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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 suspect various chronic diseases such as 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 intake (EDI) for adults and children was >1. Target Hazard Quotient for adults and children was within the safe limits. Since heavy metals are bioaccumulative, 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 wastewater 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 wastewater fed sugarcanes compared to rainwater-fed sugarcanes (Alghobar & Suresha, 2015).

Furthermore, sugarcane's potential to bioconcentrate heavy metals from soils with acceptable heavy metal 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 to manufacture local brews (O, Ruth, Jane, & Charles, 2013). Jaggery, a confectionary-like sugarcane product, is popular with school-going children (Rao & 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 their antioxidant activities (Kadam *et al*., 2008). 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 widespread in Kenya and more so in Nairobi town and its environs. There is a 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 present in the soil, water, and some crops are 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 the 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 can bioaccumulate heavy metals reported along with 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 the 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 the 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 Dagoretti forest down, streaming down Kibera slums, Mukuru slums, industrial area Maili Saba, Njiru, and joins Main Nairobi River. Nairobi basin lies at longitudes 10’S36049’E. The annual rainfall is 1000-1200mm, with a long rainy season between March and June and a short rainy season of October to December. The mean annual temperature is 170c (Foeken & Mwangi, 1995). September to mid-October is the driest period, January to mid-march is hot and dry, while June to mid-October is cool, cloudy, and dry.

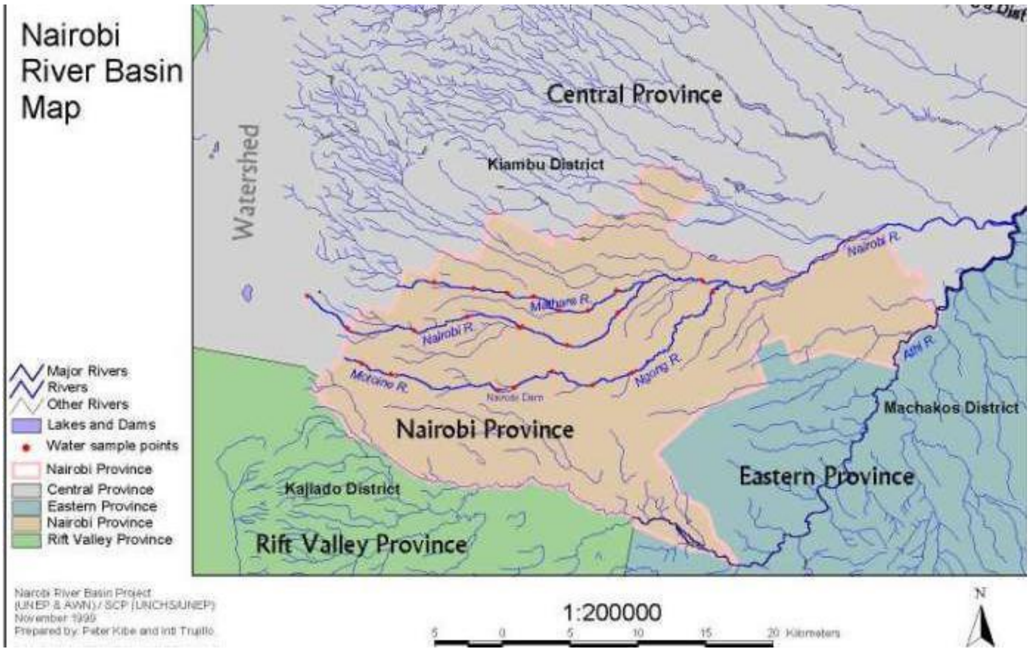

Fig 1: Nairobi river basin map 1(Source: Krhoda, 2002 Nairobi river basin project, UNEP, 1999)

2.2 Sample collection:

2.2.1 Sugarcane sample

Sampling was done on selected plots on the bank of the Nairobi River Ngong tributary in all sampling points, namely Montoine dam(the lowly polluted region) which is the river source, Industrial area(highly polluted region, and Confluence area to the main Nairobi River (fig 1). The control region 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 the industrial area of Kayole up to Mukuru Kwa Ruben and finalized on the confluence area of Chokaa to Njiru confluence. A total of 15 samples was collected in every region, making a total of 45 samples. Control area was done on an 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 was 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. (Xueli *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 Xeuli et al total of 15 samples each from the 4 sampling points were taken. Labelling was done accordingly, (Xueli *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 two 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 that 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 March 2017. The analysis was done semi-quantitatively and quantitatively, and results were presented quantitatively.

2.3.3. Sample digestion:

2.3.3.1. Soil sample

The soil sample was 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 acid respectively). 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 Copper, Chromium, and Cadmium. The analysis was done semi-quantitatively and quantitatively, and results were presented quantitatively. Standards used for quantitative 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 samples were thoroughly washed with distilled water and separated into liquid extract and bagasse using a stainless steel juicer in the 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 the addition of 2ml hydrogen peroxide and 3ml Concentrated Nitric acid heated to 190˚C in a closed digester for 4 hrs. Until the digest was a transparent liquid with no coloration or sediments. The digest was then topped to 50ml using distilled water and analysed for heavy metals using an ICP mass spectrophotometer. This method was also used for water samples.

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2.3.3.2 Stock solution:

The stock solution, calibration standards, and working standards were prepared from analytical grade single multi-elements for ICP mass (Merck) with high purity of 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 of 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 the 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, 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 the concentration of heavy metal multiplied with daily intake (DI) and resulted divided by average body weight which is 70kg for an adult and 25kg for children USEPA, 1989. For determining the 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 then multiplied by the 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

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

III. Results and Discussion

Anthropogenic activities will determine the 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 the production environment were 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 standard error of mean in each region lowly polluted region; Middle (the industrial area region) characterized by high pollution; Confluence (Lower part) of the river which is also polluted .Statistical significance at $P<0.005$ compared the control region and the other regions. ND means Not Detected.

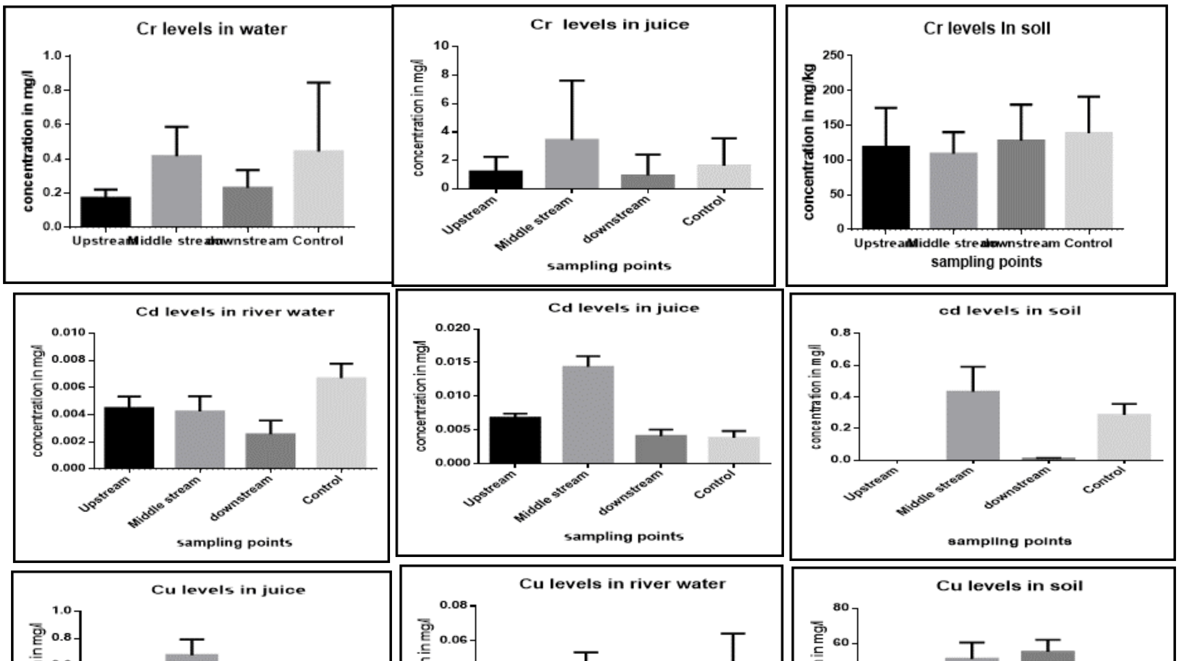

Fig 2: Comparison of heavy metal levels in Sugarcane juice, Soil and water; Sugarcane and water in mg/l; Soil in mg/kg

3.1 Juice samples:

Lowly polluted regions sugarcane juice had levels of Copper (0.2mg/l), Chromium (1.369mg/l) and Cadmium (0.003mg/l) (Table 1.0). The highly polluted region sugarcane juice had Copper(0.804mg/l),Chromium(3.481mg/l)and Cadmium(0.015mg/l) while the confluence(lower end) sugarcane juice had Copper(0.17mg/l),Chromium(1.056mg/l) and Cadmium(0.004mg/l) .Control region sugarcane juice had levels of Copper(0.217mg/l), Chromium(1.741mg/l) and Cadmium(0.004mg/l).There was statistically significant difference in some heavy metals levels in the juice for highly polluted zones. Levels of Copper and Cadmium were significantly higher ($P<0.05$) in juice from highly polluted zone of Ngong river compared with other zones and the control region (fig 1.0). There was a significant difference in Chromium levels in the juice at ($P<0.05$) in the highly polluted zone compared to other zones. The descending order of elements concentration in the juice were Cr>Cu>Cd; Cr>Cu>Cd, Cr>Cu>Cd, Cr>Cu>Cd for the lowly polluted region, highly polluted zone, confluence and the control region (Gatundu) respectively. Previous studies done on copper levels in sugarcane juice 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 value. Levels of Chromium in all sugarcane juice 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 below detectable limits-0.15mg/kg, respectively. The values for Chromium and Cadmium in juices in this study were in the range of 1.056mg/l- 3.481mg/l and 0.003mg/l- 0.015mg/l. The levels of sugarcane juice chromium levels in this study were far much less compared with the previous study which was done in China that recorded 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 Cadmium and Chromium are 0.03 and 0.1mg/. Levels of chromium in this study exceeded the recommended levels in all sampling sites indicating widespread chromium pollution.

3.2 Soil samples:

Mean levels of heavy metal concentrations in the soil were recorded as follows; the lowly polluted region soil samples had Copper(10.06mg/kg), Chromium(265mg/kg) and Cadmium(0.405 mg/kg)(Table 1.0).Levels in soil samples from the highly polluted region(Mukuru area) along the river were ; Copper(51.07mg/kg), Chromium(108.4mg/kg) and Cadmium (0.381mg/kg) while levels in soil samples from confluence region levels were; Copper (55.09mg/kg), Chromium(480.1mg/kg) and Cadmium(0.598mg/kg). Control region had ; Copper(14.3mg/kg), Chromium(217.7mg/kg) and Cadmium(1.143mg/kg) (Table 1.0) The descending order for levels were Cr> Cu>Cd, Cr>Cu> Cd, Cr> Cu> Cd , Cr> Cu>Cd for lowly polluted region, highly polluted , confluence region and Control(Gatundu) region respectively. 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. These levels were far lower than the current study indicating increased pollution levels along the river. Cadmium levels from the previous study indicated a range of below detectable limit to 0.223mg/kg. This was below the range of the current levels in this study. The industrial zone had higher values than WHO recommended limits of 200mg/kg, indicating a combination of industrial activities and agricultural activities pollution.

3.3 Water samples:

Levels of heavy metals in the water samples at lowly polluted region were below detectable limits of copper, Chromium(0.255mg/l) and Cadmium(0.005mg/l).The water sample levels at the highly polluted region were; below detectable limits of copper, Chromium(7.55mg/l) and Cadmium(0.006mg/l).Water samples levels at the confluence were; below detectable limit for Copper, chromium(0.457mg/l) and Cadmium(0.003mg/l).Control region water samples had below detectable limits of Copper, Chromium(0.95mg/l and Cadmium(0.006mg/l).The descending order for the concentration in the water samples were Cr>Cd>Cu, Cr>Cd>Cu, Cr>Cd>Cu , Cr> Cd>Cu for the low polluted region , highly polluted region, confluence and Control region respectively. Cadmium and Chromium levels in a water sample ranged between 0.00-0.15mg/l and 0.44-0.61mg/l, respectively, in a previous study done along Ngong river (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 the Ngong river indicated Copper levels in water ranging between 0.04 and 0.18mg/l (Kithiia, 2007). The levels of copper in water samples 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 the edible portion of a crop to metal concentration in the corresponding soil (Liao et al., 2016). The transfer factor for the sugarcane juice was calculated on a dry weight basis as follows; $TF=C_{juice}/C_{soil}$. Transfer factor evaluated possible heavy metal transfer from soil to the edible portion of the sugarcane plant, which could lead to potential health risk. Metals with higher transfer risk indicate more effortless transfer from the ground to the crop. (Liao *et al*., 2016). Bioavailability 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 toxic soil 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 affect metal ions transfer from soil to plants (Liao *et al*., 2016). This factors could have affected the heavy metals transfer factor.

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

The orders of transfer factor at various regions were; Cu> Cd>Cr, Cd> Cr>Cu , Cd>Cu>Cr, Cu> Cr> Cd For Lowly polluted area, highly polluted region ,lower part of the river (confluence), and control region respectively. In all the areas Cadmium and Chromium had relatively lower transfer factors compared with Copper. Less polluted zones provided a favourable environment for copper absorption and accumulation than other regions. In contrast, polluted zones provided a suitable environment for Cadmium absorption and expansion in the sugarcane juice. However, all the regions showed a transfer factor of less than 1. (Arnot & Gobas, 2006).

3.5 Consumption risk assessment:

EDI, THQ, TEDI, and TTHQ were calculated from survey questionnaires done to establish the frequency of consumption.EDI is the estimated daily intake of the metal (mg/kg/d)) (Pandey, Suthar, & Singh, 2016) determined as follows;

$EDI=C \times DI/BW$, Where C is the contaminant concentration in Sugarcane juice (250ml for an adult) and (125ml for children) taken daily for frequent customers.BW is the average body weight taken as ;15kg average body weight for children and 70kg for adults(Pandey et al., 2016).THQ is the Total Hazard quotient calculated as follows

: $THQ=EDI \times EF \times ED/ RfD \times AT$

Where EF is the exposure frequency (90days), ED is the exposure duration (59yrs), RfD (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 means, range, and standard deviation recorded were 0.804mg/l,3.481mg/l, and 0.01464mg/l for Copper, Chromium, and Cadmium.

Table 3: Total hazard quotient (TTHQ) and estimated daily intakes (EDI) for the heavy metals in adults 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 the 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 one then there is a 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 0.121 and 0.284 for adults and children, respectively (Table 3). TTHQ for children was less than 1, indicating a less serious health risk for consuming sugarcane and 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 was Cr >Cd>Cu. This means Chromium posed the greatest risk on the consumption of sugarcane juice.

IV. Conclusion

The study was carried out to determine levels of concentration in juices, soil, and water and 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 areas. This indicated that industrial, high settlement areas and agricultural activities have contributed to soil and water pollution. The industrial area, however, recorded the most elevated levels of heavy metal pollution. All heavy metals had a transfer factor of less than 1.TTHQ for adults was less than one, while for children, it was higher than 1, indicating an increased risk among children consumers. Overall the results showed the impact of anthropogenic activities on the environment and overall health risk posed to urban dwellers.

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