LEAD AND CALCIUM IN HUMAN MALES USING FINGER NAILS AND SCALP HAIR

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ABSTRACT

Lead and calcium concentrations were determined in scalp hair and finger nails of exposed and unexposed males by atomic absorption spectrometry (AAS). The mean levels of Pb in the finger nails ranged from 50-480 µg/g, and 50-240 µg/g for exposed and unexposed males respectively. The mean levels of Pb in the scalp hair ranged from 30-410 µg/g, and 30-200 µg/g for exposed and unexposed males respectively. The mean levels of Ca in the finger nails ranged from 250-1650 µg/g and 315-1860 µg/g for exposed and unexposed males respectively, while in the scalp hair the mean levels of Ca ranged from 130-1280 µg/g and 235-1275 µg/g for exposed and unexposed males respectively. The study established that there was a negative correlation between Pb and essential element Ca in both scalp hair and finger nail samples from all the male respondents in that the levels of Pb increased the levels of essential elements Ca decreased and vice versa. A significant difference ($P<0.05$) was indicated when Pb and Ca mean levels were compared. Comparing the mean lead concentration in scalp hair with finger nails a significant difference was indicated in the two tissues ($P<0.05$). Human hair and finger nails are therefore recording filaments that can reflect metabolic changes over long period of time and hence furnish a print out of post nutritional event as dietary levels of some of the essential elements.

Key words: Lead, Calcium, Scalp hair, Finger nails, Determination.

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INTRODUCTION

Lead has raised concern among heavy metals, due to relatively high toxicity and elevated quantity in the environment as a result of their widespread use (Cambra *et al.,* 1999). Further, Pb does not have any known biological use but is widely used in the industries (Mielke *et al.,* 1999; Gaw *et al.,* 2006). It is well documented that human beings may be exposed to lead through contamination of food, water, house, leaded dust, soil and through industrial activities such as metal recycling, battery industry and flaking paints (Park and Palk, 2002; Nabulo *et al.,* 2006; Ndiritu *et al.,* 2012). Nabulo *et al*. (2006) found that when Pb is released into the air, it stays there for ten days and most of it in the soil comes from particles falling from the air. Use of glazed ceramics, smoking of tobacco, source of water for drinking, consumption of exposed and highly refined foods with high fat content have also been reported to lead to increased levels of nail and hair Pb (Owago, 1999; Oyaro, 2000; Were *et al*., 2008; Mogwasi, 2009).

Calcium is one of the major essential elements that the body requires and it's the most abundant cation present in the body than any other cation (Nath 2000). The best food sources are daily products, beans, cauliflower, egg yolk, leafy green vegetables and oysters. Daily requirement is 800-1200 mg (Duruibe *et al.,* 2007) Ninety eight percent of body calcium is found in the hydroxyapatite of the mineral bones. The remaining calcium is largely found in the extra cellular fluid compartments (Singh, 2004). Calcium performs several vital functions in addition to those it serves in the skeletal system and Ca ions strongly influence the permeability of cell membranes and constitute an important secondary messenger to hormones (Cousins *et al.,* 2004). Calcium balance is determined by the relationship between Ca intake and Ca absorption and excretion (Ayodele and Bayero, 2009). A striking feature of the system is the changes in Ca absorption and excretion can neutralize a high intake or compensate for a low one (WHO, 1996). Ayodele and Bayero, 2009 observed that there is a wide variation in calcium intake among nations generally following the animal protein intake and depending largely on dairy product consumption. A high level of Ca in hair and finger nails does not mean one has an excess of Ca in the body, the phenomenon is called biologically unavailable calcium. In this case Ca precipitates into the tissues instead of remaining in blood (Nath, 2000). Several studies have considered many metals that play a critical role in maintaining of life (Satarug *et al.,* 2000), studies have shown that some metals form the structure of biological materials such as Ca in bone and Fe in haemoglobin

(Olivero-Verbel *et al.*, 2007). Oostdam *et al.* (1999) found that metalloenzymes were known to be involved in the synthesis and degradation of biological molecules. Further studies have established that displacement of certain essentials metals by toxic metals was another form of toxicity (D' Souza *et al*., 2003) for example Pb was generally known to substitute for essential metal calcium in the bone which led to toxic consequences, (Nowak and Chmielnicka, 2000). Studies have shown that Pb interferes with several enzymatic processes responsible for nerve synthesis by inhibiting the activity of cytoplasm enzymes α aminolevolinic dehydrates (α ALA) (Olivero-Verbel *et al.*, 2007).

In addition to cellular toxin, studies have shown that Pb complexes with Ca in the body causing various malfunctions in Ca metabolism which included a decreased neural transmitter release and a blockage of calcium channel (Barbosa *et al.*, 2005). It was reported that the CNS appeared to be affected the greatest degree of Pb toxicity and it has explained many neuropsychiatry symptoms associated with exposure to this heavy metals (Lanphear *et al.*, 2005). Animal studies have shown that low dietary levels in Ca increased the absorption of Pb from the gut (Ostdam *et al.*, 1999). Studies have observed that Ca intake was inversely related to both absorption and retention of Pb in infants (Wilhelm and Hafner, 1993). WHO (1996) showed that daily intake of 1200 mg Ca conferred optimal protection for pregnant and lactating women. Low dietary levels of Ca, and Fe have been found to enhance absorption of Pb and exacerbate the symptoms of poisoning (Oostdam *et al*., 1999; Satarug *et al.*, 2000).

Different methods have been used to by different researchers to determine the levels of Pb and Ca in hair and nails. Biological samples such as nails and hair tissues may have low concentration of these metals and therefore require sensitive instruments (Ju, 2002). Most common applied methods are: flame atomic absorption spectrometry (AAS) (Wilhem and Hafner, 1993; Nowak and Chmielnicka, 2000; Sukumar and Subramanian, 2003), graphite furnace atomic absorption spectrometry (GF-AAS) (Lekouch *et al.*, 1999; Paoliello *et al*., 2002), inductively coupled plasma atomic emissions spectroscopy (ICP-AES) (Wanjau *et al.*, 2004), X-ray fluorescence (XRF) (Ambrose *et al*., 2000), fluorination assisted ETV-ICP-AES (Wanjau *et al.*, 2004) and inductively coupled plasma-mass spectrometry (ICP-MS) (Chen *et al.*, 1999; Morton *et al.*, 2002; Samantha *et al.*, 2004). Mehra and Juneja (2005) employed atomic absorption spectroscopy method for determining Cd, Pb, Cr, Mn, Fe, Ni, Cu and Zn reported that the values obtained were correlated

to the personal and medical history of the subjects. This paper reports the determination of Pb and Ca in human male scalp hair and finger nails using Atomic Absorption Spectroscopy (AAS). Deficiencies of C exacerbates Pb uptake in the body (Nowak and Chmielnicka, 2000). It is important to note that human males are not exempted from diseases and ailments that are brought about by Ca deficiencies, it is therefore imperative that human males are advised accordingly on proper eating habits. This will go a long way in reducing the effects of Pb pollution besides reducing its absorption in the body.

AREA DESCRIPTION

Nairobi, cover an area of 697 km^2 with a population of over 3.1 million (CBS, 2010). Nationally it is established to have the greatest concentrations of industrial and vehicular air pollution sources (UNEP, 2006). It is reputed to be the fastest growing city of the world and lacks air quality management system (Mulaku and Kariuki, 2001). Indeed among the developing countries cities that were sampled for the study on air quality management capabilities, Nairobi was rated the worst (UNEP/WHO, 1996). The city is now regarded as a "hot zone" with highest concentration of pollutants which has been influenced by increasing industries, population, construction, heavy traffic density and deforestation of city fringes (Mulaku and Kariuki, 2001). The CBS (2010) reported that Mathira is situated in central Kenya with a population of 152,000 and covers an area of 434 Km^2. It is a rural setting with few vehicles and industries. The study sites included schools and homes situated in this agricultural region, in which coffee, tea and horticultural crops are predominant. These crops requires substantial amount of fertilizers and pesticides for their production. These chemicals have heavy metals in-put. Therefore it was the aim of this study to determine levels of lead in the fingernails and scalp hair samples of males over the age of 18 years in different environmental settings to find out whether they accumulate metals differently.

EXPERIMENTAL

Participation of all the subjects in this study was voluntary and relevant permits were obtained prior to the study. Confidentiality of the data collected and subsequent findings were assured by

using only code numbers for each participant. Participants were free to terminate participation at their convenience. Any subject who would later like to know the levels of elements in his finger nails and scalp hair samples would do so. Field visits and sampling began in June 2010 and ended in September 2010. Samples were obtained from Nairobi and Mathira.

EQUIPMENT AND REAGENTS

Atomic Absorption Spectrophotometer (AAS-Spectr AA-10, Varian- Techron, Austria) was used. Water used throughout the analytical procedures was deionised and distilled. Weighing balance used was Mettler Toledo AG-240. Digester block was 2080/DA, No 935, Volt 220-w Germany,-LIEBISCH BIELEFELD 14. Reagents used in the analysis were of high quality analytical grade. Liquid soap-an Izal product, nitric acid AR, acetone, 4-methyl pentan-2-one and perchloric acid were supplied by Hopkin and Williams, England. The plastic bottles were cleaned with non-ionic liquid soap rinsed with distilled water. They were then soaked overnight in 1:1 nitric acid and rinsed thoroughly with deionised and distilled water. All the glasswares used in this study were decontaminated by soaking them overnight in 5 % HNO_3 and rinsed thoroughly in deionised and distilled water. They were oven dried at 105 ^{o}C and stored safely. The metal standards from the stock solution (1000 µg/mL) were freshly prepared daily by serial dilution and checked for constancy of the results before taking the readings.

SAMPLING

Two hundred (n=200) males over the age of 18 years were randomly recruited. Consent was sought from parents/guardians in case the subject was still under their care. The informed and consented subjects (n=200) filled a self-administered questionnaire. This took into account the previous findings and the WHO (1996) recommendations. The questionnaire elicited information on demographic characteristics, health conditions, socio-economic background, environmental risk exposure factors and diet habits of the subjects. The diet habits considered factors such as consuming processed food, canned with high fat content and marginal proteins. The environmental risk factors included; working in industries, petrol stations, drivers and conductors, those people who spend most of their time traveling, living near the road, living in a house

painted with leaded paint or use of glazed ceramics utensils frequently, consuming exposed food from open air market or streets and taking water from leaded piping or borehole water frequently.

Purposive sampling strategy was used to select males in both urban and rural settings. The major criterion for selection of males in urban areas was influenced by the intensity of pollution, one hundred and twenty (n=120) subjects were recruited under this category. In Mathira rural, the criterion for selection was that it is in the interior far from urban influence. Therefore, eighty (n=80) subjects were recruited under this category. Each recruited subject gave his paired sample of finger nails and scalp hair.

In view of high prevalence of bacterial and fungi infections, each subject was given a labelled stainless steel nail clippers and a towel. All the fingernails and scalp hair samples of the subjects were cleaned using surgical spirit followed by non-ionic detergent. They were rinsed with water then dried with a clean towel. In order to minimize secondary contamination with metallic elements the stainless steel nail clippers were washed with analytical reagent grade HCl, diluted at 1:10 then rinsed with distilled water. Twenty eight (n=28) subjects were excluded from this study as either they dropped from the study or were unable to get sufficient samples for analysis. Fingernails and scalp hair were clipped from the same subject once in two weeks for a period of three months. The samples (n=344) were kept in labeled plastic bottles under lock and key until they were analysed.

LABORATORY PROCEDURE AND QUALITY ASSURANCE

Analysis of nail and hair samples was carried out using standard methods as reported by Mehra and Juneja (2005) and Sukumar and Subramanian (2003).

SAMPLE PRE-TREATMENT

Great care was taken to avoid external contamination of samples during analytical procedure. The fingernail and scalp hair samples were separately soaked in non-ionic liquid soap in a glass beaker for two hours and washed free from metallic debris following a standardized washing procedure (Mehra and Juneja, 2005; Sukumar and Subramanian, 2003). They were subsequently soaked in acetone for one hour and rinsed five times in deionised and distilled water. The samples were kept

in vial tubes, and oven dried at 60 ^{o}C to a constant weight. The polished fingernail and scalp hair samples were separately placed into beakers to which 10 mL of 4-methyl pentan-2-one was added and left for 45 min. They were then rinsed three times using deionised and distilled water before oven drying. The samples were weighed in triplicates and kept in the desiccators.

ACID DIGESTION AND AAS ANALYSIS

The dry 1.0000 g samples were quantitatively transferred into digesting tubes. A 6 ml aliquot of concentrated nitric acid was added and heating done until brown fumes were observed, the solution was cooled to room temperature after which 1 ml of concentrated perchloric acid was added. The digesting tubes were then covered with aluminum foil and placed on digester block in a fume chamber and subsequently heated at 180 °C (Mehra and Juneja, 2005); use of open digestion is discouraged due to air borne particles, and loss of volatile elements and production of hazardous vapour (Samatha *et al*., 2004). The samples were then allowed to digest slowly for about one hour until all the samples (nails or hair) dissolved to form a clear solution. The digested sample solution was diluted with 1 ml aliquot of 0.1 N HNO_3 and then quantitatively transferred into a 100 ml volumetric flask, and volume adjusted to the mark with distilled water. They were then put in plastic bottles, labeled and stored under lock and key awaiting AAS analysis.

Analysis of nail and hair samples was carried out using standard methods as reported by Mehra and Juneja (2005) and Sukumar and Subramanian (2003). Concentration of Pb was assayed by use of AAS in triplicates with acetylene flame (the accuracy of the AAS was checked by triplication of the samples). A series of standards were prepared by serial dilution of the stock solutions containing 1000 µg/l of the metal and were used for instrumental calibration. For quality control, standards and blank samples were analyzed for every ten samples analyzed. The main instrumental parameter for example band width, lamp current, height of the flame and wavelength for AAS were optimized for Pb. Detection limit of the instrument used for the analysis of Pb was 0.020. Adequate quality control was ensured by inter-laboratory comparisons of representative samples carried out at Kenyatta University Research Laboratory and Mines and Geology Analytical Research Department, Nairobi. The validity of method was further ascertained by linearity of calibration curves and regression equations.

DATA ANALYSIS

Statistical calculations were done using statistical SPSS program (Statistical Package for Social Sciences Version 17).

RESULTS AND DISCUSSION

The concentration of elements in scalp hair and finger nails vary widely among individuals, thus large number of samples from a population was analyzed and the results treated statistically for meaningful correlation. Pb and Ca concentration in scalp hair and finger nails were determined using atomic absorption spectroscopy (AAS). Information on demographic characteristics, health conditions, socio-economic background, environmental risk exposure factors and diet habits of the subjects were noted from the questionnaire. The age distribution pattern for the age of donors (years) is as shown in Figure 1

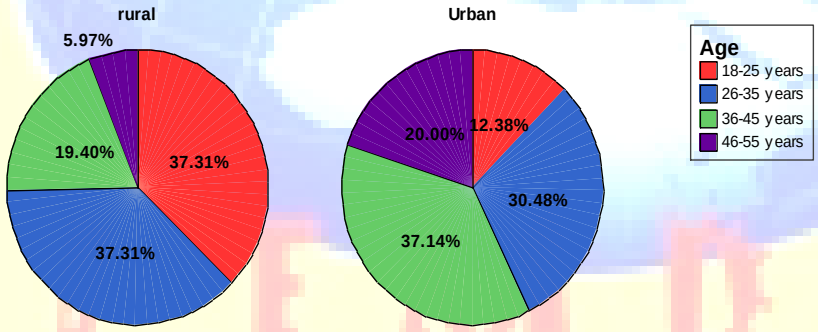

Figure 1: Age distribution of the respondents from rural and urban areas

It was established that 63.37 % of the respondents from both urban and rural were middle aged, the age category of 26-45 years. These people are energetic and hence active in their respective occupations. These findings agree with those of other researchers (Owago, 1999; Mogwasi, 2009). It is also evident that after people go to urban areas while young, they are reluctant to go back to their rural homes since in the rural areas there were fewer people aged 36-55 years. The Pb levels in the finger nails and scalp hair of males from rural and urban areas are summarized in Table 1.

Table 1: Mean Pb and Ca levels (µg/g) in scalp hair and finger nails of urban and rural subjects

	Finger nail samples		Scalp hair samples		
Element	rural	urban	rural	urban	P-value
Pb	126.25±4.05[b]	275.92±11.13[d]	79.36±3.85[a]	176.39±8.11[c]	<0.001
Ca	944.20±37.0[c]	824.76±27.36[b]	748.78±22.6[ab]	674.43±23.18[a]	<0.001

Mean levels followed by different small letters within the same row are significantly different $\alpha=0.05$, $p<0.05$(One-way Anova)

From Table 1, the mean levels of Pb was highest in the nails from urban subjects (275.92±11.13 µg/g) and lowest in the hair of the rural samples (79.36±3.85 µg/g) the mean levels of Pb in the finger nails and scalp hair samples from rural and urban areas all were significantly different. The mean levels of Pb were higher in the urban respondents than in the rural respondents in both finger nails and scalp hair samples the means were significantly different. This could be as a result of the increased environmental pollution due to mushrooming of many industries dealing with heavy metals which could be released to the environment and find their way into the bodies of human beings, vehicular density also could be a contributory factor to this high level of lead since most vehicles could still be using leaded gasoline which when emitted into the atmosphere can then be inhaled into the body. Oyaro (2000) noted that the concentration of Pb in large cities where there was heavy traffic using leaded gasoline and near highways was so high to cause toxic reactions in some individuals. There could also have Pb aerosol deposition on vegetables, fruits among other food substances which could have been grown along the roads or are sold in open places where there is heavy traffic. Besides this there is occupational exposure among other risk factors. Although the subjects in the rural area had lower levels of Pb than their urban counter parts, the levels were dangerous for human health. The elevated levels of Pb in the rural areas could be as a result of the fertilizers and a wide range of pesticides used in this highly agricultural area since most of the subjects were farmers and students.

The mean Pb levels were generally higher than those reported by Wilhelm and Hafner (1993); Rodushkin and Alexelsson (2000) Mehra and Juneja, 2005; Were *et al.*, 2008; and others in finger nails and scalp hair. The levels were higher than those reported in blood (Owago, 1999; Mogwasi, 2009). However, the mean levels were generally lower than those reported by Ayodele and Bayero (2009) 0.384±0.34 mg/g and 0.464±0.364 mg/g in hair and finger nails respectively for exposed subjects to environmental pollution.

Mean Ca levels (µg/g) in scalp hair and finger nails of urban and rural subjects

From Table 1, it can be seen that the mean levels of Ca were highest in the finger nail samples from the rural areas (944.20±37.0 µg/g) and were lowest in the hair samples of the urban respondents (674.43±23.18 µg/g). The mean levels of Ca in the rural nails, urban nails and urban hair were significantly different however, the mean levels of Ca in the rural hair was not significantly different from the mean levels of Ca in both urban nails and hair. Generally the mean levels of Ca were higher in the rural subjects as compared to the urban subjects. The reported mean Ca levels in this study can be correlated with long term dietary intake, absorption from the gastro-intestinal tract and retention (Mahaffey, 1995; Owago, 1999). Consequently, the mean Ca levels were higher in the finger nails than in the scalp hair samples. This is probably because Ca plays some physiological functions such as the development of nail bed and nail plate (Ayodele and Bayero, 2009).

The trend observed for mean Ca levels in this study was in agreement with other studies done elsewhere (Nowak and Chmielnicka, 2000; Rodushkin and Axelsson, 2000; Miekeley *et al.*, 2001; Chojnacka *et al.*, 2006). The levels of Ca were higher than those reported by Mehra and Juneja (2005), Were *et al.* (2008) and Mogwasi (2009) but were lower than those reported by Ayodele and Bayero (2009). The mean levels of Ca in the scalp hair for subjects from both rural and urban areas was lower than the levels obtained in the same kind of samples for exposed and unexposed subjects as reported by Mehra and Juneja (2005).

Influence of age on mean Pb and Ca levels (µg/g) in finger nails and scalp hair

The results of quantative analysis of Pb and Ca in the scalp hair and finger nail samples for subjects of different ages from the rural and urban settings are presented in Tables 2 and 3 respectively.

Table 2: Relationship between age and mean Pb and Ca levels in the rural area

		18-25	26-35	36-45	46-55
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
Pb	Nails	100.14±6.35^{a}	136.98±3.49^{b}	142.23±4.13^{b}	170.50±24.64^{c}
	Hair	57.44±3.94^{a}	81.32±4.01^{b}	103.31±6.80^{c}	126.25±30.10^{d}
Ca	Nails	1119.60±53.06	863.80±61.72	760.19±67.31	948.50±23.59
	Hair	805.60±30.00	707.80±42.29	718.69±56.47	747.50±37.50

Mean levels capped with different small letters within the same row are significantly different $P<0.05$(One-way Anova)

Table 3: Relationship between age and mean Pb and Ca levels in the urban setting

		18-25	26-35	36-45	46-55
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
Pb	Nails	154.46±24.37^{a}	233.94±19.37^{b}	318.28±15.03^{c}	336.43±19.37^{c}
	Hair	115.38±20.84^{a}	157.16±13.31^{ab}	193.03±13.02^{b}	212.57±17.19^{b}
Ca	Nails	966.92±77.25^{b}	908.09±56.53^{ab}	755.03±34.43^{a}	739.29±59.53^{a}
	Hair	806.15±69.11^{b}	731.53±42.24^{ab}	607.44±31.46^{a}	630.29±56.46^{a}

Mean levels capped with different small letters within the same row are significantly different $P<0.05$(One-way Anova)

The study established that the mean Pb levels in both the finger nails and scalp hair increased with increase in the age of the subjects (Tables 2 and 3). In the rural setting, the mean levels of Pb in the scalp hair samples of subjects in all the age categories studied were significantly different. However, the mean Pb levels in the finger nails of subjects in the age categories 26-35 years and

36-45 years were not significantly different. Younger subjects had significantly higher Ca levels than older subjects consequently, their Pb levels were also low an indication that higher levels of Ca led to low accumulation of nail or hair Pb. Ca levels decreased with increase in age though the decrease was not uniform in all the age categories studied.

For exposed subjects in urban areas, the mean Pb levels increased with increase in the age of the subjects, the means across all the age categories studied were significantly different. The study established that the levels of Ca decreased with increase in the levels of nail and hair Pb. There were significant differences between the levels of Ca in the subjects who were in the age bracket of 18-25 years and those who were in the age bracket 36-45 and 46-55 years. Those subjects who were in the age category of 18-25 years had higher levels of Ca than those subjects who were in the age category of 26-35 years. However, the two means were not significantly different at P <0.05 (Table 3). The study established that Mean Ca levels decreased with increase in age and as the mean Pb levels increased. This confirms that a higher Ca level discourages absorption of Pb (Ndiritu *et al.*, 2012) therefore correlation of Pb and Ca was done and the results are presented in Table 4.

Table 4: Correlation of lead with essential elements

		Pb	**Ca**
Rural	Hair	1	-0.224
	Nails	1	-0.401*
Urban	Hair	1	-0.374**
	Nails	1	-0.533**

**-Correlation is significant at 0.05 level (2-tailed)

*-Correlation is significant at the 0.01 level (2-tailed).

There was a negative correlation between Pb and Ca in both the hair and nail samples from both rural and urban areas implying that the Pb levels reduced as the levels of essential elements increased (Table 4). At 0.01 level Pb had a negative correlation with Ca (R=-0.401). The study

established that there was a negative correlation between Pb and essential element Ca in hair samples from urban subjects, R=-0.374 and -0.0019 respectively thus as the levels of Pb increased the levels of essential elements Ca. Therefore, there are other factors (nutritional and environmental) in urban area that influence the relationship between Pb and Ca in the scalp hair and finger nails.

The negative correlation observed between Pb and Ca can be attributed to the low levels of pollution in the rural environment coupled with the fact that subjects in the rural area depend on natural foods which supply high levels of essential elements in their bodies (Owago, 1999; Mogwasi, 2009). The results of this study agrees with other studies done elsewhere which reported that low dietary levels of Ca enhances the absorption of Pb and exacerbates the symptoms of Pb poisoning (Wilhelm and Hafner, 1993; Palminger *et al.*, 1995; Oostdam *et al.*, 1999; Nowak and Chmielnicka, 2000; Satarug *et al.*, 2000; Barbosa *et al.*, 2005; Were *et al.*, 2008). It is therefore possible that those with high Pb levels consumed less Ca. Correlation of Pb and Ca in combined samples of finger nails and scalp hair was done and results presented in Figures 2 and 3.

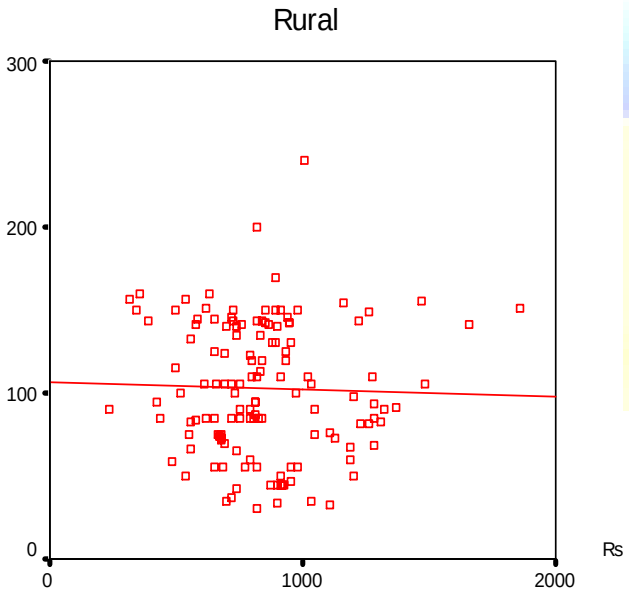

Figure 2: Correlation of Pb with Ca in combined sample of finger nails and scalp hair in rural setting

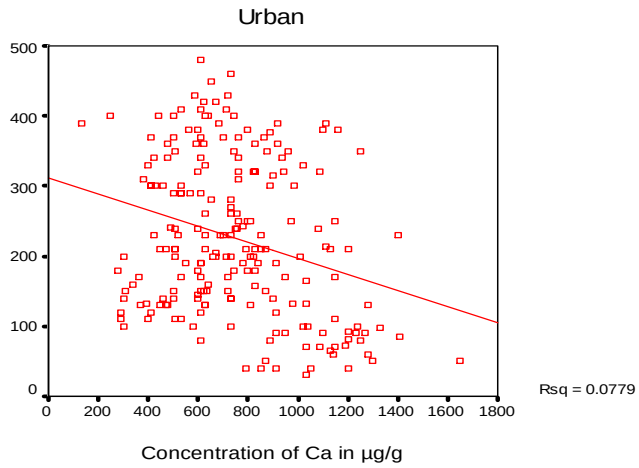

Figure 3: Correlation of Pb with Ca in combined sample of finger nails and scalp hair in urban setting

CONCLUSIONS

The levels of Pb were high in the two tissues irrespective of whether the subject was from rural or urban area and considering that the finger nails and scalp hair are points of excretion it therefore means that the levels are equally high in the body. The mean levels of Pb were significantly lower in subjects who had higher Ca levels and vice versa. Therefore scalp hair or finger nails are recording filaments that can reflect the extent of environmental pollution. The study established negative correlation between Pb levels and Ca in nails and hair from rural and urban settings. Human males and general public should be advised accordingly on proper eating habits to ensure that they have higher levels of Ca as well as other essential elements. This will go a long way in reducing the chances of Pb absorption.

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