SURVEY OF LEVELS OF SOME HEAVY METALS IN SOIL, SUGARCANE AND SOME SUGARCANE PRODUCTS FROM MUHORONI AND SOUTH NYANZA SUGAR COMPANIES NUCLEUS ESTATES, KENYA

BY

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DECLARATIONS

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This thesis is my original work and has not been previously presented for a degree in Maseno University or in any other University. The sources of information herein have been supported by relevant references.

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DEDICATION

To my beloved mother Phelgona who is ailing and my late brother Abisai who inspired me to go to school.

ABSTRACT

Sugarcane is an important cash crop in Kenya. Due to its economic value, efforts are in progress to increase its production. The efforts include intense use of fertilizers, besides the use of high-yielding varieties. The intense use of fertilizers adds heavy metals to the sugarcane growing soils. The sugarcane plants can bioaccumulate heavy metals from soils to levels above safe limits. Metal levels in soil and factors such as soil pH and soil organic matter (SOM) influence levels of metals in food crops. Cultivation of sugarcane with intense use of fertilizers reduces soil pH and SOM content. Levels of cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) accumulated in Muhoroni and South Nyanza (SONY) sugarcane estate soils and sugarcane, and soil pH values and SOM content have not been established. The objective of this survey research was to determine soil pH, SOM content and levels of some heavy metals in soil and sugarcane. Topsoil (0-30 cm) and sugarcane stems were randomly sampled from 90 sampling points in triplicate and collected in polyethene bags. Samples were digested in *Aqua regia* solution and analyzed for Cd, Cr, Cu, Pb and Zn content, and their corresponding contamination levels and bioaccumulation factors were evaluated. Levels of heavy metals in top-soils and in sugarcane were determined using Atomic Absorption Spectrophotometer. Data analysis was done using SPSS 20 and results compared with acceptable limits. The soils had medium SOM content and were strongly acidic. SOM contents were within range required by sugarcane while soil pH values were below optimum range. Levels of metals were low in soil samples and were within safe limits of European Union standards. Levels of Cd, Cu, Pb and Zn in stems were low and within WHO/FAO safe limits except for Cr. Levels of Cr, Cu and Zn in juice were low and within recommended maximum limit while the level of Cd in juice was 13 times higher than the maximum limit. Level of Pb in juice was also above recommended maximum limit. Bagasse contained low levels of metals which were within maximum permissible limit for adult livestock feeding. Bioaccumulation factors of the metals were below 1 showing that sugarcane did not bioconcentrate the metals. Sugarcane can be cultivated in these areas but measures be taken to reduce uptake of Cd and Pb. It is recommended that soil pH be increased to the range required by sugarcane.

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ABBREVIATIONS/ACRONYMS

AAS	Atomic Absorption Spectrophotometer
BAF	Bioaccumulation factor
C	Carbon
C_b	Pre-industrial concentration of metal
C_d	Degree of contamination
Cd	Cadmium
C_f	Contamination factor
Cr	Chromium
C_s	Mean content of metals from sampling sites
Cu	Copper
DAP	Di-ammonium phosphate
D.W	Dry weight
EPA	Environmetal protection agency
Er	Potential ecological risk factor
FAO	Food and Agriculture Organization
g	Gramme
Ha	Hectare
IQ	Intelligence quotient
kg	kilogram
L	litre
M	Molar/Molarity
MDL	Method detection limit
mg	Milligrams
ml	Millilitre
mm	Millimeter
nm	Nanometre
Pb	Lead
RI	Potential ecological risk index
S.D	Standard deviation
SOM	Soil organic matter
SONY	South Nyanza
USA	United States of America
USEPA	United States Environmetal protection agency
WHO	World Health Organization
μg	Microgramme
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background to the study

Heavy metal pollution of agricultural soil and food crops is one of the most severe ecological problems in the world in general and also in Kenya. While soil serves as sink for numerous waste products of natural and anthropogenic origins, it is also media for plant growth and at the same time transmitter of pollutants to the environment, sub-surface water and plants. The food chain contamination is the major pathway of heavy metal exposure for humans (Khan *et al.*, 2008). Human health may be negatively affected by the consumption of crops grown in soils contaminated with heavy metals (Zheng *et al.*, 2007). Sugarcane is one of the world's major food crops providing about 75% of the sugar used for human consumption (Sreenivasan *et al*., 1987; FAO, 2004). It is also source of make molasses used in animal food industry, production of alcohol, ferment and glycerin (Leblebici and Kavas, 2000). Kenya produced approximately 520,000 metric tonnes of sugar between May 2015 and May 2016 while the consumption averaged 840,000 metric tonnes creating a deficit of 320,000 metric tonnes (USDA, 2016). Efforts are in progress to increase the production of sugarcane to levels that meet the domestic demand and improve productivity. Such efforts include the intense use of nitrogenous fertilizers and high-yielding cane varieties.

Many African soils which are predominantly Acrisols, Nitosols and Ferralsols, are acidic due to old age (highly weathered), depletion of soil carbon and continuous cultivation (Lungu, 1987; Sumner, 2001). Inorganic fertilizers may aggravate the process, particularly with application of ammonium based fertilizers such as ammonium sulphate, ammonium nitrate and urea. Previous studies on long term (5 - 10 years) soil fertility in Kenya and West African moist Savannah soils have shown an increase in soil acidity of between pH 1.0 to 5.0 (Smaling and Baun, 1996; Vanlauuwe and Giller, 2006). Application of fertilizers affects soil chemistry, specifically by lowering soil pH hence increasing some heavy metal solubility and mobility (Duiker *et al.*, 1982). Soil pH is an important property controlling heavy metal uptake by plants (Basta *et al.*, 2005). The extent of plant metal uptake is important to the food chain because of its possible implication to the consuming general public. It is therefore important to regulate soil pH to a level that does not enhance accumulation of heavy metals by food crops. The metals whose solubility, mobility and uptake by plants increase with the decrease in soil pH include cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) (Alloway, 1995; Baker & Senft, 1995; Kiekens, 1995; Korte, 1999; Raskin and Ensley, 2000; Kabata-Pendias & Pendias, 2001; Yang *et al.*, 2006). The exceptions to the rule is chromium (Cr) which is more mobile under alkaline conditions. The problem of soil acidification is likely to increase with intensification of agriculture and increased fertilizer use. For example, there was a decline in topsoil pH from 6.6 to 5.7 after 13 annual applications of 200 kg NH_4NO_3-N ha^{-1} (Pierre *et al.*, 1971) and a significant decrease of 0.9 pH units after 17 annual applications of 168 kg NH_4NO_3-N ha^{-1} (Jolley and Pierre, 1977). Soil acidification has been reported from various areas of the world where sugarcane is grown. In comparing land cultivated with sugarcane and uncultivated land in Herbert Valley in Queensland, Australia, a significant difference of 0.3 pH units was found in the soil horizon at a depth of 0.2 - 0.3 m (Wood, 1985). The difference was attributed to the annual application of 200 kg N ha^{-1}. Sugarcane has been cultivated in Muhoroni for 51 years and in South Nyanza Sugar belt for 36 years with repeated application of fertilizers. It has not been established whether the soil pH of the sugarcane farms is within the recommended value.

Accumulation of metals by plants is greatly influenced by soil physicochemical properties among other factors (Dudka and Chlopecka, 1990). Organic matter has many negative charges due to the dissociation of organic acids, which have a high affinity to adsorb metal cations and reduce its availability (Basta *et al.*, 2005; Vangheluwe *et al.*, 2005) and thus making them unavailable to the plants. High organic matter content of the soil is very important to retain and supply plant nutrients for sugarcane (Blackburn, 1984). However, the lower the soil organic matter content in the soil, the higher the amount of the heavy metals that bioaccumulates in the plant (Halim *et al.*, 2003). Consumption of food crops that accumulate high levels of metals can lead to poisoning. Consequently, serious systematic health problems such as bellyache, kidney damage and pulmonary emphysema can develop from excessive dietary accumulation of toxic metals in human body. It is therefore adviseable to maintain high soil organic matter content. Environmental concerns and policies are key factors affecting the future of sugarcane production (Valdes, 2007). Soil organic matter declines in soils under continuous cultivation sugarcane and burning of the crop residues (Blair *et al.*, 1995; Dominy *et al.*, 2002). Similar results were reported in northeast Brazil (Ball-Coelho *et al.*, 1993) and in North Queensland, Australia, (Noble *et al.*, 2003). There is no report on soil organic matter content in Muhoroni and South Nyanza sugar estates.

The non-biodegradable nature of heavy metals and their long biological half-life make them a crucial environmental issue (Cui *et al.*, 2005). Anthropogenic sources of heavy metal contamination are widespread. Agricultural activities such as pesticide, herbicide and mineral fertilizer application (Alloway and Jackson, 1991; Gray *et al.*, 1999a) are major sources of enrichment. Large quantities of fertilizers are regularly added to soils in intensive farming systems to provide nutrients for crop growth (Wuana and Okieimen, 2011). The fertilizers that supply nutrients for crop growth contain heavy metals as impurities, which, after continued fertilizer application may increase their levels in the soil (Jones and Jarvis, 1981; Raven *et al.*, 1998). Optimum amounts of fertilizers vary greatly with soil types, climatic conditions, and the kind and length of the growing cycle. In Kenya, di-ammonium phosphate is applied to sugarcane farms at the rate of 3 bags per hectare for planting while urea is applied at the rate of 3 bags per hectare for top-dressing (South Nyanza Sugar Co., 2012). Analysis of nitrogenous fertilizers (urea and di-ammonium phosphate) used in agricultural farms in Kenya revealed that they contain heavy metals such as chromium, lead, copper, zinc and cadmium as impurities (Omwoma *et al.*, 2010; Kananu *et al.*, 2014). It was therefore suspected that these metals accumulate in Muhoroni and South Nyanza sugar estates. Mineral uptake by plants can be affected by several factors including mineral concentrations in soils (Jung, 2008). The contamination of soil by toxic metals affects soil properties and further increases plant metal levels through root uptake (Pandey and Pandey, 2009), and eventually these metals are taken up by plants parts and transfer some into the food chain. Ideally human beings take in daily requirement of minerals by eating plants that grow in mineral rich soils (Harold and Leslie, 2000; Lokeshwari and Chandrappa, 2006). Even essential metals required by organisms at lower levels become toxic at higher levels of exposure hence daily intake of heavy metals needs to be controlled. Higher soil heavy metal concentration can result in higher levels of uptake by plants (Ebong *et al.*, 2008) and inherent contamination of food crops. Inhabitants of mine areas have experienced health problems as a result of heavy metal pollution (Liu *et al.*, 2005a). For instance, soil contaminated with Pb and Cd reduced human life expectancy in Romania (Lacatusu *et al.*,

1996) and high prevalence of upper gastrointestinal cancer in Van region of Turkey was linked to heavy metal pollution of soil, fruits and vegetables (Turkdogan *et al.*, 2003). It is therefore important to continuously assess the levels of heavy metals in agricultural soil. This has been lacking in Muhoroni and South Nyanza sugar estates and Kenya in general.

Heavy metals are naturally present in soils in very low concentrations (Zhenli *et al.,* 2005). When their concentrations increase due to anthropogenic activities, heavy metals are viewed as dangerous pollutants (Adriano, 2001). Due to the disturbance and acceleration of nature's slowly occurring geochemical cycle of metals by man, most soils may accumulate heavy metals above defined guidelines. The values can be high enough to cause risks to human health, plants, animals, ecosystems, or other media (D'Amore *et al.,* 2005). Polluted soils are no longer appropriate for agricultural production because they are unable to produce healthy food. Since about 98% of human food is produced on land, soil is the main, primary source that supplies these elements to the food chain (Deckers *et al.,* 2000). To prevent and remedy the contamination, an accurate assessment of contamination level and extent at each site is needed. Such studies help to raise public awareness of soil contamination and to facilitate research on contamination and contamination control strategies. The determination of total soil metal content alone is not a very useful tool to quantify contamination and potential environmental and human health risks. Comprehensive methods for identifying and assessing severity of soil contamination by heavy metals have been introduced for the past decades. The systematical quantitative evaluation of heavy metal contamination contributes to the understanding of the potential ecological risk (Yuan *et al.,* 2011). The most commonly cited assessment indices in environmental studies include contamination factor, degree of contamination, potential ecological risk factor and potential ecological risk index (Rahman *et al.*, 2012). This method comprehensively considers the synergy, toxic level, concentration of the heavy metals and ecological sensitivity of heavy metals (Nabholz, 1991; Singh *et al*., 2010; Douay *et al.*, 2013). A case of soil pollution arose in the United Kingdom during the nineteenth century when large scale expansion of a village took place and most of the new housing was built on the sites of old mines. There were high concentrations of Zn, Pb and Cd in the soils and the vegetables grown on these soils had toxic concentrations of these elements (Alloway, 1995). The United Kingdom heavy metal poisoning case could have been avoided if the contamination level of the site was assessed. It is therefore important to use assessment indices to evaluate heavy metal pollution in farming soils (Gupta *et al.*, 2008a). However, determination of the levels of contamination of sugar farms in Muhoroni and South Nyanza has not been done.

Little is known about heavy metal accumulation in sugarcane systems although flora and fauna are affected by even low concentrations (Zhang *et al*., 2014). In a feasibility study to adopt edible sugarcane for restoration of manganese mining sites, it was established that the levels of Cd and Pb accumulated in the edible parts of sugarcane were higher than the safe limits (Li *et al.*, 2007) making the sugarcane from the area unacceptable as food. A study of 12 sugarcane varieties revealed that about 77% of Cd was in the roots and 56% of Zn were contained in the stem (Rayment *et al.*, 2002). The researchers noted that the amounts of the heavy metals that were contained in the stem were much higher for canes grown in strongly acidic soils. In another study, roots, stems and leaves of sugarcane (*Saccharum spp*.) collected from 25 sites in an area under the direct influence of the municipal landfill site and the medical waste treatment system of Ribeirao Preto (São Paulo, Brazil) showed that roots contained 0.22 ± 0.12 mg Cd, 64.3 ± 48.7 mg Cr, 140.6 ± 27.7 mg Cu, 7.9 ± 2.1 mg Pb, and 177.4 ± 64.9 mg Zn kg^{-1} dry weight (Segura-Munoz, 2006). Metal levels in stems were 80 – 90% of those found in roots, while the concentrations detected in leaves were lower than those in roots. These results show that sugarcane is able to bioaccumulate heavy metals when grown in contaminated soil. In Kenya, there exists a number of literature (Amolo *et al.*, 2014; Omwoma *et al.*, 2010) on effects of nitrogenous fertilizers regarding sugarcane productivity, soil pH and heavy metal loads in sugarcane farms. However, there is no information on heavy metal levels in sugarcane stems though it is the most important source of sugar for Kenyan people.

Heavy metal content differs in different parts of sugarcane and the accumulation of metals is higher in the roots and leaf but lower in bagasse and juice (Zhang *et al.*, 2014). The stem of sugarcane can be separated into juice and bagasse. Sugarcane juice is used in the manufacture of sweeteners such as refined and raw sugar (Harish Nayaka *et al.*, 2009), fresh juice and jaggery used as sweeteners in the preparation of alcoholic beverages and to make candy, toffees and jaggery cakes (Rao *et al.*, 2010). Bagasse is used as an animal feed but is limited by low digestibility and compressed bagasse briquettes are used as fuel in the tea industry (MUSCO, 2010). The metal contents in bagasse and juice accounts for 0.6 - 3% and 0.6 - 7% of the total metal content, respectively (Zhang *et al.,* 2014). Sugarcane grown at two Zambian sites, New Farm (Mufulira) and Chilumba (Kafue) contained, copper and lead in sugarcane juice at levels higher than legislative limits (Kapungwe *et al.,* 2007). The results of these studies indicate that sugarcane is a crop that accumulates heavy metals in the edible parts. It is therefore necessary to establish the levels of heavy metals in juice and bagasse of sugarcane grown in Muhoroni and South Nyanza sugar estates and compare with the safe limits.

Soil-to-plant transfer is one of the key processes of human exposure to toxic heavy metals through the food chain (Zhuang *et al.,* 2009). The ability to absorb heavy metals differs between crop species, and several studies have shown that significant differences in the absorption of heavy metals exist between varieties of the same species (Wu *et al*., 2003; Zhu *et al*., 2007). The efficiency of different plants in absorbing metals is evaluated by soil to plant transfer factors of the metals (Rattan *et al.,* 2005). One approach to assess soil to plant transfer of metals to plants is to calculate bioaccumulation factor values (Intawongse, 2007). Bioaccumulation factor values are major parameters determining the risk of human exposure to metal in soils (USEPA, 1992a). That is, food crops with high bioaccumulation factor values may exert more potential risk to human health (Intawongse, 2007). Therefore, based on potential toxicity effects of heavy metals as a result of consumption of contaminated plant materials, there is need to identify the bioaccumulation potential of edible plants to ensure that levels of these trace elements meet the agreed international requirements. This is lacking in sugarcane grown in Muhoroni and South Nyanza sugar estates.

Western part of Kenya comprises of six sugar management zones that include: Chemelil, Kibos and Muhoroni within the sub humid agro-ecological zone and Mumias, Nzoia and South Nyanza within the humid agro-ecological zone. Muhoroni Sugar Company limited was incorporated in 1964 as East Africa Sugar industries Limited and started operations in 1966 and has a total canable land of 16,500 hectares (MUSCO, 2010) while South Nyanza Sugar Factory was commissioned in 1979 and is ranked second to Mumias in sugar production in Kenya (KSB, 2010). Muhoroni Sugar Company limited has been in existence for 52 years while South Nyanza Sugar Factory has been in existence for 37 years. In view of the time these Sugar Companies have been in existence and the difference in agro-ecological zones, it was important to determine levels of some heavy metals in soil and sugarcane grown in the areas, and also to assess soil pH and soil organic matter content.

1.2 Statement of the problem

Sugarcane has been cultivated in Muhoroni and South Nyanza sugar estates for decades with repeated application of fertilizers. The di-ammonium phosphate fertilizer is applied at the rate of 3 bags per hectare at planting stage while urea is applied at the rate 3 bags per hectare during top-dressing. Continued application of nitrogenous fertilizers may lower soil pH to a level that can enhance heavy metal accumulation by sugarcane. It is not known if the pH of the soil in the study areas is within the recommended range. Repeated application of phosphate fertilizer can increase the levels of some heavy metals in soil to a level that may boost the amount of metals accumulated by sugarcane. However, contamination of the soil and levels of heavy metals in sugarcane grown in Muhoroni and South Nyanza sugar farms have not been established. It is adviceable to assess contamination levels of heavy metals in soil as this gives information on degree of contamination and potential ecological risk. This has not been done in Muhoroni and South Nyanza sugar farms. Cultivation of sugarcane has been reported to reduce soil organic matter content to levels below required standards. Decreased soil organic matter content reduces soil biological activity and increases the susceptibility of the soils to physical degradation. Low soil organic matter content in soil also increases bioavailability and mobility of metals in the soil. This could increase the possibility of bioconcentration of toxic heavy metals in sugarcane. The content of soil organic matter in the soil of these study areas has not been quantified.

1.3 Objectives

1.3.1 Main objective

The main objective of this study was to determine the levels of soil pH, soil organic matter, and heavy metals (Cd, Cr, Cu, Pb and Zn) in the soil, sugarcane and some sugarcane products from Muhoroni and SONY sugar nucleus estates.

1.3.2 Specific objectives

This study was undertaken to:

1. Determine the soil pH and soil organic matter content in the farm soils of Muhoroni and SONY sugar nucleus estates and compare with the recommended range for sugarcane,
2. Determine the levels of Cd, Cr, Cu, Pb and Zn in the farm soils of Muhoroni and SONY sugar nucleus estates and compare with acceptable limits,
3. Evaluate the extent of heavy metal pollution and assess the overall potential ecological risk of the studied metals in farms soil of Muhoroni and SONY sugar nucleus estates and compare with the recommended range,
4. Determine the amount of Cd, Cr, Cu, Pb and Zn in stems, juice and bagasse of sugarcane grown in Muhoroni and SONY sugar nucleus estates and compare with safe limits,
5. Evaluate bioaccumulation factors, as a function of their absorption, of the heavy metals in sugarcane grown in Muhoroni and SONY sugar nucleus estates and compare with known range in plants.

1.4 Research questions

The research was aimed at answering the following questions:

1. Are soil pH and soil organic matter content in the farm soils of Muhoroni and SONY sugar nucleus estates within the recommended range?
2. Are the levels of Cd, Cr, Cu, Pb and Zn in the farm soils of Muhoroni and SONY sugar nucleus estates within acceptable limits?
3. Are the soils of Muhoroni and SONY sugar nucleus estates contaminated with Cd, Cr, Cu, Pb and Zn?

4. Are the levels of Cd, Cr, Cu, Pb and Zn in juice, bagasse and stems of sugarcane grown in Muhoroni and SONY sugar nucleus estates within safe limits?

5. Are the bioaccumulation factors of Cd, Cr, Cu, Pb and Zn in sugarcane grown in Muhoroni and SONY sugar nucleus estates within the known range in plants?

1.5 Justification of the research

Heavy metal contamination of soil pose risks and hazards to humans through direct ingestion or contact with contaminated soil and the food chain (soil-plant-human or soil-plant-animal-human). It can also cause reduction in food quality (safety and marketability) and land usability for agricultural production causing food insecurity. There exists little information on heavy metal contamination of sugarcane in Kenya. However, sugarcane has been cultivated in Muhoroni for 52 years and in South Nyanza for 37 years with repeated application of nitrogenous fertilizers. The fertilizers contain heavy metals as impurities which accumulate in soil with repeated application and sugarcane plant may uptake the metals to a level above safe limits. Consumption of plants produced in contaminated areas is one of the principal factors contributing to human exposure to metals. Consequently, serious systematic health problems such as bellyache, kidney damage and pulmonary emphysema can develop from excessive dietary accumulation of toxic metals in human body. Consumption of sugarcane and its products is high yet the levels of the heavy metals in the sugarcane are unknown. It is therefore important to assess the levels of heavy metals in soil and sugarcane for mitigation measures to be formulated in case the levels are above safe limits.

1.6 Significance of the study

Contamination and subsequent pollution of the environment by toxic heavy metals have become an issue of global concern due to their sources, widespread distribution and multiple effects on the ecosystem. Heavy metals are generally present in agricultural soils at low levels. Due to their cumulative behaviour and toxicity, however, they have a potential hazardous effect not only on crop plants but also on human health. Increase of contaminants in agricultural ecosystem has become a social issue worldwide as it is related with public health. International agencies, such as the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), are currently advocating compliance to permission criteria of pollutants in agricultural soil and products. Therefore the results of this study would provide basic information on the level of metal contents in sugarcane and soil which might subsequently be used for the improvement of soil environment management and heavy metal pollution prevention and control in Muhoroni and South Nyanza sugar estates in particular and Kenya in general.

1.7 Limitation of the study

Different calculation methods might lead to discrepancy on pollution assessment when they are used to assess the quality of soil ecological geochemistry. So it is of great importance to select a suitable method to assess soil quality for decision making and spatial planning. As in many studies to assess soil contamination, pre-industrial reference levels were used in this study to calculate contamination indices. The pre-industrial reference levels used were determined from various European and American countries. These may not be the same to pre-industrial reference levels calculated in Kenya. More reliable data on pollution indices would have been obtained if background levels (ambient concentrations) of the metals or national criteria of the metals were used as reference values. Due to limitation of funds, it was not possible to determine the background levels of the metals to be used in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Bioavailability of heavy metals in soils

Soil chemistry is a key factor influencing heavy metal availability and mobility (GWRTAC, 1997). Bioavailable heavy metal is the fraction of heavy metal in the soil that is accessible to the food chain and to the plants (Misra *et al.*, 2009). Generally, the heavy metal bioaccumulation in plants from environment is dependent in part upon bioavailability of the metals to the plants. There is evidence that the chemical speciation of heavy metals in solution affects their availability and toxicity to plants (Parker *et al*., 1995). Heavy metal transport and availability in a competitive heterogenous system between different soil components is a function of the interactions of the different chemical species of such metals in the system, the soil conditions and the prevailing environmental conditions and not only the function of total metal content in the soil-solution or solid phase (He *et al*., 2004). To be available for uptake by plants, heavy metals must be present in the soil solution. Soluble heavy metal concentrations in soils are likely to be influenced to some extent by the total concentrations of heavy metals present in soils. Thus, in uncontaminated soils, heavy metal bioavailability is likely to be related to the nature of the soil parent material and the degree of soil weathering (McLaren, 2003). In case of contaminated soils, solutions of heavy metal concentrations are likely to increase with total contaminant loading. Heavy metal absorption from soil and translocation to edible plant parts is a potential risk for the food chain and has to be evaluated based on soil metal availability and plant efficiency for metal uptake and translocation (Alleoni *et al.*, 2005). Generally, conditions resulting in less fixation of metal in soil lead to greater bioavailability of metals. Soil properties that affect bioavailability of metals include soil pH and soil organic matter content (Nyles and Ray, 1999).

2.1.1 Soil pH

Soil pH is a measure of soil solution acidity or basicity. The letters pH stand for "power of hydrogen" and the numerical value is defined as the negative base 10 logarithm of the molar concentration of hydrogen ions:

$$pH = -\log_{10}[H^+] \qquad (2.1)$$

Descriptive terms commonly associated with certain ranges in soil pH are: extremely acidic (pH < 4.5), very strongly acidic (pH 4.5 - 5.0), strongly acidic (pH 5.1 - 5.5), moderately acidic (pH 5.6 - 6.0), slightly acid (pH 6.1 - 6.5), neutral (pH 6.6 - 7.3), slightly alkaline (pH 7.4 - 7.8), moderately alkaline (pH 7.9 - 8.4), strongly alkaline (pH 8.5 - 9.0), and very strongly alkaline (pH > 9.1) (Foth and Ellis, 1997). A pH range of 6 to 7 is generally most favourable for some plant growth because most plant nutrients are readily available in this range. However, some plants have soil pH requirements above or below this range (USDA, 1998). Sugarcane grows best in soil with pH the range of 6.5 - 7.0 (Barnes, 1974). Soil pH is an important factor governing metal speciation, solubility from mineral surfaces, transport, and eventual bioavailability of metals in aqueous solution (Baruah *et al*., 1996) and the most important property controlling heavy metal uptake by plants (Kukier *et al.,* 2004; Basta *et al.,* 2005). Several studies had been done on bioavailability of heavy metals in soil. Higher proportions of mobile fractions of Cu, Zn and Pb were detected in samples taken from soils with acidic pH (Takáč *et al.,* 2009) while negative correlations was found between soil pH and water-soluble Zn and exchangeable Cu (Dudka *et al.,* 1996). Another study of the influence of soil pH on bioavailability of metals showed that high soil alkalinity stimulated Cu uptake in *Valeriana officinalis* L while at the same time resulted in a decrease in Zn content (Adamczyk-Szabela *et al.,* 2015). The solubility of heavy metals fall into three categories: those that appear to show greatest solubility at low pH with relatively low solubility at pH levels greater than 6.4 (Cd, Pb and Zn), those that show greatest solubility at high pH with relatively low solubility at pH levels less than 6.4 (Cr), and those that produce a U-shaped curve with minimum solubility at intermediate pH and increasing solubility both greater than and less than this value (Cu) (Ashworth and Alloway, 2008). The availability of most heavy metals to plants is highest in acid soils, and decreases as the soil pH increases due to sorption onto soil colloids (McLaren, 2003). As soil pH rises, sorption of heavy metal cations increases and the solubility of metal oxides decreases.

For most agricultural soils, addition of ammonium-nitrogen (N) through chemical fertilizers has been the main cause of acidification (Rowell and Wild, 1985). This relation was first studied by Pierre (1928) in Alabama (USA) in the 1920s and several studies thereafter quantified the relation between N fertilizers and acidification. A large decrease in the topsoil pH from 7.0 to 4.1 after three annual applications of 300 kg N ha^{-1} as NH_4NO_3 was found in fertile alluvial soil where napier grass species was grown (Abruna *et al.,* 1958). The nitrogenous fertilizers lower soil pH due to oxidation by nitrification and the direct uptake of ammonium-N by plants (Oliver, 2004). In contrast, the addition of basic nitrate fertilizers such as $Ca(NO_3)_2$ causes little change in pH due to the lack of nitrification and may in some cases result in an increase in soil pH (Woods *et al.,* 2003). The nitrification reaction is carried out through the microbial conversion of NH_4^+ to NO_3^- with the consequent production of protons (H^+). The nitrification process occurs according to the following equations:

Urea: $(NH_4)_2CO_{(aq)} + 4O_{2(g)} \rightarrow 2H^+_{(aq)} + 2NO_3^-{}_{(aq)} + CO_{2(g)} + H_2O_{(l)}$

DAP: $(NH_4)_2HPO_{4(aq)} + O_{2(g)} \rightarrow 3H^+_{(aq)} + 2NO_3^-{}_{(aq)} + H_2PO_{4(aq)} + H_2O_{(l)}$

Although the production of NO_3^- through the nitrification process for nitrogenous fertilizers is a net proton accumulating reaction, the subsequent leaching of nitrate can result in a significant decline in exchangeable bases because Ca^{2+} and Mg^{2+} will move downwards as counter ions for the very mobile NO_3^-, resulting in an accumulation of protons at the point of nitrification (Haynes, 1983).

In a field study conducted to examine the development of soil acidity from long-term use of urea and its effects on selected soil properties, it was reported that following 4 years of annual application of urea, the treated soil became more acidic than the control plots with no added urea (Lungu and Dynoodt, 2008). The researchers noted that lower pH values were measured starting from the second cropping season, and at the end of the fourth season the pH had decreased 0.87 units on the plots that received 180 kg N ha^{-1}. From the third season a decrease of 0.04 pH every month was measured at the highest rate of urea application. Soil pH levels that are too high or too low lead to deficiency of many nutrients, decline in microbial activity, decrease in crop yield, and deterioration of soil health (McCauley *et al*., 2009). Assessment of heavy metal content in Nigerian agricultural soils revealed that the soils were acidic with average topsoil pH value ranging from 5.00 to 5.80 (Ahaneku and Sadiq, 2014). In Lushoto District, Tanzania, the mean pH value of agricultural soil was 6.40 ± 0.59 (Lugwisha and Othman, 2014). This soil pH was however favourable to plant growth as it was within the favourable soil pH value of 6 - 7. Soil pH values in maize farm soils in Trans Nzoia, Kenya, and control site were 5.034 ± 0.25 and 6.35 ± 0.21 respectively, (Kananu *et al.*, 2014). The difference in soil pH values was indicative of the effect of long term application of inorganic fertilizers on soil pH. Soil acidification had been reported from various areas of the world where sugarcane was grown. In Hawaii the use of sulphate of ammonia in sugarcane cultivation over a period of 20 years decreased the topsoil pH by 1.0 unit (Humbert, 1959). In Queensland, topsoil pH was 4.5 after 15 years of sugarcane cultivation compared with a pH of 5.5 in uncultivated land (Moody and Aitken, 1995). Soil acidification had also been reported from the sugarcane areas in Puerto Rico, USA (Abruna-Rodriguez and Vincente-Chandler, 1967), Fiji (Masilaca *et al.*, 1986), Philippines (Alaban *et al.*, 1990), Florida, USA (Coale and Schueneman, 1993), South Africa (Schroeder *et al.*, 1994) and Ramu valley of Papua New Guinea (Hartemink, 1998), Nzoia, Kenya (Omwoma *et al.*, 2010). However, no report on soil pH has been given for Muhoroni and South Nyanza sugar estates.

2.1.2 Soil organic matter (SOM)

Organic matter content in soil is categorized as low (< 2.0%), medium (2.0 - 3.0%), and high (above 3.0%) and the minimum quantities required for sugarcane cultivation range from 2 to 3% (Muhammad *et al.*, 2000). Soil organic matter tends to be highly reactive towards ionic and polar contaminants because ionisable functional groups within natural organic matter have propensity to bind metal ions and form stable complexes (National Research Council of the National Academics, 2003). Generally, fulvic-metal complexes are soluble, while humic-metal complexes are insoluble (McBridgem, 1995). Depending on the complex solubility, metal-organic complexes can be found either in the liquid or in the solid phase (Silveira *et al.*, 2003). Metal-organic complexes are different for each metal and in addition to forming complexes, organic matter also retain them in exchangeable forms (Aydinalp and Marinova, 2003). These two properties affect each heavy metal differently. For example Cu is bound and made unavailable chiefly through the formation of complexes, while Cd is retained in an exchangeable form and is more readily available. Researches have been done to show the effect of soil organic matter in heavy metal availability to plants. Organic amendments led to decreased heavy metal content in potato peel and tubers, and this decrease was best expressed with 10% compost and 10%

vermicompost (separately) (Angelova *et al.*, 2010). Colloids in soil to absorb heavy metals also have influence in controlling heavy metal availability to plants.

The SOM buffers the soil against major swings in pH by either taking up or releasing H^+ into the soil solution, making the concentration of soil solution H^+ more constant. The result is a stable pH close to neutral or suitable for some crops to grow (Leslie, 2002). Organic matter solubility is relatively low between pH 4.6 and 6.4 but increases markedly beyond this range to a maximum at pH 7.7 (Ashworth and Alloway, 2008). The pH influences organic-matter solubility via its effect on the pH-dependent charge of soil solids (e.g., organic matter and certain clay minerals). As pH increases, the negative charge on such surfaces is increased and repels negatively charged molecules (e.g., molecules of organic matter) into the soil solution, thereby increasing dissolved organic-matter concentration (Jardine *et al.*, 1989). When organic-matter solubility increases, it is likely to result in (a) increased concentrations of heavy metals in the soil solution and (b) increased concentrations of organically complexed forms of heavy metals in the soil solution (Ashworth and Alloway, 2008). The observation that SOM is a dominant variable affecting the spatial distribution of heavy metals is consistent with well-investigated findings (Adriano, 1986; McGrath and Lane, 1989; Friedel *et al.*, 2000; Madyiwa *et al*., 2002; Rattan *et al.*, 2005).

Soil organic matter is key for the productive capacity of many tropical soils (Woomer *et al.*, 1994). A loss of SOM negatively effects soil physical, chemical, and biological properties leading to environmental damage, since soil organic matter is a source and sink in the global carbon cycle (IPCC, 2006). Large amount of carbon is lost from crop residue via volatilization during burning (Raison, 1985). In this context, the continuous burning has been identified as one of soil degradation practice that results in a decrease in soil organic matter (Ball-Coelho *et al.*, 1993; Graham *et al.*, 2002). Converting an area of native vegetation into an agricultural plot for any crop, especially when applying traditional farming operations (plowing, soil disking), results in a decline of soil organic matter concentrations (Lal, 2002), and consequently loss of carbon in soil. In most soils, levels of soil organic matter decreased in the first years of cultivation and then slowly increased again. The increase is higher with higher levels of organic inputs (trash, vinasse). Loss of soil organic matter results in soil degradation and once organic matter is lost, recuperation is a slow process and both carbon and nitrogen are needed for its restoration (Du

Preez and Snyman, 1993). Rarely, the original soil organic matter levels are reached. Typically, the levels settle at 60% of the soil organic matter levels in soils under natural vegetation (Zhang *et al.*, 2014). Very high amount of soil organic matter (10.2 ± 6.21%) was reported in agricultural soils of Khoshk River Banks, Shiraz, Iran, (Qishlaqi and Moore, 2007) as compared to 1.294 - 4.062% reported in agricultural areas of Guadalupe, Zacatecas, Mexico, (Dávila *et al.*, 2012). Soil organic matter (1.8 - 12.4%) was reported in paddy soils from wetlands of lake victoria basin, Tanzania, (Machiwa, 2010) and in the Bundaberg area, the soils cultivated under sugarcane for 22 years contained on average 22 g C kg^{-1} whereas the C content of virgin soils was 48 g kg^{-1} (King *et al.*, 1953). In Inceptisols and Oxisols in the South African province of KwaZulu-Natal, the organic C content was 40 - 50 g C kg^{-1} under natural vegetation but it declined exponentially with increasing years under sugarcane (Dominy *et al.*, 2002). Studies on SOM dynamics under sugarcane showed that the rates of soil organic matter decline differed for different soils (clay protection), agroecologies (climate), and management (e.g., trash-harvesting, vinasse applications) (Zhang *et al.*, 2014). Studies conducted in Herbert valley, Queensland, in Australia (Wood, 1985), Brazil (Vitorello *et al.*, 1989), India (Jadhav, 1995) and Swaziland in Southern Africa (Henry and Ellis, 1995) to showed that sugarcane cultivation reduced SOM content in soil. However, no study has been done in Muhoroni and SONY to establish the soil organic matter content.

2.2 Heavy metals in soil

Heavy metals are ubiquitous in the environment. As a result of both natural and anthropogenic activities, and humans are exposed to them through various pathways (Wilson and Pyatt, 2007). Heavy metals in the soil from anthropogenic sources tend to be more mobile, hence bioavailable than pedogenic, or lithogenic ones (Kuo *et al.*, 1983; Kaasalainen and Yli-Halla, 2003). It is projected that the anthropogenic emission into the atmosphere, for several heavy metals, is one-to-three orders of magnitude higher than natural fluxes (Sposito and Page, 1984). The anthropogenic sources of heavy metals in soil include inorganic fertilizers, pesticides and atmospheric deposition (Basta *et al.*, 2005; Khan *et al.*, 2008; Zhang *et al.*, 2010). Unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, metals do not undergo microbial or chemical degradation (Kirpichtchikova *et al.*, 2006), and their total concentration in soils persists for a long time after their introduction (Adriano, 2003). Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effects on food quality, crop growth (Msaky and Calvert, 1990; Fergusson, 1990; Ma *et al*., 1994) and environmental health. Concentrations of heavy metals in food are closely related to heavy metal concentrations in soil (Krauss *et al*., 2002). The mobility and bioavailability, and hence potential toxicity of metal in the soil depend on its concentration in soil solution, the nature of its association with other soluble species, and soil ability to release the metal from the solid phase to replenish that removed from soil solution by the plants (Krishnamurti and Naidu, 2002; Huang and Gobran, 2005; Krishnamurti *et al.,* 2007). Many research works have been done to investigate effect of application of inorganic fertilizers to agricultural soils. The mean levels for Cu, Pb, Cr and Zn in maize farm soil were higher than the levels in the control site indicating that long term application of inorganic fertilizers increases the amount of heavy metals in the soil (Kananu *et al.,* 2014). Increase in heavy metal concentration due to fertilizer application on agricultural soil had also been reported from Mahidasht, Iran (Atafar *et al.,* 2010), Hesse, Germany (Czarnecki and Düring, 2015), Kara in Bodija, Nigeria (Thomas *et al.,* 2012), Nzoia, Kenya (Omwoma *et al.,* 2010). However, the levels of heavy metals in Muhoroni and South Nyanza sugar estates have not been established although fertilizers have been applied in these areas for decades.

During recent decades, Cd has been the subject of increasing interest for health authorities, crop producers, soil and environmental scientists and agronomists due to its potential toxicity to man and its relatively high mobility from soil to plant. Cadmium is found to be more mobile than Cu and Zn owing to its weak affinity with soil colloids and is easily transferred to crops (McGrath *et al*., 1999). Consumption of Cd contaminated crops is considered to contribute to the dietary Cd intake. The importance of Zn and Cu as essential micronutrients to plants as well as their occurrence as toxic pollutants at elevated concentration and wide range of interaction with other metals like Cd in the soil-plant system has promoted Zn and Cu as subject of various studies (Kabata-Pendias, 2001). Chromium has recently received much attention due to the knowledge of its importance as an essential micronutrient in human metabolic processes and also because of its carcinogenic effects. Due to its wide industrial use, consequent environmental contamination increased and has become an increasing concern in the last years (Zayed and Terry, 2003). Lead exists in many forms in the natural sources throughout the world and is now one of the most widely and evenly distributed trace metals. Lead contamination is long-term and without remedial action, high soil Pb levels will never return to normal (Traunfeld and Clement, 2001). It is well known to be toxic and its effects have been more extensively reviewed than the effects of other trace metals. Essential heavy metals that are required by organisms at lower levels and become toxic at higher levels of exposure include Cr, Cu and Zn while non-essential ones including Cd and Pb, are toxic and not required by organisms at any level (McGrath, 2001).

2.2.1 Cadmium in soil

Cadmium occurs naturally in soils, rocks and coal (Nassef *et al.*, 2006). The average concentration of Cd in the earth's crust is 0.06 - 1.1 mg kg^{-1} with a calculated worldwide mean of 0.53 mg kg^{-1} (Alloway, 1995). In natural soils the Cd concentration is influenced by the amount of Cd in the parent rock. Soils derived from igneous rocks contain the lowest Cd concentrations (< 0.1 - 0.3 mg kg^{-1}), soils derived from metamorphic rocks contain 0.1 - 1 mg kg^{-1} Cd and soils derived from sedimentary rocks contain the largest amount of Cd (0.3 - 11 mg kg^{-1}) (Adriano, 2001). High levels of Cd pollution in soil are limited to areas with specific input histories such as mining or smelting. But low to medium levels of Cd pollution in soil are a wide spread issue on agricultural soils. This pollution is mainly caused by the application of Cd containing phosphate and other fertilizers as well as by periurban atmospheric deposition (Nassef *et al.*, 2006). At these levels Cd is not toxic to plants and does not hinder plant growth or soil fertility but may potentially transfer into humans with food or water. So in many countries including Kenya, Cd is the most important metal pollutant in agricultural soil because of its toxicity risks for humans and its widespread distribution (WHO, 2010). The mean Cd levels in khat farms in Ethiopia ranged from 49.9 to 131.3 mg kg^{-1} (Atlabachew *et al.*, 2011) while in Tanzania, levels of Cd in paddy soils in wetlands of lake victoria basin was between 0.15 and 0.88 mg kg^{-1} (Machiwa, 2010). The levels of Cd in tobacco farms in Western Kenya, ranged from 1.02 to1.90 mg kg^{-1} (Kisinyo, 2016) while agricultural soils of Khoshk River Banks, Shiraz, Iran, was reported to have Cd mean level of 5.2 ± 1.49 mg kg^{-1} (Qishlaqi and Moore, 2007). The safe limit of Cd in agricultural soil is 3.0 mg kg^{-1} (EU, 2002). However, soil type generally determines heavy metal critical soil level (Verloo *et al.*, 1986). Only a small proportion of the total Cd concentration is present in the soil solution and this amounts to only 1%. However, the solubility of Cd is largely increased under acidic conditions so the proportion of Cd in the soil solution is much higher for acidic soils (Alloway, 2004; Christensen and Haung, 1999; Helmke, 1999). Cadmium is adsorbed mainly by organic matter in soils and the adsorption capacity of soils for Cd is highest at low soil pH. Therefore, the lower the soil pH and organic matter content, the higher the mobility of Cd will be (Alloway, 1995).

In its compounds, Cd occurs as the divalent cadmium (II) ion. It is directly below Zn in the periodic table and has a chemical similarity to that of Zn. This may account in part for its toxicity because Zn being an essential trace element, its substitution by Cd may cause the malfunctioning of metabolic processes. Cadmium is a heavy metal with no known essential biological functions in higher plants, animals and humans. Like other heavy metals, including those that are essential at low concentrations, Cd becomes toxic at elevated concentrations. Toxicity levels depend on the organism, physiological conditions and environmental factors. The joint FAO (Food and Agriculture Organisation of the United Nations) and WHO (World Health Organisation) expert committee on food additives has established a provisional tolerable level for Cd at 25 μg Cd intake per kg body weight per month (WHO, 2010). Sugarcane has a high ability to tolerate and accumulate Cd due to its large biomass (Xia *et al.*, 2009) and consumption of sugarcane grown in contaminated soil can be the main routes by which Cd enters the body (Manahan, 2003). Cd is highly toxic and can cause bone damage, chronic kidney disease, cancer and hypertension in human beings (WHO, 1992; Goyer, 1996; Tandi *et al.*, 2005; Abbas *et al.*, 2010). It is therefore important to regularly assess the levels of Cd in agricultural soil. This has not been done in Muhoroni and SONY sugar estates.

2.2.2 Chromium in soil

Chromium is the 17th most abundant element in the Earth's mantle (Avudainayagam *et al.*, 2003). It is one of the less common elements and does not occur naturally in elemental form, but only in compounds. It occurs naturally as chromite ($FeCr_2O_4$) in ultramafic and serpentine rocks or complexed with other metals like crocoite ($PbCrO_4$), bentorite $Ca_6(Cr,Al)_2(SO_4)_3$ and tarapacaite (K_2CrO_4), vauquelinite ($CuPb_2CrO_4PO_4OH$), among others (Babula *et al.*, 2008). The average Cr concentrations in world soils are 40 - 84 mg kg^{-1} (Adriano, 2001). Chromium is widely used in industry as plating, alloying, tanning of animal hides, inhibition of water corrosion, textile dyes and mordants, pigments, ceramic glazes, refractory bricks, and pressuretreated lumber (Avudainayagam *et al.,* 2003). The concentration of Cr in the soils may vary considerably according to the natural composition of rocks and sediments that compose them (Kimbrough *et al.,* 1999). The levels of Cr in the soil may increase mainly through anthropogenic deposition, as for example atmospheric deposition (Rosas *et al.,* 1989), also dumping of chromium-bearing liquids and solid wastes as Cr by-products, ferrochromium slag, or Cr plating baths (Kimbrough *et al.,* 1999). Chromium mean level in agricultural soils of Khoshk River Banks, Shiraz, Iran, was 124.5 ± 68.9 mg kg^{-1} (Qishlaqi and Moore, 2007) while the range was 14.42 - 19.28 mg kg^{-1} in agricultural soil of Zhengzhou, China, (Liu *et al.,* 2009). The mean level of Cr in maize farm soils in Trans Nzoia, Kenya, was 42.11 ± 3.25 mg kg^{-1} (Kananu *et al.,* 2014) and the level of the metal in wetlands of lake victoria basin, Tanzania, ranged between 11.4 - 39.4 mg kg^{-1} (Machiwa, 2010). The safe limits of Cr in agricultural soil is 150.0 mg kg^{-1} (EU, 2002).

Trivalent and hexavalent Cr are the environmentally important species (Zayed and Terry, 2003). Soil type and mineral content influence Cr oxidation states in soils (Bagdon and Hazen, 1991). Depending on environmental conditions, Cr can be reduced from VI to III or oxidised from III to VI. Both types of reactions may occur simultaneously in the same system. Chromium is most commonly found in nature as Cr-III, which is the most stable and dominant form of Cr species at low pH (< 4). Chromium-III mobility is decreased by adsorption to clays and oxide minerals below pH 5 and low solubility above pH 5 due to the formation of $Cr(OH)_3$ (Chrostowski *et al*., 1991). Most Cr-III compounds are insoluble in water and Cr-III is considered to be an essential trace element for human diets, although ingestion of large amounts can cause toxic effects (Zayed and Terry, 2003). Chromium-III helps the body to use sugar, protein, and fat and its deficiency results in impaired growth and longevity, and disturbance in glucose, lipid, and protein metabolism (Calabrese *et al.,* 1985). The second most common and stable form of Cr in the environment is Cr-VI. It is the most toxic, with toxicity ranging 100 - 1000 times higher than that of Cr-III (Godgul and Sahu, 1995). Chromium-VI is much more toxic than Cr-III because of its greater ability to enter cells and its strong oxidation potential (Katz and Salem, 1993). Once inside cells, Cr-VI reduction produces free radicals, Cr-V, Cr-IV and eventually Cr-III, which are believed to be responsible for carcinogenic effects (Stearns *et al.,* 1995; Li *et al.,* 2011). Chromium-VI occurs naturally in the rare mineral crocoite, but most Cr-VI in the environment is created by human activities (Zayed and Terry, 2003). Chromium mobility depends on sorption characteristics of the soil, including amount of organic matter present. Cr-VI has higher solubility and thus bioavailability than Cr-III, which tends to form stable complexes in the soil (López-Luna *et al.,* 2009). Thus, Cr toxicity is dependent on metal speciation, which is determinant for its uptake, translocation and accumulation.

Chromium is associated with allergic dermatitis in humans (Cheryl and Susan, 2000; Scragg, 2006) and Cr fumes have been classified by International Agency for Research on Cancer as a category 1 human carcinogen as it causes pulmonary oedema, pnemonia and increased risk of lung and prostrate cancer (Wright and Welbourn, 2009). Both Cr-III and VI are respiratory and mucous irritants while Cr-VI is linked to occupational asthma (Assem and Zhu, 2007). Chromium can be transferred from mother to child through the placenta and mother's milk (Barceloux, 1999) and increased birth and developmental defects in children have been informally noted in areas of poorly regulated chromite mining, leather tanning (using Cr) and chrome production (Blacksmith Institute, 2007). It is therefore necessary to monitor the degree of contamination of Cr in soil. This is lacking in the study areas.

2.2.3 Copper in soil

Copper occurs in the Earth's crust at concentrations between 25 - 75 mg kg^{-1}, with the abundance pattern that shows the tendency for the concentration in mafic igneous rocks (60 - 120 mg kg^{-1}) and argillaceous sediments (40 - 60 mg kg^{-1}), but it is rather excluded from the carbonate rocks (2 - 10 mg kg^{-1}) (Kabata-Pendias and Mukherjee, 2007). Toxic levels of Cu occur naturally in some soils whereas others may contain high levels of Cu as a result of the anthropogenic release of heavy metals into the environment (Yruela, 2005). Copper toxicity is a problem of both agricultural and environmental significance. Sources of Cu contamination include mining and smelting, urban, industrial and agricultural wastes, and the use of agrochemicals. Values for soil contents generally range worldwide from 1 to 140 mg kg^{-1} depending on the nature of the soil parent material and agricultural activities. The mean Cu levels in agricultural areas of Guadalupe, Zacatecas, Mexico ranged from 69.87 to 120.35 mg kg^{-1} (Dávila *et al.,* 2012) while in Sri Lanka, levels of Cu in soils collected from intensively cultivated areas was 51 ± 34 mg kg^{-1} (Premarathna *et al.,* 2010). The levels of Cu in arable agricultural soils in South Western

Nigeria, ranged from 6.17 - 20.87 mg kg^{-1} (Olatunji *et al.*, 2013) while soil in Maiduguri, Nigeria, was reported to have Cu level of 3.78 ± 0.29 mg kg^{-1} (Uwah *et al*., 2011). In Keffi, Nigeria, the levels of Cu in agricultural soil of was 9.74 mg kg^{-1} (Abdullahi, 2012) while the levels of Cu in tobacco farms in Western Kenya, ranged from 1.07 to 2.31 mg kg^{-1} (Kisinyo, 2016). The safe limit of Cu in agricultural soil is 140.0 mg kg^{-1} (EU, 2002).

Copper is present in many forms in soils, with free Cu^{2+} activity considered to be the best indicator of phytoavailability (Sauve *et al.,* 1996). Copper has a high affinity for solid phase organic matter, and is therefore not readily leached and can accumulate in the surface soil (McBride *et al.,* 1997). Copper concentrations in soil solution are generally low, with more than 98 % of Cu in solution bound to soluble organic matter in soils of neutral pH (Sauve *et al.*, 1997). Adsorption of Cu is highly pH dependent and phytoavailability of Cu increases with decreasing pH. The solubility of copper is drastically increased at pH 5.5 (Martinez and Motto, 2000). As soil pH increases, both total soluble copper, and Cu^{2+} activity decreases (Tye *et al.*, 2004). Copper is an essential micronutrient required in the growth of both plants and animals. Copper aids in the electron transfer process which also involves iron in haemoglobin, photosynthesis in plants and the terminal step of mitochondrial respiration and hence Cu influences life and supports functions like production of red blood cells and carbohydrate synthesis (USEPA, 1980). Wilson's disease is caused by the inability of body to utilize Cu and is a hereditary metabolic disorder whereby there is accumulation of Cu in some organs of the body, the most sensitive being the brain and liver (Prasad and Oberleas, 1976; USEPA, 1990). Toxic levels lead to Cu poisoning that can result in hemolytic anemia (Finelli *et al.,* 1981) hence the need to monitor its presence in soil as it is actively absorbed in the stomach and duodenum (Stokinger, 1981).

2.2.4 Lead in soil

Lead is a naturally occurring, bluish-gray metal usually found as a mineral combined with other elements, such as sulphur (i.e., PbS, $PbSO_4$), or oxygen ($PbCO_3$), and ranges from 10 to 30 mg kg^{-1} in the earth’s crust (USDHHS, 1999). Typical mean Pb concentration for surface soils worldwide averages 32 mg kg^{-1} and ranges from 10 to 67 mg kg^{-1} (Kabata-Pendias and Pendias, 2001). Besides natural weathering processes the main sources of Pb pollution are exhaust fumes of automobiles, chimneys of factories using Pb, effluents from the storage battery, industry, mining and smelting of Pb ores, metal plating and finishing operations, fertilizers, pesticides and additives in pigments and gasoline (Eick *et al.,* 1999). Lead accumulates in the upper 8 inches of the soil and is highly immobile. Lead levels in soil from Bacita sugar estate, Nigeria, ranged from 0 - 0.02 mg kg^{-1} (Adekola and Akinpelu, 2002) while in Maiduguri, Nigeria, the level of Pb was 3.58 ± 0.08 mg kg^{-1} (Uwah *et al.,* 2011). The range of Pb in agricultural soil of Zhengzhou, China, was 14.42 - 19.28 mg kg^{-1} (Liu *et al.,* 2009) while mean level of Pb in maize farm soils in Trans Nzoia, Kenya, was 37.72 ± 3.44 mg kg^{-1} (Kananu *et al.,* 2014). The level of Pb was 18.23 ± 4.627 mg kg^{-1} in urban agriculture soil in Dar es Salaam, Tanzania, (Kibassa *et al.,* 2013). The safe limit of Pb in agricultural soil is 130.0 mg kg^{-1} (EU, 2002).

The predominant insoluble Pb compounds are lead phosphates, lead carbonates (form when the pH is above 6), and lead hydroxides (Raskin and Ensley, 2000). In general, plants absorb or accumulate Pb if its concentration is high in the soil. Generally, it has been considered safe to use garden produce grown in soils with total Pb levels less than 300 mg kg^{-1} (Rosen, 2002). The risk of Pb poisoning through the food chain increases as the soil Pb level rises above this concentration. Even at soil levels above 300 mg kg^{-1}, most of the risk is from Pb contaminated soil or dust deposits on the plants rather than from uptake of Pb by the plant (Rosen, 2002). Inhalation and ingestion are the two routes of exposure, and the effects from both are the same. Uses of Pb include storage batteries, solders, bearings, cable covers, ammunition, plumbing, pigments, and caulking (Wuana and Okieimen, 2011).

Lead metal depresses immune status, causes serious damage to the brain, nervous system, red blood cells, kidneys, bone, liver, pancrease, reproductive system, teeth and gum (Fergusson, 1990; Baldwin and Marshall, 1999; Bakidere and Yaman, 2008; Martin and Griswold, 2009; Alao *et al*., 2010; Olayemi, 2010). Analysis of sperm count in lead workers has shown a decreased sperm count as well as decreased motility and lifespan of sperm, in direct relation to the level of lead in the blood (Xuezhi *et al.,* 1992). Children exposed to lead are at risk for impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration, with children under the age of six being at a more substantial risk. Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints when exposed to lead (NSC, 2009). Lead is a prime environmental pollutant and a probable human carcinogen therefore its level in the environment should be put on check. The levels of lead metal in soil from Muhoroni and SONY sugar estates have not been established.

2.2.5 Zinc in soil

Zinc occurs naturally in soil (about 70 mg kg^{-1} in crustal rocks) (Davies and Jones, 1988), but zinc concentrations are rising due to anthropogenic additions. Most Zn is added during industrial activities, such as mining, coal, and waste combustion and steel processing. The total Zn concentration of soils is related to the composition of the parent rock material and soil mineralogy. In the soil, Zn forms complexes with organic acids, humic substances and other types of dissolved organic carbon. The total Zn concentration is not used for evaluating the availability of soil Zn to the plants because only a small amount of total Zn is exchangeable or soluble (Stahl and James, 1991; Sadeghzadeh, 2013). The total Zn concentrations in soils vary between 10 to 300 mg kg^{-1} with an average of 50 mg kg^{-1} (Lindsay, 1972). But the average available Zn varied from 1 to 3 mg kg^{-1}. The safe limits of Zn in agricultural soil is 300.0 mg kg^{-1} (EU, 2002). However, the mean Zn levels in wetland soils in the Lake Victoria basin, Butabika, Uganda, ranged from 30.7 ± 3.2 mg kg^{-1} (Nabulo *et al.*, 2008) while in Adogo in Nigeria, levels of Zn in Ajaokuta local Government area of Kogi State, was 0.04 ± 0.003 mg kg^{-1} (Omono and Kakulu, 2012). The range of Zn in Bacita sugar estate, Nigeria, was 0.27 - 0.38 mg kg^{-1} (Adekola and Akinpelu, 2002) while the mean level of Zn soils from intensively cultivated areas of Sri Lanka, was 227 ± 106 mg kg^{-1} (Premarathna *et al.*, 2010). In tobacco farms in Western Kenya, the levels of Zn ranged from 0.32 to 1.02 mg kg^{-1} (Kisinyo, 2016).

The Zn concentration in the soil solution is largely dependent on pH; for example, at pH 5.0, the concentration of Zn in the soil solution is approximately 10^{-4} M (6.5 g L^{-1}), whereas at pH 8.0, this concentration is 10^{-10} M (0.0065 μg L^{-1}) (Kiekens, 1995). Liming of acid soils results in an increase in the soil pH from 5.2 to 6.8 and an approximate 10-fold decrease in the Zn concentration in plants (Parker and Walker, 1986). A high pH decreases the desorption of Zn from soil surfaces, which also reduces the availability of Zn to plants. Soil organic matter content is another factor that contributes to Zn deficiency in crops. Zinc availability to plants is often reported to be low in soils with high organic matter content due to increased adsorption of Zn by organic ligands and components.

Zinc deficiency is the most widespread micronutrient deficiency in agricultural lands around the world, causing yield decreases and diminishing the nutritional quality of agricultural plants (Sadeghzadeh, 2013). Plant-based foods are significant sources of Zn for humans (Welch and Graham, 2004). Zinc deficiency in soils also reduces Zn concentration and content in the edible portions of staple food crops and diminishes their nutritional quality (Welch and Graham, 2004). Zinc is an essential trace element required in small but critical amounts by plants and animals including humans (Alloway, 2009). Zinc can interrupt the activity in soils as it negatively influences the activity of microorganisms and earthworms, thus retarding the breakdown of organic matter (Greany, 2005). Despite being an essential trace element, Zn is toxic to most organisms above certain concentrations (Ho, 2004). The Zn DRI (Dietary Reference Intake) is 11 mg day^{-1} for males and 8 mg day^{-1} for females (WHO, 1996). Symptoms of Zn toxicity in human beings include lassitude, slower tendon reflexes, bloody enteritis, diarrhea, lowered leukocyte count and depression of central nervous system, and paralysis (Venugopal and Luckey, 1978). Zinc deficiency is the most widespread micronutrient deficiency in agricultural lands around the world, causing yield to decrease. Therefore, the amount of Zn in the agricultural soil should be constantly assessed in order to arrest micronutrient deficiency. The levels of zinc in the soils of the study areas are unknown.

2.3 Contamination levels of heavy metals in soil

Soils are usually regarded as the ultimate sink for heavy metals discharged into the environment (Banat *et al.,* 2005). Therefore the environmental problem of soil pollution by heavy metals has received increasing attention in the last few decades in both developing and developed countries throughout the world (Zhang *et al.,* 2007). With the development of ecological geochemistry survey and exploration geochemistry survey, a great deal of data related to heavy metal concentration in soils have been measured which can be used to assess the severity of soil contamination.

The content of heavy metals in soils is a joint action of both natural processes and human activity, with a prevalence of anthropogenic sources (Nriagu and Pacyna, 1988; Baize and Sterckeman, 2001). Significant increase in soil heavy metal content is found in lands under cultivation with intensive application of fertilizer. Determination of the total content of metals in soils is an important step in estimating the hazards to the vital roles of soil in the ecosystem, and also in comparison with the quality standards in terms of the effects of pollution and sustainability of the system. Contamination is delineated through a remedial investigation. The purpose of the remedial investigation is to gather enough information about the contamination, the media and the site, to determine the appropriate course of action required to remediate/mitigate the contamination. Several approaches have been used for evaluating the level of heavy metals contamination in different ecosystems. They are commonly based on the amounts of metals extracted by applying specified soil tests (Kabata-Pendias *et al.,* 1993; Grzebisz *et al*., 1997; Reimann *et al.*, 2000).

In the study of environmental contamination, a common approach to estimate how much the soil is impacted (naturally or anthropogenically) with heavy metal is to evaluate ratio of metal concentrations to reference levels (Abrahim and Parker, 2008) and pollution signals compared with established guidelines for environmental media. Pollution indices to assess heavy metal contamination are classified into three types: contamination indices, background enrichment indices and ecological risk indices. In calculating background enrichment indices, reference sample used is an average crust or a local background sample (Chatterjee *et al.*, 2007; Liu *et al.*, 2005b). Indices-based assessment of soils contaminated by heavy metals seem to be a useful geochemical method, since it “shifts” from commonly reported concentrations of particular heavy metals into unitless parameters (Diatta *et al.*, 2003). Determination of contamination levels of the heavy metals in farm soil is important for the development of proper management strategies to reduce pollution. This is lacking in Muhoroni and South Nyanza sugar estates.

2.3.1 Contamination factor and degree of contamination of soil

The contamination factor (C_f) and the degree of contamination (C_d) are used to assess soil contamination through comparison of the current concentration in the surface layer to pre-industrial value (Hakanson, 1980; Krzysztof *et al.*, 2004). Contamination factor gives an indication of the level of contamination of individual metal in soil samples. Contamination factor is calculated as shown in Eq. (2.2):

$$C_f = \frac{C_s}{C_b} \qquad (2.2)$$

where C_s is the mean content of metal from at least five sampling points and C_b is the pre-industrial concentration of the individual metal. Although the pre-industrial concentrations are for sediment layers, the same values are also employed in pollution assessment of heavy metals in soil (Wei and Yang, 2010). The assessment is based on four classification categories proposed by Hakanson (1980): $C_f < 1$, low contamination; $1 \le C_f < 3$, moderate contamination; $3 \le C_f < 6$, considerable contamination; and $6 \le C_f$, very high contamination. Pre-industrial reference levels given in Table 2.1 were applied for the calculations of contamination factor, since they have been most frequently employed in this field of study (Hakanson, 1980).

Table 2.1. Pre-industrial reference level (mg/kg) and toxic-response factor

Elements	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**
Pre-industrial reference level	1.0	90	50	70	175

Source: Hakanson (1980)

Sum of contamination factors for all elements examined represents the degree of contamination of the environment (Hakanson, 1980). The degree of contamination is aimed at providing a measure of the degree of overall contamination in surface layers in a particular sampling site. The degree of contamination (C_d) is assessed using Eq (2.3).

$$C_d = \sum_{i=1}^{n} C_{f\,i} \qquad (2.3)$$

where C_f is contamination factor of individual heavy metal. For the description of contamination degree, the following terminologies were used: $C_d < 5$, low degree of contamination; $5 \le C_d < 10$, moderate degree of contamination; $10 \le C_d < 20$, considerable degree of contamination; and $20 \le C_d$, very high degree of contamination (Hakanson, 1980).

2.3.2 The potential ecological risk factor and potential ecological risk index

The potential ecological risk factor (*Er*) and potential ecological risk index (*RI*) are used to evaluate the potential ecological risk of heavy metals (Hakanson, 1980). Although ecological risk assessment was originally used as a diagnostic tool for the purpose of controlling water pollution (Hakanson, 1980), it has successfully been used for assessing the quality of sediments and soils in environment polluted by heavy metals (Gong *et al.*, 2008). This method comprehensively considers the synergy, toxic level, concentration of the heavy metals and ecological sensitivity of heavy metals (Nabholz, 1991; Singh *et al*., 2010; Douay *et al.*, 2013). The potential ecological risk factor which represents the ecological risk of one heavy metal takes into account both contamination factor (C_f), and the "toxic-response" factor (Tr). The toxic response factor represents the potential hazard of heavy metal contamination by indicating its toxicity and the environmental sensitivity to contamination. The potential ecological risk factor is calculated using Eq. (2.4):

$$Er = \mathrm{Tr} \times C_f \qquad (2.4)$$

where C_f is contamination factor of each metal and Tr denotes the "toxic-response" factor for heavy metals (Table 2.2). The following terminologies are used to describe the risk factor: $Er < 40$, low potential ecological risk; $40 \leq Er < 80$, moderate potential ecological risk; $80 \leq Er < 160$, considerable potential ecological risk; $160 \leq Er < 320$, high potential ecological risk; and $Er \geq 320$, very high ecological risk (Hakanson, 1980).

Table 2.2. Toxic-response factor

Elements	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**
Toxic-response factor	30	2	5	5	1

Source: Hakanson (1980)

The potential ecological risk index (*RI*) of the heavy metals is quantitatively evaluated by summimg up the potential ecological risk factors (Hakanson, 1980; Zhu *et al.,* 2008). The potential ecological risk index is the potential ecological risk caused by the overall contamination. The potential ecological risk index (*RI*) can be calculated using the Eq.(2.5):

$$RI = \sum_{i=1}^{n} Er_i \qquad (2.5)$$

where, *Er* denotes the potential ecological risk factor of individual metal. The potential ecological risk index values obtained were categorised as: $RI < 150$, low risk index; $150 \leq RI <$

300, moderate ecological risk index; $300 \leq RI < 600$, considerable ecological risk index; and $RI \geq 600$, high ecological risk index (Hakanson, 1980).

2.4 Sugarcane

Sugarcane, like wheat, rice, corn and other grains, is of the grass family, Gramineae, of the genus *Saccharum* and agriculturally produced from hybrids which are the progeny of a number of *Saccharum species* commonly referred to as cane. It is one of the important commercial crops cultivated in tropical and sub-tropical regions for sugar production (Plaut *et al*., 2000). The plant originated from the island of the South Pacific area and is one of the world's major food crops providing about 75% of the sugar used for human consumption (Sreenivasan *et al*., 1987; FAO, 2004).

In Kenya, growing of sugarcane started in the early 1900s when it was introduced around Lake Victoria by the labourers engaged in the construction of the Kenya-Uganda railway and the first factory was established at Miwani in the year 1923 (Wawire *et al.*, 2006). Sugarcane varieties in Kenya include variety CO945 which occupies 35.72% of the total area under cane. Varieties CO421, CO617 and N14 occupy 28.4%, 13.29% and 10.95% of the total area respectively (KSB, 2008). Sugarcane has a vigorous root system with the maximum root depth exceeding 6 m, which can extract water and nutrients from considerable depths and make use of water up-flow from water tables (Smith *et al.*, 2005). As a result, it can combat adverse conditions and tolerate dry, salty, or contaminated environments (Wahid, 2004). The plant has a very strong tolerance to heavy metal contamination (Zhang *et al.*, 2014). The heavy metal tolerance of sugarcane could be due to the vigorous perennial root and huge biomass. The higher biomass of sugarcane could lead to a "growth dilution effect" on metals and subsequently alleviate metal phytotoxicity.

Large quantities of fertilizers are regularly added to soils in intensive sugarcane farming to provide adequate nitrogen, phosphrus, and potassium for crop growth. These fertilizers contain heavy metals that accumulate in soil with repeated application (Mortvedt, 1995) which may be bioconcentrated by the sugarcane plant to an extent that compromises quality of sugarcane products. Crops cultivated in soils polluted with toxic metals take up heavy metals and accumulate them in their edible and non-edible parts in quantities high enough to cause clinical problems both to animals and human beings consuming these metal-rich plants (Alam *et al.*, 2003). Many studies have investigated the effects of inorganic fertilizer on yield and sugarcane quality (Muhammad *et al.*, 2010), leaf nutrient content (Joris *et al.*, 2014), and the overall response to inorganic fertilizers (Abayomi, 1987). However, studies were few on toxic heavy metal accumulation by sugarcane and no such study have been done in Muhoroni and South Nyanza sugar estates. This data could directly give information on the quality of sugarcane produced to save the people who chew sugarcane or take its juice from excessive dietary accumulation of toxic metals in human body.

2.4.1 Sugarcane stem

Sugarcane stem characteristics vary considerably depending on variety. The cane stem is made up of shorter segments and joints. These joints vary in length from 5 to 25 cm. The lower joints are longer, larger in diameter, and older than the upper joints. Each joint has two distinctive parts, the node and the internode (Elsunni and Collier, 1996). At the node and immediately around it are the important stalk structures: the root band, the bud, the growth ring, and the shoulder where the leaf attaches to the stem (Clements, 1980). In a cross section in the internode there are two distinctive areas. The first one, known as the rind, is the outer dense hard layer. The inside layer, known as the pith, is the soft light colored region where the fibro vascular bundles are embedded. The rind varies in thickness and texture along the stem length. The mature stems may vary from 1.2 to 3.6 metres or more in height, and in commercial varieties are from 1.9 to 5.1 cm in diameter (Magness *et al.*, 1971). The sugarcane stems are milled to obtain the cane juice, which is subsequently used for sugar production. The residual fraction from the sugarcane stem milling is the bagasse (Canilha *et al.*, 2012).

2.4.2 Sugarcane juice

Juice is obtained by crushing sugarcane. Although juice is often regarded as a solution of sucrose in water, a variety of other extracted compounds are present. Inorganic components of juice consist of water and elements dissolved in it as ions. The mineral content of the juice depends on the cane variety and soil (McKaig and Fort, 1938). Plants grown in contaminated soils exhibit significant interspecies and intervarieties differences in accumulation of pollutionants (Molchan, 1996). The inorganic constituents of juice include silicon (0.016 - 0.101%), phosphates (0.14 - 0.40%) (Fort and Smith, 1952), potassium (0.77 - 1.31%), calcium (0.24 - 0.48%), magnesium (0.10 - 0.39%), sodium (0.0 - 0.04%), iron (0.006 - 0.04%), aluminum (0.005 - 0.17 %), copper (0.002 - 0.003%), zinc (0.003 - 0.012%), manganese (0.007%) and cobalt (0.00007%) (Sang *et al.,* 1974). Sugarcane bioconcentrates and biomagnifies toxic heavy metals such as Pb and Cd in sugarcane juice to levels that far exceed WHO recommended tolerable values (0.05 mg/L for Pb and 0.01 mg/L for Cd) for drinking water (Abdus-Salam *et al.,* 2008). This concurred with the levels of toxic Pb and Cd in sugarcane juice that ranges from 0.028 - 0.27 mg L^{-1} and 0.085 - 0.21 mg L^{-1}, respectively, (Wang *et al.*, 2012).

2.4.3 Bagasse

Bagasse is the fibrous matter that remains after sugarcane stalks are crushed to extract their juice. It represents about 12% of the original cane (Kent, 1974; Hamilton and Longe, 1980) and consists of water, fibres, and small amounts of soluble solids. Percent contribution of each of these components varies according to the variety, maturity, method of harvesting, and the efficiency of the crushing plant (Elsunni and Collier, 1996). Sugarcane bagasse typically contains approximately 40 - 50% moisture, and 1 - 3% sugar, with the remainder as fibre (Payne, 1991). Fine bagasse, known as bagacillo is used to produce filter cake which is used as a fertilizer on cane farms (Sreenivasan *et al*., 1987; Mackintosh, 2000). Bagasse is used as an animal feed but is limited by low digestibility. Steam treatment of bagasse improves its digestibility so that it can be used in the fattening of cattle (Allen *et al.,* 1997; UN Industrial Development Organisation, 2000). Bagasse is burnt for heat to produce steam as source of power to run the sugar mills, with excess heat directed to the electricity grid (Sreenivasan *et al.*, 1987; Mackintosh, 2000). In a study to select sugar cane varieties with a low heavy metal accumulation ability for the ecological remediation of contaminated farmland, the levels of toxic Pb and Cd in bagasse of sugarcane was 1.68 - 5.54 and 0.15 - 0.99 mg kg^{-1}, respectively, (Wang *et al.,* 2012). The presence of heavy metals in bagasse makes it unsuitable as a fertilizer and animal feed. For this reason, amount of heavy metals in bagasse ought to be assessed and compared with standard limits. The information obtained could also be used to formulate safe disposal of the by product in case it is found to be unsuitable for use. This data is not available for sugarcane grown in Muhoroni and South Nyanza sugar estates

2.5 Uptake and accumulation of heavy metals by plants

Plants take up essential and non-essential elements from soils in response to concentration gradients inducted by selective uptake of ions by roots, or by diffusion of elements in the soil. The accumulation level of heavy metals differs between and within species (Huang and Cunningham, 1996; McGrath *et al.,* 2002). Plants could be classified into three categories: (1) excluders: those that grow in metal-contaminated soil and maintain shoot concentration at low level up to a critical soil value above which relatively unrestricted root-to-shoot transport result, (2) accumulators: those that concentrate metals in the aerial part, and (3) indicators: the uptake and transport of metals to the shoot is regulated so that internal concentration reflects external levels, at least until toxicity occurs (Baker, 1981).

The food chain (soil-plant-human) is known as one of the major pathways for exposure of human to soil contaminants. Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general well being. Minerals are essential components of our diet that serve as cofactors in the thousands of enzyme-controlled reactions, control the action of nerves and muscles, help maintain the body's water balance, and buffer the pH (acidity) of the cell and extracellular fluids (Minerals-Learn, 2010). Plant-derived foods have the potential to serve as dietary sources for all human-essential minerals that make a significant contribution to daily mineral needs at all stages of the life cycle (Grusak, 2002). Food chain contamination is one of the important pathways for the entry of these toxic pollutants into the human body (Ferner, 2001; Ma *et al.,* 2006). Generally, too low or too high of a concentration of trace elements in human diet can affect the quality of human life. Excessive accumulation of heavy metals in human bodies creates the problems like cardiovascular, kidney, nervous and bone diseases (Jarup, 2003). Therefore the plant that has a lot of heavy metals can give greatest impact and carry the highest risk to human health. For example, in the 1920s Jintsu river basin in Japan near the town of Toyama, rice was grown in water contaminated by Zn, Pb and Cd from a mining and smelting operations. The consequences of the Jintsu river contamination were renal dysfunction, osteomalacia, skeletal deformities including severe pain (Wright and Welbourn, 2009).

Crops grown in soils contaminated with heavy metals have greater accumulation of metals than those grown in uncontaminated soil (Alloway and Ayres, 1997; Amusan *et al*., 2005; Sharma *et al.,* 2006, 2007). It is generally accepted that the metal concentration in soil is the dominant factor that influences the concentrations of heavy metals in food (Alloway and Davies, 1971; Adriano, 1986). However, high levels of heavy metals in soils do not always indicate similar high concentrations in plants. The variations in heavy metal concentrations in plants are due to variations in their absorption and accumulation tendency as a result of pH variations (Fuller, 1977; Brummer and Herms, 1983; Christensen,1984; Kabata-Pendas and Pendas, 1992). The metals Cd, Zn and Cr are relatively mobile at low pH (Kabata-Pendas and Pendas, 1992) but Cu and Pb are slowly mobile (Fuller, 1977).

The interactive effect of metals may be synergistic, antagonistic, independent, or additive in nature (Kabata-Pendias, 2001). In plants Cd and Zn may interact during plant uptake, transport from root to shoot and/or accumulation in edible tissues. The effect of Zn on Cd uptake, and vice versa and their concentrations in plants is very controversial in the literature. The interactions between the two metals can be antagonistic and synergistic or they can have no effect on each other depending on plant species, growth conditions, nutritional status of the plants, Zn status of the soil and the plants and Cd content of the soil and the plant (Köleli *et al.,* 2004; Shute and Macfie, 2006). Generally the application of Zn decreases Cd uptake and accumulation in plants due to competition for uptake, because of a common transport system on the plasma membrane (Köleli *et al.,* 2004). This antagonistic interaction has been shown in experiments involving multiple plant species such as wheat (Choudhary *et al.,* 1995; Oliver *et al.,* 1994), rice (Honma and Hirata, 1978), and soybean (Shute and Macfie, 2006). However, synergistic interactions between Zn and Cd have also been shown. Zinc and Cd interactions in hydroponically grown beans had been reported (Chaoui *et al.,* 1997). Shute and Macfie (2006) found synergistic and antagonistic interactions in soybean depending on the level of Zn and Cd applied in a pot experiment. They spiked the soil with six levels of Zn (between 50 and 2000 mg kg^{-1}) and six levels of Cd (between 2 and 100 mg kg^{-1}), plus six combination treatments. When Cd and Zn were present in low doses antagonistic interactions occurred between the two metals: Less Cd was taken up by the soybean plants compared to the single Cd treatments. For Zn, the amount of Zn taken up by plants was not significantly altered in the combination treatment compared to the single treatment at low doses. At high soil concentrations of Zn and Cd the opposite was the case. More Cd and less Zn accumulated in the soybean plant. Zinc and Cu may also interact in several ways: Zn strongly depresses Cu absorption, Cu competitively inhibits Zn absorption and Cu nutrition affects the redistribution of Zn within plants (Loneragan and Webb, 1993).

2.5.1 Uptake and accumulation of cadmium by plants

Although Cd is not essential for plant growth, it is effectively absorbed by roots and leaves (Kabata-Pendias & Pendias, 2001). It is readily absorbed by plant roots and translocated to above-ground parts. Within plants Cd is present as a free ion or as part of metal complexes. It enters the plant mainly by root uptake but foliar uptake is also possible (Santos *et al*., 2010). Human and animal life is threatened when Cd concentrations in the plant are well below phytotoxicity levels (McLaughlin *et al*., 2000) and its accumulation in food crops at sub-toxic levels is a cause for concern due to the risk it poses to the food chain and consumers (Alloway, 1995). The critical phytotoxic level for Cd is set at around 10 mg Cd/kg plant dry weight (Wallnöfer and Engelhardt, 1995). Safe limits of Cd in plant is 0.2 mg kg^{-1} (WHO/FAO, 2007). Cd levels in stem of maize (*Zea maize* L.) cultivated on soil collected from selected dumpsites in Ekiti State, Nigeria, was 5.0 - 7.8 mg kg^{-1} (Awokunmi *et al.*, 2015) while in soil treated with wastewater the level of Cd in maize stem was 0.33 mg kg^{-1} (Kobaissi *et al.,* 2014). Soil pH is considered to be one of the most important factors that influence the transfer of Cd from soil to plants, and higher pH values have been found to reduce the bioavailability and toxicity of Cd (McBride *et al.*, 1997a; Gray *et al.*, 1999b). There are many researchers who devoted themselves to the research on the relation between soil pH and Cd uptake (Hart *et al.,* 2002; Kim *et al.*, 2002; Tudorean and Phillips, 2004; McLaughlin *et al.,* 2011). The consistent conclusion is that the adsorption between soil particles and Cd increases with the increase of soil pH, while Cd bioavailability decreased. The mechanism for this phenomenon can contribute to the increases of solubility and ion competition. As the soil pH decreases, the concentrations of Fe^{2+}, Mn^{2+}, Zn^{2+}, and Ca^{2+} increase in soil solution, which enhance the competition of free ions and reduce the adsorption to soil particles (Tudorean and Phillips, 2004). Under similar chemical, physical, biological and mineralogical conditions in the soil, amounts of Cd absorbed by plants tend to increase as the concentration of Cd in the soil increases (Valadares *et al.*, 1983; Chang *et al.*, 1983). However, the increased Cd concentration in plants may or may not be proportional to the increased concentration in the soil.

2.5.2 Uptake and accumulation of chromium by plants

Chromium accumulates mainly in roots and shoots; however roots accumulate the major part and only a small part is translocated to the shoots (Paiva *et al*, 2009; Sundaramoorthy *et al.*, 2010). The essentiality of Cr for plants has not been demonstrated but it has been found that Cr deficiency in humans can cause diabetes and cardiovascular problems. Countries with high levels of soil Cr tend to have lower death rates caused by cardiovascular diseases (McGrath, 1995). Chromium is toxic for agronomic plants at about 0.5 to 5.0 mg mL^{-1} in nutrient solution and 5 to 100 g kg^{-1} in soil (Hossner, 1996). Under normal conditions, concentration of Cr in plants is less than 1 mg kg^{-1} (Mortvedt and Giordano, 1975). Safe limits of Cr in plant is 5 mg kg^{-1} (WHO/FAO, 2007). The mean level of Cr in stem of maize under long-term wastewater irrigation area in Tongliao, China, was 9.29 ± 2.26 mg kg^{-1} (Lu *et al.*, 2015) while the range of Cr in maize plant grown in selected dumpsites in Ekiti State, Nigeria, was 23.8 - 25.6 mg kg^{-1} (Awokunmi *et al.*, 2015). Cr^{3+} is only slightly mobile in very acidic conditions and its compounds are considered stable in soils where it forms strong complexes with organic matter and adsorbs onto clay particles and oxides (Romic *et al*., 2004). Soluble Cr^{6+} is readily converted to Cr^{3+} under normal soil conditions so soil Cr is not available for plant uptake and therefore unlikely to reach phytotoxic concentrations in soils (Kabata-Pendias & Pendias, 2001; McLaughlin *et al*., 2000; Korte, 1999). Organic matter in soil increases the supply of organic carbon and protons, and stimulation of microorganisms that are considered to be the major factors enhancing the reduction of Cr (VI) to the relatively immobile Cr (III) (Bolan *et al.*, 2003). There are conflicting results concerning the uptake and translocation of Cr-VI. While some authors defend that Cr-VI is reduced to Cr-III on the root surface (Ramachandran *et al.*, 1980; Zayed *et al.*, 1998), others suggest that dissolved Cr-VI is taken up by plants without reduction (Mishra *et al.*, 1995). The uptake of Cr-VI is thought to be an active mechanism performed by carriers for the uptake of essential elements such as sulphate (Cervantes *et al.*, 2001; Kim *et al.*, 2006).

2.5.3 Uptake and accumulation of copper by plants

Although human Cu needs are low, copper deficiency can occur because of its low concentration in edible plant tissues, especially when plants are grown on calcareous or alkaline soils in arid and semi-arid environments (White and Broadley, 2009). Copper concentrations in plant cells need to be maintained at low levels since this element is extremely toxic in view of its high redox properties. The copper deficiency level for plants has been reported as 1 - 5 mg kg^{-1} dry mass and the copper toxicity level in plants has said to be 20 - 30 mg kg^{-1} dry mass (Marschner, 1985). However, safe limit of Cu in plant has been reported to be 40 mg kg^{-1} (WHO/FAO, 2007). In Ekiti State, Nigeria, the level of Cu in the maize stem ranged from 9.1 - 10.1 mg kg^{-1} (Awokunmi *et al.*, 2015). The level of Cu in stem of maize planted in agricultural areas of Guadalupe, Zacatecas, Mexico, was in the range of 69.87 - 120.35 mg kg^{-1} (Dávila *et al.*, 2012) while in agricultural area near Alor Gajah, Melaka, Malaysia, the range of Cu in maize stem was 0.38 - 4.27 mg kg^{-1} (Ibrahim *et al.*, 2015). Copper is actively taken up by plant roots and is not readily transported to shoots, so high concentration of Cu accumulates in roots (Fernandes and Henriques, 1991; Ahsan *et al*., 2007). The average content of Cu in plant tissue is 10 mg kg^{-1} dry weight (Baker and Senef, 1995). The critical free Cu concentration in nutrient media (below which Cu deficiency occurs) ranges from 10^{-14} to 10^{-16} M. Plants usually find a variable supply of Cu in the soil since typically soil solution concentrations range from 10^{-6} to 10^{-9} M, but plants may still need to solubilize and reduce the metal (Yruela, 2005). Thus, for healthy plant growth and development Cu must be acquired from the soil, transported throughout the plant, distributed and compartmentalized within different tissues and its content carefully.

2.5.4 Uptake and accumulation of lead by plants

Lead is taken up by plants only in very small quantities, its largest proportion being accumulated in roots and only small portion transported into shoots (Xiong 1998; Wierzbicka 1999; Fargašová, 2004). The intensity of Pb uptake by plant biomass is affected significantly by plant species, soil pH and organic matter content (Tlustoš *et al.*, 2001). The absorption of Pb in soil follows the Langmuir relation and increases with increasing pH between 3.0 to 8.5 (Lee *et al.*, 1998a). However, in soil with a pH between 5.5 and 7.5, Pb solubility is controlled by phosphate or carbonate precipitates and very little Pb is available to plants even if they have the genetic capacity to accumulate it (Blaylock *et al.*, 1997). Lead present in the soil is always tightly bound to organic or colloidal material or in a precipitated form, all of which serve to reduce the uptake of Pb by plant roots (Sharma and Dubey, 2005). Lead accumulates in the surface layers of soils and therefore it is difficult to reliably measure the portion of soil Pb directly available to plants (Sharma and Dubey, 2005). Plants can tolerate even higher concentrations of Pb in the substrate without visual symptoms of damage (Xiong, 1997). The safe limit of Pb in plant is 5 mg kg^{-1} (WHO/FAO, 2007). Excessive accumulation of this toxic metal in plant tissues results in numerous disturbances of physiological processes (Vangronsveld and Clijsters, 1994), causing eventually inhibition of growth, decreased yield and decreased quality of cultivated crops. The mean level of Pb in maize stem grown in wastewater irrigation area in Tongliao, China, was 1.25 ± 0.26 mg kg^{-1} (Lu *et al.*, 2015). In agricultural areas of Guadalupe, Zacatecas, Mexico, the level of Pb in maize stem was 0 - 21.39 mg kg^{-1} (Dávila *et al.*, 2012). The level of Pb which causes toxicity in plants is difficult to establish but it is generally agreed that soil Pb concentrations of 100 - 500 mg g^{-1} are considered to be excessive (Voutsa *et al.*, 1996). Further more, phytotoxicity is often species and environment specific. Thus studies on Pb uptake by agricultural crops assume greater importance especially in the regions where such contamination of soils and agricultural fields is a possibility.

2.5.5 Uptake and accumulation of zinc by plants

Roots take up zinc from the soil solution as a divalent cation (Zn^{2+}), and at high pH, Zn is absorbed as a monovalent ($ZnOH^{+}$) cation (Marschner, 1995), which is a dynamic and complex process. Uptake depends on ion concentrations at the root surface, plant demand and root absorption capacity (Sadeghzadeh, 2013). Zinc reaches the plant root surface through mass flow, diffusion and root interception mechanisms. The interception of nutrients by roots is an important uptake mechanism for soil immobile nutrients such as Zn. Thus, root interception (i.e. root growth and root surface area) is also an important factor in determining plant availability of Zn (Sadeghzadeh, 2013). The availability of Zn to plants depends on several soil factors such as the concentration of Zn in solution, ion speciation and the interaction of Zn with other macronutrients and micronutrients (Li *et al.*, 2003). Safe limits of Zn in plant is 60 mg kg^{-1} (WHO/FAO, 2007). The range of Zn level in maize stem grown in agricultural area Near Alor Gajah, Melaka, Malaysia, ranged from 6.45 - 22.83 mg kg^{-1} (Ibrahim *et al.*, 2015) while in agricultural areas of Guadalupe, Zacatecas, Mexico, the range of Zn in stem of maize was 89.02-688.63 mg kg^{-1} (Dávila *et al.*, 2012). The level of Zn accumulated in maize stem grown in selected dumpsites in Ekiti State, Nigeria, was 16.8 - 21.0 mg kg^{-1} (Awokunmi *et al.*, 2015) while the mean level of Zn in stem of maize grown in soil treated with wastewater was 29.0 mg kg^{-1} (Kobaissi *et al.*, 2014). The critical limits of Zn in plants indicating deficiency are: < 10 mg kg^{-1} definite Zn deficiency, 10 - 15 mg kg^{-1} very likely, 15 - 20 mg kg^{-1} likely and > 20 mg kg^{-1} unlikely (sufficient) (Dobermann and Fairhurst, 2000). In most crop species leaf sufficiency range for Zn is 15 to 50 mg kg^{-1} in the dry matter of mature plants and in most cases 15 mg kg^{-1} Zn is considered as critical value (Benton, 2003). Zinc is therefore important for plant growth, as plants require a proper balance of all the essential nutrients for normal growth and optimum yield.

2.6 Bioaccumulation factor

Excessive accumulation of heavy metals can be toxic to most plants and to the food chain as a whole. This is because heavy metals entering the ecosystem lead to bioaccumulation and biomagnification along the food chains causing direct consequences to the components of the food chains (Nyatwere, 2014). Total concentrations of metals in soils are a poor indicator of metal toxicity since metals exist in different solid-phase forms that can vary greatly in terms of their bioavailability (Krishnamurti *et al.,* 1996; Krishnamurti and Naidu, 2002, 2008; Huang and Gobran, 2005). However, most regulations or guidelines used for protecting soil from metal pollution (to set maximum permissible metal concentrations) are still based on assessing the total concentration of metal present in the soil. Recently, some countries have started to introduce the concept of bioavailability in their regulations concerning environmental protection and remediation.

Transfer factor describes the amount of an element expected to enter a plant from its substrate, under equilibrium conditions (Sheppard and Sheppard 1985; Davis *et al.,* 1999). This theory assumes a linear relationship between the contents of a certain element in the plant with that in the soil. An approach based on soil-plant transfer provides an uncomplicated and helpful method for assessing uptake of trace metals for the purposes of assessment and testing. The degree of metal enrichment in crops can be described with enrichment factor. The concept of enrichment factor was first proposed by Zoller et al. (1974) for source diagnosis of atmospheric particulate matter in the Antarctic. In the following year, enrichment factor was used for investigating high-altitude atmospheric substances in the North Atlantic Ocean (Duce *et al.,* 1975). Recently, the application of enrichment factor in the form of bioaccumulation factor (BAF) has been expanded to research on soil as well as assessment of heavy metal pollution in environmental geochemistry (Wang *et al.,* 2006; Khan *et al.,* 2010; Brioschi *et al.,* 2013; Delgado *et al.,* 2012). To show quantitatively the transfer of an element from soil to plant, bioaccumulation factor, which is defined as the ratio of the metal content in the plant part to the metal content in the soil, and is used to estimate the ability of plant to accumulate metals in their parts (García *et al.,* 2009; Zhou *et al.,* 2016) is given by Eq. (2.6):

$$\text{BAF} = \frac{\text{Metal content in plant part (mg/kg D.W)}}{\text{Metal content in soil (mg/kg D.W)}} \qquad (2.6)$$

Bioaccumulation factor provides a useful indication of relative metal availability from soils to plants (Alloway *et al.,* 1988) and the higher the value of bioaccumulation factor, the more mobile/available the metal is. A range of generalized BAF have been suggested by Kloke et al. (1984). Kloke's bioaccumulation factors are based on the root uptake of metals, with no consideration given to aerial deposition and foliar adsorption of elements. The extent of accumulation of heavy metals by plants differ with the type of metal. Among the metals, Cd and Zn are fairly mobile and readily absorbed by plants (Mench *et al.*, 1994). In contrast, Cu and Pb are strongly adsorbed onto soil particles reducing their availability to plants (WHO, 1989, 1998). In addition, they are bound to organic matter, as well as being adsorbed by carbonate minerals and hydrous iron and manganese oxides. Bioaccumulation factor not greater than 1.0 indicates that plant excludes the heavy metal from its tissues and the factor value that is greater than 1.0 indicates that plant bioconcentrates heavy metal (Ma *et al*., 2001; Cluis, 2004; Bose and Bhattacharyya, 2008). Range of bioaccumulation factors of some heavy metals in maize plant grown in contaminated soil in agricultural areas of Guadalupe, Zacatecas, Mexico, was as follows: Cu (0 - 1.64), Pb (0 - 0.5) and Zn (0.38 - 2.02) (Dávila *et al*., 2012) while the bioaccumulation factors for some elements in sugarcane grown in soil irrigated with wastewater was Cd (1.08), Cr (0.14), Cu (0.14), Pb (0.37) and Zn (0.64) (Alghobar and Suresha, 2015). Food crops which have high soil-plant transfer are more likely to expose human to toxic heavy metals. It is therefore important to determine the bioaccumulation factors of the studied metals in sugarcane grown in Muhoroni and South Nyanza sugar estates.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study areas

The study areas (Figure 3.1) are located within the western part of Kenya, comprising two sugar management zones namely: (i) Muhoroni which lies within the sub humid agro-ecological zone; and (ii) SONY found within the humid agro-ecological zone of Kenya (Mulianga *et al.*, 2013). Muhoroni Sugar Company is located in Muhoroni sub-county of Lake region and falls within Nyando sugar belt at an altitude of 1,380 meters above mean sea level. The basin bound by latitudes 0^{o} $7^{'}$ $48^{''}$ N and 0^{o} $24^{'}$ $36^{''}$ S and longitudes 34^{o} $24^{'}$ $36^{''}$ E and 35^{o} 43’ 12” E. The Nyando basin experiences a bimodal rainfall pattern with long rains in March - May and short rains in September - November. The mean annual rainfall calculated over the 9-year period (2002 - 2010) is 1,486 ± 214 mm (Mulianga *et al.*, 2013) with a minimum and maximum mean monthly rainfall of 72 mm and 243 mm respectively (JICA, 1992). The predominant soil type in this subzone is chromic vertisols, sodic phase (Jaetzold *et al.*, 2009). Although this is a marginal sugarcane sub-zone, most farmers grow sugarcane as a cash crop. The first rainy season can expect an amount of more than 550 - 700 mm in 10 out of 15 seasons; the second rainy season > 400 - 600 mm. The 60% reliability of the growing periods during the 1st and 2nd seasons is 175 or more and 105 - 120 days, respectively.

South Nyanza Sugar Factory (SONY) is located in Rongo sub-county of Lake region and falls within South Nyanza sugar belt. It lies 160 km south of Kisumu City at an altitude of 1,454 meters above mean sea level, latitude 0^{o} $54^{'}$ South and longitude 34^{o} $32^{'}$ East. Average annual rainfall calculated over the 9-year period (2002 - 2010) is 1,869 ± 221 mm (Mulianga *et al.*, 2013). The rainfall amount is variable but reliable. The predominant soil type in this subzone is the well drained humic acrisols, partly piso- and petroferric phases (Jaetzold *et al.*, 2009). The first rainy season can expect an amount of more than 900 mm in 10 out of 15 seasons; the second rainy season more than 500 mm. With some probability the 60 % reliability of the growing periods during the 1st and 2nd seasons is more than 190 and 170 days, respectively.

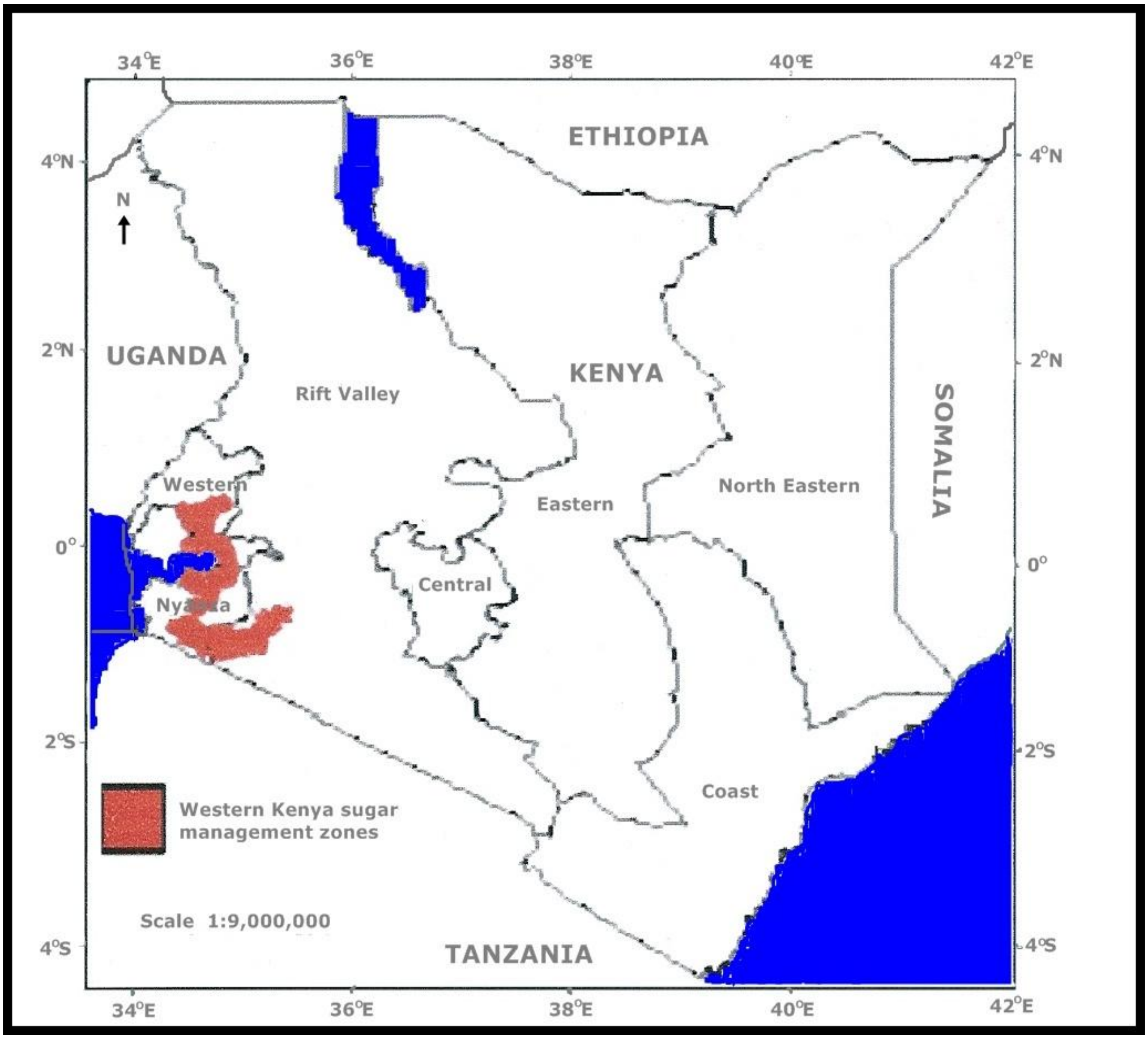

Figure 3.1. Location of study areas.
(Source: Kenya's Sugar Industry, 2005)

3.2 Sampling points

Each sugarcane estate was divided into three sites comprising of 15 plots each from which both soil and mature sugarcane stems were sampled in triplicate. The major sugarcane varieties grown in the study areas that were sampled were CO421, CO617 and CO945. Soil samples were collected at the root zone of each sugarcane sample. The criteria for picking the plots where sampling was done was maturity of the sugarcane. A zigzag sampling pattern, according to Sabbe and Marx (1987), was used to obtain a reasonably representative samples. The sampling points, as shown in Figures 3.2 and 3.3 are identified by letters and numbers. The numbers before the letters represent the site number and the numbers after the letters represent the replicate. The letter A represents variety CO421, letter B represents variety CO617 and letter C represents variety CO945.

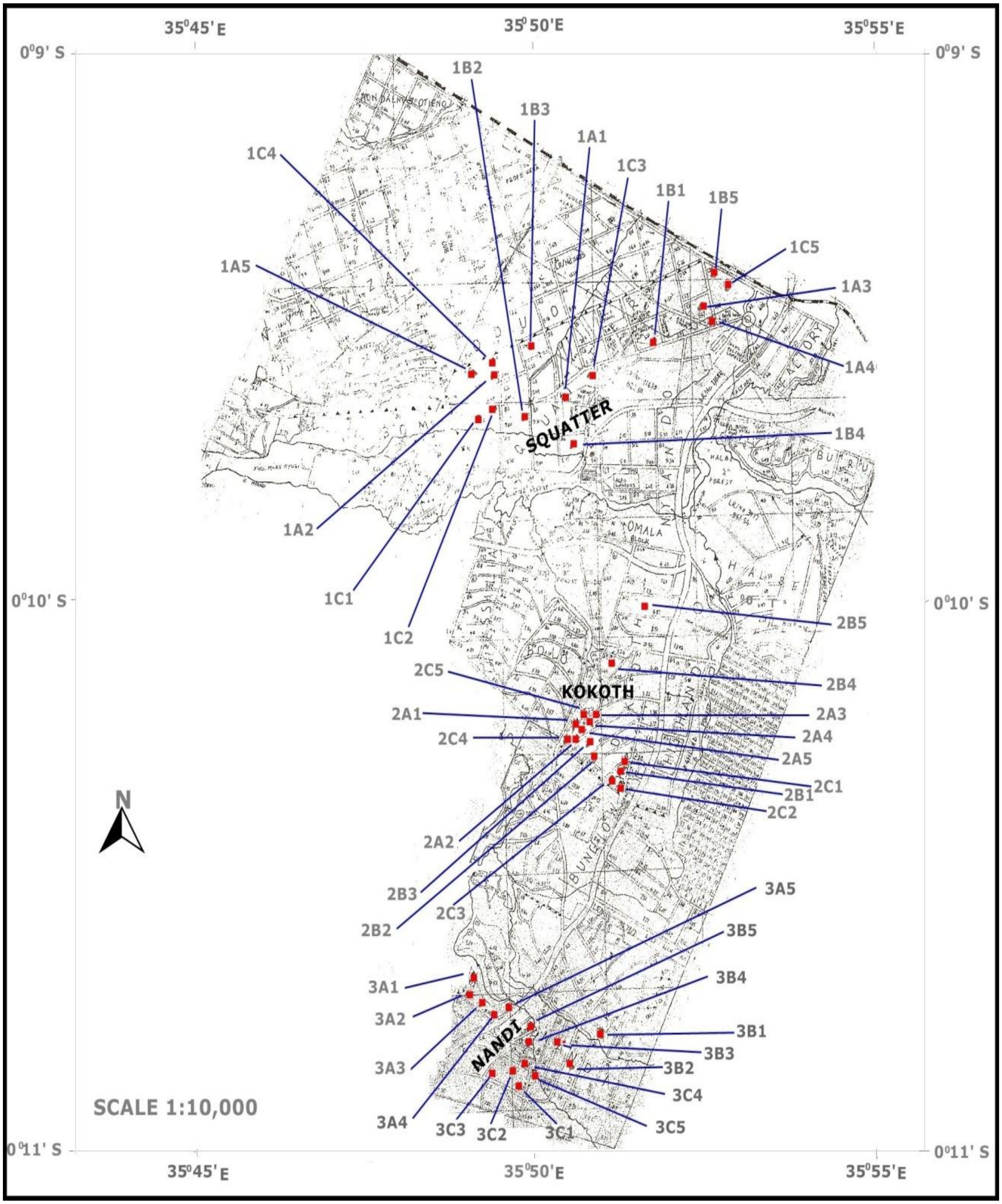

Figure 3.2. Sampling points in Muhoroni nucleus estate

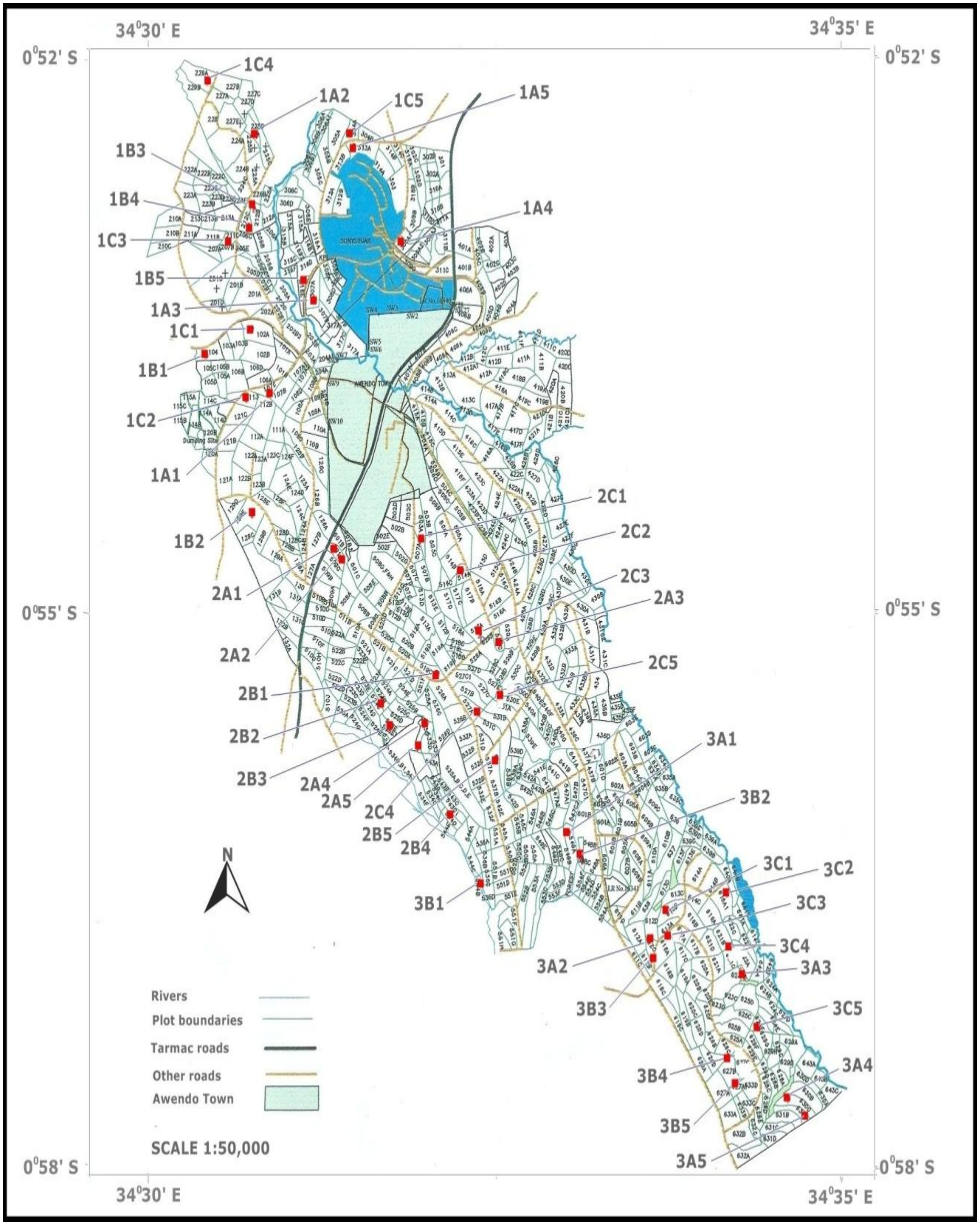

Figure 3.3. Sampling points in SONY nucleus estate

3.3 Reagents

Analytical reagent grade chemicals and reagents were used in digestion of samples and preparation of solutions and are listed in the Table 3.3.

Table 3.3: Chemicals used in metal analysis

Chemicals	%Purity	Manufacturer
Hydrochloric acid	38.0	Sigma-Aldrich Co., America
Nitric acid	70	Sigma-Aldrich Co., America
Cadmium nitrate tetrahydrate	98	Sigma-Aldrich Co., America
Chromium (III) nitrate nonahydrate	≥ 99.99	Sigma-Aldrich Co., America
Copper (II) nitrate trihydrate	≥ 99	Sigma-Aldrich Co., America
Lead (II) nitrate	≥ 99	Sigma-Aldrich Co., America
Zinc nitrate hexahydrate	99.999	Sigma-Aldrich Co., America
Strontium chloride hexahydrate	99.995	Sigma-Aldrich Co., America
De-ionised water	0.1 μm filtered	Sigma-Aldrich Co., America

Stock solutions (1000 ml) containing 1000 mg L^{-1} of the analytes were prepared from nitrate salts of cadmium, chromium, copper, lead and zinc in 1:1(v/v) Nitric acid. Working standard solutions were prepared in 1M $HNO_{3(aq)}$ by appropriate dilutions of the stock solutions to known concentrations per salt in 100 ml volumetric flask after addition of 1 ml of 1000 mg L^{-1} solution of strontium chloride. The purpose of strontium chloride was to remove interference in absorption of the specific metal by other metals at the same wavelength by acting as a buffer and to minimize ionization of the metal atoms (Ikuo *et al*., 1965). These solutions were used as standards and calibration curves were drawn from them.

3.4 Sampling and sample preparation

A total of 90 soil and 90 sugarcane samples were collected, randomly in triplicates, from the sites in the two regions. Soil (0 - 30 cm) samples were collected at the root zone of each sugarcane sample using a stainless steel auger and placed in labeled plastic bags. Each sugarcane sample was cut at the ground level using stainless steel knife. The sugarcane stem was cut into pieces and placed in labeled plastic bags. The sugarcane varieties sampled were CO421 (30 plants), CO617 (30 plants) and CO945 (30 plants). The sugarcane samples were mature (18 months old) plant crops. The mean plant height as measured by a metre rule was 260.2 ± 15.9 cm.

The soil sample was air-dried in the laboratory for 7 days and 30 g of the soil oven dried at 60^{o}C temperature to a constant weight then allowed to cool in a desiccator. The dry soil samples were crushed with a pestle and mortar, sieved through a 2 mm plastic mesh and kept in clean plastic containers before acid digestion.

Sugarcane stem was washed with de-ionized water to remove dirt. Each stem was split into two where one half was chopped into small pieces and sun dried for 3 weeks then in an oven at 60^{o}C for 24 h. Using stainless steel jar, the dry sample was powdered in an electric grinder and sieved through a 2 mm plastic mesh. The powder was kept in clean plastic containers before acid digestion.

The remaining half of sugarcane stem was crushed using a three roller crusher. The juice was filtered through a sieve of 100 microns. Sugarcane juice was collected in clean plastic bottles and kept in refrigerator at 4^{o}C before acid digestion. The bagasse was also collected in a labeled plastic bag.

The bagasse samples were dried first in sunlight for 7 days and then oven dried at 80^{o}C for 16 hours (Handson and Shelley, 1993). The samples were crushed in an electric grinder using stainless steel jar to powder form and sieved through a 2 mm mesh. The powder was kept in clean plastic containers before acid digestion.

3.5 Determination of heavy metals in soil

One gramme of dry soil sample was digested in 12.5 ml of *Aqua regia* (2.5 ml concentrated HNO_3 + 10 ml concentrated HCl), in triplicate, according to the procedure of Tack and Verloo (1999) with slight modifications. The mixture was refluxed for 3 hours in Gerhardt block digester at 95^{o}C. The digest was filtered through Whatman No. 41 filter paper and filtrate collected in a 50 ml volumetric flask. The filter paper was washed, while still in the funnel, with 5 ml of hot (95^{o}C) HCl, followed by 20 ml of hot (95^{o}C) de-ionized water. Filter paper and residue from the funnel were removed and placed in the digestion tube and 5 ml of conc. HCl added. The mixture was placed back on the heating source, and heated at 95°C for 30 minutes. The digestion tube was removed from the heating source and allowed to cool. The digest was filtered and the filtrate collected in the volumetric flask. The digestion tube was also washed with de-ionized water and the filtrate transferred quantitatively to the 50 ml volumetric flask which was made up to the volume with de-ionized water after addition of 1 ml of 1000 mg L^{-1} solution of strontium chloride. The samples were stored in acid washed polyethylene bottles at 4°C, according to Jones et al. (1991). The digest obtained was analyzed for Cd, Cr, Cu, Pb and Zn using an atomic absorption spectrophotometer (AAS). Before analysis was done, the AAS machine was calibrated.

3.5.1 Determination of soil pH

The pH of the soil sample was determined according to the procedure of Rhodes (1982). A volume of 50 ml of de-ionized water was added to 20 g of crushed soil sample and stirring well for 10 minutes. The mixture was allowed to stand for 30 minutes before stirring again for 2 minutes. The pH was measured with an electronic JENWAY glass electrode pH meter (model 350).

3.5.2 Determination of soil organic matter

Air dried soil sample (10 g) in the crucible was heated in an oven for 3 h at 105°C and the difference in weight noted as the moisture content (Okalebo *et al.*, 2002). A method, as described by Blume et al. (1990); Nelson and Sommers (1996); ASTM (2000), was used to determine soil organic matter of soil samples. In this method moisture free soil, obtained by using Okalebo et al. (2002) method, was placed in a Vulcan A-550 muffle furnace and heated to 350°C overnight. The grey-white ash sample was removed and cooled in a desiccator and weighed. Organic matter content was calculated as the difference between the moisture free sample and final sample weights divided by the moisture free sample weight times 100 %.

3.5.3 Determination of contamination factor

Using heavy metals levels determined in soil samples, contamination factor (C_f) was calculated using the equation below:

$$C_f = \frac{C_s}{C_b}$$

where C_s is the mean content of metal from at least five sampling points and C_b is the pre-industrial concentration of the individual metal.

3.5.4 Determination of degree of contamination

The degree of contamination (C_d) was assessed using equation

$$C_d = \sum_{i=1}^{n} C_{f\,i}$$

where C_f is contamination factor of individual heavy metal.

3.5.5 Determination of potential ecological risk factor

The potential ecological risk factor (*Er*) was calculated using the equations:

$$Er = Tr \times C_f$$

where Tr denotes the "toxic-response" factor for heavy metals and C_f is contamination factor of individual heavy metal.

3.5.6 Determination of potential ecological risk index

Potential ecological risk index (*RI*) which is the sum potential ecological risk factors was calculated as follows:

$$RI = \sum_{i=1}^{n} Er_i$$

3.6 Determination of heavy metals in sugarcane stem

The sugarcane sample was prepared using the same procedure of Welz and Sperling (1999) with slight modifications. Briefly, 1 g of sugarcane stem powder sample was digested in a mixture of 9 ml concentrated HNO_3 and 3 ml concentrated HCl in Gerhardt block digester at 85^oC for 3 h, in triplicate. The digest was filtered through Whatman No. 41 filter paper and filtrate collected in a 50 ml volumetric flask. The filter paper was washed with 2 ml of hot (95^oC) HCl followed by 10 ml of hot (95^oC) de-ionized water. The digestion tube was also washed with de-ionized water and the filtrate transferred quantitatively to the 50 ml volumetric flask which was made up to the volume with de-ionized water after addition of 1 ml of 1000 mg L^{-1} solution of strontium chloride. The samples were stored in acid washed polyethylene bottles at 4^oC, according to Jones et al. (1991). The digest obtained was analyzed for Cd, Cr, Cu, Pb and Zn using AAS. Before analysis was done, the AAS machine was calibrated.

3.7 Determination of heavy metals in cane juice

The juice was prepared according to the procedure of Niazi et al. (1997) with slight modification where 50 ml of sugarcane juice, in triplicate, was transferred into a 250 ml digestion tube and 6 ml of concentrated HNO_3 added. The digestion tube and its content was heated at 95^oC and the sample evaporated cautiously for 3 hours. The sample was cooled, 3 ml of concentrated HNO_3 added to it and heated again at 105^oC for 30 minutes. The sample was cooled and 10 ml of 50 % HCl added and heating continued at 105^oC for further 30 minutes. The digest was filtered through Whatman No. 41 filter paper and filtrate collected in a 50 ml volumetric flask. The filter paper was washed with 5 ml of hot (95^oC) HCl followed by 20 ml of hot (95^oC) de-ionized water. The digestion tube was also washed with de-ionized and the filtrate transferred quantitatively to the 50 ml volumetric flask. The volumetric flask was made up to the mark with de-ionized water after addition of 1 ml of 1000 mg L^{-1} solution of strontium chloride. The samples were stored in acid washed polyethylene bottles at 4^oC, according to Jones et al. (1991). The digest obtained was analyzed for Cd, Cr, Cu, Pb and Zn using AAS. Before analysis was done, the AAS machine was calibrated.

3.8 Determination of heavy metals in bagasse

The bagasse samples were digested according to procedure of Welz and Sperling (1999) with slight modifications. Briefly, 1 g of bagasse sample was digested in a mixture of 9 mL concentrated HNO_3 and 3 ml concentrated HCl in Gerhardt block digester at 85^oC for 3 h. The digest was filtered through Whatman No. 41 filter paper and filtrate collected in a 50 ml volumetric flask. The filter paper was washed with 2 ml of hot (95^oC) HCl followed by 10 ml of hot (95°C) de-ionized water. The digestion tube was also washed with de-ionized and the filtrate transferred quantitatively to the 50 ml volumetric flask which was made up to the volume with de-ionized water after addition of 1 ml of 1000 mg L^{-1} solution of strontium chloride. The samples were stored in acid washed polyethylene bottles at 4°C, according to Jones et al. (1991). The digest obtained was analyzed for Cd, Cr, Cu, Pb and Zn using AAS. Before analysis was done, the AAS machine was calibrated.

3.9 Determination of bioaccumulation factor

Using heavy metals levels determined in soil samples and in sugarcane stem samples, bioaccumulation factor (BAF) was calculated as follows:

$$\text{BAF} = \frac{\text{Metal content in plant part (mg/kg D.W)}}{\text{Metal content in soil (mg/kg D.W)}}$$

3.10 Preparation of standard stock solutions

3.10.1 Cd: 1000 mg L^{-1}

2.744 g of cadmium nitrate tetrahydrate (purity: 98%) was weighed and transferred into a beaker. The nitrate was dissolved in 20 ml of 1:1(v/v) nitric acid. The solution was transferred into a 1000 ml volumetric flask and diluted to the mark with de-ionised water. The solution was put in a polyethylene bottle and stored in a fridge.

3.10.2 Cr: 1000 mg L^{-1}

7.695 g of chromium (III) nitrate nonahydrate (puriy: $\geq$ 99.99 %) was weighed and transferred into a beaker. The nitrate was dissolved in 30 ml of 1:1(v/v) nitric acid and diluted to 1 L in a 1000 ml volumetric flask with de-ionised water.

3.10.3 Cu: 1000 mg L^{-1}

3.802 g of copper (II) nitrate trihydrate (purity: $\geq$ 99 %) was weighed and transferred into a beaker. The nitrate was dissolved in 20 mL of 1:1(v/v) nitric acid and diluted to 1 l in a 1000 ml volumetric flask with de-ionised water.

3.10.4 Pb: 1000 mg L^{-1}

1.599 g of lead (II) nitrate (purity: ≥ 99.0 %) was weighed and transferred into a beaker. The nitrate was dissolved in 10 ml of 1:1(v/v) nitric acid and diluted to 1 L in a 1000 ml volumetric flask with de-ionised water.

3.10.5 Zn: 1000 mg L^{-1}

4.551 g of zinc nitrate hexahydrate (purity: 99.999 %) was weighed and transferred into a beaker. The nitrate was dissolved in 20 ml of 1:1(v/v) nitric acid and diluted to 1 L in a 1000 ml volumetric flask with de-ionised water.

3.10.6 Sr: 1000 mg L^{-1}

3.043 g of strontium chloride hexahydrate (purity: 99.995 %) was weighed and transferred into a beaker. The nitrate was dissolved in 20 ml of 1:1(v/v) nitric acid and diluted to 1 L in a 1000 ml volumetric flask with de-ionised water.

3.11 Quality assurance

De-ionized water was used throughout the study. Glassware was properly cleaned, and the reagents of analytical grade were used. Reagent blank determinations was used to correct the instrument readings. For validation of the analytical procedure, a recovery study was carried out by spiking standard solutions containing Cd, Cr, Cu, Pb and Zn, as shown in Table 3.4, together in 1 g of soil, bagasse and sugarcane stem, and in 50 ml of juice and the recovery mixture then left to stand for half an hour before digestion. The spiked and non-spiked samples were digested side by side in triplicate.

Table 3.4. Spiking standard solutions

Sample	Cd	Cr	Cu	Pb	Zn
Soil	0.2 ml of 1000 mg L^{-1}	3.0 ml of 1000 mg L^{-1}	2.0 ml of 1000 mg L^{-1}	2.0 ml of 1000 mg L^{-1}	4.0 ml of 1000 mg L^{-1}
Bagasse	0.1 ml of 1000 mg L^{-1}	2.0 ml of 1000 mg L^{-1}	0.5 ml of 1000 mg L^{-1}	1.0 ml of 1000 mg L^{-1}	2.0 ml of 1000 mg L^{-1}
Juice	0.2 ml of 100 mg L^{-1}	0.2 ml of 100 mg L^{-1}	0.2 ml of 100 mg L^{-1}	0.3 ml of 100 mg L^{-1}	1.0 ml of 1000 mg L^{-1}
Stem	0.6 ml of 100 mg L^{-1}	0.5 ml of 1000 mg L^{-1}	1.2 ml of 100 mg L^{-1}	0.5 ml of 1000 mg L^{-1}	2.0 ml of 1000 mg L^{-1}

For calibration of the instrument, a series of five standard solutions were prepared by serial dilution of the stock standard solution (1000 mg L^{-1}) of the metals to be analyzed. The prepared metal concentrations ranged between 0.01 - 10.0 mg L^{-1} of Cd, Cr, Cu, Pb and Zn. The instrument was calibrated daily with calibration blank plus 5 calibration standards (multi-point) and relative percentage difference between five point calibration and daily calibration was calculated. Continuing calibration verification was done after analysis of every 10 samples and at the end of the analytical run. The coefficient of variation of replicate analyses was determined for the measurements of calculated analytical precision. Strontium chloride (1000 mg L^{-1}) was used to remove interference in absorbance of specific metal by other metals at the same wave length and to improve efficiency by preventing unnecessary ionization of the ions. These procedures and precautions were carried out to ensure reliability of results. The readings of heavy metal concentrations were obtained from AAS in terms of absorbance which was used to get their concentrations in ppm from the standard curve.

3.12 Instrumentation

The digestion of the samples was done by Gerhardt block digester (C. Gerhardt, Germany). Block digestion systems is ideal for simultaneous multiple digestions in 100 ml tubes. Precise temperature control permits the conditions for the digestions to be optimized thus providing reproducible results. Digestion block made of aluminum with holes for digestion tubes ensures efficient heating and insulation to retain heat within the block. Insert rack made of aluminum with integrated heat shield and window provides easy and safe observation of the samples. Determination of metal levels in the digests were carried out by using a Shimadzu AA-6200 flame atomic absorption spectrophotometer (Tokyo, Japan), with an air-acetylene flame. Hollow cathode lamps having resonance lines at 228.8, 357.9, 324.8, 283.3 and 213.9 nm were used as radiation sources for the determination of Cd, Cr, Cu, Pb and Zn, respectively (Berman, 1986). Lamp intensity and band pass was used according to the manufacturer’s recommendations. Acetylene and air flow rates were 2 and 4 L $\min^{-1}$, respectively, for all the elements. Analysis was done after calibration of the equipment with different standard concentrations of the heavy metals. An electronic JENWAY glass electrode pH meter model 350 (Camlab, UK) was used to measure pH of the soil samples and cane juice, and Vulcan A-550 muffle furnace (US dental depot, USA) was used to dry the samples.

3.13 Data analysis

The mean levels of Cd, Cr, Cu, Pb and Zn in soil, bagasse, cane juice and sugarcane stems were calculated using the statistical package SPSS 20 and the results compared with acceptable limits according to WHO/FAO. In order to assess the mobility and accumulation of the heavy metals in the soil-plant system, bioaccumulation factor for each heavy metal was calculated. Degree of pollution/contamination of study areas was assessed by calculating contamination factor, degree of contamination and potential ecological risk factor. The % spike recovery was given by the difference between concentration of analyte in spiked sample and concentration of analyte in unspiked sample divided by concentration of spike multiplied by 100.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Quality assurance

The results of the validity of the procedures used for sample treatment and analysis tested by spiking experiment are presented in Table 4.1.

$$\% \text{ Spike recovery} = \frac{(\text{Level in spiked sample} - \text{Level in un-spiked sample})}{\text{Level in spike solution added}} \text{ x100}$$

Table 4.1. Percentage recoveries in soil, bagasse, juice and stem samples

		Cd	**Cr**	**Cu**	**Pb**	**Zn**
Level of metal in spiked Samples (ppm)	Soil	0.43 ± 0.15	73.72 ± 0.21	15.97 ± 0.14	76.50 ± 0.09	142.42 ± 0.10
	Bagass	0.22 ± 0.05	11.05 ± 0.07	1.25 ± 0.03	0.09 ± 0.10	32.24 ± 0.02
	Juice	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.01	0.12 ± 0.03	6.32 ± 0.01
	Stem	0.18 ± 0.03	8.36 ± 1.04	0.80 ± 0.05	2.81 ± 0.21	31.02 ± 0.03
Level of metal in un-spiked Samples (ppm)	Soil	0.24 ± 0.05	70.81 ± 6.90	14.04 ± 2.20	73.55 ± 8.06	138.51 ± 9.10
	Bagass	0.12 ± 0.01	9.08 ± 1.09	0.76 ± 0.34	3.01 ± 0.42	30.29 ± 1.86
	Juice	0.04 ± 0.03	0.03 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	3.37 ± 0.62
	Stem	0.12 ± 0.02	7.87 ± 1.02	0.68 ± 0.15	2.33 ± 0.93	29.09 ± 2.66
Level of metal in spike Solution added (ppm)	Soil	0.20	3.00	2.00	3.00	4.00
	Bagass	0.10	0.20	0.50	1.00	2.00
	Juice	0.20	0.02	0.02	0.03	3.00
	Stem	0.06	0.50	0.12	0.50	2.00
% Spike recovery	Soil	95.00 ± 0.12	97.00 ± 1.01	96.50 ± 0.09	98.33 ± 0.21	97.75 ± 0.40
	Bagass	100.00 ± 0.68	98.50 ± 0.09	98.00 ± 0.15	97.00 ± 0.47	97.50 ± 0.13
	Juice	100.00 ± 0.97	100.00 ± 0.03	100.00 ± 0.92	100.00 ± 0.06	98.33 ± 0.34
	Stem	100.00 ± 1.08	98.00 ± 0.06	100.00 ± 0.60	96.00 ± 0.31	96.50 ± 0.52
MDL*		0.001	0.003	0.002	0.010	0.002

*MDL: Method detection limit

The result of the spike recovery test in soil was Cd (95.00 ± 0.12 %), Cr (97.00 ± 1.01%), Cu (96.50 ± 0.09%), Pb (98.33 ± 0.21%) and Zn (97.75 ± 0.40%). In Bagasse the spike recovery test was Cd (100.00 ± 0.68%), Cr (98.50 ± 0.09%), Cu (98.00 ± 0.15%), Pb (97.00 ± 0.47%) and Zn (97.50 ± 0.13%). The result of the spike recovery test in juice was Cd (100.00 ± 0.97%), Cr (100.00 ± 0.03%), Cu (100.00 ± 0.92%), Pb (100.00 ± 0.06%) and Zn (98.33 ± 0.34%) while the recovery in sugarcane stem was Cd (100.00 ± 1.08%), Cr (98.00 ± 0.06%), Cu (100.00 ± 0.60%), Pb (96.00 ± 0.31%) and Zn (96.50 ± 0.52%). These mean % recoveries were above the 95 % threshold indicating the validity and reliability of the digestion method and the AAS analysis adopted for this research. The method detection limits (MDL) of metals investigated are presented in Table 4.1. To calculate the detection limits, the equation used was MDL = $3s_B/b$, where s_B = the standard deviation of the six blank solutions; b = the slope of the calibration line (Thomas *et al*., 1994; Vandecasteele and Block, 1997). The method detection limits for all the metals were less than 0.1 which indicated that the method was suitable for the determination of trace levels.

4.2 Soil pH

The soil pH of the studied areas were 5.23 ± 0.48 and 5.30 ± 0.28 for Muhoroni and SONY respectively (Table 4.2), indicative of strongly acidic soil as categorised by Foth and Ellis (1997).

Table 4.2. Soil organic matter content and soil pH (mean ± S.D) of the topsoil samples

Site	SOM (%) (n=45)		Soil pH (n=45)	
	Muhoroni	SONY	Muhoroni	SONY
1	2.55 ± 0.50	2.11 ± 0.53	5.29 ± 0.44	5.28 ± 0.26
2	2.38 ± 0.41	2.59 ± 0.54	5.14 ± 0.60	5.41 ± 0.27
3	2.39 ± 0.45	2.27 ± 0.51	5.26 ± 0.39	5.20 ± 0.32
Mean ± S.D	**2.44 ± 0.45**	**2.32 ± 0.53**	**5.23 ± 0.48**	**5.30 ± 0.28**

S.D: Standard deviation; n: the number of samples analysed

These soil pH values of sugarcane plantation in Muhoroni and SONY were typical and comparable with a pH value (5.39 ± 0.31) of Nzoia Sugar Company estate soil as reported by Omwoma et al. (2010). The pH values were also close to (5.3 ± 0.8) in the vicinity of a Korean copper - tunsten mine as reported by Jung (2008) and pH value (5.034 ± 0.25) of maize farm soils in Trans Nzoia, Kenya, as reported by Kananu et al. (2014). The mean soil pH values reported in this study were lower than 6.40 ± 0.59 reported by Lugwisha and Othman (2014), soil pH (6.75 ± 1.49) reported by Qishlaqi and Moore (2007), and pH 6.69 ± 0.04 reported by Uwah et al. (2011). However, the soil pH values of Muhoroni and SONY were higher than soil pH (4.71 - 4.93) in agricultural area near Alor Gajah, Melaka, Malaysia, as reported by Ibrahim et al. (2015). The soil pH values of Muhoroni and SONY were below the optimum range of 6.5 - 7.0, which is required for sugarcane cultivation according to Barnes (1974). The low soil pH for both fields could be attributed to the composition of the parent rock from which the soils were formed and inherent factors affecting soil pH such as climate, mineral content and soil texture. The low soil pH in sugarcane farm soils was also attributed to fertilizer application as observed by Nartey et al. (2012). The soil pH in the study areas ought to be increased to the range required by sugarcane. The low soil pH could lead to elevated heavy metal uptake by the crop which might affect food quality and safety.

4.3 Soil organic matter content (SOM)

The soil organic matter content (Table 4.2) in Muhoroni and SONY soil samples were 2.44 ± 0.45 % and 2.32 ± 0.53%, respectively. The soil organic matter contents in the study areas were within the range (2 - 3%) required by sugarcane according to Muhammad et al. (2000). These soil organic matter contents in the study areas were below organic matter content (1.8 - 12.4%) in paddy soils from lake Victoria basin as reported by Machiwa (2010), below organic matter content (10.2 ± 6.21%) in agricultural soils of Khoshk river banks, Shiraz, Iran as reported by Qishlaqi and Moore (2007) and far much below 31.45 - 59.42% in agricultural area near Alor Gajah, Melaka, Malaysia, as reported by Ibrahim et al. (2015). The low soil organic matter content in Muhoroni and SONY soil samples was common phenomenon in land under sugarcane cultivation as observed by Dominy et al. (2002a). This was in agreement with the report given by Haynes and Hamilton (1999) who suggested that the most serious factor associated with soil degradation under sugarcane is the loss of soil organic matter, and Wood (1985) who noted that sugarcane soils can have less than half the amount of organic matter of virgin sites. The reason for this was presumably that there was a large removal of organic material from the soil-plant system in the form of harvested cane. Du Toit et al. (1994) observed general depletion pattern for organic matter and reported that there were three distinct phases: phase 1 – the rate of organic matter loss was rapid (during the first five years of cultivation); phase 2 – the rate decreased until equilibrium was reached (after about 35 years of cultivation) and phase 3 – very little or no further loss occurred. The study areas have been cultivated for over 35 years. It is therefore assumed that very little or no further loss of SOM can occur. However, the soil samples from had medium soil organic matter content in Muhoroni and SONY be maintained or increased in order to boost sugarcane productivity in these areas in order to bridge the gap of sugar deficit.

4.4 Heavy metal levels in the soil samples

The levels of Cd, Cr, Cu, Pb and Zn in soil were determined and their results were as shown in Tables 4.3, 4.4, 4.5, 4.6 and 4.7. The levels of metals in the soil varied from site to site in the two areas. Site 3 in Muhoroni contained the highest amount of Cd (Table 4.3) while site 1 contained the least level. The levels in Muhoroni were 0.20 ± 0.14 mg kg^{-1}(site 1), 0.22 ± 0.15 mg kg^{-1} (site 2) and 0.31 ± 0.13 mg kg^{-1}(site 3) while the levels in sites 1, 2 and 3 in SONY were 0.19 ± 0.08 mg kg^{-1}, 0.34 ± 0.25 mg kg^{-1} and 0.28 ± 0.14 mg kg^{-1} respectively (Table 4.3). The mean level of soil Cd in Muhoroni was 0.24 ± 0.14 mg kg^{-1} while it was 0.27 ± 0.16 mg kg^{-1} in SONY (Table 4.3).

Table 4.3. Cadmium levels (mean ± S.D mg kg^{-1} dry) in topsoil samples

	Cd levels in soil	
Area / Site	**Muhoroni**	**SONY**
1	0.20 ± 0.14	0.19 ± 0.08
2	0.22 ± 0.15	0.34 ± 0.25
3	0.31 ± 0.13	0.28 ± 0.14
Mean ± S.D	**0.24 ± 0.14**	**0.27 ± 0.16**

The metal Cd had lower levels in soil as compared to other metals in this study. This observation concurred with the result reported by Gupta et al. (2008b) that the level of Cd in soil is normally lower than those of Cr, Cu, Pb and Zn. A comparison of the levels of Cd obtained in this study with those recorded in sugarcane farms in other areas of the world showed that the levels varied from one place to another. The levels (0.24 ± 0.14 and 0.27 ± 0.16 mg kg^{-1}) of Cd in soil samples from Muhoroni and SONY were lower than 1.9 mg kg^{-1} in sugarcane soils of Brazil (da Silva *et al.,* 2016) and far much lower than 32.3 - 43.0 mg kg^{-1} in sugarcane soil irrigated with industrial effluent in rural areas of Uttarakhand, India, (Pandey *et al.,* 2016). The levels were also lower than 5.20 mg kg^{-1} in agricultural soil of Khoshk River Banks, Shiraz (Qishlaqi and Moore, 2007) and lower than 0.45 ± 0.15 mg kg^{-1} in paddy soils from wetlands of lake Victoria basin, Tanzania, (Machiwa, 2010) (Table 4.8). The levels of Cd were between 20 to 42 times lower than the 11.42 mg kg^{-1} in Dhaka soil (Ahmad and Goni, 2010) (Table 4.8). Spatial information about the prevalence of heavy metals according to the soil parent material in most arable agricultural farmland soils in Kenya is scarce. The detected levels were therefore compared with the European union standards (EU, 2002) in order to ascertain whether the investigated soils contain metal levels that may be of concern. The levels (0.24 ± 0.14 and 0.27 ± 0.16 mg kg^{-1}) of Cd in the soils in this study were within European Union standards of 3.0 mg kg^{-1} (EU, 2002) (Table 4.8). This indicated that agricultural areas in Muhoroni and SONY were safe from cadmium contamination.

In Muhoroni, site 2 contained highest amount of Cr (75.45 ± 22.09 mg kg^{-1}) while site 3 contained the least, 65.31 ± 21.72 mg kg^{-1} (Table 4.4). Site 1 in Muhoroni contained 71.65 ± 20.07 mg kg^{-1} of Cr. Site 1 in SONY contained 63.76 ± 15.15 mg kg^{-1}, site 2 contained 72.80 ± 20.32 mg kg^{-1} while site 3 contained lowest level of 58.36 ± 12.05 mg kg^{-1}(Table 4.4). The mean levels of Cr in soil samples from Muhoroni and SONY were 70.80 ± 21.29 mg kg^{-1} and 64.97 ± 15.83 mg kg^{-1}, respectively (Table 4.4).

Table 4.4. Chromium levels (mean ± S.D mg kg^{-1} dry) in topsoil samples

	Cr levels in soil	
Area / Site	**Muhoroni**	**SONY**
1	71.65 ± 20.07	63.76 ± 15.15
2	75.45 ± 22.09	72.80 ± 20.32
3	65.31 ± 21.72	58.36 ± 12.05
Mean ± S.D	**70.80 ± 21.29**	**64.97 ± 15.83**

The levels (70.80 ± 21.29 and 64.97 ± 15.83 mg kg^{-1}) of Cr in the soil samples from Muhoroni and SONY were higher than 18.8 mg kg^{-1} in sugarcane soils of Brazil (da Silva *et al.,* 2016) and higher than 24.10 - 67.58 mg kg^{-1} in sugarcane soil in rural areas of Uttarakhand, India, (Pandey *et al.,* 2016). The levels were also higher than 21.9 ± 2.58 mg kg^{-1} reported in paddy soils from wetlands of lake Victoria basin, Tanzania, (Machiwa, 2010). However, the levels of Cr in the soil samples from the study areas were lower than 124.50 mg kg^{-1} in agricultural soil of Khoshk River Banks, Shiraz, (Qishlaqi and Moore, 2007) and lower than 146.63 mg kg^{-1} in Nzoia sugar estate soil (Omwoma *et al.*, 2010) (Table 4.8). Ali and Maina (2015) also reported lower level of Cr ranging from 0.000039 – 0.000101 mg kg^{-1} in sugarcane farm in Numan, Adamawa State, Nigeria. The levels (70.80 ± 21.29 and 64.97 ± 15.83 mg kg^{-1}) of Cr in the soil from Muhoroni and SONY were within European Union standards of 150 mg kg^{-1} (EU, 2002). The agricultural areas of Muhoroni and SONY were not contaminated by chromium.

The Muhoroni sites 1, 2 and 3 contained 13.35 ± 3.85 mg kg^{-1}, 13.77 ± 4.09 mg kg^{-1} and 15.11 ± 4.67 mg kg^{-1} of Cu respectively. Site 2 of SONY contained the highest level of Cu while site 1 had the least level (11.65 ± 7.23 mg kg^{-1}). There was 15.19 ± 5.85 mg kg^{-1} of Cu in site 3 of SONY. The mean levels of Cu in Muhoroni and SONY soils were 14.08 ± 4.20 mg kg^{-1} and 14.33 ± 5.88 mg kg^{-1}, respectively (Table 4.5).

Table 4.5. Copper levels (mean ± S.D mg kg^{-1} dry) in topsoil samples

	Cu levels in soil	
Area Site	**Muhoroni**	**SONY**
1	13.35 ± 3.85	11.65 ± 7.23
2	13.77 ± 4.09	16.15 ± 4.55
3	15.11 ± 4.67	15.19 ± 5.85
Mean ± S.D	**14.08 ± 4.20**	**14.33 ± 5.88**

The levels (14.08 ± 4.20 and 14.33 ± 5.88 mg kg^{-1}) of Cu in Muhoroni and SONY soil were about 6.7 times lower than 96.90 mg kg^{-1} in agricultural soil of Khoshk River Banks, Shiraz, (Qishlaqi and Moore, 2007) (Table 4.8), about 5-fold lower than 73.34 mg kg^{-1} in Nzoia Sugar estate soil (Omwoma *et al*., 2010) and about 2.7 times lower than 39.14 mg kg^{-1} of Cu in Dhaka soil (Ahmad and Goni, 2010). Ali and Maina (2015) also reported lower level of Cu ranging from 0.00007 – 0.000105 mg kg^{-1} in sugarcane farm in Numan, Adamawa State, Nigeria. However, the levels of Cu in Muhoroni and SONY soils were higher than 12.8 ± 4.2 mg kg^{-1} in paddy soils from wetlands of lake Victoria basin, Tanzania, (Machiwa, 2010) (Table 4.8). The levels were also higher than 6.4 mg kg^{-1} in sugarcane soils of Brazil (da Silva *et al.,* 2016) and far much higher than 0.01 - 0.28 mg kg^{-1} in sugarcane soil irrigated with industrial effluent in rural areas of Uttarakhand, India, (Pandey *et al.*, 2016). However, the levels (14.08 ± 4.20 and 14.33 ± 5.88 mg kg^{-1}) of Cu in the soils in this study areas were within European Union standards of 140 mg kg^{-1} (EU, 2002) (Table 4.8). No copper contamination was established in these study areas .

The levels of Pb in Muhoroni soil were 79.66 ± 13.40 mg kg^{-1}, 68.19 ± 12.04 mg kg^{-1} and 73.09 ± 13.44 mg kg^{-1} in sites 1, 2 and 3 respectively. In sites 1, 2 and 3 in SONY, the levels were 70.81 ± 6.77 mg kg^{-1}, 74.14 ± 10.90 mg kg^{-1} and 70.54 ± 8.41 mg kg^{-1}. The mean levels of Pb in both Muhoroni and SONY were 73.65 ± 12.96 mg kg^{-1} and 71.83 ± 8.69 mg kg^{-1} respectively (Table 4.6).

Table 4.6. Lead levels (mean ± S.D mg kg^{-1} dry) in topsoil samples

	Pb levels in soil	
Area / Site	**Muhoroni**	**SONY**
1	79.66 ± 13.40	70.81 ± 6.77
2	68.19 ± 12.04	74.14 ± 10.90
3	73.09 ± 13.44	70.54 ± 8.41
Mean ± S.D	**73.65 ± 12.96**	**71.83 ± 8.69**

This study revealed that the mean levels (73.65 ± 12.96 and 71.83 ± 8.69 mg kg^{-1}) of Pb in both Muhoroni and SONY were higher than 54.71 mg kg^{-1} observed in Nzoia Sugar estate soil (Omwoma *et al.*, 2010), higher than 254.60 mg kg^{-1} in agricultural soil of Khoshk River Banks, Shiraz, (Qishlaqi and Moore, 2007) and higher than 49.71 mg kg^{-1} in Dhaka soil (Ahmad and Goni, 2010) (Table 4.8). The levels were also higher than the 21.34 - 50.35 mg kg^{-1} in sugarcane farms in rural areas of Uttarakhand, India, (Pandey *et al.*, 2016) and higher than 6.4 mg kg^{-1} in sugarcane soils of Brazil (da Silva *et al.*, 2016). Ali and Maina (2015) also reported lower level of Pb ranging from 0.000021 – 0.000062 mg kg^{-1} in sugarcane farm in Numan, Adamawa State, Nigeria. However, Machiwa (2010) reported lower level (59.8 ± 25.0 mg kg^{-1}) of Pb in paddy soils from wetlands of lake Victoria basin, Tanzania. The level (73.65 ± 12.96 and 71.83 ± 8.69 mg kg^{-1}) of Pb in the soil under study were within European Union standards of 300 mg kg^{-1}

(EU, 2002) (Table 4.8). This indicates that Muhoroni and SONY are not polluted with to lead metal.

Soil in Muhoroni contained 138.40 ± 9.39 mg kg^{-1}, 140.52 ± 10.12 mg kg^{-1}and 136.35 ± 8.10 mg kg^{-1} of Zn in sites 1, 2 and 3 respectively. In SONY, site 1 contained 145.01 ± 17.24 mg kg^{-1} of Zn, site 2 had a level of 142.55 ± 16.81 mg kg^{-1} while site 3 contained 138.39 ± 12.64 mg kg^{-1} of Zn. The mean levels of Zn in both Muhoroni and SONY were 138.42 ± 9.20 mg kg^{-1} and 141.98 ± 15.56 mg kg^{-1} respectively (Table 4.7).

Table 4.7. Zinc levels (mean ± S.D mg kg^{-1} dry) in topsoil samples

	Zn levels in soil	
Area Site	**Muhoroni**	**SONY**
1	138.40 ± 9.39	145.01 ± 17.24
2	140.52 ± 10.12	142.55 ± 16.81
3	136.35 ± 8.10	138.39 ± 12.64
Mean ± S.D	**138.42 ± 9.20**	**141.98 ± 15.56**

The levels of Zn were the highest among the measured heavy metals in the agricultural soils investigated. This concurred with the results obtained by Olatunji et al. (2013) that zinc is the most abundant metal in cultivated soils as compared to the other metals. A comparison with other studies indicated that the mean levels (138.42 ± 9.20 and 141.98 ± 15.56 mg kg^{-1}) of Zn in both Muhoroni and SONY were close to 137.54 mg kg^{-1} observed in Nzoia Sugar estate soil (Omwoma *et al.,* 2010). The levels in the study areas were higher than 117.00 mg kg^{-1} in agricultural soil of Khoshk River Banks, Shiraz, (Qishlaqi and Moore, 2007), higher than 115.43 mg kg^{-1} in Dhaka soil (Ahmad and Goni, 2010), and slightly 2-fold higher than 59.8 ± 25.0 mg kg^{-1} in paddy soils from wetlands of lake Victoria basin, Tanzania, (Machiwa, 2010) (Table 4.8). The levels were also about 8.5 times higher than 16.2 mg kg^{-1} in sugarcane soils of Brazil (da Silva *et al.,* 2016) and higher than the range 0.00003 - 0.00014 mg kg^{-1} in sugarcane farm in Numan, Adamawa State, Nigeria, as reported by Ali and Maina (2015). However, the levels were lower than 202.2 - 209.6 mg kg^{-1} in sugarcane farms in rural areas of Uttarakhand, India, (Pandey *et al.,* 2016). The levels (138.42 ± 9.20 and 141.98 ± 15.56 mg kg^{-1}) of Zn in the soils in this study were within European Union standards of 300 mg kg^{-1} (EU, 2002) (Table 4.8). This indicates that agricultural areas in Muhoroni and SONY are safe from zinc contamination at the present.

Table 4.8. Levels (mean ± S.D mg kg^{-1}dry) of metals in agricultural soil in study areas and safe limits

	Muhoroni Sugar estate soil (this study)	**SONY Sugar estate soil (this study)**	**Shiraz soil**[a]	**Lake Victoria Basin soil**[b]	**Nzoia Sugar estate soil**[c]	**Dhaka soil**[d]	**Safe limits (mg/kg)**[e]
Cd	0.24 ± 0.14	0.27 ± 0.16	5.20	0.45 ± 0.15	-	11.42	3
Cr	70.80 ± 21.29	64.97 ± 15.83	124.50	21.9 ± 2.58	146.63	53.70	150
Cu	14.08 ± 4.20	14.33 ± 5.88	96.90	12.8 ± 4.2	73.34	39.14	140
Pb	73.65 ± 12.96	71.83 ± 8.69	254.60	18.9 ± 2.5	54.71	49.71	300
Zn	138.42 ± 9.20	141.98 ± 15.56	117.00	59.8 ± 25.0	137.54	115.43	300

Sources: [a]Qishlaqi and Moore (2007), [b]Machiwa (2010), [c]Omwoma et al. (2010), [d]Ahmad and Goni (2010) and [e]European Union standards (2002)

The levels of heavy metals in the sites of each area of study showed that the metals were evenly spread throughout the entire sugarcane estates. This was an indication that the metals were introduced into the soils by anthropogenic activity. The levels of the heavy metals in the soils of these study areas were within the recommended limits although continued fertilizer application in these sugarcane plantations for decades was expected to significantly increase heavy metal content in the soil (Jones and Jarvis, 1981; Raven *et al.,* 1998). This could be ascribed to their continuous removal by sugarcane grown in these areas and leaching to the underground waters as the metals occur in their mobile-accessible-soluble forms due to low soil pH.

4.5 Contamination factor and degree of contamination of soil

Table 4.9 shows values of the contamination factor of the soil and degree of contamination due to heavy metals. The contamination factors, calculated using Eq. (2.2), of the metals in Muhoroni were Cd (0.24), Cr (0.79), Cu (0.28), Pb (1.05) and Zn (0.79) while in SONY soil the contamination factors of the metals were Cd (0.27), Cr (0.72), Cu (0.29), Pb (1.03) and Zn (0.81).

Table 4.9. Contamination and Potential ecological risk factors

	Contamination factor (C_f)		**Potential ecological risk factor (*Er*)**	
	Muhoroni	SONY	Muhoroni	SONY
Cd	0.24	0.27	7.20	8.10
Cr	0.79	0.72	1.58	1.44
Cu	0.28	0.29	1.40	1.45
Pb	1.05	1.03	5.25	5.15
Zn	0.79	0.81	0.79	0.81
C_d	3.46	3.12	🟩	🟩
RI	🟩	🟩	16.22	16.95

Rahman et al. (2012) reported contamination factors of the metals in agricultural soil around Dhaka Export Processing Zone (DEPZ), Bangladesh, as Cd (0.02), Cr (1.42), Cu (4.68), Pb (1.38) and Zn (2.95). The agricultural soil around Dhaka Export Processing Zone had higher contamination levels of all the metals except Cd. In a study to establish ecological risk of heavy metals and a metalloid in agricultural soils in Tarkwa, Ghana, Bortey-Sam et al. (2015) reported low contamination factors of the metals. The results were Cd (0.14), Cr (0.63), Cu (0.29), Pb (0.14) and Zn (0.57) which shows that the agricultural soils in Tarkwa, Ghana, had lower contamination levels of all the metals. The contamination factors (C_f) indicated moderate contamination levels ($1 \leq C_f \leq 3$) of Pb in soil in Muhoroni and SONY while contamination by Cd, Cr, Cu and Zn were low ($C_f < 1$) according to Hakanson (1980). The degree of contamination (C_d), calculated using Eq. (2.3), were 3.46 for Muhoroni and 3.12 for SONY. Bortey-Sam et al. (2015) reported lower degree of contamination (1.77) in agricultural soils in Tarkwa, Ghana. However, Rahman et al. (2012) reported a higher degree of contamination (10.45) in agricultural soil around Dhaka Export Processing Zone. The soils of Muhoroni and SONY had low degree of contamination ($C_d < 5$) according to Hakanson (1980).

4.6 Ecological risk factors

The average ecological risk factors (*Er*), calculated using Eq. (2.4), of the metals in Muhoroni were Cd (7.20), Cr (1.58), Cu (1.40), Pb (5.25) and Zn (0.79) (Table 4.9). In SONY, the average ecological risk factors were Cd (8.10), Cr (1.44), Cu (1.45), Pb (5.15) and Zn (0.81). Bortey-Sam et al. (2015) reported average ecological risk factors of these metals as Cd (4.2), Cr (1.2), Cu (1.4), Pb (0.66) and Zn (0.57). However, Wang et al. (2013) reported higher average ecological risk factors in reclaimed soils at an opencast coal mine. The average ecological risk factors in reclaimed soils at an opencast coal mine were Cd (34.02), Cr (2.03), Cu (6.05), Pb (7.02) and Zn (1.28). Cadmium presented highest ecological risks compared to other metals because of its highest toxicity coefficient. The percentage contribution of individual metal to overall potential ecological risk revealed that the most toxic metal, Cd, contributed 44.39% of the total potential ecological risk in Muhoroni and 47.79% for SONY. This concurred with the studies of Bortey-Sam et al. (2015) and Wang et al. (2013) which revealed the contribution of Cd as 52.30% and 67.50% respectively. The order of average ecological risk of the metals was Cd > Pb > Cr > Cu > Zn (Figures 4.1 and 4.2). The average ecological risk factors of all the metals in Muhoroni and SONY were below 40 (Table 4.9), indicating low potential ecological risk to local environment according to Hakanson (1980).

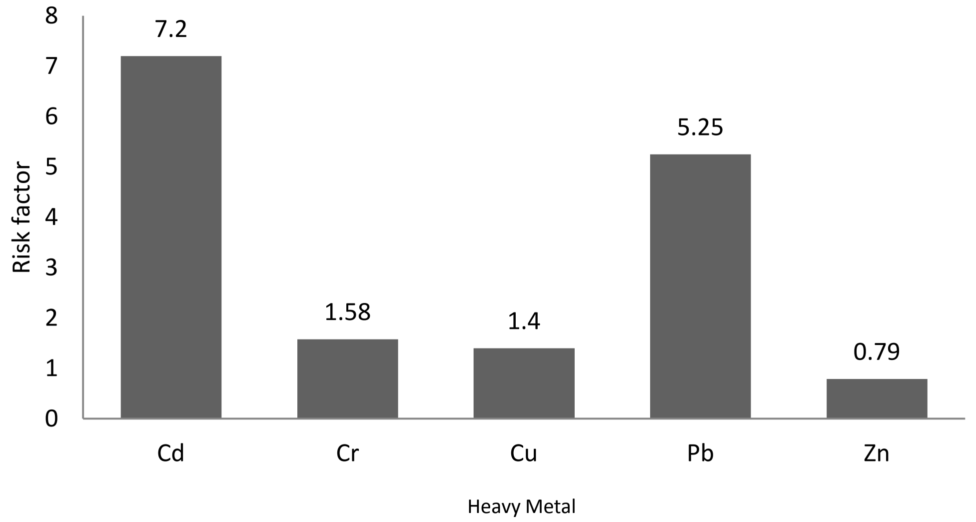

Figure 4.1. Risk indices of heavy metals in soil samples from Muhoroni

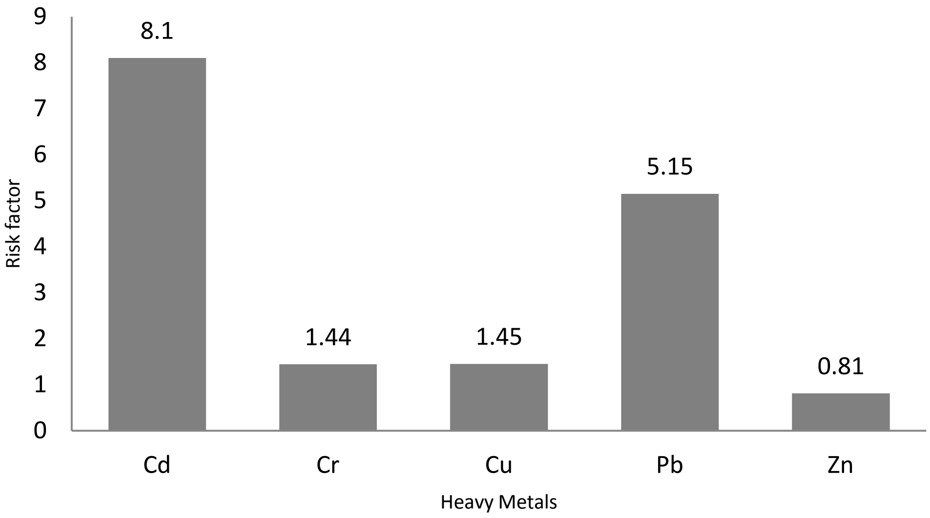

Figure 4.2. Risk indices of heavy metals in soil samples from SONY

4.7 Potential ecological risk index

In order to quantify the overall potential ecological risk of observed metals in the farm soil, potential ecological risk index (*RI*) was calculated using Eq. (2.5). The potential ecological risk index values were 16.22 and 16.95 for Muhoroni and SONY respectively. Bortey-Sam et al. (2015) reported lower potential ecological risk index 8.03 in agricultural soils in Tarkwa, Ghana. However, Wang et al. (2013) reported a higher potential ecological risk index of 50.4 in reclaimed soils at an opencast coal mine. The potential ecological risk index values were 16.22 and 16.95 for Muhoroni and SONY showing that the overall risk caused by the studied metals was low ($RI < 150$) according to Hakanson (1980). The low potential ecological risk indices in Muhoroni and SONY could be as a result of low soil pH which resulted in the decrease of the levels of the metals in the soil possibly due to leaching and absorption by sugarcane plants. Although the contamination factors of other metals were low in these soils, the moderate contamination levels of toxic Pb need to be checked as these soils could be sources of Pb poisoning. The potential ecological risk indices for SONY and Muhoroni were very close suggesting that the sources of these metals in the two areas are the same. The metals could be majorly added to the soils by agronomic inputs.

4.8 Heavy metal levels in sugarcane stems

All the sugarcane varieties in this study contained the heavy metals in their stems. The mean levels of metals in sugarcane stems are shown in Tables 4.10, 4.11, 4.12, 4.13 and 4.14. The mean level of Cd in sugarcane stems sampled from site 1 of Muhoroni was 0.10 ± 0.07 mg kg^{-1}. It was 0.12 ± 0.09 mg kg^{-1} in site 2 and 0.16 ± 0.13 mg kg^{-1} in site 3. The levels in sites 1, 2 and 3 of SONY were 0.10 ± 0.05 mg kg^{-1}, 0.21 ± 0.14 mg kg^{-1} and 0.15 ± 0.10 mg kg^{-1} respectively (Table 4.10). The mean levels of Cd in stems of sugarcane grown in Muhoroni and SONY were 0.13 ± 0.10 mg kg^{-1} and 0.15 ± 0.10 mg kg^{-1} respectively **(**Table 4.10).

Table 4.10. Cadmium levels (mean ± S.D mg kg^{-1} dry) in sugarcane stems

	Cd levels in cane stem	
Area / Site	**Muhoroni**	**SONY**
1	0.10 ± 0.07	0.10 ± 0.05
2	0.12 ± 0.09	0.21 ± 0.14
3	0.16 ± 0.13	0.15 ± 0.10
Mean ± S.D	**0.13 ± 0.10**	**0.15 ± 0.10**

The levels (0.13 ± 0.10 and 0.15 ± 0.10 mg kg^{-1}) of Cd in stems of sugarcane grown in Muhoroni and SONY were within the range of 0.001 - 0.15 mg kg^{-1} in sugarcane irrigated with industrial effluent in some rural areas of Uttarakhand, India, as reported by Pandey et al. (2016) **(**Table 4.15). Segura-Muñoz et al. (2006) reported lower Cd mean value of 0.034 ± 0.02 mg kg^{-1} dry weight for sugarcane samples from Ribeirao, Brazil. The levels of Cd in the stem of sugarcane in this study were higher than the mean level of 0.02 ± 0.007 mg kg^{-1} reported from sugarcane sold in the streets of Kumasi, Ghana, (Azanu *et al.,* 2015). The levels of Cd in the stems were below the range of 0.2 - 0.8 mg kg^{-1} in agricultural crops according to Allaway (1968). The levels were also within the safe limits of 0.20 mg kg^{-1} (WHO/FAO, 2007). Xia et al. (2009) reported that sugarcane has a high ability to tolerate and accumulate Cd due to its large biomass. However, the mean levels of Cd in sugarcane stems were lower than those in corresponding soil samples (Table 4.8) showing that sugarcane does not bioconcentrate Cd. This result disagreed with that of Abdus-Salam et al. (2008) stated that sugarcane bioaccumulates and biomagnifies some toxic metals. The low accumulation of Cd in sugarcane grown in these study areas could be as a result of reduction of the availability of the metal by precipitation, adsorption and complexation (Mench *et al*., 1994; Chen and Lee, 1997). The edible parts of sugarcane grown in Muhoroni and SONY were not contaminated with Cd.

The levels of Cr in sugarcane stems grown in sites 1, 2 and 3 of Muhoroni were 10.94 ± 1.92 mg kg^{-1}, 12.10 ± 2.20 mg kg^{-1} and 10.08 ± 1.89 mg kg^{-1} respectively. Sugarcane stems sampled from site 1 of SONY contained 6.59 ± 1.50 mg kg^{-1} of Cr. The levels in stems of sugarcane grown in sites 2 and 3 were 8.84 ± 1.87 mg kg^{-1} and 6.41 ± 1.53 mg kg^{-1} of Cr respectively (Table 4.11). The mean levels of Cr in stems of sugarcane grown in Muhoroni and SONY were 11.04 ± 2.00 mg kg^{-1} and 7.28 ± 1.63 mg kg^{-1} respectively (Table 4.11).

Table 4.11. Chromium levels (mean ± S.D mg kg^{-1} dry) in sugarcane stems

	Cr levels in cane stem	
Area / Site	**Muhoroni**	**SONY**
1	10.94 ± 1.92	6.59 ± 1.50
2	12.10 ± 2.20	8.84 ± 1.87
3	10.08 ± 1.89	6.41 ± 1.53
Mean ± **S.D**	**11.04 ± 2.00**	**7.28 ± 1.63**

This study revealed that sugarcane accumulates higher levels (11.04 ± 2.00 and 7.28 ± 1.63 mg kg^{-1}) of Cr than safe limits of 5.00 mg kg^{-1} (WHO/FAO, 2007) (Table 4.15). Pandey et al. (2016) reported a higher level of Cr ranging from 10.54 to 60.22 mg kg^{-1} in sugarcane collected from areas under irrigation with industrial effluent of Uttarakhand, India. However, Ali and Maina (2015) reported lower level of Cr ranging from 0.0000358 - 0.0000573 mg kg^{-1} in sugarcane grown in Numan, Adamawa State, Nigeria. Awokunmi et al. (2015) reported a higher level of Cr (23.8 - 25.6 mg kg^{-1}) in maize plant grown in selected dumpsites in Ekiti State, Nigeria. The levels of Cr in the sugarcane stems grown in Muhoroni and SONY were above the range of 0.2 - 1.0 mg kg^{-1} in agricultural crops according to Allaway (1968). Uptake of Cr by plants is dependent on the oxidation state of the metal. Cr(VI) has higher solubility and thus bioavailability than Cr(III), which tends to form stable complexes in the soil (López-Luna *et al.*, 2009). Hence plants grown in soil contaminated with Cr(VI) are likely to bioaccumulate more Cr than plants grown in soil contaminated with Cr(III) as long as other factors which affect uptake of metals remain constant. The bioaccumulation of heavy metals by sugarcane plants may be an entry point of heavy metals into a complex food chain that might have diverse consequences at the apex. Chromium (VI) is a danger to human health because it can alter genetic materials and cause cancer (Cheryl and Susan, 2000). The high level of Cr in the stem of sugarcane grown in Muhoroni and SONY was a cause to worry. Therefore ways needed to be formulated to reduce the absorption of Cr by the sugarcane.

The amount of Cu contained in the stems of sugarcane grown in site 1 of Muhoroni was 0.66 ± 0.22 mg kg^{-1} (Table 4.12) while the levels in sugarcane sampled from sites 2 and 3 were 0.67 ± 0.27 mg kg^{-1} and 0.65 ± 0.19 mg kg^{-1} respectively. The levels of Cu in sites 1, 2 and 3 of SONY were 0.57 ± 0.11 mg kg^{-1}, 0.85 ± 0.21 mg kg^{-1} and 0.74 ± 0.15 mg kg^{-1} respectively (Table 4.12). The mean levels of Cu in stems of sugarcane grown in Muhoroni and SONY were 0.66 ± 0.23 mg kg^{-1} and 0.72 ± 0.16 mg kg^{-1} respectively (Table 4.12).

Table 4.12. Copper levels (mean ± S.D mg kg^{-1} dry) in sugarcane stems

	Cu levels in cane stem	
Area / Site	**Muhoroni**	**SONY**
1	0.66 ± 0.22	0.57 ± 0.11
2	0.67 ± 0.27	0.85 ± 0.21
3	0.65 ± 0.19	0.74 ± 0.15
Mean ± S.D	**0.66 ± 0.23**	**0.72 ± 0.16**

The levels (0.66 ± 0.23 and0.72 ± 0.16 mg kg^{-1}) of Cu below the range 22.2 to 45.7 mg kg^{-1} dry weight in sugarcane collected from area under direct influence of municipal landfill site and medical waste treatment system of Ribeirao Petro, Brazil as reported by Segura-Muñoz et al. (2006). Pandey et al. (2016) reported higher Cu levels ranging from 3.56 - 22.38 mg kg^{-1} dry weight in sugarcane collected from areas under irrigation with industrial effluent of Uttarakhand, India. Azanu et al. (2015) reported higher Cu level of 1.052 ± 0.748 mg kg^{-1} dry weight for sugarcane sold in the streets of Kumasi, Ghana. However, Ali and Maina (2015) reported lower level of Cu ranging from 0.0000058 - 0.000017 mg kg^{-1} in sugarcane grown in Numan, Adamawa State, Nigeria. The Cu levels in stems of sugarcane grown in Muhoroni and SONY were within the safe limits of 40.00 mg kg^{-1} (WHO/FAO, 2007) (Table 4.15). The levels of Cu in the stems were within the range of 4 - 15 mg kg^{-1} in agricultural crops according to Allaway (1968). The low level of Cu in sugarcane stem could be due to the fact that the metal is not readily transported to shoots (Ahsan *et al.*, 2007). The stems of sugarcane grown in these study areas were not contaminated with Cu.

The mean level of Pb in sugarcane stems sampled from site 1 of Muhoroni was 2.41 ± 1.23 mg kg^{-1}. It was 2.36 ± 0.86 mg kg^{-1} in site 2 and 2.16 ± 0.67 mg kg^{-1} in site 3. The levels in sites 1, 2 and 3 of SONY were 2.15 ± 0.56 mg kg^{-1}, 2.38 ± 1.08 mg kg^{-1} and 1.99 ± 0.61 mg kg^{-1} respectively (Table 4.13). The mean levels of Pb in stems of sugarcane grown in Muhoroni and SONY were 2.31 ± 0.92 mg kg^{-1} and 2.17 ± 0.75 mg kg^{-1}, respectively (Table 4.13).

Table 4.13. Lead levels (mean ± S.D mg kg^{-1} dry) in sugarcane stems

	Pb levels in cane stem	
Area / Site	**Muhoroni**	**SONY**
1	2.41 ± 1.23	2.15 ± 0.56
2	2.36 ± 0.86	2.38 ± 1.08
3	2.16 ± 0.67	1.99 ± 0.61
Mean ± S.D	**2.31 ± 0.92**	**2.17 ± 0.75**

The stems of sugarcane grown in Muhoroni and SONY had Pb levels (2.31 ± 0.92 and 2.17 ± 0.75 mg kg^{-1}) above the levels (0.01 - 1.11 mg kg^{-1}) in sugarcane collected from areas under irrigation with industrial effluent of Uttarakhand, India, as reported by Pandey et al. (2016). The levels were also higher than the range 0.0000057 - 0.0000099 mg kg^{-1} reported by Ali and Maina (2015) in sugarcane grown in Numan, Adamawa State, Nigeria. Segura-Muñoz et al. (2006) reported lower Pb levels ranging from 1.76 to 2.28 mg kg^{-1} dry weight in sugarcane collected from area under direct influence of municipal landfill site and medical waste treatment system of Ribeirao Petro, Brazil. The levels of Pb in the stems in this study were higher than the levels 0.10 ± 0.032 mg kg^{-1} in sugarcane sold in the streets of Kumasi, Ghana (Azanu *et al.*, 2015) but within the range of 0.1 - 10 mg kg^{-1} in agricultural crops according to (Allaway, 1968). However, the levels of Pb in sugarcane stems in this study were within the safe limits of 5.00 mg kg^{-1} (WHO/FAO, 2007) (Table 4.15). Sugarcane grown in Muhoroni and SONY did not accumulate Pb to toxic level.

The levels of Zn in sugarcane stems grown in sites 1, 2 and 3 of Muhoroni were 28.59 ± 3.24 mg kg^{-1}, 28.04 ± 3.48 mg kg^{-1} and 28.63 ± 2.94 mg kg^{-1} respectively. Sugarcane stems sampled from site 1 of SONY contained 34.13 ± 3.94 mg kg^{-1} of Zn. The levels in stems of sugarcane grown in sites 2 and 3 were 30.50 ± 4.08 mg kg^{-1} and 32.13 ± 3.41 mg kg^{-1} of Zn respectively (Table 4.14). The mean levels of Zn in stems of sugarcane grown in Muhoroni and SONY were 28.42 ± 3.22 mg kg^{-1} and 32.25 ± 3.81 mg kg^{-1} respectively (Table 4.14).

Table 4.14. Zinc levels (mean ± S.D mg kg^{-1} dry) in sugarcane stems

	Zn levels in cane stem	
Area / Site	**Muhoroni**	**SONY**
1	28.59 ± 3.24	34.13 ± 3.94
2	28.04 ± 3.48	30.50 ± 4.08
3	28.63 ± 2.94	32.13 ± 3.41
Mean ± S.D	**28.42 ± 3.22**	**32.25 ± 3.81**

The levels (28.42 ± 3.22 and 32.25 ± 3.81 mg kg^{-1}) of Zn in sugarcane stems were within the range of 4.55 - 48.9 mg kg^{-1} in sugarcane collected from areas under irrigation with industrial effluent of Uttarakhand, India, as reported by Pandey et al. (2016). Azanu et al. (2015) reported lower mean Zn value of 0.093 ± 0.037 mg kg^{-1} dry weight for sugarcane sold in the streets of Kumasi, Ghana. Ali and Maina (2015) also reported lower level of Zn ranging from 0.0000053 - 0.0000088 mg kg^{-1} in sugarcane grown in Numan, Adamawa State, Nigeria. The levels of Zn in stems of sugarcane grown in Muhoroni and SONY were within the safe limits of 60.00 mg kg^{-1} (WHO/FAO, 2007) (Table 4.26). The levels of Zn in the stems were within the range of 15 - 200 mg kg^{-1} in agricultural crops according to (Allaway, 1968). Wagesho and Chandravanshi (2015) noted that Zn is one of the main elements that plants accumulate and pass up in the food chain. The levels of Zn in the edible parts of sugarcane grown in Muhoroni and SONY were desirable as Zn is an essential metal whose deficiency is a fact in crops and humans (Hotz and Brown, 2004) and is currently listed as a major risk factor for human health and a cause of death globally (Cakmak, 2008).

Table 4.15. Levels (mean ± S.D mg kg^{-1} dry) of metals in sugarcane stems from study areas and maximum permissible limits

Location	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**
Muhoroni (n=45)	0.13 ± 0.10	11.04 ± 2.00	0.66 ± 0.23	2.31 ± 0.92	28.42 ± 3.22
SONY (n=45)	0.15 ± 0.10	7.28 ± 1.63	0.72 ± 0.16	2.17 ± 0.75	32.25 ± 3.81
Levels in stems[a]	0.001 - 0.15	10.54 - 60.22	3.56 - 22.38	0.01 - 1.11	4.55 - 48.9
Safe limit[b]	0.20	5.00	40.00	5.00	60.00

Source: [a]Pandey et al. (2016); [b]WHO/FAO (2007)

The metals were accumulated in the cane stems in different levels. This was because the difference in heavy metal accumulation in plants depends on variations in their absorption and accumulation tendency as a result of pH and soil organic matter variations (Fuller, 1977; Brummer and Herms, 1983; Christensen, 1984; Kabata-Pendas and Pendas, 1992; Halim *et al.*, 2003).

4.9 Heavy metal levels in sugarcane juice

All the heavy metals investigated were detected in sugarcane juice with Zn being the most abundant metal in the juice of sugarcane. Results of the levels of heavy metals in the sugarcane juice grown in different sites within Muhoroni and SONY are given in Tables 4.16, 4.17, 4.18, 4.19 and 4.20. Juice of sugarcane grown in sites 1, 2 and 3 of Muhoroni contained 0.04 ± 0.02 mg L^{-1}, 0.04 ± 0.02 mg L^{-1} and 0.04 ± 0.03 mg L^{-1} respectively. Juice of sugarcane sampled from site 1 of SONY contained 0.04 ± 0.03 mg L^{-1} of Cd. The levels in juice of sugarcane grown in sites 2 and 3 were 0.04 ± 0.02 mg L^{-1} and 0.05 ± 0.03 mg L^{-1} of Cd respectively (Table 4.16). The mean levels of Cd in Muhoroni and SONY were 0.04 ± 0.02 mg L^{-1} and 0.04 ± 0.03 mg L^{-1} respectively (Table 4.16).

Table 4.16. Cadmiuum levels (mean ± S.D mg L^{-1}) in sugarcane juice

	Cd levels in juice	
Area / Site	**Muhoroni**	**SONY**
1	0.04 ± 0.02	0.04 ± 0.03
2	0.04 ± 0.02	0.04 ± 0.02
3	0.04 ± 0.03	0.05 ± 0.03
Mean ± S.D	**0.04 ± 0.02**	**0.04 ± 0.03**

The levels of heavy metals in cane juice were compared with the recocommended limits of drinking water according to WHO and with the results of studies carried out in other parts of the world. The levels (0.04 ± 0.02 and 0.04 ± 0.03 mg L^{-1}) of Cd in the cane juices from Muhoroni and SONY were the same to 0.04 ± 0.01 mg L^{-1} in cane juice of variety S9CO957 from Bacita, Nigeria (Adekola and Akinpelu, 2002) but lower than 0.06 ± 0.01 mg L^{-1} in cane juice of variety S27BCO997 (Table 4.21). The levels were also lower than 0.085 - 0.21 mg L^{-1} as reported by Wang et al. (2012) and 0.006 mg L^{-1} reported by Damodharan and Reddy (2014). However, the levels were twice as high as 0.01 - 0.02 mg L^{-1} from Ilorin Metropolis, Nigeria (Abdus-Salam *et al.*, 2008) (Table 4.21). The levels (0.04 ± 0.02 and 0.04 ± 0.03 mg L^{-1}) of Cd in the cane juices from Muhoroni and SONY were about 13 times higher than recommended maximum limit of 0.003 mg L^{-1} (WHO, 2011). This observation was consistent with the findings of Adekola and Akinpelu (2002) and Abdus-Salam et al. (2008) who reported higher level of Cd than the recommended maximum WHO limit (Table 4.21). The result of this present study also concurred with that of Mbabazi et al. (2010) who reported that sugarcane juice from relatively high heavy metal polluted soils accumulated higher cadmium levels than the maximum permissible limits. The level of metals in juice depends mainly on physicochemical properties of the soil and the amount of the metals in the soil. It also depends of the type of the metal. The high level of Cd in the cane juice might be due to tolerance of sugarcane to the metal and high biological absorption coefficient (Lee *et al*., 1998b). The other factor, according to Ashworth and Alloway (2008), was the greatest solubility of Cd at low soil pH (less than 6.4) in Muhoroni and SONY. Human beings should be advised to minimize the chewing of the sugarcane from Muhoroni and SONY in order to avoid high accumulation of Cd in their body parts.

Sugarcane sampled from site 1 of Muhoroni contained 0.03 ± 0.02 mg L^{-1} of Cr in juice. The levels in juice of sugarcane grown in sites 2 and 3 were 0.03 ± 0.02 mg L^{-1} and 0.04 ± 0.02 mg L^{-1} of Cr respectively The levels of Cr in juice of sugarcane grown in sites 1, 2 and 3 of SONY were 0.04 ± 0.03 mg L^{-1}, 0.05 ± 0.04 mg L^{-1} and 0.05 ± 0.04 mg L^{-1} respectively (Table 4.17). The mean levels of Cr in Muhoroni and SONY were 0.03 ± 0.02 mg L^{-1} and 0.05 ± 0.04 mg L^{-1} respectively (Table 4.17).

Table 4.17. Chromium levels (mean ± S.D mg L^{-1}) in sugarcane juice

	Cr levels in juice	
Area Site	**Muhoroni**	**SONY**
1	0.03 ± 0.02	0.04 ± 0.03
2	0.03 ± 0.02	0.05 ± 0.04
3	0.04 ± 0.02	0.05 ± 0.04
Mean ± S.D	**0.03 ± 0.02**	**0.05 ± 0.04**

The levels (0.03 ± 0.02 and 0.05 ± 0.04 mg L^{-1}) of Cr in sugarcane juice grown in Muhoroni and SONY was lower than 0.25 mg L^{-1} reported by Tirmazi et al. (2007). The level (0.03 ± 0.02 mg L^{-1}) of Cr in the cane juice from Muhoroni was below the recommended maximum limit of 0.05 mg L^{-1} (WHO, 2011) while 0.05 ± 0.04 mg L^{-1} of Cr in the cane juice from SONY was equal to the recommended maximum limit of 0.05 mg L^{-1} (WHO, 2011). However, the levels of Cr in cane juice from Muhoroni and SONY were within the recommended maximum limit of 0.1 mg L^{-1} (USEPA, 2011). The levels of Cr in cane juice were low because of precipitation and adsorption on soil organic matter as reported by Chen and Lee (1997) that soil organic matter makes metals unavailable to the plants. The other reason for low levels of Cr could be because of low solubility of Cr at the prevailing soil pH due to the formation of $Cr(OH)_3$ (Chrostowski *et al*., 1991). Although the levels of Cr in juice were equal to the recommended maximum limit according to WHO (2011), continued chewing of sugsarcane grown in the study areas should be minimized to avoid accumulation of the metal in the body. On the other hand, the level of this metal in juice should be assessed regularly because the level may rise above the maximum limit.

The amount of Cu contained in the juice of sugarcane grown in site 1 of Muhoroni was 0.03 ± 0.01 mg L^{-1} while the levels in sugarcane juice sampled from sites 2 and 3 were 0.04 ± 0.02 mg L^{-1} and 0.04 ± 0.01 mg L^{-1} respectively. The levels of Cu in juice of sugarcane sampled from sites 1, 2 and 3 of SONY were 0.04 ± 0.02 mg L^{-1}, 0.04 ± 0.02 mg L^{-1} and 0.05 ± 0.03 mg L^{-1} respectively (Table 4.18). The mean levels of Cu in Muhoroni and SONY were 0.04 ± 0.01 mg L^{-1} and 0.04 ± 0.02 mg L^{-1} respectively (Table 4.18).

Table 4.18. Copper levels (mean ± S.D mg L^{-1}) in sugarcane juice

	Cu levels in juice	
Area Site	**Muhoroni**	**SONY**
1	0.03 ± 0.01	0.04 ± 0.02
2	0.04 ± 0.02	0.04 ± 0.02
3	0.04 ± 0.01	0.05 ± 0.03
Mean ± S.D	**0.04 ± 0.01**	**0.04 ± 0.02**

The levels (0.04 ± 0.01 and 0.04 ± 0.02 mg L^{-1}) of Cu in sugarcane juice in this study were slightly higher than 0.03 ± 0.01 mg L^{-1} in cane juice of variety S27BCO997 from Bacita, Nigeria (Adekola and Akinpelu, 2002). The levels were however the same with 0.04 ± 0.01 mg L^{-1} in cane juice of variety S9CO957 from Bacita, Nigeria. The levels were 10-fold lower than 0.4 mg L^{-1} reported by Damodharan and Reddy (2014). The levels (0.04 ± 0.01 and 0.04 ± 0.02 mg L^{-1}) of Cu in sugarcane juice in this study were below the WHO recommended maximum limits of 2.00 mg L^{-1} (WHO, 2011) (Table 4.37). The result concurred with that of Wang et al. (2012) who reported that sugarcane is a poor accumulator of some metals such as copper in the juice. Copper in soil is normally bound to soil organic matter and become unavailable through the formation of complexes (Aydinalp and Marinova, 2003). The juice of sugarcane grown in Muhoroni and SONY was not contaminated with copper.

The mean level of Pb in sugarcane juice sampled from site 1 of Muhoroni was 0.05 ± 0.02 mg L^{-1}. It was 0.06 ± 0.02 mg L^{-1} in site 2 and 0.05 ± 0.02 mg L^{-1} in site 3. The levels in sites 1, 2 and 3 of SONY were 0.06 ± 0.01 mg L^{-1}, 0.05 ± 0.01 mg L^{-1} and 0.05 ± 0.02 mg L^{-1} respectively (Table 4.19). The mean levels of Pb in Muhoroni and SONY were 0.05 ± 0.02 mg L^{-1} and 0.05 ± 0.01 mg L^{-1} respectively (Table 4.19).

Table 4.19. Lead levels (mean ± S.D mg L^{-1}) in sugarcane juice

	Pb levels in juice	
Area Site	**Muhoroni**	**SONY**
1	0.05 ± 0.02	0.06 ± 0.01
2	0.06 ± 0.02	0.05 ± 0.01
3	0.05 ± 0.02	0.05 ± 0.02
Mean ± S.D	**0.05 ± 0.02**	**0.05 ± 0.01**

The levels (0.05 ± 0.02 and 0.05 ± 0.01 mg L^{-1}) of Pb in cane juice from Muhoroni and SONY were the same to 0.05 mg L^{-1} in cane variety CP88-1762 as reported by Xie et al. (2014) but higher than 0.03 ± 0.01 and 0.02 ± 0.01 mg L^{-1} in cane juice from Bacita, Nigeria (Adekola and Akinpelu, 2002) and 0.04 mg L^{-1} reported by Damodharan and Reddy (2014). However, the levels were between 2 - 7 times lower than 0.15 - 0.42 mg L^{-1} in cane juice from Ilorin Metropolis, Nigeria (Abdus-Salam *et al.*, 2008) (Table 4.21). The levels (0.05 ± 0.02 and 0.05 ± 0.01 mg L^{-1}) of Pb in sugarcane juice from Muhoroni and SONY was above the recommended maximum limit of 0.01 mg L^{-1} (WHO, 2011) (Table 4.21). This observation corroborated that of Abdus-Salam et al. (2008) who reported higher level of Pb than WHO recommended maximum limit. The result concurred with Mbabazi et al. (2010) who reported that sugarcane juice from relatively high heavy metal polluted soils accumulated higher lead levels than the maximum permissible limits. The result of this study was contrary to Ashworth and Alloway (2008) who reported that Pb showed greatest solubility at high pH with relatively low solubility at pH levels less than 6.4. Chewing of the sugarcane from Muhoroni and SONY should be minimized in order to avoid dietary accumulation of toxic Pb in their body parts.

The levels of Zn in juice of sugarcane grown in sites 1, 2 and 3 of Muhoroni were 3.38 ± 0.79 mg L^{-1}, 3.39 ± 0.91 mg L^{-1} and 4.02 ± 0.90 mg L^{-1} respectively. Juice of sugarcane sampled from site 1 of SONY contained 3.58 ± 0.78 of Zn. The levels in juice of sugarcane grown in sites 2 and 3 were 3.67 ± 0.93 mg L^{-1} and 3.77 ± 0.87 mg L^{-1} of Zn respectively (Table 4.20). The mean levels of Zn in Muhoroni and SONY were 3.60 ± 0.87 mg L^{-1} and 3.67 ± 0.86 mg L^{-1} respectively (Table 4.20).

Table 4.20. Zinc levels (mean ± S.D mg L^{-1}) in sugarcane juice

	Zn levels in juice	
Area / Site	**Muhoroni**	**SONY**
1	3.38 ± 0.79	3.58 ± 0.78
2	3.39 ± 0.91	3.67 ± 0.93
3	4.02 ± 0.90	3.77 ± 0.87
Mean ± S.D	**3.60 ± 0.87**	**3.67 ± 0.86**

Table 4.21. Levels (mean ± S.D mg L^{-1}) of metals in sugarcanecane juice from study areas and maximum permissible limits

	Muhoroni (n=45)	**SONY** (n=45)	**Bacita**[a] (S9CO957)	**Bacita**[a] (S27BCO997)	**Ilorin Metropolis**[b]	**Max. limit (mg L^{-1})**[c]
Cd	0.04 ± 0.02	0.04 ± 0.03	0.04 ± 0.01	0.06 ± 0.01	0.01 - 0.02	0.003
Cr	0.03 ± 0.02	0.05 ± 0.04	-	-	-	0.05
Cu	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	-	2.00
Pb	0.05 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.15 - 0.42	0.01
Zn	3.60 ± 0.87	3.67 ± 0.86	0.31 ± 0.06	0.40 ± 0.10	0.16 - 1.54	NGL*

Source: [a]Adekola and Akinpelu (2002), [b]Abdus-Salam et al. (2008) and [c]WHO (2011).
*NGL: no guideline, because it occurs in drinking water at concentrations well below those at which toxic effects may occur.

The levels (3.60 ± 0.87 and 3.67 ± 0.86 mg L^{-1}) of Zn in sugarcane juice in this study were higher than 0.31 ± 0.06 and 0.40 ± 0.10 mg L^{-1} in juice from Bacita, Nigeria (Adekola and Akinpelu, 2002) and 0.16 - 1.54 mg L^{-1} in juice from Ilorin Metropolis, Nigeria, (Abdus-Salam *et al.*, 2008) (Table 4.21). The levels were also higher than 0.20 mg L^{-1} reported by Tirmazi et al. (2007) and 0.6 mg L^{-1} reported by Damodharan and Reddy (2014). Although no guideline is set by WHO (2011) for zinc level in drinking water, the levels (3.60 ± 0.87 and 3.67 ± 0.86 mg L^{-1}) of Zn in the juice in this study were above the maximum admissible limit of 0.5 mg L^{-1} (USEPA, 2011). Ashworth and Alloway (2008) reported that Zn shows greatest solubility at low soil pH with relatively low solubility at soil pH levels greater than 6.4. There is no harm in chewing sugarcane grown in Muhoroni and SONY since the levels are lower than the dietary reference intake of 11 mg day^{-1} for males and 8 mg day^{-1} for females according to WHO (1996).

4.10 Heavy metal levels in bagasse

The levels of the five metals in bagasse are given in Tables 4.22, 4.23, 4.24, 4.25 and 4.26. Sugarcane sampled from site 1 of Muhoroni contained 0.11 ± 0.01 mg kg^{-1} of Cd in bagasse. The levels in bagasse of sugarcane grown in sites 2 and 3 were 0.11 ± 0.02 mg kg^{-1} and 0.12 ± 0.03 mg kg^{-1} of Cd respectively. The levels of Cd in bagasse of sugarcane grown in sites 1, 2 and 3 of SONY were 0.15 ± 0.02 mg kg^{-1}, 0.14 ± 0.03 mg kg^{-1} and 0.14 ± 0.03 mg kg^{-1} respectively (Table 4.22). The mean levels of Cd in bagasse grown in Muhoroni and SONY were 0.15 ± 0.02 mg kg^{-1} and 0.14 ± 0.03 mg kg^{-1} respectively (Table 4.22).

Table 4.22. Cadmium levels (mean ± S.D mg kg^{-1} dry) in bagasse

	Cd levels in bagasse	
Area / Site	**Muhoroni**	**SONY**
1	0.11 ± 0.01	0.15 ± 0.02
2	0.11 ± 0.02	0.14 ± 0.03
3	0.12 ± 0.03	0.14 ± 0.03
Mean ± S.D	**0.11 ± 0.02**	**0.14 ± 0.03**

Little information on the level of heavy metals in bagasse was available hence the levels of the metals studied were compared with the maximum permissible limit for adult livestock feeding (WHO, 1996). The levels (0.11 ± 0.02 and 0.14 ± 0.03 mg kg^{-1}) of Cd in bagasse of sugarcane grown in Muhoroni and SONY were above the level in bagasse of sugarcane grown in cadmium-contaminated soil (Xie *et al.,* 2014) (Table 4.27). However, the levels were below 0.15 - 0.99 mg kg^{-1} in bagasse from Guangxi Zhuang region, China (Wang *et al.,* 2012). The levels of Cd in the study areas were below 0.5 mg kg^{-1} maximum permissible limit for adult livestock feeding (WHO, 1996) (Table 4.27). The levels of this trace metal in cane juice were lower than the levels in bagasse in this study. The present results were in agreement with the previous study of Barzegar et al. (2005) who reported that Cd is primarily accumulated in bagasse. Although the levels of Cd in bagasse were below maximum permissible limit for adult livestock feeding according to WHO (1996), bagasse from the study areas be used as animal feeds sparingly due to toxic nature of the metal and also due to appreciable amount of it in bagasse bearing in mind that the metals accumulates in body parts of animals.

The amount of Cr contained in the bagasse of sugarcane grown in site 1 of Muhoroni was 10.85 ± 0.76 mg kg^{-1} while the levels in bagasse of sugarcane sampled from sites 2 and 3 were 10.93 ± 0.89 mg kg^{-1} and 11.28 ± 0.70 mg kg^{-1} respectively. The levels of Cr in bagasse of sugarcane sampled from sites 1, 2 and 3 of SONY were 7.28 ± 1.31 mg kg^{-1}, 7.35 ± 1.93 mg kg^{-1} and 7.16 ± 2.17 mg kg^{-1} respectively (Table 4.23). The mean levels of Cr in bagasse grown in Muhoroni and SONY were 11.02 ± 0.78 mg kg^{-1} and 7.26 ± 1.80 mg kg^{-1} respectively (Table 4.23). The level of Cr in bagasse accounted for the highest amount of the metal in sugarcane stem.

Table 4.23. Chromium levels (mean ± S.D mg kg^{-1} dry) in bagasse

	Cr levels in bagasse	
Area / Site	**Muhoroni**	**SONY**
1	10.85 ± 0.76	7.28 ± 1.31
2	10.93 ± 0.89	7.35 ± 1.93
3	11.28 ± 0.70	7.16 ± 2.17
Mean ± S.D	**11.02 ± 0.78**	**7.26 ± 1.80**

The levels (11.02 ± 0.78 and 7.26 ± 1.80 mg kg^{-1}) of Cr in bagasse were below 50 mg kg^{-1} maximum permissible limit for adult livestock feeding (WHO, 1996) (Table 4.27). The bagasse of sugarcane grown in Muhoroni and SONY was not contaminated with chromium.

The mean level of Cu in bagasse of sugarcane sampled from site 1 of Muhoroni was 0.62 ± 0.13 mg kg^{-1}. It was 0.64 ± 0.15 mg kg^{-1} in site 2 and 0.63 ± 0.14 mg kg^{-1} in site 3. The levels in sites 1, 2 and 3 of SONY were 0.71 ± 0.17 mg kg^{-1}, 0.72 ± 0.19 mg kg^{-1} and 0.67 ± 0.16 mg kg^{-1} respectively (Table 4.24). The mean levels of Cu in bagasse grown in Muhoroni and SONY were 0.63 ± 0.14 mg kg^{-1} and 0.70 ± 0.17 mg kg^{-1} respectively (Table 4.24).

Table 4.24. Copper levels (mean ± S.D mg kg^{-1} dry) in bagasse

	Cu levels in bagasse	
Area / Site	**Muhoroni**	**SONY**
1	0.62 ± 0.13	0.71 ± 0.17
2	0.64 ± 0.15	0.72 ± 0.19
3	0.63 ± 0.14	0.67 ± 0.16
Mean ± S.D	**0.63 ± 0.14**	**0.70 ± 0.17**

The levels (0.63 ± 0.14 and 0.70 ± 0.17 mg kg^{-1}) of Cu levels were 7-fold above the level in bagasse of sugarcane grown in cadmium-contaminated soil (Xie *et al.,* 2014). However, the levels of Cu were below 20 mg kg^{-1} maximum permissible limit for adult livestock feeding (WHO, 1996) (Table 4.27). The low level in bagasse could be because soluble Cu accounts for only a very small proportion of the total amount in soil since it has a high affinity for solid phase organic matter (McBride *et al.,* 1997b). The levels of Cu in bagasse were not high enough to be toxic to animals.

The levels of Pb in bagasse of sugarcane grown in sites 1, 2 and 3 of Muhoroni were 2.67 ± 0.81 mg kg^{-1}, 2.45 ± 0.67 mg kg^{-1} and 2.65 ± 0.86 mg kg^{-1} respectively. Bagasse of sugarcane sampled from site 1 of SONY contained 2.03 ± 0.63 mg kg^{-1} of Pb. The levels in bagasse of sugarcane grown in sites 2 and 3 were 2.30 ± 0.67 mg kg^{-1} and 2.08 ± 0.92 mg kg^{-1} of Pb respectively (Table 4.25). The mean levels of Pb in bagasse grown in Muhoroni and SONY were 2.59 ± 0.78 mg kg^{-1} and 2.14 ± 0.74 mg kg^{-1} respectively (Table 4.25).

Table 4.25. Lead levels (mean ± S.D mg kg^{-1} dry) in bagasse

	Cu levels in bagasse	
Area / Site	**Muhoroni**	**SONY**
1	2.67 ± 0.81	2.03 ± 0.63
2	2.45 ± 0.67	2.30 ± 0.67
3	2.65 ± 0.86	2.08 ± 0.92
Mean ± S.D	**2.59 ± 0.78**	**2.14 ± 0.74**

The levels (2.59 ± 0.78 and 2.14 ± 0.74 mg kg^{-1}) of Pb were within the range (1.68 - 5.54 mg kg^{-1}) in bagasse from Guangxi Zhuang region, China (Wang *et al.,* 2012) but below 0.09 mg kg^{-1} reported in bagasse of sugarcane grown in cadmium-contaminated soil (Xie *et al.,* 2014). The levels (2.59 ± 0.78 and 2.14 ± 0.74 mg kg^{-1}) of Pb were below 10 mg kg^{-1} maximum permissible limit for adult livestock feeding (WHO, 1996) (Table 4.27). This result is probably due to the low Pb translocation in plants and its preferential accumulation in roots (Alloway, 1990). The bagasse from the study areas contained levels of toxic Pb that may contaminate the food chain when given to livestock due to bioaccumulation of the metal in animals.

Bagasse of sugarcane grown in sites 1, 2 and 3 of Muhoroni contained 26.46 ± 2.75 mg kg^{-1}, 26.90 ± 2.42 mg kg^{-1} and 26.55 ± 2.75 mg kg^{-1} of Zn respectively. Bagasse of sugarcane sampled from site 1 of SONY contained 30.38 ± 1.68 mg kg^{-1} of Zn. The levels in bagasse of sugarcane grown in sites 2 and 3 were 30.29 ± 2.00 mg kg^{-1} and 30.64 ± 1.99 mg kg^{-1} of Cd respectively (Table 4.26). The mean levels of Zn in bagasse grown in Muhoroni and SONY were 26.64 ± 2.64 mg kg^{-1} and 30.44 ± 1.89 mg kg^{-1} respectively (Table 4.26).

Table 4.26. Zinc levels (mean ± S.D mg kg^{-1} dry) in bagasse

	Zn levels in bagasse	
Area / Site	**Muhoroni**	**SONY**
1	26.46 ± 2.75	30.38 ± 1.68
2	26.90 ± 2.42	30.29 ± 2.00
3	26.55 ± 2.75	30.64 ± 1.99
Mean ± S.D	**26.64 ± 2.64**	**30.44 ± 1.89**

Table 4.27. Levels (mean ± S.D mg kg^{-1} dry) of metals in bagasse from study areas and maximum permissible limit

	Cd	**Cr**	**Cu**	**Pb**	**Zn**
Muhoroni (n=45)	0.11 ± 0.02	11.02 ± 0.78	0.63 ± 0.14	2.59 ± 0.78	26.64 ± 2.64
SONY (n=45)	0.14 ± 0.03	7.26 ± 1.80	0.70 ± 0.17	2.14 ± 0.74	30.44 ± 1.89
Guangdong[a]	nd	-	0.09	1.73	31.52
MPL*	0.5	50	20	10	50

Source: [a] Xie et al. (2014)
*MPL: Maximum permissible limit for adult livestock feeding (WHO, 1996)

The levels (26.64 ± 2.64 and 30.44 ± 1.89 mg kg^{-1}) of Zn were slightly below 31.52 mg kg^{-1} reported in bagasse of sugarcane grown in cadmium-contaminated soil (Xie *et al.*, 2014). The levels of Zn in the bagasse in this study were also below 50 mg kg^{-1} maximum permissible limit for adult livestock feeding (WHO, 1996) (Table 4.27). Bagasse from the study areas was safe for use as livestock feed.

4.11 Bioaccumulation factors of the heavy metals by sugarcane stems

Bioaccumulation factor (BAF) is a parameter used to describe the transfer of trace elements from soil to plant body. It is calculated using Eq. (2.6). The bioaccumulation factors of the metals in cane stems are given in Table 4.28. The BAF of Cd in sugarcane grown in sites 1, 2 and 3 of Muhoroni were 0.51 ± 0.02, 0.53 ± 0.03 and 0.50 ± 0.02 respectively. In site 1 of SONY the BAF of Cd was 0.52 ± 0.04 while the BAF in sites 2 and 3 was 0.53 ± 0.02. The mean BAF of Cd in sugarcane grown in Muhoroni (0.51 ± 0.02) and SONY (0.53 ± 0.03) were lower than 1.08 of Cd in sugarcane crop irrigated with wastewater as reported by Alghobar and Suresha (2015) (Table 4.29). However, BAF of Cd in sugarcane grown in these study areas were higher than 0.007 reported in sugarcane (Saccharum *Officinarum* L.) irrigated with treated effluents of sugar industry (Damodharan and Reddy, 2014).

Table 4.28. Bioaccumulation factors (mean ± S.D) of the heavy metals in the stems

		Site		
Area	**Metals**	**1**	**2**	**3**
Muhoroni	Cd	0.51 ± 0.02	0.53 ± 0.03	0.50 ± 0.02
	Cr	0.16 ± 0.01	0.16 ± 0.02	0.15 ± 0.01
	Cu	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.01
	Pb	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
	Zn	0.21 ± 0.03	0.21 ± 0.02	0.21 ± 0.02
SONY	Cd	0.52 ± 0.04	0.53 ± 0.02	0.53 ± 0.02
	Cr	0.16 ± 0.01	0.16 ± 0.03	0.16 ± 0.03
	Cu	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.02
	Pb	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.00
	Zn	0.24 ± 0.02	0.21 ± 0.01	0.23 ± 0.01

Since BAF can be used to estimate the ability of plant to accumulate metals in their parts (Zhou *et al.,* 2016), the above results indicated that Cd has higher ability to transfer from soil to the edible parts of sugarcane than the other four heavy metals in agricultural soil of Muhoroni and SONY sugar estates. This can be attributed to the competition between Cd^{2+} and Ca^{2+}. It is easier for Ca^{2+} to be replaced by Cd^{2+} than other metals because of their same ionic radius and valence (Kim *et al.,* 2002; Hart *et al.,* 2002). In addition, Ca is an essential element for crops and it can enter plant tissues by active transport, while most heavy metals (as non-essential elements) can only enter plant tissues through passive methods (e.g., concentration diffusion and permeation) (Costa and Morel 1993). Differently, Cd can also enter plant tissues through Ca channels (Tester 1990). This concurred with Xia et al. (2009) who reported that sugarcane has a high ability to tolerate and accumulate Cd due to its large biomass. Lee et al. (1998b) also indicated that Cd^{2+} is more bioavailable to plant than other heavy metals, such as Zn, Cu, and Pb. The higher BAF of Cd than that of Zn was consistent with the observation of McBridge et al. (2003), Liu et al. (2005b), Kashem et al. (2007), Kumar et al. (2009) and Zhuang et al. (2009) but in contrast to that of Ma and Rao (1997) who observed that the mobility index for Zn is higher than for Cd. The low pH and SOM content in Muhoroni and SONY soils were expected to enhance bioaccumulation of Cd in the sugarcane. However, strong antagonistic effect of Zn on the accumulation of Cd (McKenna *et al.,* 1993) made Cd to have BAF below the range of 1 - 10 suggested by Kloke et al. (1984) (Table 4.29). Such a mitigating effect of Zn on Cd in the edible part of sugarcane may decrease Cd toxication.

Table 4.29. Bioaccumulation factors (mean ± S.D) of the heavy metals in the sugarcane stems from these study areas, another area and suggested ranges

Metal	**Muhoroni** (n=45)	**SONY** (n=45)	**Vidyaranyapuram area**[a]	**Suggested range**[b]
Cd	0.51 ± 0.02	0.53 ± 0.03	1.08	1.00 - 10.00
Cr	0.16 ± 0.01	0.16 ± 0.02	0.14	0.01-0.10
Cu	0.05 ± 0.01	0.05 ± 0.01	0.14	0.01 - 0.10
Pb	0.03 ± 0.01	0.03 ± 0.01	0.37	0.01 - 0.10
Zn	0.21 ± 0.02	0.23 ± 0.01	0.64	1.00 - 10.00

[a]Alghobar and Suresha (2015); [b]Kloke et al. (1984)

The BAF of Cr in sugarcane grown in sites 1, 2 and 3 of Muhoroni were 0.16 ± 0.01, 0.16 ± 0.02 and 0.15 ± 0.01 respectively. Sugarcane sampled from site 1 of SONY had BAF of 0.16 ± 0.01 for Cr while the BAF of Cr in sugarcane grown in sites 2 and 3 was 0.16 ± 0.03 (Table 4.28). Bioaccumulation factors (0.16 ± 0.01 and 0.16 ± 0.02) (Table 4.29) of Cr were higher than 0.14 reported in sugarcane crop irrigated with wastewater as reported by (Alghobar and Suresha, 2015). The bioaccumulation factors (0.16 ± 0.01 and 0.16 ± 0.02) of Cr in sugarcane reported in this study were above the range 0.01 - 0.10 suggested by Kloke et al. (1984) (Table 4.28).

The BAF of Cu in sugarcane grown in site 1 of Muhoroni was 0.05 ± 0.02 while the BAF of Cu in sugarcane sampled from sites 2 and 3 were 0.05 ± 0.01 and 0.05 ± 0.01 respectively. The BAF of Cu in sugarcane grown sites 1, 2 and 3 of SONY were 0.05 ± 0.01, 0.05 ± 0.01 and 0.05 ± 0.02 respectively (Table 4.28). The BAF of Cu (0.05 ± 0.01) in sugarcane grown in Muhoroni and SONY was the same as BAF of 0.050 for Cu in sugarcane (Saccharum *Officinarum* L.) irrigated with treated effluents of sugar industry as reported by Damodharan and Reddy (2014). The BAF of Cu in sugarcane grown in Muhoroni and SONY was within the range 0.01 - 0.10 given by Kloke et al. (1984) (Table 4.29). The bioaccumulation factors of Cu in this study were lower than 0.14 reported in sugarcane crop irrigated with wastewater as reported by (Alghobar and Suresha, 2015) (Table 4.29). Uptake of Cu by plants and its toxicity are contingent on nutritional condition of plant, Cu^{2+} concentration in soil, length of exposure, and genotype of a species (Nicholls and Mal, 2003). A plethora of research studies such as (Sheldon and Menzies, 2005) in Rhodes grass (*Chloris gayana* Knuth), (Yau *et al.*, 1991) in clove (*Syzygium aromaticum* L.), (Alaoui-Sossé *et al.*, 2004) in cucumber (*Cucumis sativus*), and (Assareh *et al.*, 2008) in some *Eucalyptus* species indicated that copper has a propensity for the accumulation in the root tissues with little upward movement towards shoots because it is strongly adsorbed onto soil particles reducing their availability to plants (WHO, 1989,1998; CCME, 1999a). Low BAF of Cu was also coupled by the fact that Cu generally has low mobility in plants (Kabata-Pendias and Pendias, 2001)

The mean BAF of Pb in sugarcane sampled from site 1 of Muhoroni was 0.03 ± 0.01. It was 0.03 ± 0.02 in site 2 and 0.03 ± 0.01 in site 3. The levels in sites 1, 2 and 3 of SONY were 0.03 ± 0.02, 0.03 ± 0.01 and 0.03 ± 0.00 respectively (Table 4.28). The BAF (0.03 ± 0.01) of Pb in sugarcane reported in this study was within the range of 0.01 - 0.10 suggested by Kloke et al. (1984) (Table 4.29) and was close to 0.039 adopted as conservative soil-to-plant BAF of Pb by Ontario Ministry of Environment (CCME, 1999b). It was also close to 0.037 in sugarcane (Saccharum *Officinarum* L.) irrigated with treated effluents of sugar industry reported by Damodharan and Reddy (2014). However, the BAF of Pb in this study was lower than 0.37 reported in sugarcane crop irrigated with wastewater as reported by (Alghobar and Suresha, 2015) (Table 4.29). Although the soil in this study areas contained higher levels (73.65 ± 12.96 and 71.83 ± 8.69 mg kg^{-1}) of Pb than Cd (0.24 ± 0.14 and 0.27 ± 0.16 mg kg^{-1}), Cr (70.80 ± 21.29 and 64.97 ± 15.83 mg kg^{-1}) or Cu (14.08 ± 7.20 and 14.33 ± 5.88 mg kg^{-1}) , Pb metal had the lowest BAF in sugarcane because of low soil mobility and tendency of uptake by plants (Lasat, 2002). The result in this study concurred with Kabata-Pendias and Pendias (2001) who reported that only 3% of Pb absorbed via the root accumulate in the shoot.

The BAF of Zn in sugarcane grown in sites 1, 2 and 3 of Muhoroni were 0.21 ± 0.03, 0.21 ± 0.02 and 0.21 ± 0.02 respectively. The factors of Zn in sugarcane sampled from site 1 of SONY was 0.24 ± 0.02 of Zn. The BAF of Zn in sugarcane grown in sites 2 and 3 were 0.21 ± 0.01 and 0.23 ± 0.01 respectively (Table 4.28). BAF (0.21 ± 0.02 and 0.23 ± 0.01) of Zn in this study were higher than 0.008 in sugarcane (Saccharum *Officinarum* L.) irrigated with treated effluents of sugar industry as reported by Damodharan and Reddy (2014). However, Alghobar and Suresha (2015) reported a higher BAF (0.64) of Zn in sugarcane crop irrigated with wastewater (Table 4.29). The bioaccumulation factors of Zn were below the range 1 - 10 given by Kloke et al. (1984) (Table 4.29). Zinc is reported to be among the metals that are notoriously mobile (Citeau *et al.,* 2003). The low BAF of Zn in sugarcane in this study concurred with the findings of (Root *et al.,* 1975; Jalil *et al.,* 1994; Grant and Bailey, 1998; Sharma *et al.,* 1999; Jiao *et al*., 2004; Wu *et al.*, 2006; Akay and Koleli, 2007; Lambert *et al.,* 2007; Jain *et al.,* 2008) who reported antagonistic effects of Cd, Cu and phosphorus to Zn that contributes to low accumulation of Zn in plants.

The descending order of mean BAF of the five heavy metals in the cane stems was Cd > Zn > Cr > Cu > Pb (Table 4.29). This trend was similar to the order (Cd > Zn > Cu > Pb) of BAF of food crops for heavy metals (Zhuang *et al.,* 2009), except for Cr which was not investigated. Bioaccumulation factor classifications can be divided into four categories which are; BAF from 1.0 - 10 known as high accumulator plant, BAF between 0.1 - 1.0 known as moderate accumulator plant, BAF between 0.1 - 1.0 known as moderate accumulator plant, BAF between 0.01 - 0.1 known as low accumulator plant and BAF < 0.01 known as non-accumulator plant (Behrouz *et al.,* 2008). BAF mean values for Cd (0.51 ± 0.02 and 0.53 ± 0.03), Cr (0.16 ± 0.01 and 0.16 ± 0.02), Cu (0.05 ± 0.01), Pb (0.03 ± 0.01) and Zn (0.21 ± 0.02 and 0.23 ± 0.01) in sugarcane cultivated in Muhoroni and SONY respectively, indicating that the sugarcane plants are moderate accumulator of Cd, Cr and Zn, and low accumulator of Cu and Pb. This result showed that high levels of heavy metals in soil do not necessarily enrich the metals in sugarcane stem, suggesting that there is a mechanism existing in the plant to filter harmful levels of heavy metals from entering the plant (Stijve and Besson, 1976).

CHAPTER 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

- The soil organic matter contents in Muhoroni and SONY sugar estates were within the range required by sugarcane. However, the soil pH values were lower than the optimum value of 6.5 - 7.0 which is required for sugarcane cultivation. The low soil pH might enhance uptake of heavy metals by sugarcane.

- The levels of metals Cd, Cr, Cu, Pb and Zn in the farm soils of Muhoroni and SONY sugar estates were within safe limits of European Union standards.

- The contamination factors indicated moderate contamination by Pb in soil in the study areas while contamination by Cd, Cr, Cu and Zn were low according to Loska et al. (2004). The soils had low degree of contamination according to Duong and Lee (2011) and the average ecological risk factors of all the metals in were below 40 indicating low potential ecological risk to local environment according to Hakanson (1980). The potential ecological risk index values were 16.22 and 16.95 showing low overall risk caused by the studied metals according to Shi et al. (2010).

- The amounts of Cd, Cu, Pb and Zn in the sugarcane stems were within WHO/FAO safe limits. However, the levels of Cr in the stems of the sugarcane were above the safe limit. The levels of Cr, Cu and Zn in sugarcane juice were within recommended maximum limit. However, the level of Cd in the sugarcane juice was 13 times higher than WHO recommended maximum limit. The levels of Pb in sugarcane juice were also above the recommended maximum limit. The high levels of toxic Cd and Pb in sugarcane juice was a cause to worry. The levels of Cd, Cr, Cu, Pb and Zn in bagasse were below the WHO maximum permissible limit for adult livestock feeding.

- The bioaccumulation factors of Cd, Cr, Cu, Pb and Zn in sugarcane grown in Muhoroni and SONY sugar estates were below 1.

5.2 Conclusions

- The soil samples from Muhoroni and SONY had medium organic matter content and they were strongly acidic.

- The levels of metals Cd, Cr, Cu, Pb and Zn were low in the soil samples.

- There was no contamination the sugarcane farms by Cd, Cr, Cu, Pb and Zn.

- The levels of Cd, Cu, Pb and Zn in sugarcane stems were low. However, the levels of Cr were relatively high. All the heavy metals investigated were detected in sugarcane juice. The levels of Cr, Cu and Zn in juice of sugarcane were low. However, the levels of Cd and Pb in juice of the sugarcane were high. The bagasse of the sugarcane contained low levels of Cd, Cr, Cu, Pb and Zn.

- There was no bioconcentration of Cd, Cr, Cu, Pb and Zn in sugarcane grown in Muhoroni and SONY sugar estates**.**

5.3 Recommendations

In view of the results, it was recommended that;

- The soil organic matter content be maintained or increased to boost sugarcane productivity in these areas in order to bridge the gap of sugar deficit. The soil pH be increased to the range required by sugarcane. The low soil pH could lead to elevated heavy metal uptake by sugarcane which might affect food quality, quantity and safety. Regular application of small quantities of lime to prevent the development of topsoil and subsoil acidity is recommended for this soil. Once the pH is increased to optimum range, non-acidifying fertilizers such as calcium ammonium nitrate, which prevents further soil acidification, should be applied.

- The levels of Pb metal in the soil of Muhoroni and SONY be reduced in order to check on its contamination level.

- Cultivation of sugarcane in Muhoroni and SONY sugarcane estates to continue as there was no potential ecological risk established.

- Mitigation measures be taken to reduce the levels of Cr in sugarcane stems, and toxic Cd and Pb in the sugarcane juice. Bagasse obtained from the sugarcane grown in Muhoroni and SONY be used as livestock feeds.

- The major sugarcane varieties in Muhoroni and SONY be cultivated but measures should be taken to reduce uptake of Cd, Cr and Pb. This could be done by increasing soil organic matter content and soil pH to 6.5 - 7.0

5.4 Suggestion for future research

- There is need for further research to screen sugar crystals in order to ascertain heavy metals levels in this widely used sugarcane product to avoid intoxication of human beings given that the levels of toxic Cd and Pb in the juice were higher than WHO recommended maximum limit.

- Research work should be done on the sugarcane varieties to assess varietal differences with respect to bioaccumulation of heavy metals.

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APPENDICES

Appendix I: Sampling points

Sampling points in Muhoroni nucleus estate

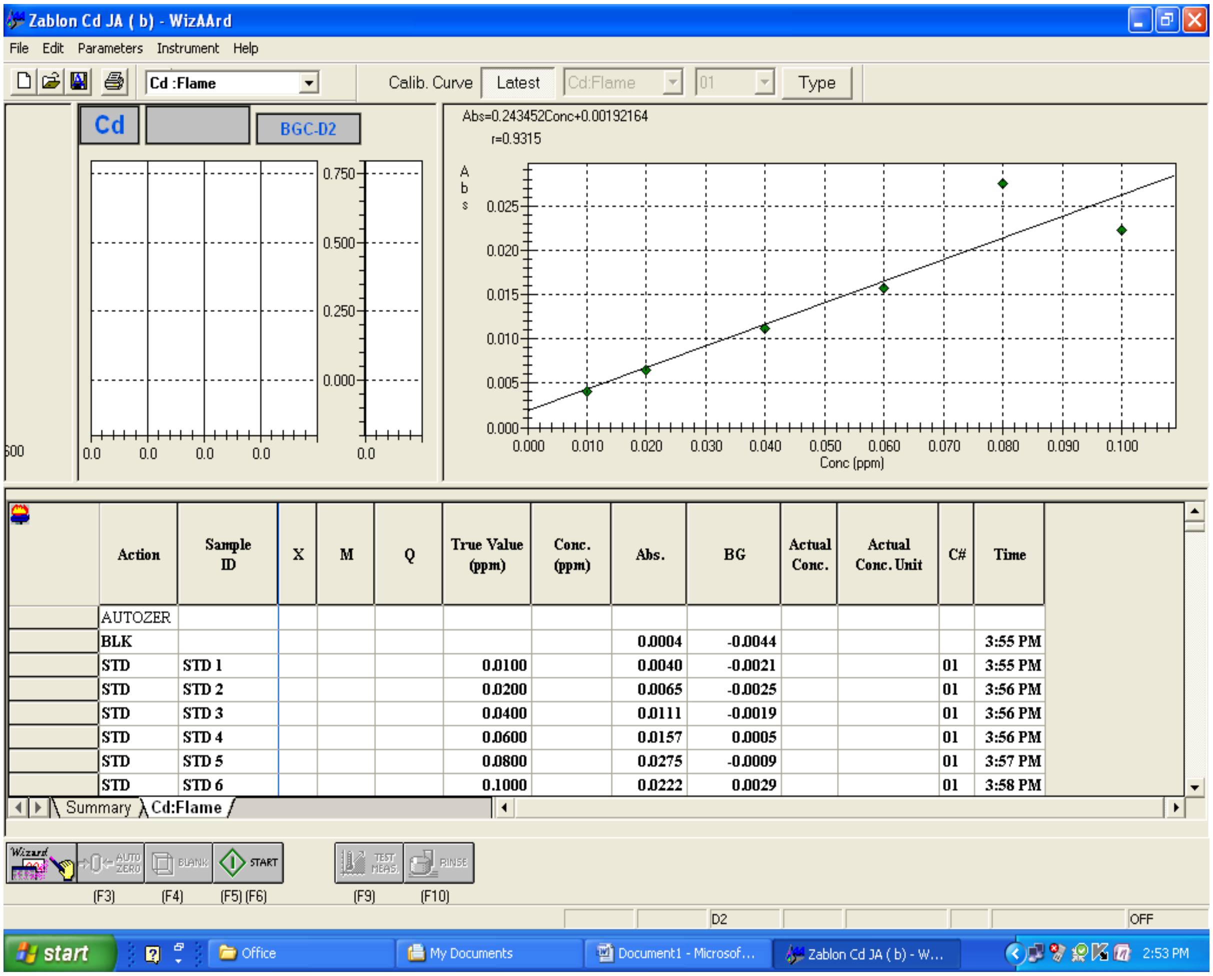
Sampling points	Latitude	Longitude
1A1	0^{0}9’38” S	35^{0}50’27” E
1A2	0^{0}9’35” S	35^{0}49’24” E
1A3	0^{0}9’27” S	35^{0}52’30” E
1A4	0^{0}9’29” S	35^{0}52’42” E
1A5	0^{0}9’35” S	35^{0}49’16” E
1B1	0^{0}9’32” S	35^{0}50’59” E
1B2	0^{0}9’40” S	35^{0}48’30” E
1B3	0^{0}9’32” S	35^{0}49’30” E
1B4	0^{0}9’42” S	35^{0}50’36” E
1B5	0^{0}9’24” S	35^{0}52’42” E
1C1	0^{0}9’40” S	35^{0}49’12” E
1C2	0^{0}9’38” S	35^{0}49’24” E
1C3	0^{0}9’35” S	35^{0}50’54” E
1C4	0^{0}9’34” S	35^{0}49’24” E
1C5	0^{0}9’25” S	35^{0}52’54” E
2A1	0^{0}10’13” S	35^{0}50’38” E
2A2	0^{0}10’15” S	35^{0}50’36” E
2A3	0^{0}10’12” S	35^{0}50’54’ E
2A4	0^{0}10’13” S	35^{0}50’51” E
2A5	0^{0}10’13” S	35^{0}50’42” E
2B1	0^{0}10’19” S	35^{0}51’18” E
2B2	0^{0}10’17” S	35^{0}50’54” E
2B3	0^{0}10’15” S	35^{0}50’51” E
2B4	0^{0}10’07” S	35^{0}51’09” E
2B5	0^{0}10’00” S	35^{0}51’39” E
2C1	0^{0}10’17” S	35^{0}51’24” E
2C2	0^{0}10’20” S	35^{0}51’18” E
2C3	0^{0}10’20” S	35^{0}51’09” E
2C4	0^{0}10’15” S	35^{0}50’24” E
2C5	0^{0}10’12” S	35^{0}50’45” E
3A1	0^{0}10’41” S	35^{0}49’03” E
3A2	0^{0}10’42” S	35^{0}49’00” E
3A3	0^{0}10’43” S	35^{0}49’12” E
3A4	0^{0}10’45” S	35^{0}49’24” E
3A5	0^{0}10’44” S	35^{0}49’36” E
3B1	0^{0}10’47” S	35^{0}51’00” E
3B2	0^{0}10’50” S	35^{0}50’30” E
3B3	0^{0}10’48” S	35^{0}50’18” E
3B4	0^{0}10’48” S	35^{0}49’54” E
3B5	0^{0}10’46” S	35^{0}49’54” E
3C1	0^{0}10’53” S	35^{0}49’45” E
3C2	0^{0}10’51” S	35^{0}49’39” E
3C3	0^{0}10’53” S	35^{0}49’21” E
3C4	0^{0}10’50” S	35^{0}49’48” E
3C5	0^{0}10’51” S	35^{0}50’00” E

Sampling points in SONY nucleus estate

Sampling points	**Latitude**	**Longitude**
1A1	0^{0}53’53” S	34^{0}40’51” E
1A2	0^{0}52’28” S	34^{0}40’45” E
1A3	0^{0}53’21” S	34^{0}41’11” E
1A4	0^{0}53’02” S	34^{0}41’48” E
1A5	0^{0}52’32” S	34^{0}41’28” E
1B1	0^{0}53’39” S	34^{0}40’23” E
1B2	0^{0}54’03” S	34^{0}40’44” E
1B3	0^{0}52’51” S	34^{0}40’45” E
1B4	0^{0}52’58” S	34^{0}40’42” E
1B5	0^{0}53’15” S	34^{0}41’07” E
1C1	0^{0}53’32” S	34^{0}40’44” E
1C2	0^{0}53’53” S	34^{0}40’42” E
1C3	0^{0}53’04” S	34^{0}40’34” E
1C4	0^{0}52’10” S	34^{0}40’25” E
1C5	0^{0}52’28” S	34^{0}41’27” E
2A1	0^{0}54’44” S	34^{0}41’19” E
2A2	0^{0}54’48” S	34^{0}41’22” E
2A3	0^{0}55’15” S	34^{0}42’32” E
2A4	0^{0}55’41” S	34^{0}41’59” E
2A5	0^{0}55’48” S	34^{0}41’56” E
2B1	0^{0}55’25” S	34^{0}42’05” E
2B2	0^{0}55’35” S	34^{0}41’40” E
2B3	0^{0}55’42” S	34^{0}41’45” E
2B4	0^{0}56’10” S	34^{0}42’10” E
2B5	0^{0}55’52” S	34^{0}42’30” E
2C1	0^{0}54’40” S	34^{0}41’59” E
2C2	0^{0}54’50” S	34^{0}42’13” E
2C3	0^{0}55’10” S	34^{0}42’26” E
2C4	0^{0}55’38” S	34^{0}42’22” E
2C5	0^{0}55’10” S	34^{0}42’33” E
3A1	0^{0}56’07” S	34^{0}43’01” E
3A2	0^{0}56’43” S	34^{0}43’37” E
3A3	0^{0}56’54” S	34^{0}44’18” E
3A4	0^{0}57’34” S	34^{0}44’37” E
3A5	0^{0}57’40” S	34^{0}44’46” E
3B1	0^{0}56’53” S	34^{0}42’23” E
3B2	0^{0}56’15” S	34^{0}43’07” E
3B3	0^{0}56’50” S	34^{0}43’38” E
3B4	0^{0}57’22” S	34^{0}44’10” E
3B5	0^{0}57’31” S	34^{0}44’14” E
3C1	0^{0}56’33” S	34^{0}43’44” E
3C2	0^{0}56’39” S	34^{0}44’10” E
3C3	0^{0}56’39” S	34^{0}43’44” E
3C4	0^{0}56’45” S	34^{0}44’09” E
3C5	0^{0}57’11” S	34^{0}44’23” E

Apendix II: Multi-level calibration curves used for the AAS (AA-6200 Shimadzu)

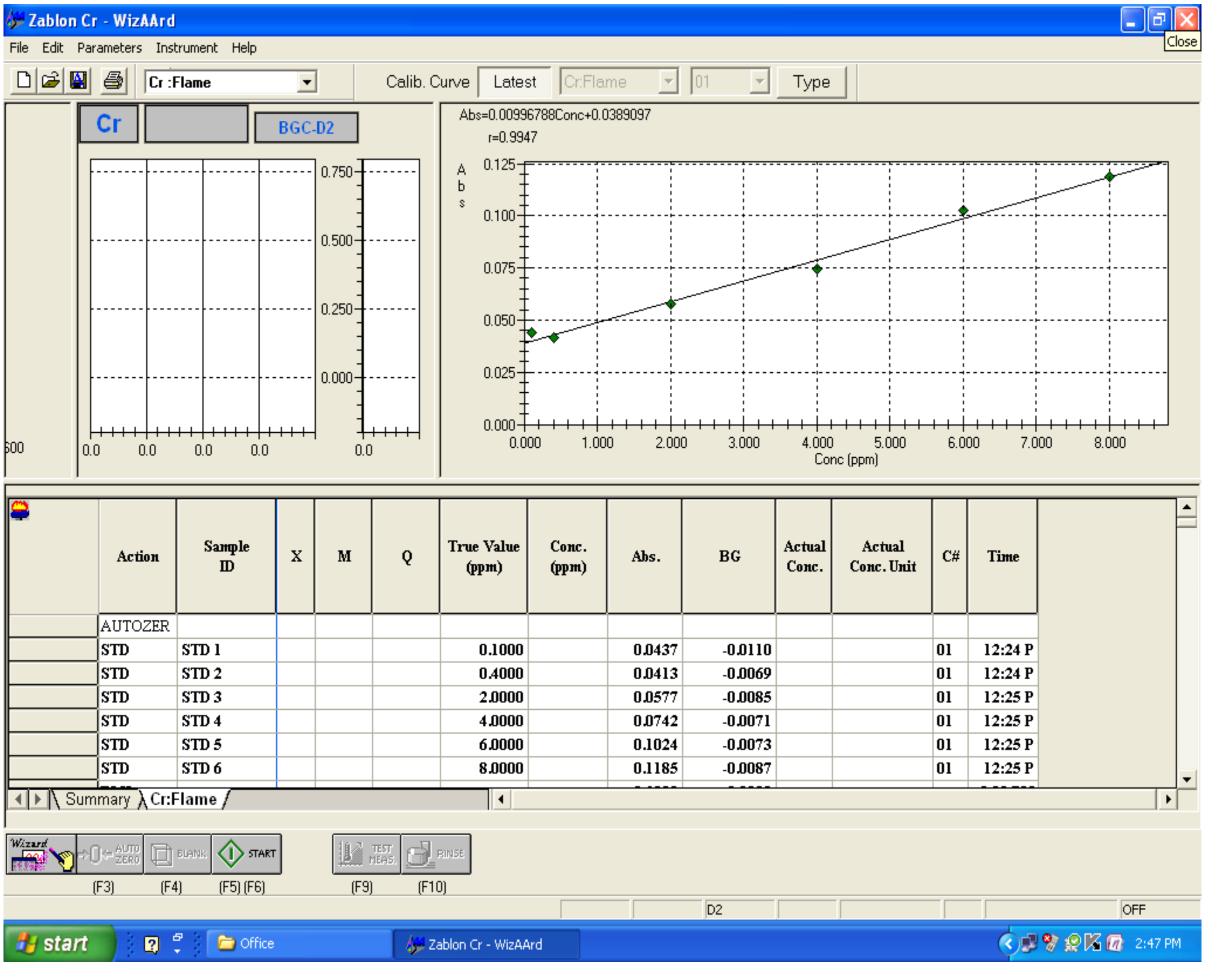
Calibration curve for Cadmium



Abs=0.243452Conc+0.00192164
r=0.9315

	Action	Sample ID	X	M	Q	True Value (ppm)	Conc. (ppm)	Abs.	BG	Actual Conc.	Actual Conc. Unit	C#	Time
	AUTOZER												
	BLK							0.0004	-0.0044				3:55 PM
	STD	STD 1				0.0100		0.0040	-0.0021			01	3:55 PM
	STD	STD 2				0.0200		0.0065	-0.0025			01	3:56 PM
	STD	STD 3				0.0400		0.0111	-0.0019			01	3:56 PM
	STD	STD 4				0.0600		0.0157	0.0005			01	3:56 PM
	STD	STD 5				0.0800		0.0275	-0.0009			01	3:57 PM
	STD	STD 6				0.1000		0.0222	0.0029			01	3:58 PM

Calibration curve for Chromium

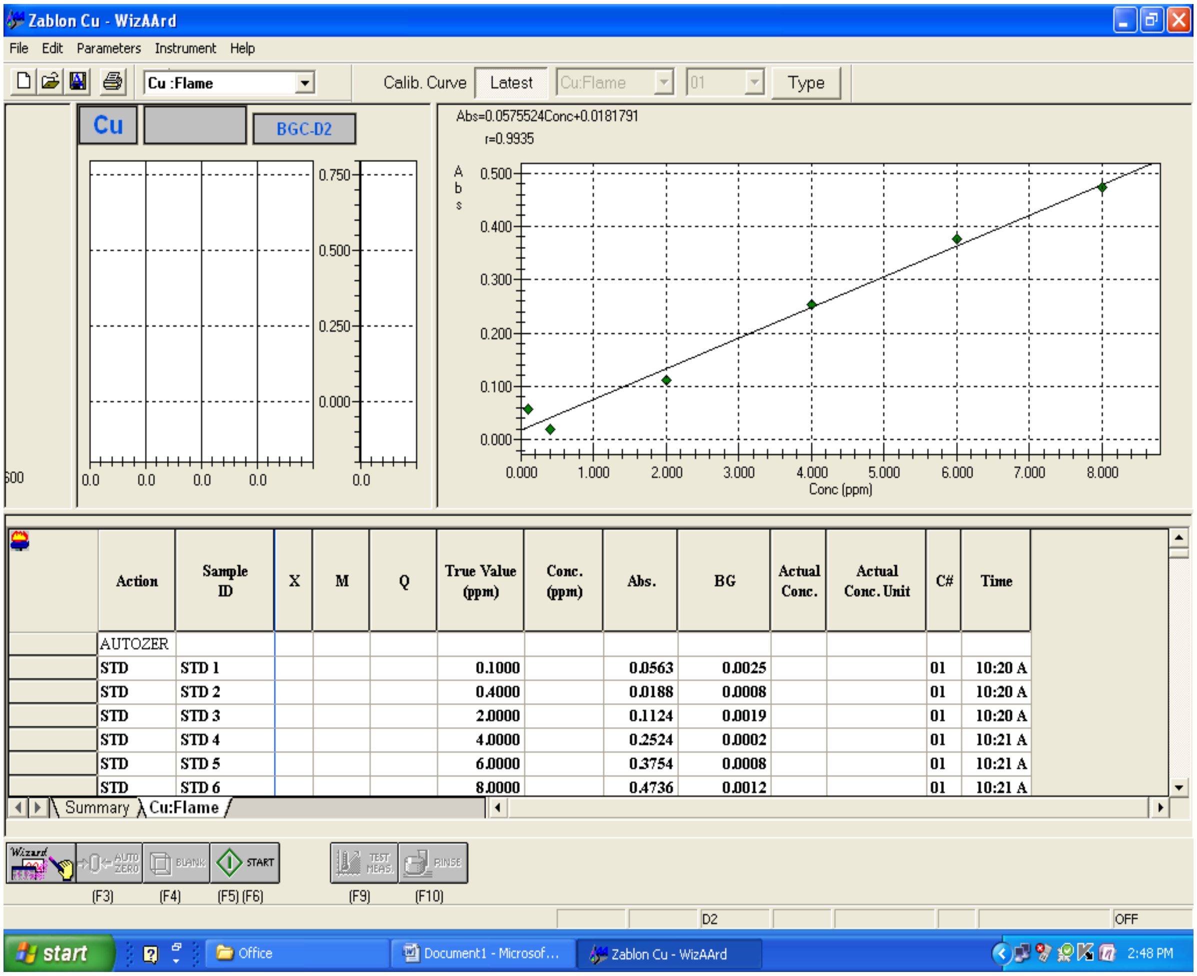


Abs=0.00996788Conc+0.0389097

r=0.9947

Action	Sample ID	X	M	Q	True Value (ppm)	Conc. (ppm)	Abs.	BG	Actual Conc.	Actual Conc. Unit	C#	Time
AUTOZER												
STD	STD 1				0.1000		0.0437	-0.0110			01	12:24 P
STD	STD 2				0.4000		0.0413	-0.0069			01	12:24 P
STD	STD 3				2.0000		0.0577	-0.0085			01	12:25 P
STD	STD 4				4.0000		0.0742	-0.0071			01	12:25 P
STD	STD 5				6.0000		0.1024	-0.0073			01	12:25 P
STD	STD 6				8.0000		0.1185	-0.0087			01	12:25 P

Calibration curve for Copper

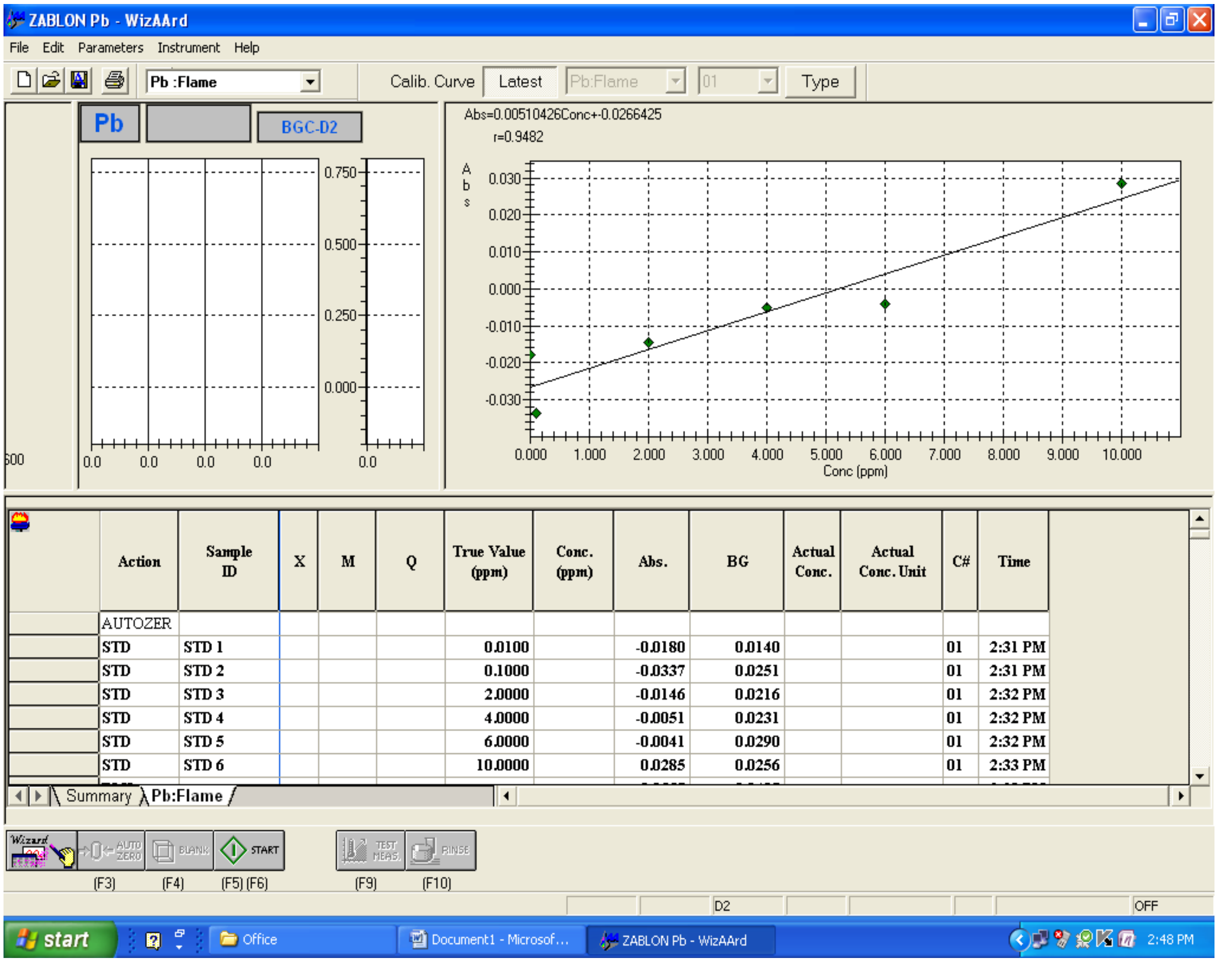


Abs=0.0575524Conc+0.0181791

r=0.9935

	Action	Sample ID	X	M	Q	True Value (ppm)	Conc. (ppm)	Abs.	BG	Actual Conc.	Actual Conc. Unit	C#	Time
	AUTOZER												
	STD	STD 1				0.1000		0.0563	0.0025			01	10:20 A
	STD	STD 2				0.4000		0.0188	0.0008			01	10:20 A
	STD	STD 3				2.0000		0.1124	0.0019			01	10:20 A
	STD	STD 4				4.0000		0.2524	0.0002			01	10:21 A
	STD	STD 5				6.0000		0.3754	0.0008			01	10:21 A
	STD	STD 6				8.0000		0.4736	0.0012			01	10:21 A

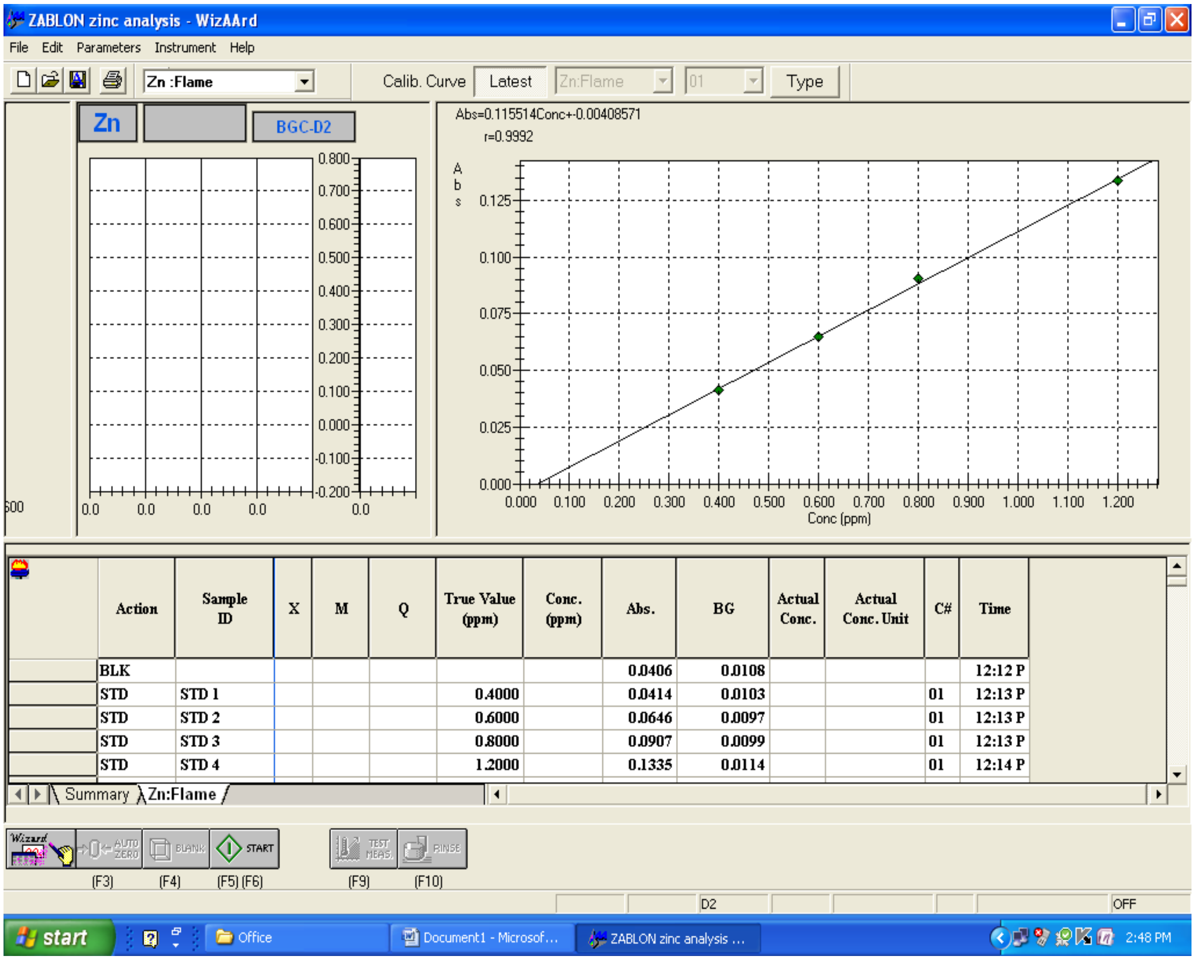
Calibration curve for Lead



Abs=0.00510426Conc+-0.0266425
r=0.9482

Action	Sample ID	X	M	Q	True Value (ppm)	Conc. (ppm)	Abs.	BG	Actual Conc.	Actual Conc. Unit	C#	Time
AUTOZER												
STD	STD 1				0.0100		-0.0180	0.0140			01	2:31 PM
STD	STD 2				0.1000		-0.0337	0.0251			01	2:31 PM
STD	STD 3				2.0000		-0.0146	0.0216			01	2:32 PM
STD	STD 4				4.0000		-0.0051	0.0231			01	2:32 PM
STD	STD 5				6.0000		-0.0041	0.0290			01	2:32 PM
STD	STD 6				10.0000		0.0285	0.0256			01	2:33 PM

Calibration curve for Zinc



Abs=0.115514Conc+-0.00408571

r=0.9992

Action	Sample ID	X	M	Q	True Value (ppm)	Conc. (ppm)	Abs.	BG	Actual Conc.	Actual Conc. Unit	C#	Time
BLK							0.0406	0.0108				12:12 P
STD	STD 1				0.4000		0.0414	0.0103			01	12:13 P
STD	STD 2				0.6000		0.0646	0.0097			01	12:13 P
STD	STD 3				0.8000		0.0907	0.0099			01	12:13 P
STD	STD 4				1.2000		0.1335	0.0114			01	12:14 P