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### MEASUREMENT OF LEAD CONCENTRATION IN THE HAIR OF SCHOOL CHILDREN IN THE MANZINI REGION, SWAZILAND

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The concentration of lead in the scalp hair of 257 school children within the age range of 6-12, living in the Manzini region in Swaziland, was measured using a graphite furnace atomic absorption spectrometer. Mean lead concentration ranged between  $7.75-9.19 \mu g/g$ , and  $4.95-5.95 \mu g/g$  for the urban and rural schools, respectively. The mean lead concentrations in the hair for urban school children were significantly higher than those of the rural school children. The rapid industrialization within the city centre with increasing number of vehicles using leaded petrol may be a contributing factor to the observed levels. Although the mean lead concentration was higher in girls ( $7.59 \mu g/g$ ) than in boys ( $6.62 \mu g/g$ ), this difference was not statistically significant.

Keywords: Lead; hair; school children; Swaziland

#### INTRODUCTION

The widespread distribution, contamination and multiple effects of heavy metals in the environment have become a global problem especially in the African continent [1]. Among the offensive metals is lead. Studies have been conducted on the determination of this metal in the African environment [2-5]. Modern sources of lead contamination are widespread. They range from lead mining and smelting operations, battery manufacture to automobile emissions. A particu-

larly insidious source of contamination is the use of lead pigments in paint which are probably still widespread in developing countries. Today petrol containing antiknock additive lead has become a major source of atmospheric lead especially in cities having high traffic density. The rapid industrialisation in developing countries, including Swaziland, has lead to a rapid increase in vehicles on the roads. In Swaziland, the roads in the Manzini region are witnessing a rapid rise in traffic density as a result of industrialisation in the last 10 years. According to the Swaziland Central Motor Registry [6], the number of newly registered vehicles/year has increased from 1,022 in 1992 to 1346 in 1996.

Swaziland imports her petrol which contains about 0.7-0.9 g Pb/L. This range is higher than the range, 0.15-0.4 g/L permitted in USA and Europe. Most of the vehicles in Swaziland still use leaded petrol at present, thus contributing to an increase in lead concentration in the air and dust in the Manzini region. In Egypt [7], South Africa [8] and Mexico [9] leaded petrol has become the major source of atmospheric lead.

The history of health hazards associated with lead is well documented. Several studies [10, 11] have shown children to be more sensitive to lead toxicity than adults. The blood lead level associated with signs and symptoms of lead poisoning in children is at  $25 \,\mu g/dL$  compared to 100  $\mu g/dL$  in adults [12]. Also the blood-brain barrier in children is easier to penetrate by lead than in adults [13]. Children also absorb about 50% of ingested dietary lead, compared to only 10% by adults [14].

The use of hair as a biopsy material has been investigated by a large number of workers [15–17, 24]. Since then, many other reports on the assay of lead and other trace elements in hair samples as useful means for monitoring human exposure to environmental pollution by metals have been reported [16]. Significantly, elevated hair levels of lead in urban children were observed as compared to rural subjects [17]. In healthy persons, the concentration of lead in scalp hair may be from two to five times greater than that in bone, about 10–50 times higher than that in blood, and from 100 to 500 times greater than that excreted in urine [18]. Hence, hair concentrates more lead per unit weight than any other tissue or body fluid. Kopito *et al.*, found the average lead level in scalp hair of normal children to be 24  $\mu$ g/g [19]. LEAD IN HAIR

Hair has been chosen for this study because it is easily collected, an important consideration in studying young children. It is also relatively inert and very easily transported, stored and analysed. However, one major problem in the use of hair samples for estimating lead exposure is the possibility of external contamination. Therefore, a sample preparation technique is required to remove the contamination.

The present study was designed to measure the lead concentrations in the scalp hair of urban and rural school children aged 6-12 in the Manzini region in Swaziland. This work is part of an on-going programme into the study of heavy metal pollution in Swaziland.

#### EXPERIMENTAL

#### 1. Apparatus

For graphite furnace atomic absorption spectrophotometric (GFAAS)analysis, a GBC graphite furnace atomic absorption spectrometer comprising a model 960 AA basic instrument with an autosampler PAL 3000 was used. A Varian hollow cathode lamp as well as pyrolytically coated graphite tubes were applied. The lamp current was set at 5 mA, and the measurements were performed at the 217 nm wavelength, using a slit width of 1.0 nm. The analytical signal corrected for background and the background signal were recorded with a GBC FS3000 data station, and the absorbance profiles were out-put through an Epson-LX 400. The measurement of the absorbance signals was carried out by the peak area, and the inert gas (oxygen-free nitrogen) was interrupted during the atomisation stage. The temperature program used is given in Table I. Eppendorf automatic micropipets were used in preparing solutions. All glasswares used were of pyrex brand and were acid washed and thoroughly, rinsed with deionised water several times and then oven dried before use.

#### 2. Reagents and Solutions

The acetone (Fluka-pruss) used for washing the hair samples was of high purity (99.99%). For hair digestion, nitric acid (Fluka-pruss) of

Stage	Temperature [°C]	Time [s]	Ramp [s]	
Drying	120	20	30	
Ashing	500	30	30	
Atomizing	1900	5	0	

TABLE I GFAAS-temperature program

very high quality (99.9%) and very low lead grade was used. All lead standard solutions were prepared from a stock lead standard solution (Fisher Scientific) with a concentration of 1000  $\mu$ g/ml. Deionised water was used for washing the hair samples and preparing standard solutions.

#### 3. Sampling Procedure

Hair samples were collected from 257 school children within the Manzini region. About 50 mg of chemically untreated hair was collected from each child. For cutting hair, high quality surgical scissors was employed and hair samples were collected in new polyethene sachets.

A questionnaire requesting information on age, sex, parents' occupation, place of residence and smoking habits of house-hold were also obtained from the school children.

#### 4. Sample Treatment

*Washing of hair.* 25 mg of each hair sample was washed using the procedure recommended by the International Atomic Energy Agency [20]. This involves soaking, agitating and washing the hair in acetone, water, water, water, acetone for 10 minutes in each solvent. The solvent was decanted and the samples were air-dried. The efficiency of this washing procedure was checked using the dose-washing test.

**Dose-washing test.** One hair sample was divided into two portions and placed into separate test tubes. To one portion (Dosed Sample), 0.08 mg Pb was added, and to the other portion (Undosed Sample) equivalent amount of deionised water was added. Both portions were washed by the washing procedure described earlier. They were dried,

#### LEAD IN HAIR

digested using 2 ml of nitric acid (99.99%) evaporated to dryness and the analyte solution prepared from the residue obtained.

Hair Digestion. 25 mg of the cleaned and dried hair samples were placed in acid washed and oven dried pyrex test tubes. The samples were digested using 2 ml nitric acid (99.99%) at  $100^{\circ}$ C in a thermostated oven. After the complete dissolution of the hair strands, the remaining acid was evaporated to dryness. The residue left was dissolved in 2 ml deionised water to make the analyte solution. The efficiency of the digestion was checked using the lead recovery test.

*Lead recovery test.* Some washed and dried hair samples (from two urban and two rural schools) were treated with known amounts of lead standards, while another set of the same hair samples were without added lead standard. Both sets were digested and the analyte solution prepared as described earlier. The analyte solutions' absorbances were measured and the percentage lead recovery calculated.

Atomic absorption spectrometer analysis.  $20 \,\mu$ l of both analyte and standard solutions were injected into the graphite tube by the auto-sampler. Measurements were conducted in triplicates.

*Statistical analysis.* Statistical differences were evaluated by one-way analysis of variance (ANOVA) at 95% confidence level.

#### **RESULTS AND DISCUSSION**

#### 1. Dosing-washing Test

The results of lead analysis of hair samples washed with acetone and water are shown in Table II. The dosing-washing test was designed to test the efficacy of the hair washing procedure employed in this study in removing any exogenous lead from the hair samples. As can be seen in Table II, the overall mean absorbance of the dosed' and undosed' samples are very close (1%).

This indicates that most of the dosed lead was removed during the washing steps. Thus, the washing steps adopted in this study were capable of removing any external contamination from the hair samples.

Sample	Mean absorbance	% RSD	Overall mean absorbance	
undosed 1	0.384	2.5	0.360	
undosed 2	0.231	1.7		
undosed 3	0.465	4.3		
dosed 1	0.384	3.9	0.364	
dosed 2	0.231	1.3		
dosed 3	0.477	0.9		

TABLE II Dose-washing test

#### 2. Lead Recovery Test

Table III shows the lead recovery test which was conducted to verify if any lead was lost during the acid digestion and drying steps. The high percentage values shown in Table III indicate that very little lead was lost during the digestion and drying steps.

#### 3. Sample Analysis

The summary of lead content in the hair of school children in the Manzini region is shown in Table IV. The results are presented by age group and urban and rural schools. From Table IV, the mean lead concentrations were higher in the urban schools than in the rural schools for all the ages. The differences were found to be statistically significant (p < 0.05). The significant difference may be explained by the fact that the urban schools are situated mostly within Manzini city centre which is experiencing increasing number of vehicular traffic from both within and from increasing visitors from neighbouring

Sample code number	Absorbance of sample without Pb standard	Absorbance of added Pb standard	Absorbance of sample with added Pb standard		Percentage (%) recovery
	A	В	C	C-A	C-A/B
Urban 1	0.187	0.323	0.495	0.308	95
Urban 2	0.471	0.323	0.729	0.321	94
Rural 1	0.129	0.323	0.430	0.301	93
Rural 2	0.159	0.323	0.464	0.305	99

TABLE III Lead recovery test

Age group	Urban school n	Rural school n	Urban schools mean conc. of lead $(\mu g/g) \pm SD$	Rural schools mean conc. of lead $(\mu g/g) \pm SD$	P <sup>b</sup>
6-7	40	38	$8.62 \pm 6.38$	$5.94 \pm 5.19$	significant
8-9	53	49	$9.19 \pm 7.77$	$5.95 \pm 6.03$	significant
10-12	44	33	$7.75\pm7.35$	$4.95 \pm 4.57$	significant

TABLE IV Mean lead concentrations<sup>a</sup> in hair of school children in the Manzini region

<sup>a</sup>Arithmetic mean  $(\mu g/g) \pm arithmetic standard deviation.$ <sup>b</sup>ANOVA P value.

countries. There is thus, a continuous heavy flow of traffic inside Manzini. Also most of the school children live within the city centre. The air-borne particulate matter and dust of Manzini city have been found to contain up to 3.0 µgPb/m<sup>3</sup>(unpublished data).

The mean lead concentrations for 6-7 and 8-9 age groups are higher than 10-12 age group for both the urban and rural schools. This may indicate that the lead level in children's hair decrease with age. This agrees with previous studies which demonstrated higher lead concentrations in children's than in adults' hair [21, 22]. However, this trend was not observed for 6-7 and 8-9 age groups. The concentration of lead in the samples ranged from 0.04 to 33.89  $\mu$ g/g, with the highest value being recorded for a girl whose father was a mechanic and a smoker.

The average values for the urban and rural schools were 8.56  $\mu$ g/g and  $5.43 \,\mu g/g$  respectively. These results are comparable to those reported by Schuhmacher [16], Barry [21] and Chenard [22]. However, the value of 8.56  $\mu$ g/g obtained for the urban school in this study is by far less than that reported by Ahmad et al. [17] in urban school children.

Lead levels in hair with respect to different influencing factors are given in Table V. The mean hair lead concentrations for males (6.62  $\mu g/g$ ) and females (7.58  $\mu g/g$ ) were found not to be significant (p > 0.05), although the mean lead concentration for females is higher than those of the males. This agrees with the results reported [23, 24], although the population investigated in those studies were mostly adults. In contrast, other authors [16] found significant differences between the sexes. As can be seen from Table V, there is no significant relation between household smoking habits and lead

Factor	actor n Mean lead concentration in hair $\pm SD(\mu g/g)$		
Sex			
Male	112	$6.62\pm5.67$	NS
Female	145	$7.59 \pm 7.25$	
Smoking			
habits			
smokers	96	$7.39\pm7.03$	NS
non	961	$6.75 \pm 6.25$	
smokers			
<sup>b</sup> Parents'			
occupation			
Group I	132	$7.75 \pm 7.12$	NS
Group II	125	$6.15 \pm 5.76$	

TABLE V The effect of various factors on the lead concentration in the hair of school children in the Manzini region

<sup>a</sup>ANOVA P value; NS, not significant.

<sup>b</sup>Occupational group I includes jobs such as, drivers, metal workers, motor mechanics, farm workers, painters, printers and related occupations which are likely to involve exposure to lead. Group II includes civil servants, teachers and related occupations which are not likely to lead to any exposure to lead.

concentrations in the hair of school children studied. A similar result is also exhibited by the occupation of parents factor.

The results of the present study, which is a representative of an urban/rural population, indicate that the hair mean lead concentrations of the school children in Manzini region are presently below the toxic levels. According to Kopito *et al.* [19], the mean concentration in the hair of 16 children with lead intoxication was  $282 \ \mu g/g$  with a range of  $42-975 \ \mu g/g$ . These values are by far much higher than those obtained in the present study. However, there is a need to continuously monitor and to a large extent, prevent children from lead exposure, since lead toxicity in children can result in learning disabilities [19, 25]. The recent introduction of leaded free petrol in Swaziland is a move towards the right direction.

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