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Original Article

Stripping Voltammetric Determination Of Zinc, Cadmium, Lead And Copper In Blood Samples Of Children Aged Between 3 Months And 6 years

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Abstract: Blood samples of 160 children, ranging age between 3 months and 6 years were selected from five different parts of Amritsar district of Punjab (India) and were analyzed for Zn, Cd, Pb and Cu using anodic stripping voltammetry. Large variations in the results have been correlated to the area inhabited, age differences and other factors. It was found that the areas, more prone to environmental stress, had shown more quantities of these metals in blood samples in comparison to those which were taken from safer sites. Similarly the younger children lesser exposed to environmental pollution had shown comparatively lesser quantity of these metals in comparison to older objects.

Key Words: Stripping voltammetry, Blood, Zinc, Cadmium, Lead, Copper.

Introduction

Metals like cadmium and lead are not known to be essential for the functioning of biological systems and the general view is that wherever possible the exposure to these metals should be kept as low as possible. Cadmium in blood reflects recent exposure rather than the body burden.[1] A WHO study group (1980) agreed that a value of 10 μg/L of cadmium in whole blood should be accepted as a tentative non-adverse effect level and in 1972 a FAO/WHO Expert Committee recommended that the maximum intake level of lead should be restricted to 3 mg/week (0.05 mg/kg of body weight) for adult; however, no corresponding values for children were suggested, which clearly indicated that children should be kept as free from these metals as possible because children have a great potential for lead and cadmium exposures and are uniquely susceptible to their toxic effects. It has been estimated that 3 to 4 million children in USA are at an elevated risk of lead poisoning.[1] Effects in children generally occur at lower blood levels than in adults.

Since the blood brain barrier is not fully developed in young children, the developing nervous system accumulates the ingested lead, resulting in neurobehavioral disorders. As the lead crosses the placental barrier, the developing fetus is also at a high risk of lead poisoning from mother's blood. Moreover the greater prevalence of iron deficiency in young children also increases the gastrointestinal absorption of lead. However in case of cadmium, placenta acts as a fairly effective barrier and hence the new born are virtually free of cadmium. Accumulation in the body increases with age and at 50, non-occupationally exposed people may have 10-50mg body burden of the cadmium.[2]

Zinc and copper occur in almost all food stuffs. The amount of these metals in various foods varies with the amount of these metals present in the soil on which they are grown. Zinc is indispensable to all forms of life and is now known to be integral component of large variety of proteins and enzymes. Copper in blood is bound to serum albumin.[3] The main physiological processes in which copper participates are 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.[4]

The level of metals in the blood is considered as an index of biologically active metal in the body, reflecting also the environmental exposure of a population. Hence concentration of metal in the blood is a significant factor for child health. Population studies that relate metal concentration in blood to environmental exposure may yield useful information for any health care program anchored on pollution control measures. In India there is a scarcity of data on exposure assessment of toxic trace metals for children.

Materials and Methods:

From June 2002 to May 2004, one hundred and sixty (160) children, aged between 3 months and 6 years, were selected at the Govt. Hospital, Amritsar for estimating impacts of atmospheric pollution on the human health, children being more sensitive to the pollutants and more at risk as compared to the adult individuals. Their blood is considered as an index of biologically active metal in the body, reflecting direct interaction of atmospheric pollution of a population; hence were analyzed for four heavy metals, viz: Zn, Cd, Pb and Cu. Data about 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. Selections of the samples were made in such a way that effects of industrial pollution, age and sex could be studied and correlated.

Apparatus and Reagents

The measurements of zinc, cadmium, lead and copper were performed with Metrohm (μ-Autolab, Type II) equipped with static mercury dropping electrode. The three electrode system consists of a working hanging mercury dropping electrode (HMDE), a platinum auxiliary electrode and a Ag/AgCl saturated with KCl as reference electrode. All the reagents used were of Analytical Grade. A stock solution of 1000 ppm of each metal was prepared by taking

99.99% of pure metal (Aldrich). Solutions of low concentrations were prepared by serial dilutions with de-ionized double distilled water.

Sample Preparation:: Approximately 2ml 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 samples (1ml) were then digested with nitric acid and perchloric acid. Digested samples were made upto 5ml using 0.25% nitric acid. Special care was taken to avoid all contaminations.

Voltammetric Measurement: 0.5 ml of digested sample was transferred into the voltammetric cell containing 20ml of ammonium acetate buffer (pH 8.3). The solution in the cell was aerated for 5 minutes by purging pure nitrogen gas. Anodic stripping was performed in differential pulse mode after selecting pre-concentration time of 180s, a scan rate of 4 mV/s and pulse amplitude of 50 mV. The concentrations of all the metals in the samples were determined using standard addition method. Reagent blanks were taken along with each batch of samples and the metal concentration observed in these blank samples were subtracted from the corresponding batch of field samples. Blank samples always showed extremely low levels of trace metals.

Quality Assurance: The reliability of the procedure has been checked by analyzing various standard reference materials. Standard reference materials like animal blood (A-2), fish tissue (MA-B-3/TM) and Hay (V-10) from the International Atomic Energy Agency (IAEA), Vienna, were analyzed for zinc, cadmium, lead and copper. The results agree within ±.000007% with certified values (Table 5). The validity of the method was further ascertained by cross method check, spike recovery and replicate analysis.

Results And Discussion:

Effect Of Location: It has already been well documented by many workers that living locality has a great impact on occurrence of heavy metals in blood samples. Locations with industries and other such sources that emit various metals to the environment are known to contribute a great deal of metals to the inhabitants through a variety of routes.[5] In our study we had chosen following five sites from different locations of Amritsar (Fig.1):

[1] **Focal Point** is an industrial pocket of various large and small-scale units, producing a wide range of products like lead batteries, dyeing and processing, pharmaceuticals, paint, rubber, bulk drugs, etc. contributing a wide variety of pollutants to the city.

[2] **Majitha Road** is an industrial area situated on the main highway with mostly textile and chemical units. The polluted underground water (due to leaching) had already been found to be a problem in this segment[5].

[3] **Khankot Village,** present on the outskirt of the city, is chosen as a safer region with a view to compare the results with other polluted sites.

[4] **Azad Nagar** is a safer area located far away from industries and main high-way.

[5] **Green Avenue** is relatrively free from industries, but has a dense vehicular traffic and hence was chosen to study the effect of vehicular pollution.

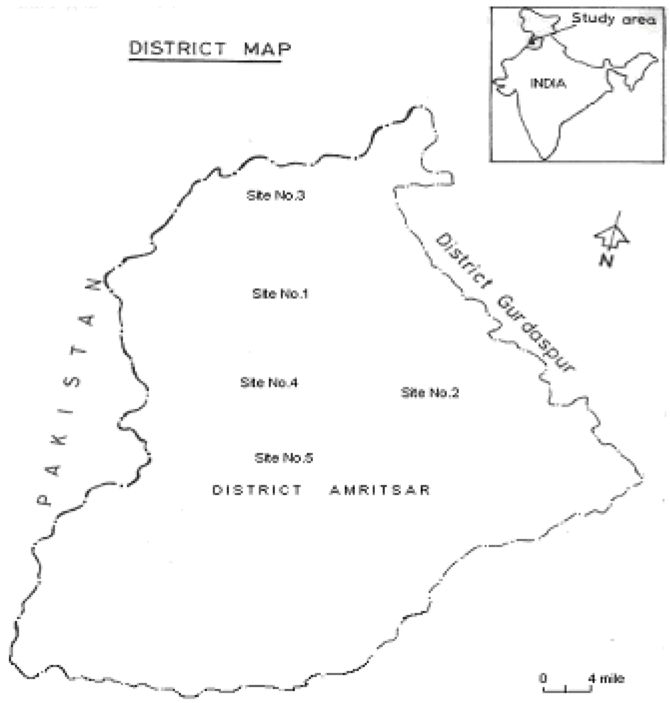

Fig.1 Map of Amritsar District with the location of sites from where the blood samples were collected.

Table 1 shows the concentration of heavy metals in the blood samples collected from different locations of Amritsar. It can be seen from Table 1 that all the samples which were taken from sites 1 and 2 showed higher quantities of lead and cadmium than the other areas. This was due to the presence of metal based industries that discharge a large amount of lead and cadmium containing effluent. However, quantities of zinc and copper in these two locations showed an inverse trend. This could be related to low level of hemoglobin and malnutrition in these children (Table 2). It is seen that the children with malnutrition or with hemoglobin level less than 6 mg/L have low level of zinc and copper. Similar types of correlations were found by some workers.[3] However, it was difficult to correlate the effects of pollutants and concentrations of hemoglobin and malnutrition.

Table 1: Area-Wise distribution of levels of Zn, Cd, Pb and Cu (μg/dL) in blood samples of children aging between 3 months and 6 years

Site No.	Location	No. of Children	Zinc		Cadmium		Lead		Copper	
			GM	GSD	GM	GSD	GM	GSD	GM	GSD
1	Focal Point	30	129.3	1.08	0.09	2.84	9.7	1.57	59.6	1.27
2	Majitha Road	35	227.9	1.86	0.11	1.57	12.6	1.48	70.3	1.58
3	Village Khankot	30	466.7	2.37	0.01	2.36	2.9	1.67	107.0	2.16
4	Azad Nagar	35	499.4	2.86	0.01	1.68	2.0	1.98	100.4	1.88
5	Green Avenue	30	470.0	2.91	0.03	1.70	9.3	1.63	102.8	1.36

GM = geometric mean; GSD = Geometric standard deviation

It can be further seen from the Table 1 that samples from site no. 3, 4 and 5 which are comparatively safer, showed lesser concentration of cadmium and lead in comparison to site 1 and 2. However, higher concentration of zinc and copper at site no. 3, 4, and 5 can be related to the fact that all these samples were having the largest concentration of hemoglobin and they were having higher body weights. So it can be said that all those 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 can also be seen that site no. 3 has higher concentration of copper and low concentration of zinc in comparison to site no. 4 which is due to the fact that zinc interferes with the copper and iron metabolism being a metabolic antagonist of both copper and iron.[6] Similarly on comparing site no. 4 and 5, one can see that the concentration of zinc has decreased from 499.4 to 470 μg/dL causing an increase in level of copper in the same samples from 100.4 to 102.8 μg/dL.

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.[7] Based on epidemiological and experimental data Chamberlain suggested that an increase in air lead of 1μg/metric cube would result in an increase in blood lead of 15-30 μg/L. Similar results are observed for the site no.5 showing higher concentration of lead probably due to higher vehicular population and thus higher amount of lead in the air.

Table 2: Clinical Parameters of Children

Site No	Location	No. of Children	Grade of Malnutrition*	Range of Haemoglobin (gm%)	Average Weights (Kg)
1	Focal Point	30	M	4.5-6.9	6.4
2	Majitha Road	35	S	5.5-8.7	5.9
3	Village Khankot	30	N	9.3-12.0.	14.5
4	Azad Nagar	35	N	8.9-11.7	15.7
5	Green Avenue	30	N	7.9-11.5	9.0

* M-mild; S-severe; N-normal

Effect Of Age: All the 160 samples were categorized into six different age groups as shown in Table 3. It is evident from Table 3 that all the children whose age were between 3 months and 1 year (group1) have lower concentration of all these metals (except lead) as compared to the children of other groups.

Table 3: Age-Wise distribution of concentration of Zn,Cd, Pb and Cu (μg/dL) in different blood samples

Group No.	Age Group	Number of Samples	Range of Zinc	Range of Cadmium	Range of Lead	Range of Copper
1	3M-1Y	48	107-127	0.01-0.02	6-8.7	57-68
2	1Y-2Y	36	253-370	0.01-0.03	2.2-4.9	55-70
3	2Y-3Y	15	327-401	0.02-0.05	3-5.4	63-79
4	3Y-4Y	17	405-439	0.03-0.06	4.4-10.2	74-83
5	4Y-5Y	12	390-417	0.01-0.02	2.9-5.9	59-80
6	5Y-6Y	32	452-499	0.07-0.11	7-12.6	90-107

M= Month, Y = Year

Children aged between 5 and 6 years show maximum 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 exposure in older children. Exception of lead can be related to other factors; most of the samples from group 1 were taken from the site no. 5 where a heavy vehicular pollution could be responsible. It is also known that lead passes through the placenta easily and fetal blood has almost the same blood lead concentration as maternal blood.[8] Therefore exceptionally higher concentration of lead in the blood of all the infants from site no.5 may be because of higher exposure of their mothers to the vehicular pollution. However, the concentration of cadmium in the infants (group 1) remained lower because in this case placenta acts as a fairly effective barrier to cadmium and hence new born are virtually free from cadmium. Accumulation of cadmium in the body increases with age, as shown in Table 3 and was well documented by some other workers.[9] It is also known that cadmium is always found in association with zinc, so all the samples the show higher concentration of zinc, had also shown higher concentration of cadmium. However, a possible reason can not be sorted out for the cases where samples had shown higher concentration of cadmium but lower concentration of zinc. A more elaborative study involving many more sample analysis is needed to explain this finding with regard to cadmium and zinc.

Although, there is a direct correlation of concentration of blood levels of metals with age because of higehr exposure, there is a little deviation from this trend in children aged between 4 and 5 years (Table 3).

It is observed that all the twelve children belonging to group no.5 were from site no.3, which is very safe site and hence this deviation in regular trend can be answered. So 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 near industrial areas are increasing their blood's metal content with passage of time.

Effect Of Sex: The effect of sex on the uptake of these metals are presented in Table 4.

Table 4: Sex-Wise distribution of metals (µg/dL) in different blood samples

Sex of children	No. of samples	Range of Zinc	Range of Cadmium	Range of Lead	Range of Copper
Male	100	127-499	0.02-0.11	2.8-12.6	57-107
Female	60	107-439	0.01-0.09	2.2-12	55-90

Though, the variation appears small with regard to cadmium and lead among both sexes, a larger variation can be seen with zinc and copper. Both lead and cadmium are found in slightly higher concentration in males in comparison to female children. Similar types of results for lead and cadmium have been found by some workers. The results of WHO / UNEP study indicated that males tend to have higher blood lead levels than females. All the male children showed higher concentration of zinc and 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.[10-11]

Table 5: Zn, Cd, Pb and Cu in Standard Reference Materials obtained from IAEA (µg/g)

Sample	Metal	Certified Concentration	DPASV Method
Animal blood (A-2)	Zn	89 ± 9	90 ± 5
	Pb	0.97 ± 0.22	1.10 ± 0.1
	Cu	45 ± 4	42 ± 3
Fish tissue (MA-B-3/TM)	Zn	109.2	108.0
	Pb	4.62	4.21
	Cu	3.08	3.16
Hay (V-10)	Zn	24.0	24.6
	Cd	0.03	0.027
	Pb	1.6	1.6
	Cu	9.4	9.3

Conclusion

We conclude that, all those children who are residing in and around industrial areas are 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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