Monitoring exposure to heavy metals through maize consumption using human hair among male adults in Eldoret Municipality, Kenya

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Abstract
This paper reports on findings using hair as biomonitor marker for heavy metals (Pb, Cd, Cr and Cu) from geological source and exposure through regular maize consumption among selected male adults in Eldoret Municipality who had resided for five continous years. Concentration of Pb and Cu in anthropogenic pathways, while Cd and Cr reflected accumulation from the human activities. Usually higher concentration of heavy metals hair samples suggest longer term exposure. The estimated intake of Cd and Cr from maize in one zone associated with high concentration of the metals from geological source was appreciably above the respective recommended daily allowance (RDA), signifying possible health risks to humans. Significant correlations between Pb, Cd and Cu in hair and heavy metals from maize consumed suggested maize consumption as possible pathways of exposure of heavy metals in humans. Possible health risks from heavy metals were likely due to consumption of higher quantities of maize contaminated from geological and human activities.

Key words
Long-term exposure, Short-term exposure, human hair, maize consumption, heavy metals

INTRODUCTION

Heavy metals are natural constituents of the Earth's crust. The term "heavy metals" refers to any metallic element that has a relatively high density and applies to the group of metals and metalloids with atomic density greater than $4g/cm^3$(Batayneh, 2010). Human activities have drastically altered the balance and biochemical and geochemical cycles of some environments. Heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr) and copper (Cu) occur naturally in water, soil and biota and are known to be toxic to most organisms when present in high concentrations in the environment (Bayayneh, 2010). Their concentrations depend on the local geology, globally distributed pollution (Cui et al., 2004; 2005; Zheng et al., 2007a; Khan et al., 2008; Hang et al., 2009) or anthropogenic routes (Wilson and Pyatt, 2007; Zheng et al., 2007b), including consumption of food from contaminated environments (Airey, 1983; Wang et al., 2005; Zheng et al., 2007b; Sridhara et al., 2008, Whyte et al., 2009; Zhuang et al., 2009; Metian et al., 2009, Oyoo-Okoth et al, 2010). Concentration of heavy metals in soils has been an issue of great interest in the recent past as it finds its importance in agricultural and nonagricultural areas. As heavy metals, Pb, Cd, Cr and Cu, have a potentially harmful effect to human health, persist in soils for a very long time and may enter the food chain in significantly elevated levels (Grzebisz et al., 2001). There is increasing demand of environmental and food safety stimulated research regarding the risks associated with environmental exposure and consumption of foods contaminated with heavy metals (D'Mello, 2003, Batayneh, 2010).

The use of human hair as biomarker for monitoring the exposure to heavy metals in man is linked with the availability of suitable analytical procedures, sensitive enough to quantify the content of the respective elements in the biological specimen tested. Since concentration of metals in human hair reflects their mean level in human body during a period of 2–5 months (Aharoni and Tesler, 1992), its use is far from being the universal tool for monitoring longer exposures to environmental pollutants (Evans, et al,1987), but provides indication of exposure throw food consumption(Birungi, et al., 2007, Oyoo-Okoth, 2010). Nail analysis is a useful alternative for longer exposure period ranging between 12 and 18 months (Chen et al., 1999; Were et al., 2008, Oyoo-Okoth, 2010). The element content of hair and nails tends to vary from one geographical region to another, depending on the natural background conditions such as element concentration in water and food and eating habits

(Eads and Lambdin, 1973; Chattopadhyay and Jervis, 1974; Teraoka, 1981). However, the use of hair for biological monitoring from maize consumption has not been fully studied to form the correlation with the exposure levels in the study area. A full understanding of the extent of any observed variability of metals from maize consumption to predict metal variation in hair is thus called for.

MATERIALS AND METHODS

Study Area

This study was conducted in Eldoret Municipality which is 312 Kilometers from Nairobi, the capital city of Kenya. Eldoret Municipality, with a latitude of 0.52 (0° 31' 0 N) and a longitude of 35.28 (35° 16' 60 E), is a populated place located in the area of Rift Valley in Kenya that is a part of Africa (Figure 1). The sampling stations were located at an average radius of eight kilometers. The main area of concern in this study was the sub-urban region covering EATEC, Yamumbi, Kahoya and Hawaii

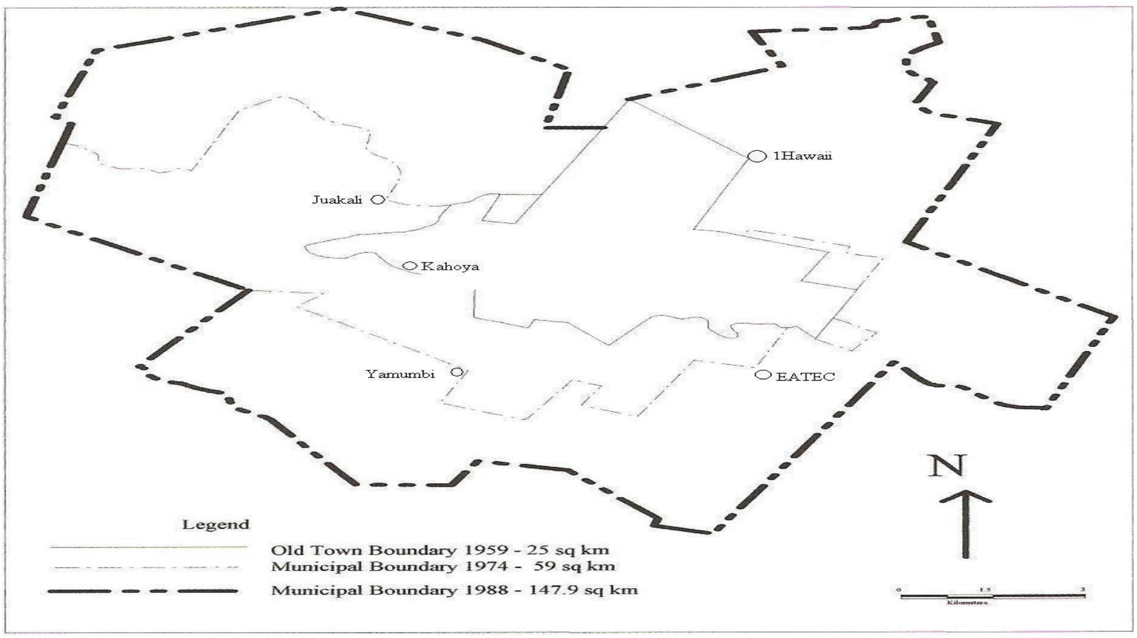

Fig 1 Sampling zones

Sampling design and procedure

The sample consisted of 100 male adults below 40 years, who reside in the farms in the four zones chosen from whence the maize and drinking water were collected within the Eldoret Municipality. The Helsinki 1996 protocols, which underline appropriate ethical considerations for studies involving human volunteer participants were followed and permission to carry out this study granted by the Moi University Institute of Ethical Research Committee (IREC). Hair was cut from the upper region at the back of the head, using stainless steel scissors. At least 0.5 g sample of hair was collected and stored in pre-washed polythene containers. Maize was sampled for this study because they are the ones normally consumed by the local residents as the main staple food. A total of 100 maize and drinking water samples were obtained from the study area between June 2008 –July 2009 from zones 1, 2, 3, and 4. The maize samples were kept in cool boxes at 0^o and immediately transported to the laboratory for metal analysis, using metal-free techniques in the Biochemical Laboratory, School of Environmental Studies, Moi University.

Further, determination of the quantity of heavy metal intake per adult per day from maize was carried out. The net maize consumption per day was estimated, using food frequency questionnaires. The weights of the adults were determined, using standard beam balance to an accuracy of 0.1 kg. Based on the amount of maize, the metal concentration in maize, and body weight of the male adults , the estimated daily intake (EDI) of metal from maize was calculated, using the formula

$$EDI = \frac{C_{heavymetal} \, x W_{maize}}{Bw}$$

where $C_{heavymetal}$ (mg/g, on fresh weight basis) is the concentration of heavy metals(maize) ,W_{maize} represents the daily average consumption of maize /water, B_W is the body weight (Fordyce, 2000).

Comparison with recommended daily allowance (RDA) was undertaken for male adults, using a mean body weight of approximately 70 kg (NRC, 1989). Hair samples were first washed with distilled water on a stirrer for 15 min in a beaker, and then washed with acetone-water-water-water-acetone as recommended by the International Atomic Energy Agency (IAEA, 1985). The washed samples were placed in glass beakers and individually allowed to dry at 50^{o}C overnight in a drying oven. Before washing the hair samples, any visible dirt on the surface was thoroughly washed using MilliQ water.

Metal analysis

All samples were analyzed in the Biochemical Laboratory, Moi University. The maize, and hair samples were crushed and homogenized using a Fritsch, Pulverisette 5, planetary mill (Fritsch GmbH Laborgerate, Idar-Oberstein, Germany) for 5 min at 400 rpm. About 0.2000 g of maize and hair samples were accurately weighed in Teflon (& polytetra-fluor-etheen (PTFE), DuPontTM) high pressure vessels. Then 4.0 ml concentrated nitric acid (65%), 1.0 ml concentrated hydrochloric acid (37%) and 1.0 ml ultra pure water was added to the samples. Six samples of each item were placed in the carrousel of a Paar Microwave oven (Anton Paar GmbH – Graz – Austria. The samples were digested, using a microwave oven (Anton Paara GmbH Karntner Straße 322A-8054 Graz/Austria) at a maximum temperature set at 220 ^{o}C and pressure at 75 bar for 15 min. After cooling, the obtained clear solutions were quantitatively poured in 50 mL volumetric flasks and diluted to the mark with ultra pure demineralized water (Barnstead NanoPure, Thermo Fisher Scientific Inc, Barnstead International, Iowa USA). Finally the diluted solutions of samples were transferred into acid cleaned polyethylene bottles. All elements were determined by means Atomic Absorption Spectrometry(AAS) using the PE calibration standards. The concentrations of heavy metals in maize and hair were calculated as mg/g dry weight. The method limit of detection for the selected metals were calculated as three times the standard deviation for the digestion blanks (n=5). Triplicate injections into AAS showed good reproducibility which were generally <5%. Due to the unavailability of appropriate and reliable certified human hair standards for the validation of our methods, standard addition method was performed with several samples, which were spiked with standards and subjected to the same digestion and analysis procedures. Satisfactory recoveries were obtained for the elements (70–99%) and procedural replication showed RSD < 10% for all the analyzed elements (n=5). During analysis of the maize samples, satisfactory certified standard reference material were unavalaible.

Statistical analyses

All analysis was performed, using SPSS for Windows Release 13.0 (SPSS Inc). The W test (Gilbert, 1987) developed by Shapiro and Wilz was used to test log–normal distribution of the data for hair of our studied population. To meet the criterion of normality before statistical procedure, all nonparametric data were log-transformed, using the equation, x' =log (x +1) (Zar, 1996). All data among sampling sites were calculated as geometric means (GMs). Comparison of heavy metal concentrations in hair and maize samples in different sampling sites was done, using One-way ANOVA. Whenever the null hypothesis was rejected, a multiple comparison test (Tukey HSD test) was used following to determine the differences between individual groups. The relationships between the heavy metal concentrations in the hair samples for individual male adults were analyzed using the linear regression model. The similarity/dissimilarity of the heavy metals in hair, maize and drinking water samples were graphically presented in a non-parametric multi-dimensional scaling ordination (NMDS), which represented matching similarities calculated in a triangular matrix of similarity coefficient computed between every pair of metal samples (Clark and Warwick, 1998). The reliability and validity of the MDS solution was determined by calculating the index of fit (R-square), which is the proportion of the variance of the optimally scaled that can be accounted for by the MDS procedure (goodness of fit). Stress value was also determined to indicate quality of MDS, which indicated the badness of fit (proportion of the variance of the optimally scaled data not accounted for by the MDS model). All the levels of the statistical significance were set at P<0.005, unless otherwise stated.

Ethical statement

During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of Moi University Institutional Research and Ethics Committee (IREC), (Formal approval no.: FAN: IREC 000209) were strictly followed.

Results

First the data were tested for distribution to better interpret the results. All the elements in hair and maize samples showed log– normal distribution, which indicates that the data were sufficient to provide the information about the shape of the target population.

The maize and hair samples were obtained in each of the four zones with 25 sampling sites each. Generally, heavy metal concentration in maize and hair were of the order Pb>Cu >Cr >Cd. In maize, concentrations of Pb in the sampling sites was significantly higher (P<0.05) in Zone 2 than the other three zones (Table 1). The differences in concentration of these metals between sites, where they occur in highest concentration, and where they occur in the lowest concentration were more than three-fold. The Cu concentration was lowest in Zone 3 which was significantly different from the other zones(p<0.05). Concentrations of Cd and Cr were highest in zone 3and 4 respectively than other zones, while similarity of Cr metal was discerned in zones 1and 3.

Table 1. Metals concentration in maize samples within Eldoret Municipality

		Maize	
Metal	Zones	Dry season	Wet season
Pb	EATEC	1.6060 ± 0.0305^{b}	1.5880 ± 0.0299^{b}
	Yamumbi	1.9810 ± 0.0260^{c}	1.8670 ± 0.0315^{b}
	Kahoya	0.5600 ± 0.0228^{b}	0.5100 ± 0.0311^{a}
	Hawaii	1.6620 ± 0.0122^{b}	1.5800 ± 0.0290^{b}
Cu	EATEC	0.2540 ± 0.0192^{a}	0.3300 ± 0.0136^{b}
	Yamumbi	0.1492 ± 0.0091^{b}	0.2112 ± 0.0176^{b}
	Kahoya	0.0187 ± 0.016^{ab}	0.0236 ± 0.0124^{b}
	Hawaii	0.2058 ± 0.0150^{a}	0.1986 ± 0.0160^{b}
Cr	EATEC	0.0419 ± 0.0406^{b}	0.0332 ± 0.0165^{a}
	Yamumbi	0.0225 ± 0.0294^{c}	0.0506 ± 0.0275^{c}
	Kahoya	0.0423 ± 0.0159^{b}	0.0382 ± 0.0172^{b}
	Hawaii	0.0627 ± 0.0312^{a}	0.0545 ± 0.0126^{a}
Cd	EATEC	0.0472 ± 0.0017^{a}	0.0434 ± 0.0016^{a}
	Yamumbi	0.0385 ± 0.0026^{c}	0.0319 ± 0.0023^{b}
	Kahoya	0.0496 ± 0.0011^{a}	0.0362 ± 0.0015^{a}
	Hawaii	0.0398 ± 0.0014^{a}	0.0321 ± 0.0013^{a}

In human hair, concentrations of Pb in the sampling sites was significantly higher (P<0.05) in Zone 2 than the other three zones (Table 2). The differences in concentration of these metals between sites, where they occur in highest concentration, and where they occur in the lowest concentration were not significantly different (p> 0.05), except in zone 2. The Cu concentration was highest in Zone 3 which was significantly different from the other zones(p<0.05). Concentrations of Cd and Cr were highest in zone 2 than other zones, while similarity of Cr metal was discerned in zones 1and 3.

Table 2. Metals concentration in human hair samples within Eldoret Municipality

		Human Hair	
Metal	Zones	Dry season	Wet season
Pb	EATEC	0.6060 ± 0.0305^{b}	0.5880 ± 0.0299^{b}
	Yamumbi	0.8681 ± 0.0260^{c}	0.7670 ± 0.0305^{b}
	Kahoya	0.6879 ± 0.0228^{b}	0.55992 ± 0.0315^{a}
	Hawaii	0.5620 ± 0.0122^{b}	0.5800 ± 0.0290^{b}
Cu	EATEC	0.0840 ± 0.0192^{a}	0.0730 ± 0.0136^{b}
	Yamumbi	0.0792 ± 0.0091^{b}	0.0772 ± 0.0116^{b}
	Kahoya	0.2497 ± 0.0173^{b}	0.2036 ± 0.0144^{b}
	Hawaii	0.1275 ± 0.0150^{a}	0.1196 ± 0.0164^{b}
Cr	EATEC	0.0412 ± 0.0421^{b}	0.4326 ± 0.0132^{a}
	Yamumbi	0.0543 ± 0.0204^{c}	0.0506 ± 0.0205^{c}
	Kahoya	0.0054 ± 0.0109^{b}	0.0052 ± 0.0152^{b}
	Hawaii	0.0052 ± 0.0302^{a}	0.0055 ± 0.0122^{a}
Cd	EATEC	0.0256 ± 0.0017^{a}	0.0234 ± 0.0017^{a}
	Yamumbi	0.0265 ± 0.0026^{c}	0.0219 ± 0.0023^{b}
	Kahoya	0.0296 ± 0.0011^{a}	0.0262 ± 0.0015^{a}
	Hawaii	0.0194 ± 0.0014^{a}	0.0178 ± 0.0013^{a}

There were positive relationships between all the heavy metals in maize and hair. However, increased concentrations of Pb, Cd and Cu in hair were better estimated (>56%) by increased concentration of heavy metals in human hair, but not for Cr. Concentrations of Pb, Cd and Cr in the samples of maize were found to be elevated in zone 3. The concentration of Pb in maize in zone 3 was four-fold in magnitude than concentration of this metal in maize in zone 4. Though significant differences (P <0.05) in the Cd and Cr were observed in maize among sampling sites, the concentrations of these heavy metals in maize between the zones having the highest concentration and zone with the lowest concentration exceeded three-fold. Copper concentration was not significantly different among zones (P >0.05).

The estimated daily heavy metal ingestion among male adults eating maize from the study area. showed daily ingestion rates of maize 0.15, 0.27, 0.32, 0.24, 0.35 kg male adult per day in sites 1, 2, 3, 4, 5, respectively. The mean weights of the male adults were: 69.4, 70.5, 70.0, 70.8, 70.1. Estimated daily intake of all heavy metals from consumption of maize was significantly higher ($P<0.05$) in zone 3, albeit Cu intake from maize consumption was similar in zones 2 and 3. Daily ingestion of Pb in zone 3 was fourfold the amount of Pb ingested in other zones. The differences in daily intake of Cd from maize consumption in zones 3 and 1 were eight-fold while the differences in concentration of between sites 3and 4 were threefold.

Generally, when all the individuals' heavy metals measured in hair, nails and heavy metals estimated from maize consumption were compared, there was metal specific similarity in concentration of Cu, Cd and Pb in hair, nail and maize consumed. The variability of Cd in the three matrices was wide variabilities of Cu and Pb. Concentration of Cr obtained from maize consumption was positively related to Cr measured in the nails only.

Discussion

In Africa, studies of metal pollution are scarce (Banza et al., 2009), yet there are growing evidence that problems of heavy metals are posing increasing risks to the residents in the continent (Nriagu, 1992). Soils and water catchments areas in some areas have remarkable quantities of mineral elements, which are yet to be exploited. These metals cause enrichment of the soils and water, thus food crops and aquatic organisms accumulate high metal body burdens. This study which investigated the heavy metal risks from environment and maize among male adults consuming large quantity maize in the diets in the Eldoret Municipality seems to be an adequate tool with a approach to the corresponding pollution risk. As far as we know no data are available regarding the human exposure to metals in this area, which is increasingly getting pollutants from the farming activities and from possible geological sources rich in minerals.

In the hair samples, concentration of the heavy metals in the present study were comparable with or even higher than those published in previous studies among the non conventionally exposed (Rodushkin and Axelsson, 2000; Wang et al., 2005; Were et al., 2008; Wang et al., 2009).

Male adults within the study area, the concentration of Pb in the present study were lower than the occupationally exposed residents of Taizhou, but the concentration of Cd, Cr and Cu in the present study were higher than the occupationally exposed residents of the area (Wang et al., 2009). Similarly, the present concentrations of Cd and Pb in hair samples are higher than those reported in human hair in Nairobi, Kenya (Were et al., 2008). In comparison to non-occupationally exposed residents, the present study of all toxic metals were found to be higher in concentration with an element such as Pb being 20 time higher and Cd being 10 times higher (Rodushkin and Axelsson, 2000), and thus the content of toxic metals suggested high levels of exposure to heavy metals. The concentration of heavy metals in the nails could be a possible indicator of chronic exposure of the humans in the Eldoret unicipality.

The correlation trends in human hair were interpreted as elemental competition for sorption in the active Zones since Pb and Cd have no known functions in the body, while Cu are required in low quantity. The positive relationships between metals in hair could also indicate exogenous sources of metals, which reflect environmental exposures. A major problem in the use of hair as a biomonitor of environmental exposure is the inability to separate endogenous and exogenous deposition of metals, because proportions of substance from the environmental media are incorporated and strongly bound to the hair structure (Kempson et al., 2006). It is also possible that washing of the hair samples removed all the exogenously deposited contaminants in hairs, and thus the observed metals are from the physiological body systems and diet. The health risks associated with maize consumption have been documented (Fordyce, et al, 2002). Maize samples in the present study had elevated metal concentrations in zone 3. Daily dietary metal intake through maize consumption was calculated based on the average metal content in maize and eating frequencies. The daily metal intake in maize was also found to be higher in zone 3. It is evident from when compared to the recommended daily dietary allowance (RDA) for Pb of 250 mg/day and Cu 3250-325,000 mg/day by FAO and USA (CAC, 1984; NRC, 1989; Chen and Chen, 2001; USEPA, 2002) that RDA was never exceeded at any zones. However, RDA for Cd (57–72 mg/day) and Cr (150 mg/day) was exceeded by the male adults in zone 3, suggesting that maize consumption was likely to expose the male adults to risks of metal toxicity. The overall risks of Cd and Cr in zone 3 was accounted by the higher consumption of maize in these areas and the higher metals from maize consumption. As yet, no studies have been conducted to quantify the geological metal sources in this area. Because the maize is easily available and being the most staple food retailing at US$ 1/kg, it was consumed more by the residents than the other forms of foods.

The interaction between metals in hair and maize examined by NMDS indicated similarity between specific elements in maize, and hair matrix (at least for Pb, Cd and Cu). This could suggest metal uptake from maize consumption (at least to some degree) as suggested in other similar studies (Fordyce, 2002, Fordyce and Janson, 2000). The high variability in Cd determined in hair and from ingested maize could suggest that other than maize consumption, there are other possible sources of Cd in the humans especially the geological sources. However, Cr was found to display similarity between hair and maize samples only, which could suggest that if the uptake of Cr was from maize consumption, then it was due to long-term metal exposure. Because of the different types of foods likely to be ingested, soils and water, it was not easy to quantify the exact amounts of element ingested from the maize, which was complicated by the metal interactions. It could also be possible that in multi-elemental samples, in the cells, differential sorption patterns occur across the hair follicle (Wang et al., 2009) that would ultimately influence the concentration of the other present metals.

CONCLUSIONS

There were evident heavy metal contaminations in sites. Furthermore, there were close associations established between the specific metals in hair. It was suggested that consumption of higher quantity of maize could pose a potential long term health risk to local residents in the study area. The present study demonstrated that determination of metals in human hair, and relating this with heavy metals estimated from consumption of maize has potential utility as a biomarker of exposure to heavy metals.

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