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Detection of cu, cd and cr in Sugarcane Grown Along Ngong Tributary of Nairobi River

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Abstract: *Heavy metals induce oxidative stress within bodies and are suspect of various chronic diseases suchas cancer, mental retardation, hepatoxicity and kidney failure. The study aimed at establishing heavy metal contamination and safety of consumption of sugarcane grown along Ngong tributary of Nairobi River. The Sugarcane crop was sampled randomly from three distinct regions along the river based on pollution activities in the dry month of January 2017 and their juices extracted. Levels of copper (Cu), Cadmium (Cd) and Chromium (Cr) were determined by modified AOAC official method 999.10 using Inductively Coupled Plasma mass spectrophotometer. Health risk assessment was done by FAO, 2006 and FAO/WHO2011 official methods based on a survey conducted in slums along the tributary and various owners of sugarcane farms. The Cu, Cd and Cr levels in sugarcane juices were between, 0.2-0.8mg/l, 0.003-0.015mg/l and 1.056-3.481mg/l respectively indicating significant differences ($p<0.05$) of Cu and Cd in sugarcane juices in the highly polluted industrial zone along the river. The total estimated daily intakes (EDI) for adults and children was >1. Target Hazard Quotient for adults and children were within the safe limits. Since heavy metals are bioaccumalative in nature it is recommended that utmost care and analysis be conducted before consumption.*

Key Words: *Heavy metals, Sugarcane, Health risk*

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I. Introduction

Sugarcane is one of the crops cultivated along banks of all tributaries of Nairobi River, and mainly watered by waste water draining to the river, river water and to a less extent seasonal rain waters. Nairobi river basin has heavy metals polluting units ranging from agricultural chemicals, unregulated informal settlements, automotive wastes and heavy industrial waste (Hide, Kimani, & Thuo, 2001). Higher soil concentration of lead, chromium, manganese and iron than those recommended by WHO and isolated high levels of mercury and aluminum have been detected in Ngong River, a tributary of Nairobi River (Budambula & Mwachiro, 2006). Some studies show variations in specific heavy metals bioaccumulation in some sugarcane varieties (W. xueli et al., 2012). There is significant bioaccumulation of heavy metal in waste water fed sugarcanes as compared to rain water fed sugarcanes (Alghobar & Suresha, 2015). Furthermore, sugarcane potential to bioconcentrate heavy metals from soils with acceptable heavy metal’s concentration limits is high. (Adekola, 2008). In Kenya's major towns such as Nairobi, Sugarcane hawking is an economic generator for youth and urban poor (Kaluli et al, 2011). In these towns Sugarcane, and its products are sold along streets, in markets, schools, hospitals, bus stops and within slums and suburbs (Hide et al., 2001). Sugarcane can be consumed raw or juiced to process jaggery used in manufacture of local brews (O, Ruth, Jane, & Charles, 2013). Jaggery, a confectionary like sugarcane product is popular to school going children (Rao, Das, & Das, 2007). Direct sale and consumption of sugarcane juice which is a mixture of Sucrose, flavonoids, polyphenols, amino acids, and minerals is also increasing with increased awareness of its health benefits (Kadam, Ghosh, De, & Suprasanna, 2008). Polyphenols in this juice are beneficial to humans due to its antioxidant activities (Kadam et al., 2008). Molasses another sugarcane product, is used in silage production for dairy animal feeds as it aids in fermentation of the feed converting them into a form more available for absorption (Valli et al., 2012)). Consumption of sugarcane and its products is therefore widespread in Kenya and more so in Nairobi town and its environs. There is high risk of heavy metals intoxication by consumption of sugarcane grown along Nairobi River, Ngong tributary and products made from these sugarcanes as studies have confirmed heavy metals presence in soil, water and some crops grown along this tributary as well as the well-known behavior of sugarcane in heavy metals rich soils. Heavy metals are not degraded in the body system but accumulate and damage body tissues such as central nervous system, excretory system, respiratory system, skeletal system among others. This lead to several health problems such as acute renal failure, autism spectrum disorders, several cancers, hepatoxicity and genotoxicity (Zahir, Rizwi, Haq, & Khan, 2005). Though sugarcane grown along Ngong tributary of Nairobi River has potential to bio accumulate heavy metals reported along this river system, there is no information or studies indicating the same. This study, therefore, aims at determining levels of heavy metals in sugarcane planted along the Ngong tributary of Nairobi River and its products.

II. Materials and Methods

2.1: Study site:

Nairobi River, Ngong tributary which forms Nairobi basin was the study point in 3 specific points depending on extent of pollution namely Montoine dam which is the source of this tributary, industrial area region and confluence to Nairobi River. The stream is a 37.5km stretch from Montoine swamp and Ndagoretti forest down, streaming down Kibera slums, Mukuru slums, industrial area Maili Saba, Njiru and joins Main Nairobi River. Nairobi basin lies at longitudes 1^{0}'S36^{0}49'E.the annual rainfall is 1000-1200mm, with long rainy season between March and June and short rainy season of October to December. Mean annual temperature is 17^{0}c (Foeken & Mwangi, 1995). September to mid- October is most dry period, January to mid-march is hot and dry while June to mid-October is cool, cloudy and dry.

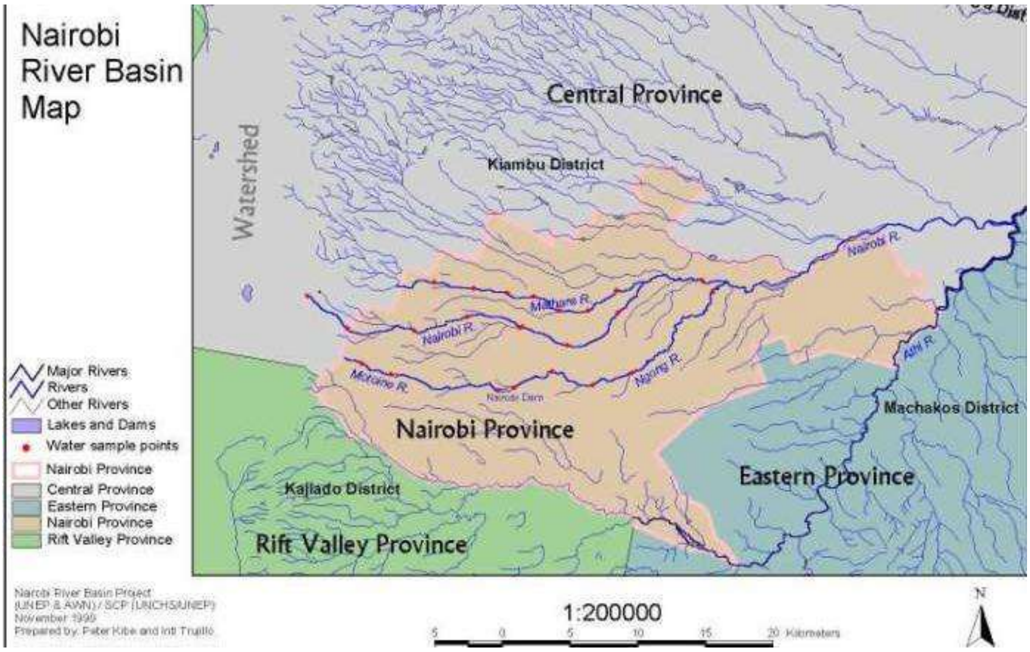

Fig 1: Nairobi river basin map 1(Source: Krhoda, 2002 Nairobi river basin project, UNEP, 1999)

2.2 Sample collection:

2.2.1 Sugarcane sample

Sampling was done on selected plots on the bank of Nairobi River in all sampling points namely Montoine dam which is the river source, Industrial area and Confluence area to the main Nairobi River (fig 1). The source area was selected as there were no industrial activities and expected agricultural contaminants. Sugarcane was collected randomly for every region covering a distance of 10km.This was replicated in industrial area of Kayole upto Mukuru kwa Ruben and finalized on the confluence area of Chokaa to Njiru confluence. A total of 15 samples was collected on every region making a total of 45 samples. Control area was done on area about 60km away and shares a different geographical pattern including soil and climate on river Thebere in Gatundu south of Thika district. The only expected contamination is from agricultural activities. The uprooted samples were washed with deionised water, dried, cut, and kept on a labelled polyethylene paper and transported by a cool box for analysis. (E. Vol et al., 2012)

2.2.2 Soil sample:

Soil was scooped at a depth of 10cm and 20cm on the uprooted sugarcane roots according to E. vol, a total of 15 samples each from the 4 sampling points were taken. Labelling was done accordingly. (E. Vol et al., 2012)

2.2.3 Water sample:

Water sample adjacent to the point of sampling was collected at a depth of 20cm (Greaney, 2005) in replicates and collected on a clean PTFE plastic bottle acidified to pH of less than 2 by addition of concentrated analytical grade Nitric acid and stored for analysis (Adekola, 2008).

2.3 Heavy metals analysis:

2.3.1 Sample Cleaning

All glassware and plastic to be used during sampling, sample storage and analysis were immersed overnight in concentrated Nitric acid, thereafter cleaned with 0.1N and rinsed with d water, soaked overnight with concentrated nitric acid and rinsed again with lots of de-ionised water and left to dry in the rack as described in the AOAC official method 999.11. Pre cleaning was done again for every analysis. The samples were transported in pretreated form and using cool boxes in the month of March 2017. The analysis was done semi quantatively and quantatively and results presented quantively. Standards used for both semi quantative and quantative determination were single multi elements standards (Merck) used for Inductively Coupled plasma spectrophotometer.

2.3.3. Sample digestion:

2.3.3.1. Soil sample

The soil sample were selected through cone and sample method ground to a fine powder .0.01g was digested using the open conventional digestion method using Aqua regia solution (1:2 Nitric acid &hydrochloric acidsrespectively). The digestion was done for 4 hours on a hot plate at a temperature of 80℃, filtered using whatman filter and topped to 50ml.The digest was analysed for Cu, Cr, and Cd. The analysis was done semi quantatively and quantatively and results presented quantively. Standards used for both semi quantative and quantative determination were single multi elements standards (Merck) used for Inductively Coupled plasma spectrophotometer. Reference standards were from WHO standards.

2.3.3.2 Sugarcane sample and water samples:

Sugarcane sample were thoroughly washed with distilled water and separated into liquid extract and bagasse using a stainless steel juicer in JKUAT fruit workshop. The juice extract was stored and eventually transported to Taiwan using cooling boxes for analysis. Digestion was done by putting 1ml of a sample to a Teflon tube with addition of 2ml hydrogen peroxide and 3ml Conc Nitric acid heated to 190˚C in a closed digester for 4 hrs. Until the digest was a clear liquid with no coloration or sediments. The digest was then topped to 50ml using distilled water and analysed for heavy metals using ICP mass spectrophotometer for heavy metals. This method was also used for water samples.

2.3.3.2 Stock solution:

Stock solution, calibration standards, and working standards were prepared from analytical grade single multi elements for ICP mass (Merck) with high purity approximately 99.9%, The standards which was 100ppb was made into 10ppb, 20ppb 50ppb and 200ppb to draw the calibration curve by topping up 1ml, 2ml 5ml and 20mls of standard solution to 10mls each respectively.

To eliminate background interferences blanks consisting the digesting solution for every analysis without the sample were used and digested together with other samples and finally topped to 50ml for analysis. Also 10µg of stock solution was used to spike.

2.4 Transfer factor (TF):

BCF has been defined as plant ability to accumulate a known heavy metal relative to concentration of the same element in soil (Ghosh and Singh, 2005)., expressed as follows:

BCF= Heavy metal concentration in plants ÷ Heavy metal concentration in soil (dry weight basis) In this case only the sugarcane juice which was the edible part of sugarcane was used.

2.5 Consumption pattern and risk assessment:

The consumption and health risk assessment was done by a method by EPA (2010) as used by M.A alghobar et al 2015 as follows; EDI (Estimated daily intake) was represented by concentration of heavy metal multiplied with daily intake (DI) and results divided by average body weight which is 70kg for an adult and 25kg for children USEPA, 1989.For determining Total hazard quotient (THQ), the estimated daily intake was multiplied 350 days exposure frequency (EF), multiplied by Exposure duration(ED) per recommended daily values and the multiplied by average life expectancy period in days (59yrs×360days).Total estimated daily intake (TEDI) and total average hazard quotient were determined by totaling specific heavy metals estimated risks and health quotient (Alghobar, 2015)

2.6 Statistical Analysis

Analysis of heavy metal concentration between the soil samples, water samples and sugarcane were determined using ANOVA test at 95% level of confidence. Descriptive statistics that included theMean, standard deviation (SD), range and standard errors were used using Graphpad (version 6) and Duncan test at 95% level of confidence. Reference recommended levels were the WHO levels (up to 2011)

III. Results and Discussion

Anthropogenic activities will determine extent to which heavy metals will be deposited in the soil and water. Soil properties and plant characteristics will influence heavy metal absorption, translocation and bioaccumulation. The levels of heavy metals in sugarcane juice and production environment was determined.

Table 1.0: Heavy metals concentration in sugarcane juice, soil and water in the production environment.

		Sugarcane juice (mg/l)			**Soil(mg/kg)**			**Water (mg/l)**		
		Cu	**Cr**	**Cd**	**Cu**	**Cr**	**Cd**	**Cu**	**Cr**	**Cd**
Low(dry season)	Mean	0.2±0.04^{a}	1369±0.24^{a}	0.03±0.001^{a}	10.06±1.7^{a}	**265±86^{bc}**	0.405±0.067^{a}	ND	0.255±0.08^{a}	**0.005±0.0003^{b}**
	Min	0.042	0.428	ND	2377	17.5	0.014	ND	0.01	0.004
	Max	0.64	4.061	0.009	20.46	546.9	0.825	ND	0.448	0.006
Middle(dry season)	Mean	**0.804±0.09^{b}**	**3.481±1.29^{b}**	**0.015±0.001^{b}**	**51.07±9.4^{b}**	108.4±31^{a}	0.381±0.053^{a}	ND	**7.55±7.031^{c}**	**0.006±0.001^{b}**
	Min	0.203	0.111	0.005	22.15	11.51	0.106	ND	0.034	0.005
	Max	1.349	12.37	0.027	108.6	204.5	0.744	ND	42.69	0.01
Confluence(dry season)	Mean	0.170±0.05^{a}	1.056±0.34^{a}	0.004±0.001^{a}	**55.09±6.9^{b}**	**480.1±273.6^{c}**	**0.598±0.16^{b}**	ND	0.457±0.17^{a}	0.003±0.001^{a}
	Min	0.008	0.297	ND	28.6	33.52	ND	ND	0.114	0.0002
	Max	0.593	4.246	0.01	93.51	2086	1.228	ND	1.277	0.006
Control(dry season)	Mean	0.217±0.05^{a}	1.741±0.49^{a}	0.004±0.001^{a}	14.3±6.27^{a}	**217.7±66.59^{b}**	**1.143±0.186^{c}**	ND	**0.950±0.87^{b}**	0.006±0.001^{b}
	Min	0.055	0.054	ND	5.29	2.283	0.636	ND	ND	0.004
	Max	0.645	6.505	0.012	76.78	497.4	2.46	ND	5.285	0.014
WHO/USEPA(2000)		**2**	**0.1**	**0.03**	**100**	**200**	**100**	**2**	**0.1**	**0.03**

Values are mean±standard error of mean in each region; Low= River origin which is lowly polluted region; Middle=the industrial area region which is characterized by high pollution; Confluence=Lower part of the river which is also polluted and settlement area for pollutants. Statistical significance $^{AP<}$0.005 compared with control group, ^{bp} compared with control group.ND=Not Detected.

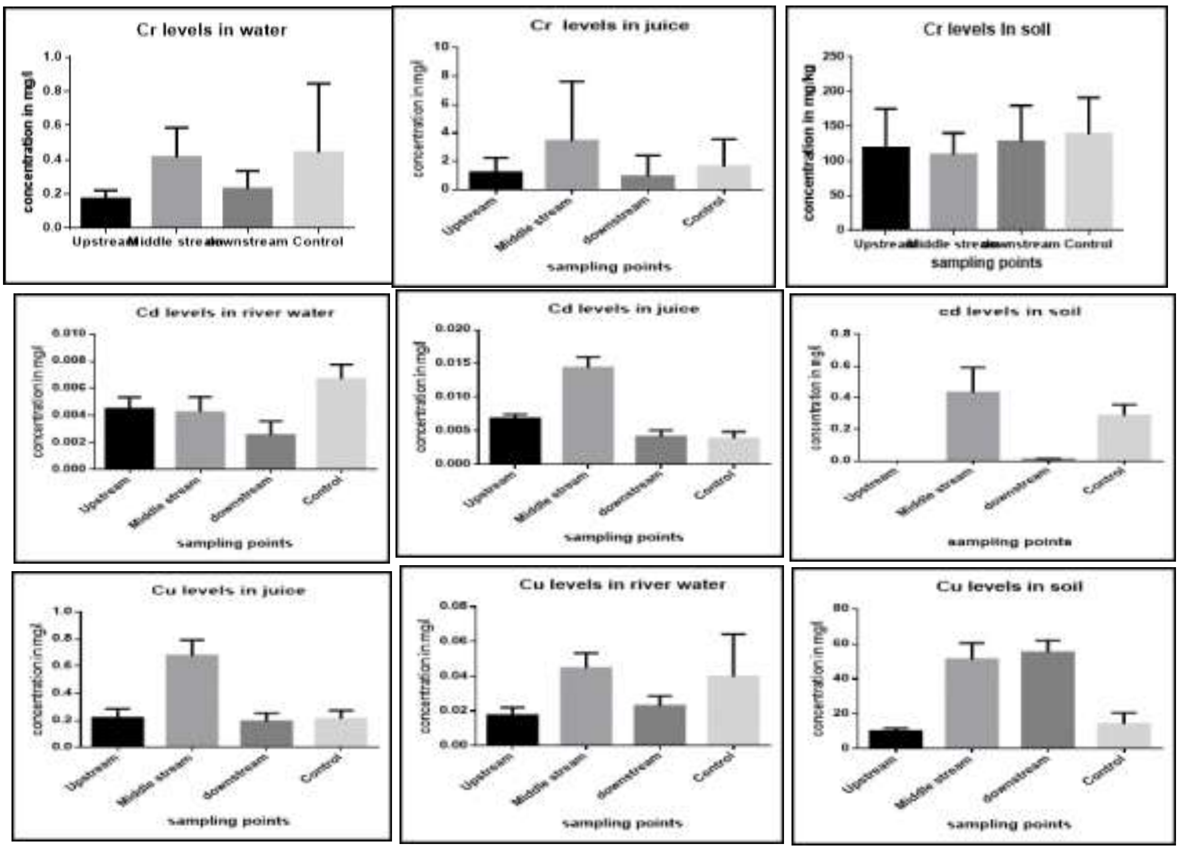

Fig 2: Comparison of heavy metal b levels in Sugarcane juice, Soil and water; Sugarcane and water in mg/l; Soil inmg/kg

3.1 Juice samples:

The descending order of elements concentration were for the low polluted region was Cr>Cu>Cd, at the high polluted zone the concentration order was Cr>Cu>Cd. At the confluence the level was Cr>Cu>Cd. The order at the control region (Gatundu) was Cr>Cu>Cd. For low polluted regions the levels of concentration were Cu (0.2±0.046mg/l), Cr (1.369±0.24mg/l) and Cd (0.003±0.001mg/l) (table 1.0). At the highly polluted region the levels were Cu(0.804±0.09mg/l), Cr(3.481±1.29mg/l) and Cd(0.015±0.001mg/l). For the confluence (lower end), the levels were Cu (0.1703±0.05 mg/l), Cr(1.056±0.34mg/l) and Cd(0.004±0.001mg/l). For control region the levels were Cu(0.217±0.05mg/l), Cr(1.741±0.49mg/l) and Cd(0.004±0.001mg/l). The ANOVA analysis for the four regions showed statistically significant difference in levels of some heavy metals in highly polluted zones. Levels of Cu and Cd were significantly higher($P<0.05$) in the highly polluted zone of Ngong river compared with other zones and the control region (fig 1.0). There was significant difference in levels of Cr at ($P<0.05$) in highly polluted zone compared to other zones. Previous studies done on copper levels indicated a range of 0.31-0.27mg/l (Kamau, 2016). The range in this study was 0.170-0.804mg/l indicating a slightly higher values which can be explained by varying levels of pollution at different times. Levels of Cr in all samples exceeded WHO recommended limits 0.1. This included the sugarcane juice from the control region and the river source (low polluted region). This could be explained by the fact that chromium pollution could not only emanate from industrial pollution but also agricultural pesticides and other agronomical pollutions that characterize the level of pollution in the low polluted region and the control region. Levels in the industrial zone region and control region (Gatundu) were higher than acceptable limits previous studies on chromium and cadmium indicated levels of 10.54-60.22mg/kg and BDL-0.15mg/kg respectively. The values for Cr and Cd in this study were in the range of 1.056mg/l- 3.481mg/l and 0.003mg/l- 0.015mg/l. The levels of chromium in this study were far much less compared with the previous study done in China reason being higher levels of chromium pollution as well as varying soil chromium composition. The levels of cadmium were within the same range. WHO recommended limits for Cd and Cr are 0.03 and 0.1mg/l. Levels of chromium in this study exceeded the recommended levels in all sampling sites indicating widespread chromium pollution.

3.2 Soil samples:

The descending order for low polluted region Cr> Cu>Cd, the highly polluted was Cr>Cu> Cd. The confluence region was Cr> Cu> Cd (fig 1).Control(Gatundu) region had the following levels of concentration ; Cr> Cu>Cd .Mean levels of heavy metal concentrations were recorded as follows; the lowly polluted region had Cu(10.06±1.7mg/kg) Chromium(265±86mg/kg) and Cd(0.405±0.067 mg/kg)(table 1.0).levels at the highly polluted region(Mukuru area) along the river were as follows; Cu(51.07mg/kg), Cr(108.4±31mg/kg) Cd (0.381±0.053mg/kg)(table 1.0) .The heavy metal levels at the confluence region were recorded as follows; Cu(55.09±6.9mg/kg), Cr(480.1±273.6mg/kg) and Cd(0.598±0.16mg/kg). Control region had the following levels of heavy metals; Cu(14.3±6.27mg/kg), Cr(217.7±66.59mg/kg) and Cd(1.143±0.186mg/kg). Studies done in India showed Cu(39.75mg/kg), Cr(47.73mg/kg) and Cd(0.1mg/kg). Previous studies done along Ngong tributary recorded copper levels ranging from 6.43-45.61mg/kg (Mutune et al, 2014). This was within the levels found in the current studies. Chromium levels in the previous study recorded levels of 0.03-0.14mg/kg. This levels were far lower than the current study indicating increased pollution levels along the river. Cadmium levels from the previous study indicated range of BDL to 0.223mg/kg.This was below the range of the current levels in this study. Levels in industrial zone, at the river confluence and control region had higher values than WHO recommended limits of 200mg/kg indicating a combination of both industrial activities and agricultural activities pollution.

3.3 Water samples:

The descending order for low polluted region was Cr>Cd>Cu>.For highly polluted region the order was Cr>Cd>Kg.The order for confluence was Cr>Cd>Cu.Control region had Cr> Cd>Cu. Levels of heavy metals at lowly polluted region the levels were Cu(BDL), Cr(0.255±0.08mg/l) Cd(0.005±0.0003mg/l) At highly concentrated region the levels were Cu(BDL),Cr(7.55±7.031mg/l) and Cd(0.006±0.001mg/l) At the confluence the levels were Cu(BDL),Cr(0.457±0.17mg/l) and Cd(0.003±0.001mg/l).Control sample was Cu(BDL) Cr(0.95±0.87mg/l and Cd(0.006±0.001mg/l). Cadmium and Chromium range of 0.00-0.15mg/l and 0.44-0.61mg/l respectively were recorded in previous study (J. Kaluli et al, 2014). The Cadmium levels were within the same range. However, values for Chromium in the current study are slightly higher than the previous study indicating an increased pollution level. A study done before along Ngong river indicated levels of Cu of 0.04-0.18mg/l (Kithiia, 2007). The levels of copper in the current study indicated low copper pollution along the river.

3.4 Heavy metals transfer from soil to crops:

3.4.1 Transfer Factor (TF)

Transfer factor refers to the ratio of metal concentration in edible portion of crop to metal concentration in corresponding soil (Liao et al., 2016). The transfer's factor for the sugarcane juice was calculated on dry weight basis as follows; $TF=C_{juice}/C_{soil.}$Transfer factor evaluated possible heavy metal transfer from soil to edible portion of the sugarcane plant which could lead to potential health risk. Metals with higher transfer risk indicates easier transfer from soil to the crop. (Liao et al., 2016). Bio availability is a factor of soil properties, metal speciation and crop genetic features. (Liao et al., 2016). High soil pH and total organic carbon can also stabilize soil toxic elements resulting in their decreased leaching. Root cell wall, water transport in the xylem as well as ions transport system in the endoderm membranes cytoplasm membrane will also affects metal ions transfer from soil to plants. (Liao et al., 2016)

Table 2: Transfer factors of heavy metals of heavy metals in sugarcane juice

Transfer factors of heavy metals in sugarcane juice				
Metals	Low	Middle	Confluence	Control
	TF	TF	TF	TF
Cu	0.020	0.016	0.003	0.015
Cr	0.005	0.032	0.002	0.008
Cd	0.008	0.038	0.007	0.00

Values = mean from each region; Low= River origin which is lowly polluted region; Middle=The industrial area region which is characterized by high pollution;

Confluence=Lower part of the river which is also polluted and settlement area for pollutants. The order of transfer factor at various regions were; For Low polluted region the order was Cu> Cd>Cr. The order at the highly polluted region was Cd> Cr>Cu. For the lower part of the river (confluence), the order for transfer factor was Cd>Cu>Cr .For control region the order was Cu> Cr> Cd .In all the regions Cd and Cr had relatively lower transfer factor compared with Cu.Less polluted zones provided favourable environment for copper absorption and accumulation compared with other regions while polluted zones provided favourable environment for Cadmium absorption and accumulation in the sugarcane juice .However all the regions showed transfer factor of less than 1 .(Arnot & Gobas, 2006).

3.5 Consumption risk assessment:

Values of EDI, THQ, TEDI and TTHQ were calculated from survey questionnaires done to establish frequency of consumption.EDI is the estimated daily intake of the metal (mg/kg/d)) (Pandey, Suthar, & Singh, 2016) determined as follows; EDI=C × DI/BW, Where C is the contaminant concentration in Sugarcane juice (250ml for adult) and (125ml for children) daily for frequent customers.BW is the average body weight. 15kg is the average BW for children and 70kg for adults (Pandey et al., 2016). THQ is the Total Hazard quotient calculated as follows: THQ=EDI × EF × ED/ R_fD × AT WhereEF is the exposure frequency (90days), ED is the exposure duration (59yrs), R_fD (recommended daily intake) taken from USEPA guidelines, 1989 and AT is the average exposure time (period over which exposure is averaged; 59×365 days) EPA (2010). The heavy metal content of the juice for all the regions were analysed and means, range and standard deviation reported as follows. Heavy metal concentration mean at the industrial area region were 0.804mg/l,3.481mg/l and 0.01464mg/l for Cu, Cr and Cd.

Table 3: Total hazard quotient (TTHQ) and estimated daily intakes (EDI) for the heavy metals inadults and children **EDI values in mg/kg/day**

		THQ	EDI **mg/kg/day**			THQ	EDI
Adults	Cu	0.001	0.003	Children	Cu	0.003	0.007
	Cr	0.119	0.012		Cr	0.278	0.029
	Cd	0.001	0.0001		Cd	0.003	0.0001
Totals		**0.121**	**0.015**			**0.284**	**0.036**

Target hazard quotient (THQ) is a ratio of determined dose of a pollutant to a reference dose level, calculated by total estimated daily intakes (EDIs) to indicate the pollution level due to contaminant exposure (Liao et al., 2016). If total THQ(TTHQ) is more than 1 then there is serious exposure risk detrimental to health. (Liao et al., 2016). The total EDIs for adults and children were 0.015mg/kg/d and 0.036mg/kg/d respectively (table 3). TTHQ values were 0.121and 0.284 for adults and children respectively (table 3). TTHQ for children were less than 1 indicating less serious health risk for consuming sugarcane as well of those of adults. However further studies need to be considered for other heavy metals, microbiological and across seasons. The order of THQ values for heavy metals were Cr >Cd>Cu. This means Cr pose the greatest risk for consumption of the sugarcane.

IV. Conclusion

The study was carried out to determine levels of concentration in juices soil and water as well as their transfer factor and total hazard quotient. The results showed higher than recommended levels of Cr and Cd in the industrial /settlement regions compared with Cr in low polluted regions. This indicated that both industrial, high settlement areas and agricultural activities have contributed to soil and water pollution. Industrial area however recorded highest levels of heavy metal pollution. All heavy metals had transfer factor of less than 1. TTHQ for adults was less than 1 while for children was higher than 1 indicating a high risk among children consumers. Overall the results showed the impact of anthropogenic activities on the environment and widespread health risk posed to urban dwellers.

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