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Anodic stripping voltammetry determination of Pb, Cd, Zn, and Cu in blood samples of children in some areas of Ibb governorate

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Abstract

Anodic stripping voltammetric determination of Pb, Cd, Zn and Cu in blood samples of 132 children was developed. The children's ages range was between 6 months and 6 years and the samples were collected from different areas of Ibb governorate (Yemen). We used anodic stripping voltammetry for analyzing and determination of cited metals in the blood of all children. Large variation in the results have been correlated to the area inhabited, age differences and other factors. It was found that areas, which have environmental struts such as waste solid and waste water, had more quantities of these metals in blood samples in comparison with other safer sites. This is may be due to the lower concentration of Cd and Pb in samples. Also the results indicate that both Pb, Cd found in high mean concentration in blood samples of males children than in females children similarly the effect of age indicate that all younger children (between 6 months and 1 year) had shown comparatively lesser quantity of these metals in comparison with elder children (between 4 – 6 yeas).

Keywords: Anodic stripping voltammtery; Determination; Heavy metals; Children; Blood.

1. Introduction

The increasing pollution with many heavy metals which are harmful for the growth of the living organisms has been the subject of considerable interest [1]. The development of the industry and expansion of the chemical compounds used in different branches of industry are leading to the environmental spread of heavy metals [2]. To many people, heavy metal pollution is a problem associated with areas of intensive industry. However, roadways and automobiles now are considered to be one of the largest sources of heavy metals [3].

The population of women exposed to lead either at work or in their environment show a correlation between high lead content in the blood and the low birth weight of their children [4]. It was also shown that lead toxicity may cause mental and psychomotor retardation of children without encephalopathy [5, 6]. Cadmium toxicity may cause premature birth, low birth weight and even disturb production of chronic gonadotrophin by the placenta and impair development of the near born vascular system [7]. It was demonstrated that Cadmium effects on the genes were involved with growth regulation of initiated cells [8].

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Since the blood brain barrier is not fully developed in young children, their developing nervous system accumulates the ingested lead resulting in neurobehavioral barrier. As the lead crosses the placental barrier, the developing fetus is also at high risk of lead poisoning from mother's blood [9]. Moreover the normal level of cadmium in blood is under $5 \mu\text{g L}^{-1}$ but acute toxic effects observed when this level exceed $50\mu\text{g L}^{-1}$ [10]. Greater prevalence of iron deficiency in young children also increases the gastrointestinal absorption of lead [11]. However in case of Cadmium placenta acts as a fairly effective barrier and hence the new born are virtually free of Cadmium. It was also shown that accumulation in the body increases with age and at the age of 50, non-occupationally exposed people may have 10-50 mg body burden of Cadmium [12].

Zinc is an important nutritive factor as well as a co-factor for many metalloenzymes. It is naturally present in blood at a high level $10^{-5} \text{ mol L}^{-1}$ [13], and necessary for the growth and division of cells, especially during the states of life when growth rates are high [14, 15]. Copper indicates the mobilization in the mother's body during pregnancy. Its deficiency may cause disorders [16]. Copper in blood is bound to serum albumin [17]. The main physiological processes in which copper participates in the formation of blood and the utilization of iron in hemoglobin synthesis, the synthesis and cross-linking of elastin and collagen in the aorta and major blood vessels, etc. So both copper and zinc are known to be beneficial for humans when present in low concentration only; but Lead and Cadmium are known to be toxic even at very low concentration [18, 19].

The homeostasis of a particular mineral involves different mechanisms depending on the organ involved; with participation of body tissue fluids, subject to modulation and high mobilization. The study of biological fluids and other materials, such as blood plasma and fecal material, is relevant as function indicative of underlying normal biochemical processes, living conditions and potential diagnostic tool to identify a disease [20]. Levels of metals in the blood are considered as an index of biologically active metal in the body, reflecting also the environmental expose of a population. Most metals are toxic at high concentration while other provokes deleterious effects at low concentrations. Heavy metals are pollutants of biological interest due to their biotoxicity [21].

Differential pulse stripping voltammetry (DPSV) is relatively inexpensive and is one of the most sensitive and selective techniques in the determination of trace amounts of metals at natural levels [22-24]. In the present work we study the use of differential pulse anodic stripping voltammetry for the evaluation and determination of trace metals in child blood whose age between 6 months and 6 years, in some areas of Ibb governorate, Yemen.

2. Experimental

2.1. Apparatus

Stripping voltammetric experiments were carried out with a Metrohm 746VA (Herisau, Switzerland). Trace Analyzer connected to a Metrohm 747VA multimode electrode used in the hanging mercury drop electrode (HMDE) regime. A platinum rod and saturated Ag/AgCl electrodes were used as auxiliary and reference electrodes respectively. pH was measured with a digital pH-meter JENWAY, model 3310. Dissolved oxygen was removed from the samples by purging with purified nitrogen (99.99%) through the measuring vessel for 5 min. During the experiments, nitrogen was passed over the solution to prevent oxygen interference.

2.2. Chemicals

The tartaric acid, CH_3COOH , CH_3COONa , $\text{Pb}(\text{NO}_3)_2$, $\text{Cd}(\text{NO}_3)_2$, $\text{Cu}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$ and HNO_3 used were from Merck, Darmstadt, Germany. De-ionized water was used to prepare all solutions. The metal stock solutions (1 g L^{-1}) were prepared in $0.005 \text{ mol L}^{-1} \text{ HNO}_3$. The

working standard solutions were prepared daily by suitable dilution of this stock solution in the required matrix. The acetate buffer solution pH 4.7 was used as the supporting electrolyte. All glassware were stored in 8 mol L⁻¹ nitric acid for 1 week and rinsed thoroughly with de-ionized water.

2.3. Sampling and sample pretreatment

From October 2007 to may 2008, one hundred and thirty two (132) Yemeni children, aged between 6 months and 6 years, were collected at the governmental hospitals in Ibb city for estimating the impacts of water, food and atmospheric pollutions on the human health. Their bloods are considered as an index of biologically active metal in the body reflecting direct effect of pollutions on populations. Blood samples were analyzed for four heavy metals of Cd, Pb, Zn and Cu. Data of hemoglobin, malnutrition and weight measurements of the same children was collected in order to correlate these parameters with the occurrence of heavy metals in blood samples.

2.0 mL blood samples were taken from each child with special care by vein puncture using disposable syringes and needles and placed into heparinized pretreated clean polypropylene tubes. The sample was then digested with 4ml of 70% nitric acid and 1ml of 70 % perchloric acid 60 %, heating to dryness. The residue was wet with 1 mL nitric acid 65 % and 9 mL distilled water, and then the crucible was boiled on a hot plate to the complete dissolution of the residue. The sample was then quantitatively transferred to 25 mL volumetric flask and diluted to the mark.

2.4. Voltammetric determination

A 0.5 mL of digested sample was directly transferred to the voltammetric cell containing 20 ml of acetate buffer pH 4.7. The solution in the cell was aerated for 120 s by purging pure nitrogen gas. A fresh mercury drops working electrode (HMDE) was extruded, then the stirrer and electrolyze were started for 90 s at -1200 mV vs Ag/AgCl .The stripping voltammogram for zinc, cadmium, lead and copper was recorded after arrest period of 10 s after stirrer stopping. The potential was swept using differential - pulse anodic stripping voltammetry (DPASV) deposition Potential of -1200 mV, deposition time of 240 s, a scan rate of 60 mVs⁻¹, and a pulse amplitude of 50 mV. The standard additions technique was used to give the concentrations of Cd, Pb, Cu and Zn simultaneously when a sweep potential was applied between -1200 mV and 200 mV (vs. Ag/AgCl electrode).

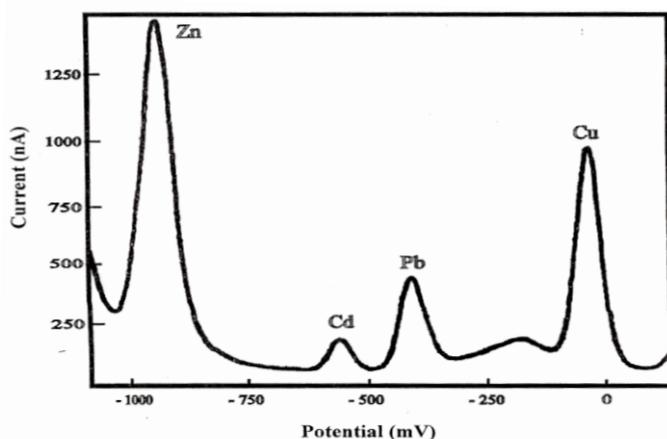


Fig. 1. Typical voltammogram of 0.5 mL digested after dilution to 20 mL. Deposition potential: 1200 mV, deposition time 240 s for Cd, Pb, Cu and 120 s for Cu, Zn; scan rate 60 mV s⁻¹; pulse amplitude 50 mV.

The stripping voltammetric measurement was repeated must be done at least twice, 100 μL of standard metal solutions was added (standard addition calibration method), and the new voltammogram was recorded again. Voltammogram of these elements is shown in Fig. 1. The concentrations were calculated after the second standard addition, using linear regression method.

2.5. Statistical analysis

Statistical analysis of data was carried out using SPSS (version 16) program. The means and standard deviations and prevalence were obtained by descriptive statistics. Data were analysed using the one way analysis of variance (ANOVA) followed by T- test to assess difference of continuous variable between two or more groups. Statistical significance was assigned for $p < 0.05$.

3. Results and Discussion

The calibration curves were linear in the concentrations range of 0.5-600 $\mu\text{g L}^{-1}$ with a correlation coefficient lies between 0.9955-0.9992 for the four elements. Based on the calibration curve, the limits of detection were also determined. The detection limits is defined as that concentration for which the signal-tonoise ratio (SD/N) equals 3 [25]. The detection limits were obtained as 1.25 $\mu\text{g L}^{-1}$ Pb, 0.18 $\mu\text{g L}^{-1}$ Cd, 2.60 $\mu\text{g L}^{-1}$ Cu and 3.11 $\mu\text{g L}^{-1}$ Zn. The precision of the proposed method was estimated by calculating the relative standard deviation (RSD %) which were 3.51 %, 4.18 %, 2.79 % and 3.24 %, respectively.

3.1. Effect of location

The living locality has impact on occurrence of heavy metals in blood samples, locations with industries waste solid, wastewater, vehicular and other such sources that emit various metals to the inhabitants through a variety of routes [26]. In our study, we had chosen four sites from different locations of Ibb governorate Fig.2. Table 1 shows the concentration of heavy metals in blood samples collected from different locations of Ibb governorate.

Table 1

Effect of area distribution of Pb, Cd, Cu and Zn ($\mu\text{g L}^{-1}$) in blood samples of children aging between 6 months and 6 years.

Location	n	Pb $\mu\text{g L}^{-1}$	Cd $\mu\text{g L}^{-1}$	Cu $\mu\text{g L}^{-1}$	Zn $\mu\text{g L}^{-1}$
		(P=0.003)	(P=0.002)	(P=0.001)	(P=0.003)
		X \pm SD	X \pm SD	X \pm SD	X \pm SD
		Min - max	Min - max	Min - max	Min - max
Almashana	36	50.56 \pm 16.52*	3.90 \pm 1.12*	1674.52 \pm 556.44	2143.07 \pm 876.67
		24.81 - 94.24	1.39 - 9.64	1000.60 - 2736.88	1132.60 - 4055.14
Aldehar	36	40.48 \pm 17.35*	3.41 \pm 1.28*	2391.85 \pm 780.50	2740.27 \pm 1201.37
		17.42 - 96.02	1.10 - 9.86	1115.20 - 2442.10	1016.70 - 5166.62
Refibb	36	61.66 \pm 26.12	5.14 \pm 0.73	1962.47 \pm 847.84*	1230.12 \pm 374.30*
		19.30 - 98.25	1.32 - 9.18	1118.50 - 4541.52	1022.62 - 2040.14
Alodian	24	32.23 \pm 13.47	2.46 \pm 0.58	2258.54 \pm 976.70*	2320.12 \pm 946.32*
		17.61 - 46.98	1.20 - 6.32	1136.60 - 4350.95	1011.03 - 4830.65

X = mean, SD = Standard deviation, P = significance, *P < 0.05 significance.

It can be seen from Table 2 that all the samples mean that were taken from Almashana and Refibb 50.56 ± 16.52 , $61.66 \pm 26.12 \mu\text{g L}^{-1}$ for Pb and 3.90 ± 1.12 , $5.14 \pm 0.73 \mu\text{g L}^{-1}$ for Cd. The results showed significantly higher ($p < 0.05$) of lead and cadmium than the other areas. This was due to the presence of metal based pollution of waste solid, wastewater, groundwater and vehicular [27-29], main high way that discharge large amount of lead and cadmium. However, quantities of zinc and copper in these two locations showed an inverse trend. This could be related to low level of hemoglobin malnutrition in these children, Table 2. It's seen that the children with malnutrition or with hemoglobin level less than 6 mg L^{-1} have low level zinc and copper. Kandzierska was found similar types of correlations; it was difficult to correlate the effects of pollutants and concentrations of hemoglobin and malnutrition [18].

Table 2
Clinical parameters of children.

Site. No.	Location	n	Grade of Malnutrition	Rang of Haemoglobin (gm%)	Average of Weight (Kg)
1	Almashana	36	Made	5.8 – 8.6	7.2
2	Aldehar	36	Normal	8.4 – 11.4	13.7
3	Refibb	36	Made	7.5 – 10.7	8.6
4	Alodian	24	Normal	8.6 – 12.1	15.2

It can be seen from the Table1 that samples from Aldehar and Alodian, which are comparatively safer, showed lesser concentration of cadmium and lead comparison to Almashan and Refibb. However, higher concentration of zinc and copper at Aldehar and Alodian (2391.85 ± 780.50 , $2258.54 \pm 976.70 \mu\text{g L}^{-1}$ for Zn and 2740.27 ± 1201.37 , $2320.12 \pm 946.32 \mu\text{g L}^{-1}$ for Cu showed significantly higher ($p < 0.05$), can be related to the fact that all these samples were having the largest concentration of hemoglobin and they were having the largest body weights [30].

Table 3
Effect of sex children distribution of Concentration Pb, Cd, Cu and Zn ($\mu\text{g L}^{-1}$) in blood samples.

Sex of children	n	Pb $\mu\text{g L}^{-1}$ (P=0.027)	Cd $\mu\text{g L}^{-1}$ (P=0.017)	Cu $\mu\text{g L}^{-1}$ (P=0.012)	Zn $\mu\text{g L}^{-1}$ (P=0.018)
		X± SD	X± SD	X± SD	X± SD
		Min - max	Min - max	Min - max	Min - max
Male	78	51.36 ± 21.26	4.59 ± 1.37	2078.23 ± 765.8	2514.6 ± 1107.33
		17.61 - 96.02	1.20 – 9.86	1000.60 – 4350.95	1011.03- 5166.62
Female	54	45.42 ± 18.15	3.35 ± 0.91	2146.5 ± 968.07	2143.24 ± 812.29
		15.14-98.25	1.10 – 8.28	1115.20 -4541.52	1145.93- 4055.14

X = mean, SD = Standard deviation, P = significance, P< 0.05 significance

So it can be said that all these samples which were taken from anemic and malnourished children had shown lower concentration of zinc and copper in comparison to those that were normal. It is well documented that a small increase in the lead levels in the air can lead to a

higher concentration in blood level of lead [31]. Similar results are observed for Aldehar showing higher concentration of lead probably due to higher vehicular population and this higher amount of lead in the air.

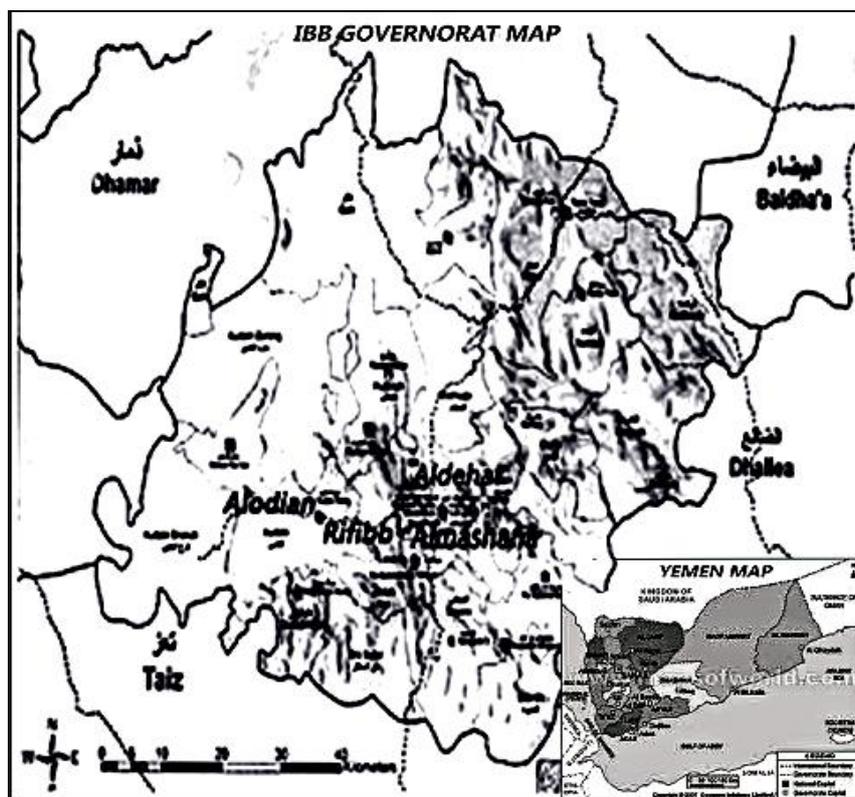


Fig. 2. Map of Ibb government with the location of sites from where the blood sample were collated.

3.2. Effect of sex

Effects of sex on the uptakes of these metals are presented in Table 3. Though, the variation appears small with regard to lead and cadmium among both sexes, a larger variation can be seen with zinc and copper. lead, cadmium and zinc are found in higher concentration in males than female children 51.36 ± 21.26 , 4.59 ± 1.37 and $2514.6 \pm 1107.33 \mu\text{g L}^{-1}$, respectively. The results of our study metals showed significantly ($p < 0.05$) that males tend to have higher blood than females. All males' children showed higher concentration of copper than the female children, and this may be due to lower levels of hemoglobin and lesser number of red blood cells in female children. Similar results were obtained by many workers [24, 32].

3.3. Effect of Age

All the 132 samples were categorized into four different age groups as shown in Table 4. It is evident from Table. 4 that all the children whose age were between 6 months -1 year and 1-2 years have lower mean concentration of all these metals except lead (35.83 ± 13.37 , $22.28 \pm 4.46 \mu\text{g L}^{-1}$). The results showed significantly higher ($p < 0.05$) among the age group (6 months -1 year and 1-2) years compared to other age groups. The children aged between 4-6 years show maximum mean concentration of metals except lead. The results show that there is a direct correlation between the age and concentration of the metals in the blood and this may be due to the longer length of expose in older children [33, 34]. Exception of lead can be related to other

factors, most of the samples from aged between 4-6 years were taken from Aldehar, Almshana and Refibb where a heavy vehicular pollution could be responsible.

Table 4

Effect of age of children distribution of concentration Pb, Cd, Cu and Zn ($\mu\text{g l}^{-1}$) in blood samples.

Age	n	Pb $\mu\text{g L}^{-1}$ (P=0.009)	Cd $\mu\text{g L}^{-1}$ (P=0.007)	Cu $\mu\text{g L}^{-1}$ (P=0.002)	n $\mu\text{g L}^{-1}$ (P=0.001)
		X \pm SD	X \pm SD	X \pm SD	X \pm SD
		Min - max	Min - max	Min - max	Min - max
6 M –	31	35.83 \pm 13.37*	3.43 \pm 1.72*	2209.73 \pm 864.80	2646.05 \pm 968.50*
1Y		20.5 – 56.5	1.01 – 5.29	1115.20 – 4350.95	1811.32 - 4830.65
1Y -	20	22.28 \pm 4.46*	2.43 \pm 1.05*	2275.70 \pm 1157.28	3138.12 \pm 1309.84
2Y		17.61– 28.85	1.41 – 5.30	1225.14 – 4541.52	2372.56 - 5166.62
2Y –	35	48.36 \pm 10.21*	3.95 \pm 2.15	2383.56 \pm 526.99	2846.90 \pm 1185.92
4Y		29.93- 65.45	1.20 – 9.64	1353.86 – 3497.64	1758.04- 5055.74
4Y –	46	68.82 \pm 23.01	6.53 \pm 2.17	1774.40 \pm 576.1	2063.95 \pm 771.63*
6Y		26.06 - 98.25	1.50 – 9.86	1000.60 – 3226 2	1011.03 - 3814.13

M = Month, Y= Year, X = mean, SD = Standard deviation, P = significance, *P< 0.05 significance

It was also known that lead passes through the placenta easily and fetal blood has almost the same blood lead concentration as maternal blood [31]. Therefore, exceptionally higher concentration of lead in the blood of all the infants from Almshana, Refibb and Aldehar may be because of higher exposure of their mother's to the vehicular pollution. However, The mean of cadmium in the infants ages between 6 months -1 year and 1-2 years remained lower 3.43 ± 1.72 , $2.43 \pm 1.05 \mu\text{g L}^{-1}$ because in this case placenta acts as a fairly effect barrier to cadmium and hence new born are virtually free from cadmium. Accumulation of cadmium in the body increase with age, as shown by other workers [32, 35]. It is also known that cadmium is always found in association with zinc, so all samples show higher concentration of cadmium but lower concentration of zinc [36, 37].

Although, there are a direct correlation of concentration of blood levels of metals with age because of higher exposure. It can be concluded that all the younger children, because of their lesser exposure are at lower risk to the pollutants than their elder counterparts and all those, which are living areas pollutant, are increasing their bloods metal content with passage of time.

4. Conclusion

We conclude that, all those children who are residing in and around pollution areas (waste soil, wastewater and dense vehicular) at higher risk of the environmental stress than those from safer sites. The increase in concentration of metals in children's blood explain the accumulating nature of heavy metals; this may reach a dangerous level if an immediate measures are not taken to shift from the location.

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