumulate. They enter our bodies via food and drinking water. Some heavy metals (e.g Cu, Se, Zn) are essential, if not at higher concentrations,

for maintaining the metabolism of the human body (Troeh *et al.,* 1999; Bothwel *et al., 2003).*

High concentrations of arsenic in drinking water have been documented in specific parts of Argentina, Canada, China, Japan, Mexico, and the USA. The problem is particularly acute in Bangladesh, where an estimated 30 million people drink arsenic-poisoned water (WHO, 1997). Some 62% of wells supply arsenic-contaminated water above WHO's (World Health Organization) limits with some containing as much as 400 times the limit (Bagla *et al.*, 1996) leading to cardiovascular problems, skin cancer, other skin effects and kidney damage.

It has been reported that Cu, Fe, Cr, Mn, Ni and Zn are part of the elements of major toxicological concern. (Troeh *et al.*, 1999). They are essential trace elements but are also toxic in the environment. In order to assess their accumulation or deficiency in biological or environmental samples, sensitive, reproducible and accurate analytical methods are required. Inductively coupled plasma mass spectrometry (Fu *et a.l.*,1998), atomic absorption spectrometry (Folin *et al.*, 1991), potentiometry with ion-selective electrode (Smith *et al.*, 1984), uv-visible spectrophotometry (Kojlo *et al.,* 1990), X-ray fluorescence spectrophotometry (Muddukrishna *et al.,* 1991) are well established techniques for this purpose. Some of these techniques are limited because of expensive instrumentation, high cost for routine analysis and problem of low sensitivity.

In this work, a simple, sensitive and novel spectrophotometric method has been developed for the determination of Cu, Ni and Fe in cattle manure and algae using para-phenylenediamine extracted from a mineral dye.

EXPERIMENTAL
Source of the Material
The white rock-like mineral dye (Yombo-fitta) was obtained from Emir's market in Ilorin, Kwara State. Nigeria. The original source of the sample has been traced to the southern part of Ghana (Adebayo *et al.,* 2005).

The environmental samples used include algae from Asa-dam in Ilorin, Kwara state, and cattle manure from a cattle house at Agbo-oba, Ilorin. Nigeria. All the reagents used were of analytical grade.
Extraction of para-phenylenediamine
The organic component of the dye was extracted by re-crystallisation method following the procedure used by Adebayo (2007).

Determination of λ_{max} of the dye extract

The absorbance of the standard solution of the dye extract previously prepared by dissolving 0.1g of the dye in 100ml of solution, were scanned between 310-600 nm to determine the λ_{max} using UV-VIS Spetrophotometer,model Aquamate V4.60.

Determination of λ_{max} of the metal-dye extract complex

10 ml was measured from 1000 ppm of salts of each of the element and 10 ml each of 1000ppm dye extract solution were added. Each solution was adjusted to specific pH. The absorption spectra for these solutions were determined over the spectra range of 310-600 nm using distilled water as blank with the aid of uv-visible spectrophotometer. The pH and wavelength that corresponds to where maximum absorption occurs was then determined. This procedure was repeated for all the metal-dye extract solutions.

Preparation of different concentrations of metal solutions for the calibration curve

A 100 ppm solution of each of the metal ion was prepared by taking 10 ml of their stock solution (1000 ppm) and made up to 100 ml in a standard volumetric flask. A range of concentrations from 0.1 ppm to 1.0 ppm of the individual metals were prepared by measuring 0.1 ml to 1.0 ml in a series of 100 ml standard flasks and made up to the mark with distilled water (Charles, 1974).

Determination of absorbance of different concentrations of metals at an observed λ_{max}

The modified method of Suresh et al, 2004 was adopted for the determination. 1 ml of metal ions (0.1 ppm) was transferred into 10 ml standard flask, then 5 ml of buffer solution (pH 4, 6 and 2 for Cu, Ni and Fe respectively) was added followed by 1 ml of dye extract stock solution and the mixture was made up to the mark with distilled water, shaken and left for 15 minutes. The colour change was observed. The absorbance of the solution was then measured at the λ_{max} previously determined for the metal. Procedure was repeated for metal ion concentration of 0.2 ppm, to 1.0 ppm.

The above procedure was repeated for other metal ions. A plot of absorbance against concentration was made for different metal ions which obeyed Beer's law and the molar absorptivity (ε) determined from Beer's law, A= εbc. Where A is the absorbance, ε is the molar absorptivity ($Lmol^{-1}cm^{-1}$), b is the path length of the

sample (path length of cuvette in which sample is contained in centimeter) and c is the concentration of the metal in solution (ppm). The calibration curve was used to determine the concentration of Cu Ni and Fe in the environmental samples.

Digestion of environmental samples
The specimen environmental samples used were Cattle manure and algae

Digestion of cattle manure: The sample was dried at 65 ^{O}C for 48 hours and finely grounded to pass through 0.5 mm sieve. Wet Digestion was carried out by weighing 0.1 g of sample into 100 ml conical flask and adding 10 ml of HNO_3/$HClO_4$/H_2SO_4 (10:4:1)mixture The sample was allowed to stand in the mixed acid solution for 2 hours, followed by digestion for 3 hours on a hot plate set at 75 ^{O}C until the digest solution became clear. The flask was then cooled, the contents filtered and transferred to a 50 ml volumetric flask for dilution to required volume with deionized water (Egrinya *et al.,* 2004). Digestion was done in triplicate.

Digestion of algae: After air-drying of the sample, it was dried to a constant weight at 60 ^{O}C in an air oven. Digestion of the sample was done in triple acid mixture (5:1:1 HNO_3:$HClO_4$:H_2SO_4) (Kalesh *et al.,* 2005). Digestion was done in triplicate.

Determination of the concentration of heavy metals in environmental samples by ultra-violet spectrophotometry
1 ml of digested algae solution was transferred into 10 ml standard flask, 5 ml of buffer solution (pH 4, 6 and 2 for Cu, Ni and Fe respectively) was added followed by 1 ml of dye extract stock solution. The mixture was made up to the mark with distilled water, mixed well and left for 15 minutes. The absorbance of the resulting solution was measured at the individual λ_{max} of 313.0, 316.5 and 391.0 nm for metal-dye solution previously determined. The resulting absorbance was traced on the individual metal-dye complex calibration curve in other to determine the concentration of each metal in the sample. The above procedure was repeated for other environmental samples.

Determination of the concentration of heavy metals in environmental samples by atomic absorption spectrophotometry

The concentrations of the metal in the digested environmental samples were determined by Atomic Absorption Spectrophotometry (AAS) after appropriate dilutions. All determinations were carried out in triplicates in order to obtain reliable average values and minimize variability. Standards were also prepared for each metal.

Determination of the concentration of heavy metals in environmental samples by x-ray fluorescence spectroscopy

The raw samples after drying in the oven and grinded into powder were taken for XRF analysis to analyze the concentrations of elements present.

Validation of the method

The developed method was validated using atomic absorption spectrophotometry and x-ray spectrophotometry determination of the metals from the same samples and compared the result obtained with the developed method using the test of significance at $p < 0.005$.
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RESULTS

Table 1: Λ_{max} For Metal Dye Extract Complex Solution at Different pH

Metal-dye extract complex	λ_{max}	pH
Cu-dye extract complex	316.5	4
Ni- dye extract complex	391.0	6
Fe- dye extract complex	313.0	2

Table 2: Heavy Metals Concentration (ppm) in Environmental Samples by UV Spectrophotometry (Developed Method)

Heavy metals	Algae	Cattle manure
Cu	30.15±4.1	15.50±2.1
Ni	51.90±9.4	25.15±5.5
Fe	1055.25±8.8	429.15±17.2

Table 3: Heavy Metals Concentration (ppm) In Environmental Samples by Atomic Absorption Spectrophotometry

Heavy metals	Algae	Cattle manure
Cu	555.0±439.4	148±14.4
Ni	447.51±129.2	240±13.8
Fe	9415±2065.2	3895±651.7

Table 4: Heavy Metals Concentration (ppm) In Environmental Samples by X-Ray Fluorescence (XRF)

Metals	Algae	Cattle manure
Cu	41±1	36±1
Ni	215±11	104±1
Fe	1136.1±0.0039	3650±24

Table 5: Values of the Coefficient of Variation ($^O/_O$ RSD) for Different Metals in Environmental Samples by Ultra-Violet Spectrophotometry

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Metals	Algae	Cattle manure
Cu	13.6	13.5
Ni	18.1	21.9
Fe	0.8	4.0

Table 6: Values of the Coefficient of Variation ($^O/_O$ RSD) for Different Metals in Environmental Samples by Atomic Absorption Spectrophotometry.

Metals	Algae	Cattle manure
Cu	79.2	9.7
Fe	21.9	16.7
Ni	28.9	5.8

Table:7: Statistical Comparison of the Developed Method with AAS (T-Test and F-Test).

Metals	t-value	F-value
Cu	1.613	0.001
Ni	2.912	0.017
Fe	2.129	0.013

Table 8: Statistical Comparison of the Developed Method with XRF (T-Test and F-Test).

Metals	t- value	F- value
Cu	1.951	8.253
Ni	2.123	0.058
Fe	1.275	0.062

DISCUSSION

The yield of para-phenyldiamine extracted from 1.0 g of mineral dye has been reported (Adebayo, 2007). The dye extract in aqueous solution has been found to absorb at the wavelength of 464.5 nm. The λ_{max} of the aqueous solution of the dye extract has also been reported (Adebayo *et.al.*, 2007) to indicate absorption in the visible and ultraviolet region. A plot of Absorbance (from UV readings) against Concentration were made for different metal-dye extract complexes at the observed

λ_{max} for each solution as shown in Table 1. The calibration curve was used to determine the concentration of these heavy metals in cattle manure and algae.

Table 2 shows the concentration of some heavy metals in algae and cattle manure. The determination was done in triplicate with the three different digestates of the samples. The concentrations of the heavy metals increases in the order Fe>Ni>Cu in both algae and cattle manure. The concentration of Fe was not only very high in algae but also in cattle manure.

The determination was done in triplicate with the three different digestate of the samples. The result in Table 3 revealed that the concentration of heavy metals in algae and cattle manure increases in the order Fe>Cu>Ni and Fe>Ni>Cu with each sample still have high concentration of Fe.

From the XRF result in Table 4, the concentration of the heavy metals increases in the order Fe>Ni>Cu for algae and cattle manure where the concentration of Fe is highest in both algae and cattle manure.

The concentrations of Cu and Ni among others have been reported in cattle manure in England and Wales (Nichoslon *et al*., 1999). It was reported that the concentration of these metals in cattle manure were (16.4mg/kg) for Cu and (2.0mg/kg) for Ni (Nichoslon *et al.*, 1999).

Accumulation of relatively high metal concentrations in cattle manure may be due to their feeding behaviour. Cu (mostly in the form of copper sulphate) is added to feeds to suppress bacterial action in the gut and to maximize feed utilization by the cattle (Nichoslon *et al.,* 1999).

The concentration of metals in the surrounding river or lake and position of algae in the place can contribute to its metal contents. The concentration of Ni and Cr were determined in algae of karala as Ni (10.13 ugg^{-1}) and Cr(13.86 ugg^{-1}) has been reported (Soltan *et al.,* 2005). These concentrations were lower than those determined by the developed method for algae. Since wide variations occur in algae cell walls depending upon the division and species, there can be species-dependent variations in various cell wall biopolymers and their chemical functional groups that can bind metal ions. This may be the reason for the observed difference in metal accumulation. The variation may also arise from the changes in the specific environmental conditions surrounding each species (Barreiro *et al.*, 2002). Species wide variations in metal accumulation can also occur as a result of difference in the surface area available for absorption, permeability of cells, number and nature of binding sites and metabolic

rate (Brown *et al.,* 1998). It can also be as a result of combined effect of environmental and growth factors. The increasing temperature and light conditions can increase the rates of photosynthesis and respiration which promote the uptake of metals by algae. The increasing salinity also enhances metal assimilation (Lobban *et al.*, 1997).

Although the concentrations of Ni and Cr have been determined in marine algae of southwest coast of India, Cu and Fe were not determined. The concentration of Ni determined was higher than that found in algae in this present study (Kalesh *et al.*, 2005). These variations may be related to the differences in the algae species and the environmental conditions of various places. There can be differences in the metal accumulation between different species of algae, which is driven by physiological or biological differences between algae cells. Different geographical regions may differ considerably in their environmental conditions and this may give rise to variations in the metal accumulation by algae from different places. Alterations in the physicochemical parameters such as pH, redox potential, ionic strength, salinity, presence of organic and particulate matter, etc can result in the transformation of the chemical forms of metals and can greatly influence their availability to algae (Loban *et al.,* 1997). The life stages of algae also contribute to the geographical variability in the metal contents of algae (Kalesh *et al.*, 2005).

The concentrations of Cd and Pb have been determined in cattle manure from four agricultural areas of Bursa, Turkey (Firdevs, 2005). But the concentrations of Ni, Cu, and Fe were not determined.The method has been used to determine these metals.

Validation of the developed method

To evaluate the developed method, the results obtained were compared with those obtained from the AAS and XRF methods (Table 3 and 4) using coefficient of variation ($^{o}/_{o}$ RSD), t-Test and F-Test for different metals in environmental samples. The coefficient of variation ($^{o}/_{o}$ RSD) for the atomic absorption spectrophotometry is higher than that of ultra-violet spectrophotometry (Tables 5 and 6). This implies that the developed method is more precise than AAS.

The developed method for the determination of traces of some heavy metals were also validated by comparing the result for the concentration of these metals from ultra-violet spectrophotometry with that of the concentration from atomic absorption spectrophotometry (AAS) and XRF statistically using t and F- test and compared at

p<0.005(Table 7 and 8). The results indicated that there were no significant difference between the devloped method and AAS and XRF.

CONCLUSION

The determination of traces of heavy metals in environmental samples has been simplified by spectrophotometric measurements of the dye complex formed with para-phenylenediamine. The developed method is more sensitive for some metals than atomic absorption spectrophotometry making it an alternative method for the determination of these metals. The method was also used for the quantitative determination of Cu, Fe, and Ni in cattle manure and algae conveniently.

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