

Interlaboratory Comparison Exercise for the Determination of Copper and Lead in Ghana

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Abstract

Determination of metals in the environment is of great interest due to their harmful effect on human health. In Ghana, monitoring of these metals in food is undertaken and regulated by the Food and Drugs Authority. The recommended maximum residual limit of copper (Cu) and lead (Pb) in food is 2 and 0.01 mg/L, respectively. This project presents a survey on various laboratories where metal analysis can be conducted in Ghana and the results of an inter laboratory comparison exercise for the determination of heavy metals of concern to human health. In all, 15 laboratories were identified in Ghana and four of these laboratories participated in the comparison exercise using atomic absorption spectrometry (AAS) for metal determination. Each was asked to analyze four test samples, two standards of 0.5 ppm and 2 ppm. Of the 15 laboratories identified, six (40 %) were in Greater Accra Region, six (40 %) in Ashanti Region, two in Western Region and one in the Eastern Region of Ghana. The recovery obtained by the four laboratories ranged from 37.4 to 113.5%. The exercise revealed that only 50% of the laboratories met the minimum acceptable recovery target; hence their results could be accurate.

Keywords: Copper, Lead, Heavy metals, Recovery rate, Laboratories, Ghana

Introduction

The presence of metals in natural ecosystems is related with natural factors such as geographic location, type of soil (Ramos *et al.*, 1999; Blasser *et al.*, 2000) and anthropogenic inputs associated with agricultural practices, mineral exploration, industrial processes and solid waste management (Bilos *et al.*, 2001; Keane *et al.*, 2001; Alumaa *et al.*, 2002). Some heavy metals such as iron, zinc, copper, cobalt and manganese are necessary for proper physiological function at low levels. However, at higher concentrations these metals can be toxic (WHO, 2011). Elements such as arsenic, cadmium, lead, mercury, nickel, etc. have a wide spectrum of toxicity that includes neurotoxic, hepatotoxic, nephrotoxic, teratogenic and mutagenic effects among others (Domingo, 1994; Bucheim *et al.*, 1998; Hudnell, 1999; Kelley, 1999; Lai *et al.*, 1999). Unfortunately, plants, animals and even humans have been exposed to these toxic heavy metals through vehicle emissions, industrial waste, dental amalgams, and a variety of other routes.

An analytical method for the determination of heavy metals is composed of three steps: Sampling which depends on the matrix, pretreatment of the sample also depends on the analyte of interest and consists of acidification, acid digestion, filtration, preconcentration,

etc. and the last step is analysis (Christian *et al.*, 2014). Determination of heavy metals in environmental samples is difficult and the results obtained often vary according to the chosen analytical technique. The main analytical techniques that have been used to determine heavy metals in environmental matrices are: Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP/AES), Inductively Coupled Plasma Mass Spectrometry (ICP/MS), Neutron Activation Analysis (NAA), X-ray fluorescence (XRF) and Ion Chromatography (IC) (Azanu *et al.*, 2016; Voica *et al.*, 2012). The choice of method for a particular application should take into account factors such as their sensitivity, precision, accuracy (recovery) and relative cost (mainly instrumental) of the techniques currently in use (Thompson *et al.*, 2002; U.S. Food and Drug Administration, 2001). The function of Analysts is to attain a specific result as near to the true value by employing analytical procedure. The state of confidence that the Analyst may enjoy with his/her results will be very low unless he/she has knowledge of the accuracy and precision of the method used (Christian *et al.*, 2014). Recovery is among the most significant critical parameters for judging the analytical procedures by their achieved result (Ravisankar *et al.*, 2015). In Ghana monitoring of metals in environmental samples is difficult due to the availability of instruments and the results obtained may be questionable because of inadequate quality control measures in place at laboratories. The objective of the survey was to identify various laboratories where metal analyses are carried out in Ghana. Again, the aim of the interlaboratory experiment was to gain more confidence in results obtained from laboratories in Ghana and also to have Certified Standard Reference Materials (CRMs) for water.

Materials and Methods

Location

The study was done in Ghana, which comprises of 10 administrative regions. Ghana lies along the Gulf of Guinea in West Africa, within longitudes 305'W and 1010'E and latitudes 4035'N and 110'N. It covers an area of about 238,540 Km² and shares borders with Côte d'Ivoire to the west, Burkina Faso to the north, Togo to the east, and to the south is the Atlantic Ocean. According to Ghana Statistical Service (2010) the total population of Ghana in 2010 stood at around 24 million. The annual intercensal rate of growth for the country has declined from 2.7 percent in 2000 to 2.4 percent in 2010.

Sample size

Every endeavor to, in this preliminary work, to identify laboratories in Ghana that use atomic spectrophotometer was made but due to the difficulty in identifying the laboratories in various regions, time, cost and some laboratories refusal to participate in the exercise, the data reported in this study are for four regions. The author acknowledges that some laboratories within the regions might not have been captured, unintentionally, due to the reasons stated earlier. During the search (from February to June, 2018) for laboratories where metals are determined, 15 laboratories were found and agreed to be interviewed (sample size, n = 15). The inclusion criteria were; ability to give verbal consent, and willingness to participate in the study. Agreement to be interviewed was obtained from the

respondents. This study was limited to laboratories where the instruments are currently in use and metals are determined with the instrument (where there is no lamp for the metal or standard solution it was ignored).

Questionnaire Development

In preparation for the survey, questions were formulated based on responses received from interviews conducted with an F-AAS operator in a laboratory in Kumasi, Ghana. Subsequently, a 10-item prototype questionnaire was developed, reviewed for clarity and appropriateness, and then pretested on two AAS operators. After pretesting, a revised 12-item questionnaire was used in the survey. The questionnaire contained a mixture of closed-ended and open-ended, with closed-ended questions predominating. Some of the questions include name and location of the laboratory, model of equipment, type of equipment, cost for the determination of a metal and calibrations.

Materials and Reagents for Inter-Laboratory Study

De-ionized water ($5 \mu\text{Scm}^{-1}$) was produced in house with a Milli-Q water gradient system (Millipore, Bedford, MA, USA). Ultra-pure HNO_3 (60%, analytical reagent grade) was purchased from Lab-Scan (Gliwice, Poland). A 1000 mg/L Pb and Cu stock standards were purchased from Merck KGaA (Darmstadt, Germany).

Spiking mixture standard solutions: spiked standard solutions ($0.5 \mu\text{g/mL}$) for Cu and Pb were prepared by diluting 100 μl of each metal's stock solution ($1000 \mu\text{g/ml}$) to 200 ml with 0.3% HNO_3 . Spiked standard solutions ($2.0 \mu\text{g/mL}$) of Cu and Pb were prepared by diluting 400 μl of each metal's stock solution ($1000 \mu\text{g/mL}$) to 200 ml with 0.3% HNO_3 . Spiking solutions were stored in freezer at -5°C before sending them for analysis. Metal determinations were carried out by flame atomic absorption spectrometer. The model, instrument detection limit and cost of analysis of various laboratories engaged in the comparative study are presented in Table 1.

Table 1: Characteristics of laboratories participating in the inter-comparison exercise

Lab	Cost of analysis (GH per metal)	Frequency of calibration	Model of Instrument	Instrument detection limit (ppm)	
				Cu	Pb
Lab 1	15	Everyday	AAS 900T	0.02	0.04
Lab 2	25	before every analysis	Analyst 200	0.01	0.05
Lab 3	20	before every analysis	Novaa 400P	0.2	1.0
Lab 4	10	every week	Buck scientific 210VGP	0.01	0.01

Data Analysis

Data from the questionnaire were tabulated and analyzed using descriptive statistics. Analysis of cost differences on type of metal analyzed, frequency and duration of calibration was done with one-way analysis of variance (ANOVA) with post hoc comparisons testing using Dunnett C after a Bonferroni adjustment. The p-value was considered statistically significant at $p < 0.05$. Analysis was performed with the Statistical Package for the Social Sciences (SPSS, Version 20, Chicago, Ill, United States). For the inter-laboratory study, Accuracy was determined by comparing the measured concentration with the certified values (prepared concentration) and was expressed as percentage recovery; R (%). A significance test (t-test) was applied to test if the recovery is significantly different from 100%.

Results and Discussion

Identification of Laboratories with AS Instrument

Table 1 shows the number of laboratories identified in four regions of Ghana. In all, 15 laboratories were identified, six (40 %) in Greater Accra region, six (40 %) in Ashanti region, two in Western region and one in the Eastern region in Ghana (Table 2). The common metals analyzed by all the laboratories are Cu, Pb, Fe, Mn, Mg and Zn. The type of atomic spectrometric instrument used by all the laboratories was F-AAS representing 100%. Additional atomic spectrometric techniques like electro-thermal were identified in two laboratories, hydride generation in two laboratories and Inductive Coupled plasma -AAS was found in one laboratory. The predominant models of instrument found were Agilent, Varian, Analyst, Novaa and Buck scientific. The cost of analyzing a metal ranged from 10 to 60 Ghana cedis (GHC) per metal (Table 2). In terms of the cost of analysis of metals in the laboratories, 20%, 33.3%, 13.3%, 13.3%, 6.7%, and 13.3% of the interviewed laboratories belonged to the amount (cost) categories of GHC10 to GHC19; GHC20 to GHC29; GHC30 to GHC39; GHC40 to GHC49; GHC50 to GHC59 and GHC60 to GHC69, respectively. Sixty (60) percent of the laboratories identified, calibrate their equipment before conducting any analysis – 13.3% weekly, 6.7% every month, 6.7% every six month and 13.3% annually. One-way analysis of variance (ANOVA) of cost analysis frequency, type of metal analyzed frequency and duration of calibration frequency showed a significance variation ($p = 0.007$). However, the post hoc comparisons testing using Dunnett C after a Bonferroni adjustment revealed that the significance is between cost of analysis frequency and type of metal analyzed frequency; and type of metal analyzed frequency and duration of calibration frequency. No significant difference ($p = 0.2$) existed between cost of analysis frequency and duration of calibration frequency.

Table 2: Selected questionnaire items and number of respondent

Selected Questionnaire items	Total Sample (n = 15)	
	No.	%
Location of lab (Region)		
Ashanti	6	40.0
Greater Accra	6	40.0
Western	2	13.3
Eastern	1	6.7
Type of Atomic Spectrometer		
F – AAS	15	100.0
ET – AAS	2	13.3
HG – AAS	2	13.3
ICP – AAS	1	6.7
Metals that can be analyzed		
Essential metals	13	86.7
Trace metals	12	80.0
Toxic metal	9	60.0
How often calibration of instrument is done		
Before every analysis	9	60.0
Every week	2	13.3
Every month	1	6.7
Every six month	1	6.7
Yearly	2	13.3
Cost of analysis (GHC per metal)		
10 – 19	3	20.0
20-29	5	33.3
30-39	2	13.3
40-49	2	13.3
50-59	1	6.7
60-69	2	13.3

Respondents were asked which technique they use in determining toxic metals like As, Hg, Pb and Cd. The results indicated that 20% determine As with F-AAS and 13.3% use HG-AAS. For Hg, only 26.7% of all the laboratories determine this metal and also with F-AAS (Table 3). The ideal technique for determining As and Hg is HG-AAS (Christian *et al.*, 2014). In all, 86.7% of laboratories indicated using F-AAS to determine Pb, 6.7% with ET-AAS and 6.7% with ICP-AAS. Of all the labs identified, only 26.7% analyze Cd, comprising of 20% using F-AAS and 6.7% using ICP-AAS.

Table 3. Respondents' responses when asked "what type of atomic spectrophotometer used"

Toxic metal	F-AAS No. of respondents (%)	ET-AAS No. of respondents (%)	HG-AAS No. of respondents (%)	ICP-AAS No. of respondents (%)
As	3(20.0%)		2 (13.3%)	
Hg	4 (26.7%)			
Pb	13 (86.7%)	1 (6.7%)		1 (6.7%)
Cd	3 (20.0%)			1 (6.7%)

Recovery studies discourse

The acceptable target limit of recovery for any analytical method is 60 to 105% (Venn, 2008). The lowest recovery (378%) and the highest recovery (95.2%) were observed for Cu at a concentration close to the instrument LOD (0.5 ppm). The result revealed that only Lab 2 recovery met the acceptable target. The recovery obtained for Cu using spiked concentration of 2 ppm ranged from 374 to 106.9% (Table 4). At this concentration all the labs except Lab 4 met the acceptable target.

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Result for the analysis of 0.5 and 2 ppm spiked water samples revealed that, Lab 1 had recovery of 27.4%, 73.7% for copper while Pb recorded recovery of 156.6% and 100.6%, respectively (Table 4). The recovery of Cu and Pb metals at higher concentration (2 ppm) meet the acceptable limit for recovery but the recovery for lower concentration (0.5 ppm) of Cu and Pb metal did not meet the acceptable target. The recoveries obtained for Lab 2 at the two spiked concentration of 0.5 and 2 ppm were 95.2% and 106.9% for Cu, and 72.8% and 113.5% for Pb, respectively. Except the recovery of Pb metal at higher concentration (2 ppm) which did not meet the acceptable target, the rest fell within the acceptable range of recovery. For Lab 3, the recovery values were 57.42% and 98.8% for Cu, and 50% and 60.1% for Pb, respectively. At higher concentration of spiked samples, the recoveries for both Cu and Pb were within the acceptable target but not so for the lower concentration of spiked water samples. A similar trend was observed for Cu spiked recovery done at Lab 3, where recoveries of 89.4 and 92.7% were recorded using spiked concentrations of 4 ppm and 8 ppm, respectively (Abubakar, 2017). Finally, Lab 4 recovery values are 378% and 374.5% for Cu, and 339.6% and 384.9% for Pb (Table 4) which are far beyond the acceptable target. Close observation of the result received from Lab 4, indicate that the concentration values were falsified. The reason for this claim is that the bottles containing the spiked samples were labelled as 2 ppm and 8 ppm. This label was on all the samples sent to the

four laboratories for the analysis, hence for Lab 4 to report concentration close to what was written on the sample bottle could indicate falsification of data. Again, Pb recovery study performed by Abubakar, (2017) at Lab 4, found recoveries of 26% and 17.9% at 4 ppm and 6 ppm spiked concentration. These values being far below accepted limit indicate that results obtained from Lab 4 could be altered or unreliable. A significance test (t-test) applied to test if the recoveries were significantly different from 100% revealed no significant difference with p-values ranging from 0.37 to 0.66.

Table 4. Results of concentration and recovery for Cu and Pb spiked samples

LAB	RECOVERY (%)			
	Copper		Lead	
	Low conc. (0.5ppm)	High conc. (2ppm)	Low conc. (0.5ppm)	High conc. (2ppm)
Lab 1	0.14 ppm (27.4%)	1.47 ppm (73.7%)	0.78 ppm (156.6%)	2.01 ppm (100.6%)
Lab 2	0.48 ppm (95.2%)	2.14 ppm (106.9%)	0.32 ppm (72.8%)	2.27 ppm (113.5%)
Lab 3	0.29 ppm (57.4%)	1.98 ppm (98.8%)	<1 ppm (50%)	1.20 ppm (60.1%)
Lab 4	1.89 ppm (378%)	7.49 ppm (374.5%)	1.70 ppm (339.6%)	7.70 ppm (384.9%)

Conclusion

The most commonly used atomic spectrometric instrument in laboratories in Ghana to determine metals is F-AAS representing 100% and the common metals analyzed by most laboratories in Ghana identified are Cu, Pb, Fe, Mn, Mg and Zn. The recovery results showed at higher concentration (2 ppm), recoveries for metals studied were within the acceptable target but not so for the lower concentration of spiked water sample. The ability of Lab 2 to provide recovery results which were within the acceptable limit shows that internal quality control and proper instrumentation measures might have been put in place to obtain reliable results. Contrariwise, metal analysis at Lab 4 is cautioned since the result from the laboratory might be unreliable. It is recommended that the study be extended to other laboratories and number of metals increased and certified reference materials should be used instead of spiked standard solution. The laboratories should perform analytical validation routinely to ensure quality of data.

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