University of Venda

SCHOOL OF ENVIRONMENTAL SCIENCES

DEPARTMENT OF ECOLOGY AND RESOURCE MANAGEMENT

EVALUATION OF POLLUTION POTENTIAL AND BIOREMEDIATION OF TANNERY-BASED CHROMIUM WASTES IN SUB SAHARAN AFRICA: THE CASE OF DUMP SITES IN BEIT ORE TANNERY IN SOUTH AFRICA AND DOGBONE TANNERY IN KENYA

By

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DECLARATION

DECLARATION I, Ongon'g Richard Oruko, student number 17007722, hereby declare that this thesis submitted to the Department of Ecology and Resource management, School of Environmental Sciences, University of Venda, for the PhD in Environmental Sciences is my own work and has not been previously submitted, in whole or in part, to any university for any degree; and all reference materials contained herein have been duly acknowledged.

23 /02/2021

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Student's signature Date

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We, the promoters, certify that this declaration is correct.

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Co-promoter's signature Date

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DEDICATION

This thesis is dedicated to the Almighty God**.** This is because, if God could wait long enough for snails to enter Noah's ark; His door of grace could not close for me until He gave me an opportunity to reach this far in my academic life. Thank you Lord.

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ABSTRACT

The leather tanning technology is one of the oldest industry in humankind's civilisation history. It converts raw hides and skins into non-putrescible products used in making various leather materials. Globally, Africa accounts for 4% of the world leather production and 3.3% of value addition in leather industry. This value addition, has been accompanied with varied nature and large magnitude of tannery chromium effluent and solid wastes discharge from leather manufacturing. Leather processing wastes are now becoming of great environmental and public health concern in the world but more seriously in Sub Saharan Africa. This study aimed first at investigating chromium status, trace metals contamination and ecological risk assessment of tannery waste disposal of selected tannery waste dumpsites in Kenya and South Africa. Then, it assessed the potential health risk associated with the consumption of edible vegetables grown on Cr (VI) polluted soils. It further investigated the anaerobic bacterial profile of donkey dung assisted anaerobic bioreactor remediation of tannery based chromium wastes. Preliminary study of the use of donkey dung as a sorbent for Cr (VI) sequestration was also performed.

This study was carried out in Dogbone (DB) and Beit Ore (BO) tanneries in Kenya and South Africa, respectively. Sampling for soils was done twice to accommodate two major seasons. The dumpsites soil was sampled at a depth of 10 - 20 cm for physico-chemical and heavy metals content analyses in both dumpsites in February, March, July and December of 2018. Wastewater samples were collected between February and March of 2018 at both tanneries. The following plants were sampled at BO dumpsites; *Spinacea oleracea, Dactyloctenium aegyptenium, Cynodon dactylon, Alternanthera caracasana,* and *Corchorus tridens.* In DB dumpsites; *Amaranthus dubius Thell, Cynodon nemfluensis vanderst* and *actyloctenium aegyptenium* were sampled. The donkey dung used in this study was obtained from Vuwani village of Vhembe district, Limpopo province, South Africa.

To achieve the specific objectives of this study, the presence of selected heavy metals (copper, iron, nickel, zinc, cadmium, arsenic, lead, silver, cobalt, manganese) and chromium oxidation status (total chromium and hexavalent chromium) in effluents, soils and plants were investigated.The reduction of total chromium and Cr (VI) by bacteria were assessed using Inductively coupled plasma-optical emission spectrophotometer and Ultra violet visible spectrophotometer, respectively. The occurrence of bacterial community profile in bioreactor and presence of methane gas generated were analysed with Illumina MiSeq System and gas chromatography machine, respectively. The donkey dung was characterised using Fourier

transform infrared spectrometer, Thermogravimetric analysis, Scanning electron microscope, Fluorescence excitation emission matrix and Brunau-Emmett-Teller.

The results of the first objective investigated chromium status, heavy metals contamination and ecological risk assessment of tannery waste disposal in Kenya and South Africa, and showed that the physico-chemical parameters in sampled soils may have favoured the transformation of Cr (III) to Cr (VI) at BO and DB dumpsites. The levels of Cr (VI) in sampled soils were found to be 0.31 and 0.4 mg/kg in BO and DB, respectively, and exceeded the WHO guidelines of 0.05 mg/kg for Cr (VI) in soils. The results from various pollution indices tools (like geo-accumulation index, contamination degree, the pollution load index, the ecological risk assessment and the potential ecological risk) showed high contamination of soils by tannery waste in relation to the control sites. The chromium contents in edible vegetables like *Amaranthus dubius Thell* from DB was 12.97 mg/kg Cr while *Spinacea oleracea* from BO was 12.08 mg/kg Cr above expected level of 1 mg/kg Cr. The study indicated that the sites were ecologically contaminated and the edible vegetables wildly growing were contaminated with Cr, thus posing a health risk to tannery workers and nearby populations who consume such vegetables.

The second objective of the study was, the potential health risk associated with edible vegetables grown on Cr (VI) polluted soils, which showed that *Vigna angularis* was the only edible vegetable that could germinate in highly polluted soil contaminated with Cr (VI) level at 456 mg/kg. The highest total chromium (ChT) factor, bioaccumulated in the roots was found in *Phaseolus vulgaris* at 0.8. The highest ChT translocation factor in the stem was that of *Cicer arietinum* and *Vigna angularis* at 0.30. The same plants translocated the highest ChT factor to the leaf at 0.7. A child or an adult consuming such contaminated *Cicer arietinum* vegetables is likely to take in between 508 and 785 mg/day of ChT. This is above the World Health Organisation (WHO) guidelines of 220 and 340 mg/day, respectively. The highest Hazard Quotient was found in *Cicer arietinum* at 8.7 and 13.4 for adults and children, respectively. The same species of plants also recorded the highest Hazard index (17.4 and 27.2) for adults and children, respectively. This imply that consumers of the edible vegetables grown in Cr (VI) rich soils may be exposed to health risks, and the children are more vulnerable to these adverse effects.

The third objective of the study was to investigate the anaerobic bacterial profile of donkey dung assisted by anaerobic bioreactor and remediation of tannery chromium wastes. There was 99% removal efficiency of total chromium concentrations. This was observed in solutions

mixed with donkey dung in the bioreactors within 30 days of the experiments as compared to 60% in the control. Methane gas was generated in DB bioreactors under alkaline pH but not in acidic pH in BO bioreactors. Abundant bacteria phyla in BO samples were *Firmicutes* (48.28%) while *Proteobacteria* (51.70%) were prevalent in DB. The Bacilli (40.29%) class dominated BO when *Gammaproteobacteria* (20.44%) class was abundant in DB. The genus of *Delftia, Streptococcus, Staplococcus* and *Cutibacterium* dominated BO while the genus of *Enterobacter, Selenviibrio, Pseudomonas* and *Alcaligen* dominated DB. These microbes may have removed total Cr by either chromosol, plasmid, enzymatic, dissimilatory, persister cell and small colony variant through diverse metabolic mechanisms.

The last part of this study (not included in the body of the thesis but attached as Appendix A 1.0), characterised and preliminarily tested the performance of *Equus africanus asinus* dung on the sorption of synthetic Cr (VI) and real tannery effluents from DB and BO tanneries. The results of the adsorption study showed that when potassium dichromate solution was used as synthetic Cr (VI) wastewater and treated with donkey dung powder, there was a removal efficiency of 93.3% for Cr (VI). However, when the dung was applied to real tannery chromium effluents from BO and DB, the removal efficiency was found to be 83.6% and 85.5%, respectively. The adsorption data fitted better to the pseudo-second order kinetic model (R^2 = 1). Thus, the interaction of the cationic species with the donkey dung powder was predominantly via chemisorption.

In conclusion, this study has extensively elucidated on pollution potential and bioremediation of tannery-based chromium effluent wastes in BO tannery in South Africa and DB tannery in Kenya. The study has contributed comprehensively on how to assess ecological and health potential risk due to ineffective treatment of chromium effluent and indiscriminate dumping of their solid wastes within tanneries. Due to that, this study has for the first time advance and enhance existing knowledge on the eco-friendly bioremediation technique of tannery chromium effluent using raw organic donkey dung as a bio-stimulant source of naturally occurring microbes and an adsorbent to reduce high concentrations before discharge into the ecosystems.

Keywords: Adsorption of Cr (VI), Anaerobic bioreactors, Bioremediation technique, Ecological pollution, Edible vegetables, *Equus africanus asinus dung,* potential health risks, Removal of chromium.

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LIST OF ABBREVIATIONS

Ace- Abundance based Coverage Estimator

Al^{3+}- Aluminium III Oxide

ANOVA- Analysis of variance

AOAC- Association of official analytical chemists

APHA- American Public Health Association

As- Arsenic

ASTM- America standard test methods

ATP- adenosine triphosphate

ATR- Attenuated total reflection

ATSDR- Agency for Toxic Substances and Disease Registry

BCF/ BF - Bioconcentration factor

Beamhouse- pre-tanning stage (soaking, liming, unhairing, deliming, and bating)

BET- Brunau-Emmett-Teller

BJH- Barrett Joyner and Halenda

BO- Beit Ore (pseudo Bath Ore)

BO_{15},- 15 gm of dung

BO_{30},- 30 gm of dung

$BO_{c,}$ - 0-control

BOD- Biological oxygen demand

C/N - Carbon/Nitrogen

$Ca(OH)_2$ -Calcium hydroxide

Ca-Calcium

CaO- Calcium oxide

CBP- Chromium-binding proteins

CCA- Canonical Correlation Analysis

Cd- Cadmium

CD- Degree of Contamination

CDI- Chronic daily intake

CEC- Cation Exchange Capacity

CF- Contamination Factor

C_f - Toxic response factor

CH_3- Methyl group

CH_3COONH_4 - Ammonium acetate

Chao 1- Corresponding lower bound of species richness

CHR- Chromate ion transporter

chrBACF- Chromate gene cluster of BACF

CrT- Total chromium

ChT- Total chromium

COD- Chemical oxygen demand

COO- Carboxyl ion

Cr (III)- Trivalent form of chromium

Cr (VI)- Hexavalent form of chromium

Cr- Chromium

$Cr(OH)_3$- Chromium hydroxide

CRDs- Chromate-resistant determinants

CrO^- - Chromium oxide

CrO^{2-} - Chromium dioxide

CrO_4^{2-}- oxyanion chromate

Crust and finishing- Post tanning (Fatliqouring, dyeing and finishes)

CTLS- Chrome tanned leather shavings

Cu- Copper

CW- Cell wall

D- Dry

$DB_{15,}$ - 15 gm of dung

$DB_{30,}$ - 30 gm of dung

$DB_{c,}$ - 0-control

DB-Dogbone (pseudo Dongo Bonde)

DIC- Daily intake of chromium

DNA- Deoxyribonucleic acid

DPC- -Diphenylcarbazide

DSC- Differential scan calorimetry

DWAF- Department of water affairs and forestry

E- East

EC- Electrical conductivity

EDS- Energy-dispersive X-ray spectroscopy

EDTA- Ethylenediaminetetraacetic acid

EF- Enrichment factor

E^{i}_{R} - Ecological risk index

EMCA- Environmental management and coordination Act

EPA-Environment protection Agency

EPS- Exopolysaccharides

EXPS- Extracellular polysaccharides

FA- free ammonia

FAAS- Flame atomic absorption spectrophotometer

FAO- Food Agricultural Organisation

FDR- False discovery rate

FDI - Foreign direct investment

Fe- Iron

FEEM- Fluorescence excitation-emission matrix

FMN- Flavin mononucleotide

FTIR- Fourier transform infrared spectrophotometer

GC- Gas chromatography

H_2O_2 – Hydrogen peroxide

H_2S- Hydrogen sulphide gas

H_2SO_4- Sulphuric acid

HA- Humic acid

HCl- Hydrochloric acid

$HCrO_4^-$ - Anionic hydrochromate

Hg- Mercury

HMs- Heavy metals

HNO_3- Nitric Acid

HQ- Hazard quotient

HRI- Hazard risk index

HS- Humic substances

ICP-OES- Inductively coupled plasma Optical Emission Spectrophotometer

I_{geo} - Geo-accumulation index

ISO- International organisation for standardisation

K- Potassium

$K_2Cr_2O_7$ - Potassium dichromate

KOH-Potassium hydroxide

LDA- Linear discriminant analysis

LEfSe –Linear effect size

M- Molarity

Mg- Magnesium

$Mg(OH)_2$ – Magnesium hydroxide

Mn-Manganese

MQ- Millique

N- Nitrogen

Na- Sodium

Na_2CO_3- Sodium bicarbonate

NACOSTI- National Commission for Science, Technology and Innovation

NADP- Nicotinamide adenine dinucleotide

NaOH- Sodium hydroxide

NEMA- National environmental management authority

ngsShoRT- Next-generation sequencing short reads

NH- Amide group

Ni-Nickel

OH- Hydroxyl

OM-Organic matter

ORP- Oxidation-reduction potential

OTUs- Operational Taxonomic Units

Pb-Lead

PBS- Phosphate buffered saline

PCoA- Principal coordinates analysis

PCR- polymerase chain reaction

PDD- Powdered donkey dung

pH- Hydrogen ion concentration

pH-H_2O- Hydrogen ion concentration- water

pH-KCl- Hydrogen ion concentration-potassium chloride

PLI- Pollution load index

PRI- Potential ecological risk index

Pty- Private

QA/QC - Quality assurance/quality control

R- Rainy

R^2 – Coefficient correlation

RAIS- Risk Assessment Information System

RfD- Reference Dose

RoKGKNBS- Republic of Kenya Government, Kenya National Bureau of statistic

RoKGMoA- Republic of Kenya government, Ministry of Agriculture

ROS- Reactive oxygen species

RSD- Relative standard deviation

rRNA- Ribosomal Ribonucleic acid

S- South

S- Summer

SA-South Africa

SCV- Small colony variant

SDG- sustainable development goal of United Nations

SEM- Scanning electron microscopy

Si- Silica

SS- Suspended solids

SSA- Sub Saharan Africa

STATA – statistica analysis

Tanyard- Tanning stage (pickling, tanning, basification, neutralisation and retanning)

TCA- Tricarboxylic acid cycle

TDS- Total dissolve substances

TEs- Trace elements

TF- Translocation factor

TGA- Thermogravimetric analysis

TI_{PH}- Stress tolerance index for plant height

T^{i}_{r} - The contaminant agents

TN- Nitrogen content

TOC- Total organic carbon

TON- Total organic nitrogen

Total Cr- Total chromium

Tukey's HSD- Honest significant difference

UNISA- University of South Africa

USA- United States of America

USEPA- United States Environmental Protection Agency

UV Visible spectrophotometer- Ultraviolet Visible spectrophotometer

VFA- Volatile fatty acid

W – Winter

WHO-World Health Organisation

WRC- Water Research Commission

XOCs- Xenobiotic organic compounds

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LIST OF UNITS AND SYMBOLS

%G- Germination percentage

°C- Degree centigrade

°F- Degree Fahrenheit

27F- Forward primers

518R- Reverse primers

%- Percentage

mg/kg- Milligram per kilogram

mg/L -Milligram per litre

m^3- Metre cube

mS cm^{-1}- Micro-Siemen per centimetre

meq $100g^{-1}$- Milliequivalent one hundred per gram

µm- Micrometre

mg/day^{-1} – Milligram per day

Kg- Kilogram

mg- Milligram

g – Gram

ton- 1000 kilogram

mm/year- millimetre per year

m- Metre

cm- Centimetre

M- Molar

mm- Millimetre

mL- Millilitre

hr – Hour

Min – Minute

L- Litre

kW- Kilowatts

L/min^{-1} – Litre per minute

rpm – Revolution per minute

µL- Microliter

nm- Nanometre

mol L- Molar litre

mg/g – Milligram per gram

kP_a – Acid dissociation constant

L/mol – Litre per molar

L/mg - Litre per milligram

µl - Microliter

mg g^{-1} min^{-1} – Milligram per gram per minute

mg g^{-1} – Milligram per gram

t – Time

s- Second

bp- Base pairs

nts- Nucleotides

N- Normality

mV- Millivolt

µS/cm- Micro-Siemen per centimetre

Å- Angstrom

m^2/g^{-1}- Metre per square per gram

cm^3/g^{-1}- Centimetre per cubic per gram

CHAPTER ONE

INTRODUCTION

1.1: Preamble

This chapter presents comprehensive background, statement of the problem, motivation, the main objective, specific objectives, hypotheses and the assumptions of the study. It also gives the descriptive summary of the study areas such as location, climate, topography, altitude, soil, population, economic activities, and research outputs. Thesis outline has also been incorporated in this chapter.

1.2: Background Information

In the recent years, there have been expansion of leather tanning industries in Sub Saharan Africa. However, their expansions have been accompanied with pollution loads that are detrimental to the surrounding ecosystems, posing ecological and health risks. The current conventional methods of managing these wastes are proving to be ineffective. The world is also shifting to eco-friendly technologies like bioremediation. The most important principle of bioremediation is that micro-organisms, plants and animals can be used to destroy hazardous contaminants or transform them to less harmful forms (Gupta *et al.*, 2014; Saranraj and Sujitha, 2013). The technique is closely related to the concept of ecosystem.

An ecosystem is a dynamic complex of plants, animals, micro-organisms communities and their non- living environment interacting as a functional unit (Globalchange, 2017; Cleland, 2011). The biotic components of an ecosystem include producers, consumers and decomposers, while the abiotic includes the non-living parts like light, temperature, pH, minerals, among others. The functions of an ecosystem include the cycling of matter/nutrients and the transfer of energy. Energy transfer passes from the sun to primary producers to consumers and to decomposers in the food web. The transfer of nutrient is basically cyclical. The elements which form the molecules of which the organisms are made of remain in circulation when these molecules pass from one trophic level to the next (Globalchange, 2017; Cleland, 2011). The decomposers play a vital role in returning the elements from higher level to soil, water and air, from where these nutrients become available for use by primary producers. These two functions can be interfered with by factors such as ecosystem degradation, pollution and species extinct (Globalchange, 2017; Cleland, 2011). The most commonly encountered interference in the leather tanning industry is pollution load effects.

Tannery is one of the oldest industries that tan raw hides and skins into leather (Mwinyihija, 2010). The world leather production is currently running at between 21 and 22 billion square feet, according to the report of Food Agricultural Organisation (FAO, 2016). Today, about 3.5 million tons of various chemicals are used for leather production globally. On average consumption, 800-850 kg of solid wastes per ton of raw hides is discharged by the leather industry (Kanagaraj *et al.,* 2010). The raw hides and skins are tanned into leather using minerals, vegetables, oils, resins, aldehydes and other organic materials. Trivalent chromic sulphate is the most commonly-used mineral tanning agent. Typically, full chrome tanned leather contains between 2.5-5.5% of chromium by weight (Mwinyihija, 2010; Font *et al.*, 1998). However, poor uptake of 50-70% chromium tannin during commercial chrome tanning method results in material waste on the one hand and creates ecological imbalances on the other (Sivaram and Barik, 2019). Famielec and Ciurowa (2011), reiterated that, the disposal of chromium in tannery waste is so significant that about 30,000 metric ton per year are produced worldwide. Therefore, the environmental effect of such wastes goes beyond the potential contamination of the environment and calls for proper management. This is because, chromium wastes result in loss of bacterial populations in chromium-contaminated soils, impacts negatively on elemental cycling, inorganic remediation efforts, plant growth and soil structure (Renella *et al.,* 2005).

In the last three decades, environmental and public health impacts of chromium wastes from tanneries have been a subject of extensive scientific and technical discourse. There has been also, an increased interest in eco-friendly technologies to minimise the production and management of chromium wastes from leather industries to ensure process safety and environmental protection (Aftab *et al.*, 2017; Elumalai *et al*., 2014; Kanth *et al.*, 2009; Kim *et al*., 2013; Zuriaga-Agustí *et al*., 2015). It is estimated that Africa owns a fifth of the global livestock population, but only accounts for 4% of world leather production and 3.3% of value addition in leather (FAO, 2015; Silva and Gurría, 2016). However, with increased investment and improvement in institutional environment, there has been a sudden growth in Sub Saharan Africa (SSA) leather industry. There is an emergence of many tanneries in the region to gain increased benefit from processed leather commodities. Such trends are expected to increase as will be observed in future as many SSA economies transform their agro-processing sectors (Abtew, 2015; Kiraye *et al.*, 2019; Mwinyihija, 2015). In addition to that, from 1960s to date, many countries in Africa have tried to restrict the exportation of unprocessed hides and skins to promote exportation of more valuable, semi-processed and finished leathers (Mwinyihija, 2015; Favazzi, 2002). Despite the expected economic benefits from leather industry, the

nature and the magnitude of wastes discharged from leather manufacturing and processing are of great environmental and public health concern worldwide because of poor disposal.

Poor management and indicriminate disposal of tannery-based Cr (III) wastes end up exposing the public to the suspected effects of Cr (VI) (Mwinyihija, 2010). Some of the burnt wastes are believed to end up in water bodies through seepage, while others accumulate on the open lands near the dumpsites (Mwinyihija, 2012). Some native heavy metals in soil or those introduced to the dumpsite from tanning process, like iron and manganese, in high concentration, may cause oxidation of Cr (III) to Cr (VI). Sometimes these metals leach deep into the ground where they cause contamination to underground water aquifers. Groundwater samples collected near tanneries in recent years have shown the presence of arsenic, chromium, lead, and zinc (Ecopol, 2017). This poses health risk to the people who live and use these ecosystems to grow kales, maize, tomatoes, cassava, arrow roots, sugarcane, Napier grass and bananas, according to Mwinyihija (2010).

The available researched data from Aziz tannery dumpsites, sediments from Sagana river and effluent from tanneries in South Africa, have confirmed the occurrence of high concentrations of total chromium, according to Mwinyihija *et al*. (2006); Oruko *et al.* (2014); Swartz *et al.* (2017); Mwondu *et al*. (2019). However, a few of these studies have evaluated the presence of Cr (VI) in effluent, soils and plants. Therefore, there is need to evaluate the occurrence and levels of total chromium, Cr (VI), Cr (III) states and other selected metals in wastewater, soils and food crops/pastures from selected dump sites. This should also include their ecological and health risk in Kenya and South Africa as well as to find out the best possible bioremediation techniques for their reduction in the polluted environments. The removal of toxic and mobile Cr (VI) from effluents and polluted soils, will help achieve the United Nations sustainable goals numbers 3, 6, 9, 14 and 15 which advocate for the good health, clean water, sustainable industry, life below water and protection of terrestrial ecosystems by reversing land, water degradation and halting species diversification loss according to the United Nations sustainable development goals (SDGs) (2015).

1.3: Statement of the problem

The environmental impacts of tannery wastes containing wastewater, hazardous chemicals such as chromium, synthetic tannins, oils, resins, biocides, detergents and the careless disposal of solid wastes and gaseous emissions create a negative image of the leather industry, although the industry has a significant economic influence (Dixit *et al.*, 2014). Furthermore, environmental impacts of Cr (III) wastes from tanneries have also been a

subject of extensive scientific and technical dispute. This has made the leather industry to continue gaining a negative image in society with respect to its pollution potential. The leather industry is facing severe challenges, especially with chromium wastes (Sharma, 2012).

Tannery chromium waste products have the possibility of oxidising from Cr (III) to Cr (VI) under uncontrolled composites dumpsites. These sites produce various soluble compounds that are suspected to be capable of converting Cr (III) wastes into potentially dangerous Cr (VI) state, thereby posing a real threat to the ecosystems (Kolomaznix *et al.*, 2008). Worldwide, the amount of tannery- based chromium contaminated soils has risen in recent years. This has been occasioned by the predominant state in the tannery wastes which is assumed to be thermodynamically stable Cr (III) according to Cervantes *et al.* (2001). However, there have been detection of significant levels of toxic Cr (VI) in the soil, surface and underground water in different parts of the world. These detections raise critical questions relating to current management and disposal of tannery- based wastes containing Cr (III) (ATSDR, 2000). Chromium (VI) is known to pollute, degrade, bioaccumulate and persist in the environment (Sharma, 2012).

In a landmark judgment of 1996, the supreme court of India ruled that tanners in Tamil Nadu compensate owners of agricultural lands that had been allegedly damaged on account of untreated effluents containing Cr (III) wastes that were discharged into their land (Buljan, 2005). This was because, economically important plants, including medicinal plants and food crops grown in those lands, were found to be contaminated with high levels of Cr (VI) (Gupta *et al.*, 2007). The accumulation of pollutants, particularly Cr (VI) in plants have been reported to alter their growth, affect their food and medicinal values (Gupta *et al.*, 2007). The effects of Cr (VI) on the growth of maize in soil has also been reported to involve reduced growth and protein content (Chibuike *et al*., 2014). In addition, Cr (VI) was found to compete with various elements of similar electronic structure in the plants; hence, it seems that Cr (VI) has an advantage at the entry point into the plant all system (Shanker *et al*., 2005). It is also estimated that 40% of tannery workers in India have health problems because they are in constant direct contact with tanning chemicals such as chromium (Tarantola, 2014). A researcher from India explained that he had to cancel a research project, he wanted to carry on the effects of chrome as a cause of illness among tannery workers. This was due to pressure and resistance from the industry owners (Tarantola, 2014).

The United States Environmental Protection Agency (USEPA) has also classified Cr (VI) among heavy metals that are carcinogenic in human beings. It also degrades the ecosystem

where it is disposed (USEPA, 1997). Heavy metals like Cr (VI) cannot be biologically transformed to more or less toxic products and hence persists in the environment indefinitely (Manimita *et al*., 2015). Cr (VI) is also significantly toxic, even in trace amounts and can cause diseases in humans and animals as they cause irreversible changes in the body, especially in the Central Nervous System (Manimita *et al.*, 2015). In Bangladesh, most of the tanneries are located in Hazaribagh neighbourhood of the capital city Dhaka. The land area adjacent to these tanneries together with rivers that pass through them are heavily polluted with pollutants emanating from these tannery wastes. This has affected manual labourers who have worked in some of the tanneries all their life. They have developed chemical- caused skin diseases in their arms and hands. This was further confirmed by medical doctors practicing in the area, who reported that, between six and eight patients attending their clinic from tanneries suffer from skin diseases or asthma (Tarantola, 2014). Tanning pollutants are also suspected of causing allergies, bronchitis and pneumonia (Kolomaznik *et al.*, 2008).

In Italy and Sweden, 20-30 % cancer risk was found among tannery workers (Kolomaznik *et al.*, 2008; Mwinyikione, 2010). Other than being carcinogenic and mutagenic, Cr (VI) accumulation may lead to birth defects and a decrease in reproductive health (Manimita *et al.*, 2015). Cr (VI) poisoning can also result in a cute tubular necrosis of the kidney and death in individuals (Engwa *et al.,* 2019). Wastes containing Cr (III) are regularly dumped in open ground composite dump sites in tanneries in Kenya and South Africa.

This current management has been based on the assumption that Cr (III) wastes are stable in the soil and do not migrate to other ecosystems. Instead, these dumpsites are suspected to pose hazard to the underground water and human populations next to these areas (Mwinyihija, 2010). In Kenya, there is a sharp dispute between scientists, tanners and environmentalists concerning the occurrence of Cr (VI) in chromium effluent from tanning process and soils near open ground dump sites within tanneries. One school of thought argues that Cr (VI) can only be transformed under controlled heavy heating conditions, while the other argues that factors favouring its transformation exist in the effluent and soil at those sites. The most recently documented study carried out on tannery-based solid wastes management in Aziz tannery in Nairobi found that the total chromium levels in soil near such dump site was 2633.38 mg/kg above NEMA-set standard of 0.1 mg/kg (Oruko *et al.,* 2014). A study by the Water Research Commission (WRC) of South Africa also detected total chromium up to a maximum of 141.3 mg/L, above the 0.5 mg/L set limit by the South Africa Water and Sanitation Department, in different Cr treated wastewater (Swartz *et al.*, 2017).

To mitigate the disputes and reduce possible negative impacts of Cr (VI) from these dumpsites, there is a need to carry out further investigations. Since Cr (VI) persists longer in water and soil, in future, food crops/pastures grown near these dump sites might experience Cr (VI) accumulation at high proportions and cause health defects in humans. Thus, novel scientific interventions such as bioremediation technology are required to remove or reduce Cr (VI). Bioremediation is known to be cost-effective, environmentally-friendly and sustainable. This study, therefore, proposes a suitable bioremediation technology for total Cr removal in wastewaters and soils at these dumpsites.

1.4: Motivation

This study was motivated by the fact that all tanneries generate wastes during tanning process. In Kenya and South Africa; National Environmental Management Authority (NEMA) and Department of Water Affairs and Forestry (DWAF) classified tannery waste especially chromium waste as hazardous. This requires that chromium wastes are treated and disposed only to permitted landfill sites (DWAF, 1998 and EMCA, 1999). However, these designated sites are scarce, expensive to design, construct and manage. Besides that, most of them are located far from the tanning industries. The current management of tannery wastes involve environmental risk as tannery owners face increasing pressure from environmental activists, resistance from community living adjacent to those tanneries and dumpsites coupled with strict enforcement of regulations from the relevant authorities. The literature reviewed for the purpose of this study has also revealed that there are several management challenges with chromium wastes involving ecological and health risks in the sub Saharan Africa. The challenges still require extensive exploration and research of eco-friendly strategies to manage them.

Furthermore, several studies have been done globally on strategies to reduce chromium pollution from tanning industry (Thanikaivelan *et al.,* 2005; Xiahui, 2016). They include; complete or partial replacement of chromium as a tanning agent, use of alternative to chromium tanning, combination tanning process, use of minimal chrome, recycling and recovery of tannery chromium wastes (Kanagaraj et al., 2015; Thanikaivelan *et al.,* 2005). However, most of the leather industries in the sub Saharan Africa region are yet to embrace these strategies. A reported survey found that they are still tanning hides and skins using traditional methods of chrome tanning which release a lot of chromium waste sludges. In addition to that, 70% of them use physical and chemical treatment methods of lime precipitation, sun drying and dumping in illegal dumpsites. But, these treatments results are

not efficient because they are applied rudimentarily ending up with more pollution of the ecosystems. In the end, they are still increasing the challenges of managing these wastes according to Miller (2004). Therefore, sustainable management of these wastes are now required. This is supposed to be done by discharging and disposing treated and non-hazardous wastes to designated municipal sewers and secure landfill sites. This is expected to ease the burden of these wastes to already overburdened dumpsites such as Dandora in Kenya and Holfontein in South Africa.

The current practice of illegal discharge or open dumping and burning of chromium wastes within and without the tanning premise is unsustainable and unhealthy. It poses health risks to the workers and people living next to these tanneries. As companies try to adopt global standard of practice, some tanning industries in Kenya and South Africa are implementing ISO 14001 standards. ISO 14001 is based on continuous improvement of the environmental management system and compliance with all the relevant environmental legislation and stipulated principles of various Acts. By coming up with eco-friendly bioremediation techniques to reduce the toxicity of these wastes, the tanning facility commitment to sound and reasonable waste management is validated and confirmed.

This study is therefore required to aid tanning industries in sub Saharan Africa to find out where they are not fulfilling their duty of care as spelt out in their National Environment Management Acts, 1998, 2008, respectively (Act 107 of 1998; Act no. 59 of 2008 and EMCA, 1999). Our preliminary observation found out that some of the tanneries have not employed qualified personnel who can help them achieve these task as stipulated in the Acts. The successful implementation of duty of care in regular operation of the tanneries will prevent the current illegal disposal of potentially hazardous chromium wastes into the environment. Chromium wastes management is in line with good housekeeping. This is the management of chromium sludge and other solid waste disposal as everything has its right place and should be placed as a such. Chromium wastes is already a global concern that African tanneries must address, not only to avoid future challenges in marketing their leather products, but also to ensure process safety and environmental protection.

Lastly the health of workers and the environment are always “distant after-thoughts” for many tanners (Tarantola, 2014). In Kenya, there is growing public concern that cases of cancer are on the increase among the residents of Nairobi County and its environs. The sources of cancer are clearly not known (Ministry of Health, Kenya, 2017). In South Africa, there is an increasing environmental awareness of the need to reduce quantitative use of Cr (III) and recycle its

solids and effluents (Swartz *et al.,* 2017). In Kenya and South Africa some vegetables are currently naturally growing around such internal dumpsites, while others are grown nearby by tannery workers as food crops for consumption, as attached in Appendix Figure 1.1. Little is known if these could be one of the contamination pathways for Cr (VI) and possibly sources of increased cases of cancer in Kenya (Oruko *et al*., 2014). Therefore, there is still an urgent need to develop more eco-friendly clean-up technologies for discharged Cr (VI) wastes and their contaminated sites (Molokwane *et al*., 2008).

1.5: Main and specific objectives of study

This study is aimed at investigating the ecological and health risks of Cr status and other metals in tannery chromium effluents, soils and plants in selected tanneries dumpsites in Kenya and South Africa and develop appropriate eco-friendly bioremediation technologies.

The specific objectives include:

i) To investigate chromium status, other metals contamination and ecological risk of selected tannery waste dumpsites in Kenya and South Africa.

ii) To evaluate the potential health risk associated with edible vegetables grown on Cr (VI) polluted soils.

iii) To investigate the bacterial profile in donkey dung-assisted anaerobic bioreactor remediation of tannery chromium wastes.

1.6: Hypotheses

i) Chromium and other heavy metals wastes contamination increase potential ecological risks on soils and plants from selected tannery waste dumpsites in Kenya and South Africa.

ii) Selected edible vegetables that germinate and grow in Cr (VI) polluted soils pose high health risk to their consumers.

iii) The introduction of organic matter improves nutrient availability and introduces *ex situ* microbes that improve Cr bioremediation efficiency.

1.7: Assumptions in the study

i) Chromium bioavailability risk is dependent on the level of soil contamination.

ii) Donkeys are adapted to similar feeds and feeding habits, hence no significant difference in dung characteristics.

iii) The bacteria species resident in *Equus africanus asinus* gut and dung are similar.

iv) Weather and seasonal variation do not significantly influence the bioaviabililty of chromium in plants.

1.8: Study Areas

1.8.1: Location: - The study was conducted in two locations; Dogbone and Beit Ore tanneries in Kenya and South Africa, respectively. The Dogbone tannery is situated in the eastern part of Nairobi County and falls within Mowlem area, Embakasi west. It is at latitude 1°15'49.96''S and longitude $36^0$53''52.12''E as shown in Appendix Figure 1.2. Dogbone tannery is a private company. It is a semi processing leather industry that was started in the year 1986. It was first located in industrial area of Nairobi before relocating to its current location. DB mostly undertakes contract tanning up to wet blue meant for the export market in Asia and Europe, with occasional crust and finished leather operations. It does contract tanning for Alpharama Limited Company and small leather sellers. The small leather sellers, sell their crust and finished leather products to shoe cobblers, leather bags and other leather goods makers at Kariakor market in Starehe Sub County and from other parts of the country.

The tanning capacity of the Dogbone tannery is 12 tons of raw hides per day. The tannery generates unquantified amount of wastewater, green trimmings, lime fleshings, chrome sludge, wet blue trimmings, shavings and buffing dust. Wastewater from beamhouse (pretanning stage) and tanyard (tanning stage) are released into the oxidation ponds. Wastewater containing residual chrome sludge are then pumped into the raised tank. In the tank wastewater is precipitated with lime to form chromium sludge (chromium oxide cake). The sludge is then released into the drying beds while the semi purified water is released into the equalisation pond for further treatment. When the sludges are dried, they are packed into sacks and dumped into the composite dumpsite inside the tannery together with other solid wastes like green trimmings, lime fleshings, wet blue trimmings and shavings and the general wastes from the tannery (Appendix Figure 1.3).

Beit Ore (BO) tannery is located in South Africa. It is situated along Kopa road, Annadale surburb, Ladanna area, Polokwane town, Limpopo Province. It is within the Latitude of 23°52′03″S and Longitude 29°26′38.90″E as depicted in Appendix Figure 1.2. The tannery is a semi processing leather industry. It was started in the year 1982. It tans both domestic

and game animal hides with hair-on. They also buy “green hides” and preserve them using brine and wet salting methods. Some of these preserved hides are exported raw while others are processed into leather using chromium sulphate salt or combination tanning with either vegetable or synthetic tannin.

The tanning capacity of Beit Ore tannery is 1 ton of raw hides per day. The tannery generates unquantified amount of wastewater, green trimmings, wet blue trimmings, shavings and general wastes. BO tannery waste effluents containing residual chromium are recycled before being treated together with municipality wastewater at their wastewater treatment plant. However, the company is planning to construct their waste treatment plant as per the National Environment Management Act (Act 107/1998; Act no 59 of 2008) of South Africa. Currently, their recycled effluents are discharged into the municipal sewers’ lines. The green trimmings, wet blue trimmings and shavings are packed into large sacks and kept inside the tannery compound before being disposed outside the tannery to designated landfills.

1.8.2: Climate: - Embakasi area (Dogbone location) in Nairobi has a tropical climate with two rainy periods. The long rainfall is received between March - June and the short rainfall occurs in September- November. The mean annual rainfall ranges between 300 -1000 mm/year. The mean daily temperature ranges between 12 - 26°C. It is usually dry and cold between July and August, but hot and dry in January to Mid-March. The average monthly relative humidity varies between 36 - 55% (RoKGMoA, 2010; Ikaira, 2006). Despite its position on the Tropic of Capricorn, Polokwane town (Beit Ore location) climate is tempered by its position on a plateau 1230 m above sea level. Average temperatures reach around 21 – 22 °C (70– 72 °F) in January and fall to 11 °C (52 °F) in July. Polokwane has a dry climate with a rainy summer season and a pronounced dry winter season. The average annual rainfall is 495 mm (19.5 inches), with December or (less often) January being the wettest months and July the driest (Atlas of Southern Africa, 1984).

1.8.3: Topography, altitude and soil: - Since Embakasi lies on the Eastern side of Nairobi, it is generally low and flat. It has an altitude range of 1400-1600 m. The soil of the area is vertisol type (black cotton soil). It varies from deep to very dark grey to black firm and slightly calcareous with cracking clay in many places. The profile variation is composed of humic top soil and gravely calcareous deeper soil (Ikaira, 2006; RoKGMoA, 2010). In the Limpopo basin, where Polokwane is located, soils of the escarpment foot slopes and the lowveld itself are mixtures of Vertisols, Planosols, Solonetz, Lixisols, Luvisols, Phaeozems, Cambisols, Arenosols, Regosols and Leptosols. All these soils have a neutral or alkaline soil reaction, a

high base status and medium or high cation exchange capacity values. However, the textures and other properties, such as soil depth, colour and structure show a wide variation. Regosols (weakly-developed soils) dominate the lowveld between the eastern escarpment and the Lebombo range. Leptosols occur wherever the terrain is hilly (FAO-ISSS-ISRIC, 1998).

1.8.4: Population and Economy: - Human activity in Embakasi sub-county is varied and ranges from farming, trade to industrial activity. The total human population of the area is 376, 546, out of which 187,023 are females and 189,523 are males (RoKGKNBS, 2009). Polokwane's economy is essentially built on its function as a central service hub for the entire Limpopo Province, and to a lesser degree, for the neighbouring provinces and countries (Maluleke *et al.,* 2014). The South African census of 2011, showed the population of Polokwane City as 130,028 with 43,846 households. Black Africans were found to constitute 74.4%, coloured 3.7%, Indian/Asian 3.1%, white/ European 18.2% and others 0.6% (Atlas of Southern Africa, 1984; Maluleke *et al*., 2014).

1.9: Research outputs

This thesis has the potential to generate five peer reviewed articles, of which three has been published. A provisional patent has been filed from the outcome of this study. One related work has also been published. Other research output and the published articles are listed from sections 1.9.1 to 1.9.5.

1.9.1: Authored book chapters

Richard O. Oruko, John O. Odiyo and Joshua N. Edokpayi (2019). The Role of Leather Microbes in Human Health. DOI: http://dx.DOI.org/10.5772/ intechopen 81125.

R.O. Oruko, J.N. Edokpayi, H.J.O. Ogola, T.E. Volenzo, J.O. Odiyo (2021). Integration of sustainable development goals into leather tanning industries in sub-Saharan Africa. Sustainable Development in Africa: Fostering sustainability in one of the world's most promising continents https:// link.Springer.com/book/10.1007/978-3-3-030-74693-3.

1.9.2: Refereed /peer reviewed journal articles

R.O. Oruko, R. Selvarajan, H.J.O. Ogola, J.N. Edokpayi, J.O. Odiyo (2019). Contemporary and future direction of chromium tanning and management in sub Saharan Africa tanneries. Process Safety and Environmental Protection 133, https://DOI.org/10.1016/j.psep.*2019.11.013. 0957-5820/*©. 2020

Richard O. Oruko, Joshua N. Edokpayi, Titus A.M. Msagati, Nikita T. Tavengwa, Grace Ijoma and John O. Odiyo (2021). Investigating the chromium status, heavy metal contamination, and ecological risk assessment via tannery waste disposal in sub Saharan Africa (Kenya and South Africa) Environmental Science and Pollution Research Https://Doi.Org/10.1007/S111356-021-13703-1.

Richard Oruko Ongon'g, Joshua N. Edokpayi , Titus A. M. Msagati, Nikita T. Tavengwa, Grace N. Ijoma and John O. Odiyo (2020). The potential health risk associated with edible vegetables grown on Cr (VI) polluted soils. International Journal of Environment Research and Public Health, 17, 470; DOI: 10.3390/ijerph17020470 www.mdpi.com/journal/ijerph.

1.9.3: Related work in peer reviewed journal

T. Lukhele,H.J.O Ogola,R.Selvarajan, R.O. Oruko, H. Nyoni, B.B. Mamba, T.A.M. Msagati (2021). Metagenomic insights into taxonomic diversity and metabolic potential of bacterial communities associated with tannery waste- contaminated soils. International Journal of Environmental Science and Technology.http:/DOI.org/10.1007/s 13762-021-03298-y.

1.9.4: Peered review proceedings

R.O. Oruko, J.O. Odiyo and J.N. Edokpayi (2018). Bioremediation technology as a method for reducing soil pollution from tannery based chromium wastes in the sub Saharan Africa. Proceedings on ICSMNR Conference, 2018. ISBN 978-0-620-82267-1176. Pp 38-43. Edited by Edokpayi *et al*. (2018).

1.9.5: Conference presentation

R.O. Oruko, J.O. Odiyo and J.N. Edokpayi (2018). Bioremediation technology as a method for reducing soil pollution from tannery based chromium wastes in the sub Saharan Africa. ICSMNR08017. 15 -17th October, 2018, Bolivia Lodge, Polokwane, South Africa (Oral presentation).

R.O. Oruko, J.O. Odiyo and J.N. Edokpayi., (2018). Historical, contemporary and future direction of chromium tanning management: challenges and environmental legislations in sub Saharan Africa tanneries. ICSMNR08017. 15 -17th October, 2018, Bolivia lodge, Polokwane, South Africa (Poster presentation).

1.10: Thesis outline

The thesis has seven chapters in total. The first three chapters deal with introduction, literature review and methodology. The first chapter covers specifically background information, statement of the problem, motivation of the study, objectives and their hypothesis, the assumption in the study, description of the study areas and research outputs of the study. Chapter two presents extensive literature review which includes the relevant concepts, the scientific work that has been done in this field of study, the methods applied, the findings and implications. In addition, it covers the tannery wastes disposal challenges and put them in context by showing clear gaps currently existing in chromium wastes management in examples of sub-Saharan Africa tanning industries. Chapter three gives full details of all overarching methodologies used in this study, statistical analysis and the ethical statements related to it.

The above chapters are followed by results and discussion chapters written in paper formats with more details from chapter four to chapter six. Results and discussion of chapter four, and five are presented in combined format while results and discussion of chapter six are presented separately. Each of these follow proposed journal format. In principle, chapter four presents the full details on “Investigations on chromium status, other metals contamination and ecological risk of selected tannery waste dumpsites in sub Saharan Africa”, based on the hypothesis that “Chromium and other heavy metals wastes contamination increase potential ecological risks on soils and plants from selected tannery waste dumpsites”. Chapter five presents “The potential health risk associated with edible vegetables grown on Cr (VI) polluted soils”, based on the hypothesis “Selected edible vegetables that germinate and grow in Cr (VI) polluted soils pose high health risk to their consumers”.

Chapter six presents “Investigation of the bacterial profile in donkey dung-assisted anaerobic bioreactor remediation of tannery chromium wastes”, based on the hypothesis that “The introduction of organic matter improves nutrient availability and introduces *ex situ* microbes that improve Cr bioremediation efficiency”. Chapter seven, which is the last chapter gives the general conclusion and recommendations for future work. All the chapters have raw data presented in the appendices. The preliminary study on characterisation and test of a novel green adsorbent prepared from Equus africanus asinus dung for the removal of hexavalent chromium from tannery effluents”, based on the hypothesis that “Equus africanus asinus dung adsorbs synthetic hexavalent chromium and real tannery effluents is only attached as an Appendix A1.0 in this thesis.

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CHAPTER TWO

LITERATURE REVIEW: BIOREMEDIATION OF TANNERY- BASED CHROMIUM WASTES IN SUB SAHARAN AFRICA

2.1: Preamble

This chapter presents detailed literature review for this thesis. The review covers short introduction on tannery pollution, historical advances in tanning, comparison of tanning technologies, composition of tannery wastes, environmental and health impacts and current technologies used in tannery waste management and their limitations. More review touched on physico- chemical properties, ecological risk assessment and assessment of potential health risk, bioremediation using animal dung, bacterial interspecific Interactions, population adaptation, bacterial chromate resistance, enzymatic chromate reduction and the knowledge gap that informed this study.

2.2: Introduction

Pollutants from the tanneries affect the ecosystems and cause health problems to plants, animals, microbes and human inhabitants residing near the tanning industries (Oruko *et al*., 2014). Of particular interest is the, chromium wastes pollution within and without the tanning industries in sub Saharan Africa. They are becoming an environmental and health concern as depicted by Figure 2.1. Amin *et al*. (2013) adds that the mobility of Cr in the ecosystems is very complex and normally leads to pollution of the environment, thus the need for review to assess its impacts in sub Saharan Africa.

2.3: Historical advances of tanneries in Africa

The first tanning of leather in Africa is credited to *Australopithecus habilis* who was noted as wandering around East Africa some two million years ago (Infogalactic, 2016). He possessed a diet with substantial meat consumption. The skins he produced at that time were used to make shelters. The types of skins used in these structures were warmed by fire which created a curing effect by the smoke drying the skins gradually (Thomson, 2011). After that, before the coming of the Europeans, leather tanning in Africa was dominated by artisanal crude tanning practise which were full of trials and errors. The tanning of a hide or skin also took a long period of time to accomplish. With time, the art of tanning leather became a skill which was only possessed by a few gifted individuals, family or community (Siyanbola *et al*., 2012).

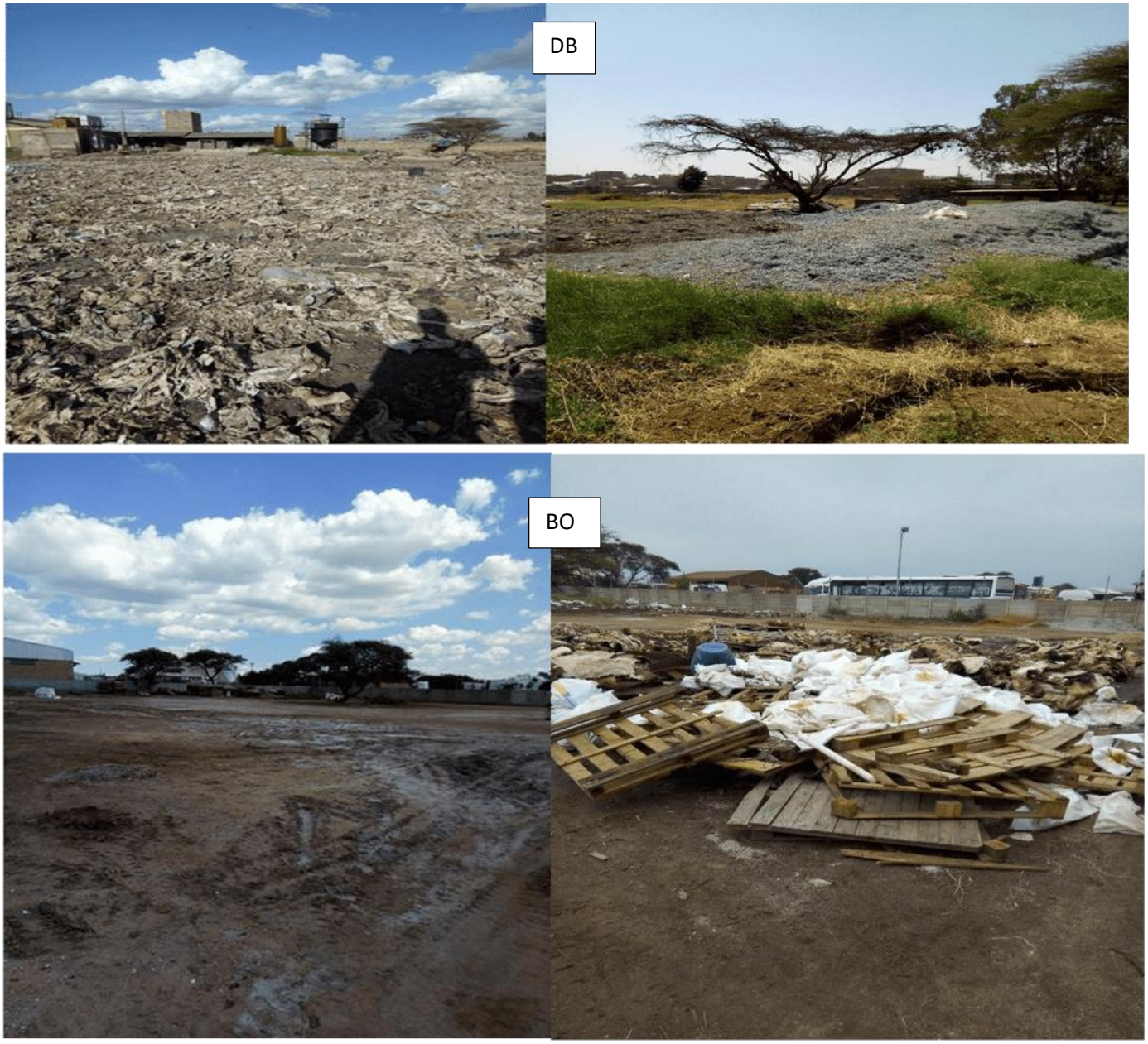

Figure 2.1: Chromium and other wastes dump inside Dogbone tannery in Kenya and Beit Ore tannery in South Africa

The technique and art of tanning were later passed on from one generation to another along the lineage. These craftsmen were using manual labour, simple earthen pots and pits. The pits were dug and located near rivers as source of water used to tan hides and skins using leached plants barks, leaves, soil paste and burnt ash (Brempong *et al.,* 2020). When the Europeans and Arabs came to Africa they contributed to the introduction of modern and mechanised commercial hides and skins trade and tanning technology. The tanning was

supplemented with manual labour, using chromium salt, sulphated vegetable and synthetic tannins. They were done with large cemented pits, vats and heavy rotating drums using electricity. This increased the exploitation and consumption of raw hides and skins and expanded leather processing in Africa during the colonial era (Umar *et al*., 2015).

Colonisation of Africa by Europeans in the nineteenth century provided increased access to raw materials for the European tanning industries as it coincided with their industrial revolution era (Azeta *et al*., 2016: Falcao and Araujo, 2018). This was the period, with a fast-increasing demand for leather and leather products in Europe. Exploitation of these raw materials exported into Europe later required installation of heavy tanning equipment's in Africa. This was to pre-tan the skins before export (value addition). This continued throughout most of the twentieth century (Mwinyihija, 2016). The rise of political resistance and independence struggle among emerging African young nations was later associated with the expansion of domestic commercial tanning and leather products manufacturing industries. The large tanneries were seen as political, social and economic priorities for the generation of employment, centres of national cohesion and creation of wealth for the indigenous inhabitants (China and Ndaro, 2016). Due to that fact, during the 1960s and 1970s, many newly independent countries in Africa tried to restrict the exportation of unprocessed hides and skins. In fact, most of them, promoted the exportation of more valuable, semi-processed and finished leathers using fiscal approach (Fitawek and Kalaba, 2019). This further led to the growth of more commercial tanneries with increased tanning capacity and installation of more advance and automated machines in the region. For that reason, by 2020, sub Saharan African countries have constructed and retained 175 tanneries, 650 leather goods companies and 850 footwear companies (ALLPI, 2020). Thus, African leather industry development can generally be categorised into three levels depending on the economic development status of a country: Advance/more developed economy – Egypt, Morocco, Tunisia and South Africa. Fairly/moderately developed economy – Eastern, North eastern and Southern Africa countries including Kenya, Ethiopia and Zimbabwe. Relatively under-developed economy – most of West African countries excluding Nigeria (Mwinyihija, 2015). Currently, the global leather market is shifting to eco-friendly tanning techniques which require sophisticated tanning equipment. It is likely that African tanneries will still undergo further technological transformation and improvement to maintain its global market and role.

2.4: Comparison of tanning technologies used in African tanneries and Developed countries

Many tanneries in Africa have installed tanning capacities ranging from 1 ton to over 40 tons per day. However, they face various challenges like lack of space to install waste management facilities. They also lack technical experience and economic means to build up individual wastewater treatment plants (Mwondu *et al.,* 2020). But their counterparts in developed economies have moved from single isolated locations to clusters design. This is known as leather parks and leather cities (Kiraye *et al*.**,** 2018). In a number of African countries, tanneries are situated in urban areas. Here, they use the competitiveness of such locations in terms of availability of resources like cheap labour, large market and quick ties with the international markets through exit ports (Carol and Page, 2017). With time, this kind of localisation has been found to be a limiting factor for the expansion development of these tanneries into modern standards. The end result is that, the quality of life of the inhabitants living near them have been adversely affected (Kibret and Tulu, 2014). For a number of tanneries in Africa, some feasibility studies have been made. This is to investigate the possibility of transferring them inside industrial parks. The upcoming industrial parks are planned to be located outside the urban areas. Currently, the cluster of industrial parks is practiced in developed countries like China, Italy and Spain. But the programme has not become operational in many countries in Africa except in Ethiopia, Egypt and Morocco (ALLPI, 2020; UNIDO, 2017).

From the industrial point of view, the types of tanneries found in Africa range from the automated and well-equipped ones to the small and outdated ones. They are known in leather industry as commercial and rural tanneries, respectively. Worse still, they are equipped with second hand machineries that breakdown frequently. In most cases they consume a lot of electricity which increases the cost of production. Those in the developed countries are now opting to install solar power plants to lower carbon footprint and mitigate climate change while moving towards electronic and digital automation with highly qualified labour force (Mwinyihija, 2015; Gupta *et al.,* 2018). Africa is also believed to have suffered a decrease of its stake of the global leather trade in the last twenty-five years of the 20th century. This was because of lack of latest technical knowledge (research) related to latest tanning techniques unlike developed countries (UNIDO, 2010). Currently in developed countries, they are moving away from conventional wet blue to wet white leather which is still common in Africa tanneries.

The development of leather tanning industry has also been adversely affected due to political changes and civil unrest in the region. Investors are not keen to invest in expensive heavy

equipment in the leather industry of most countries in sub Saharan Africa. This is due to frequent and unpredictable political unrest among other factors (UNIDO, 2010). Africa leather industries are also still faced with the use of second-hand footwear imports and counterfeit footwear from industrialised countries. These are undermining potential growth of the fledgling tanning industries in the region (Magezi, 2017).

An additional matter of concern is infrastructure and transportation. Relocating raw materials, chemicals, tanning equipment and other components of the industry from one African country to another and even within the same country, is still very problematic. This is due to poor roads and railway networks. This increases the price of hide and skin gathering in the field as well as the cost of transporting processed leather from inland to the ports for export to international markets (UNIDO, 2010; Mwinyihija, 2015). In the end they affect the competitiveness of leather produced in Africa at global markets. These hindering factors are minimised or lacking in developed countries (Lwesya, 2018). To date, the continent of Africa has also not done well in foreign direct investment (FDI) in the leather industry. This could improve their level of technologies to compare with their counterparts in the developed countries. At the moment, the most probable nation or group to lead such a change by investing in the sector are China and wealthy African expatriates. But it is not yet known if the two will invest in the tanning industries in Africa (Banga *et al*., 2015; Lwesya, 2018).

For a long time, most of the African tanneries have been producing semi-finished leather (in pickle or wet-blue). These are usually shipped to more industrialised countries like Spain, Italy, China and India for further processing and finishing (Kiraye *et al*., 2018). From 90s to date, the requirement of importer nations is to buy leather at a more advanced stage like crust or semi-finished products. The requirement of finished leather from the shoe industries of North Africa have also given a new push to tanning industry in the continent. The increase in demand of semi-finished to finished tannery products have further placed strain on the environment in the region. They have increased generation of wastewater and solid wastes containing chromium from the tanning process (Coetzee *et al.,* 2020). In order for Africa tanners to compete effectively in the global leather market, some major issues should be addressed. They include; scale of production, site, plant layout, skilled labour improvement and leather waste management. The others are training, research, installation and utilisation of modern equipment, information and communications infrastructure and participation in global value chains which developed countries have addressed (Banga and Willem te Velde, 2018). However, before implementing these recommendations, the contamination of the environment

with chromium wastes still remains the major problem. Thus, the chromium leather tanning industries in the region require urgent attention (China *et al*., 2020*;* Manimita *et al*., 2015).

2.5: Composition of tannery wastes

The leather tanning industry has the reputation of being one of the filthiest and vile smelling of all processing industries. Each operation in the tanning process generates unquantified amount of different types of wastes in Africa (Teklay *et al*., 2018). Each subcategory has its own processes and operations that vary to some extent from another subcategory. The two most polluted waste streams in a tannery are the beamhouse wastes and the tanyard wastes. Beamhouse wastes are highly alkaline and have high organic contents. They are the wastes with high tons of biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total dissolved solids (TDS). All these originate from beamhouse, which is a pretanning stage in leather processing. In addition to those, they also contain dirt, manure, fleshings, grease, residual hair, proteins and fatty oils (Liu *et al.,* 2020; Oruko *et al*., 2014).

The tanyard wastes are highly acidic and contain high concentrations of chromium, vegetable and synthetic tannins (Lofrano *et al.,* 2013). According to Dixit *et al*. (2015) chromium and other wastes are generated from tanyard, crust and finishing yard during the tanning process. The types/forms of wastes range from chrome sludge, splits, wet blue shavings, trimmings, crust trimmings, buffing dust and vegetable tanning extracts. Others are mineral acids, alum, sodium chloride, unfixed tan liquors and accidental spill over of chromium chemicals (Oruko *et al.,* 2013). But in most cases post tanning and finishing operations contribute to very low amount of neutral salts and COD. But, they are high in azo dyes, biocides, fat liquors, acid dyes, solvent coatings, pigments wastewater streams and traces of heavy metal pollution (Hansen *et al*., 2020). The waste streams are characterised by the occurrences of polymeric binders, heavy-metal-based pigments, solvents, nitrocellulose and other topcoat materials, as described by Ortiz-Monsalve *et al*. (2019) and Piccin *et al*. (2016). Oruko *et al*. (2014) explain that wastes quantities and treatments vary from one tannery to another. They depend on the level of housekeeping, technology employed, expertise available, legislation requirements for their management and disposal regulations of each country. These chromium wastes can be transformed in the soil and water by physico-chemical parameters. Thus the need to monitor and assess their impacts in such ecosystems.

2.6: Physico-chemical and heavy metals properties on tannery waste dumpsites

Tanneries are some of the agro-based industries that are fast growing in sub Saharan Africa region. However, they generate undocumented yet substantial amount of hazardous wastes. Islam *et al*. (2014) reports that nearly 30 m^3 of wastewater is produced when one ton of raw skin/hide is tanned. Such wastewaters are extremely polluted in terms of biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), conductivity, sulphate, nitrates, sulphide and chromium (Sawalha *et al*., 2019). In most emerging countries of sub Saharan Africa, tannery effluents are sometimes released straight into waterways without treatment (Terfie and Asfaw, 2015). According to Sugasini and Rajagopal (2015), effluents with high BOD content disturb the survival of beneficial microbes and gill breathing faunas of the receiving waterbodies. The high COD values also, confirm toxic state of the wastewaters. The above physico-chemical parameters are sometimes accompanied with occurrence of biologically resistant organic matters. The high level of ammonia-N in those effluents may also be toxic to a broad range of aquatic organisms. The nitrates presence may also cause long term eutrophication effects on receiving water bodies. The high salinity and total dissolved substances (TDS) in tannery effluent could also cause physiologically stressful environments for non-tolerant aquatic organisms. This is due to changes in osmotic conditions (Ugya and Aziz, 2016).

Other reported studies indicate that increase in salinity causes changes in biotic communities. They also limit biodiversity, eliminate less tolerant species and cause species loss in some ecosystems. Variations in the ionic composition of water can result to alien species invasion of some unique ecosystems (Chamier *et al*., 2012). The spills or releases of tannery pollutants into the environment also increase the total carbon content presence in the soil (Moses *et al.,* 2016). However, when tannery effluents are treated with conventional methods, they form large bulk of sludge. These sludges are normally dried on drying beds inside these tanneries. Later on they are packed in polyethylene sacks and dumped in tannery dumpsites. But, one of the major emerging environmental challenge in the tanning industry in the globe and more so in sub-Saharan Africa (SSA) is the inappropriate dumping of chromium contaminated sludge. They are generated as a by-product of wastewater treatment. At the composite dumpsites these sludge wastes release leachates which contaminate the ecosystems further.

Leachate is a hazardous liquid that originates from solid waste when it comes into contact with water. Tannery dumpsite leachate is a major cause of soil pollution. They are caused by the occurrence of chemical wastes or other alteration in the natural terrestrial environment (Webler

et al., 2019). Generation of leachate from unofficial landfill (dumpsite) in SSA is a compound mixture of physical, chemical and biological processes (Segundo *et al.,* 2020). Leachate discharge causes serious environmental problems such as percolation through subsoil. They, then cause pollution of ground and surface water resources through run-off and infiltration. The risk of groundwater pollution is a severe environmental impact from dumpsites in tanneries. This is because, most dumpsites are without engineered liners, leachate collection or treatment systems (Magaji, 2020; Idowu *et al.,* 2019).

In composite dumpsites, leachate can be divided into four groups. Leachate could be grouped as containing organic and inorganic compounds, xenobiotic organic compounds (XOCs) and/or heavy metals (Zhao *et al*., 2018). They can also be classified according to the nature of hazardous substances present in them (Matejczyk *et al.*, 2011; Vaccari *et al*., 2019). These pollutants from the tanning industries can be detrimental to the health of living organisms and the surrounding in general (Moses *et al.,* 2016). For example, hazardous XOCs and heavy metals do bio accumulate and persist in the ecosystems. In addition some of them can be toxic, corrosive, flammable, reactive, carcinogenic, teratogenic, mutagenic and ecotoxic (Krstev *et al., 2012*). Therefore, heavy metals like chromium are some of the typical components and essential parameters for the characterisation of tannery waste leachates. Thus, in recent decades, monitoring of heavy metals in tannery dumpsite leachate affected sites has commonly been prescribed by the municipal and environmental authorities. But, they are never routinely performed by dumpsite operators in SSA (Oduro-Appiah *et al.*, 2013; Morita *et al*., 2020).

Ohwoghere–Asuma and Aweto (2013) report some studies that found that leachates seepage and percolation are sources of groundwater and surface water pollution in the neighbourhood of landfill sites. Consequently, unofficial landfill in SSA tanneries could be constituting potential health hazards and environmental problems. In spite of these glaring negative effects of landfills (dumpsites), they remained the cheapest and most widely accepted methods. This is for temporary disposal of tannery wastes in most areas of the world including SSA (Sabour *et al.,* 2020). Moses *et al.* (2016) reports that, different researchers have carried out studies on dumpsites leachate pollution of soil. They concluded that heavy metals concentrations are always higher than the recommended standards. It is also suspected that leachate from these unengineered landfills are leading to contamination of nearby soils. This in turn, leads to contamination of groundwater and poisoning of the food chain (vegetables, fruits and tubers) around such sites (Longe *et al*., 2010).

Heavy metals pollution in kitchen garden is now becoming one of the worldwide challenges. They challenge food production and the long term maintance of life in urban centres (Sam and Taylor, 2014). The pollutants accumulated in plants not only interfere with the growth and quality of crop yields but also threaten the health of consumers (Wortman and Lovell, 2013). The most common heavy metals pollutants are Cd, As, Cr, Cu, Hg, Pb, Ni, and Zn. However, some of these metals such as Zn, Cu, Mn, Ni, and Co are micronutrients essential for plant growth. But, others such as Cd, Pb, As, and Hg have no recognised biological functions and are very lethal to human health (Govind and Madhuri, 2014). Therefore, there could be a need to evaluate the quality of soils around tannery dumpsites in SSA. Such undertaking could provide information on the concentration of heavy metals and contamination of surrounding soils. For that reason, Mofor *et al*. (2017) proposed that physico-chemical and heavy metals properties of such adjacent soils need to be regularly monitored, determined and correlated to one another. When the soil physico-chemical condition is acidic, the presence of nitrogen, phosphorus, and potassium is decreased (Tale and Ingole, 2015). This is probably due to the fact that at low pH values, oxides and hydroxides of iron and aluminium become soluble and tend to fix these elements in soils (Njoyim *et al.,* 2016b). If these nutrients are not immediately taken up by plants, they may leach into deeper soil horizons (Kramer and Chadwick, 2018). The Electrical conductivity (EC) value as a physico-chemical property reflects the quantity of soluble salts in soil. Thus, it provides an indication of soils levels of salinity. Low values of electrical conductivities are indicators that soils are good for plant growth (Horneck *et al*., 2011).

If a soil has high Carbon/Nitrogen (C/N) ratio, then that indicates that the organic matter (OM) content as a physico-chemical property was poorly mineralised (immobilisation was highly favoured) (Tsozue *et al*., 2016). Soils with high C/N ratios could slow down decomposition rate of OM and organic N. This is, by restraining the soil microbial activity's ability with lesser mobilisation of N. Soils with low C/N ratio on the other hand, do hasten the process of microbial decay of OM and N (Zechmeister-Boltensternnyma *et al*., 2015). Cation Exchange Capacity (CEC) as a physico-chemical property of soil can begin from below 5 meq $100g^{-1}$ in sandy low organic matter soils to above 15 meq $100g^{-1}$ in fine textured soils and those high in organic matter. Low CEC soils are more prone to cation nutrient loss through leaching (Spargo *et al*., 2013). Base saturation is the percentage of the soil CEC that is composed of basic cations (calcium, magnesium, potassium, sodium). Soil with low pH and basic cation value of below 50%, might show acidity in nature (Njoyim *et al.,* 2016b).

Soil texture is also another significant physico-chemical parameter. It affects water and nutrient holding capacity, drainage, aeration, vulnerability to compaction, irrigation, planting practices and erodibility of a given soil (Jindaluang *et al*., 2013). For example, coarse-textured soils such as sand, loamy sand or sandy loam, have low water holding capacity, releases water quickly and are limited in nutrients, especially nitrogen and potassium. Medium-textured soils usually have good drainage, water and nutrient holding capacity. Fine textured soils such as clay loam and clay, have high water and nutrient holding capacity. However, they are usually poorly drained and are difficult to manage when wet (Mofor *et al*., 2017; Jindaluang *et al*., 2013). Closely related to physico-chemical parameters in dumpsite soils is the heavy metals occurrence.

The occurrence of heavy metals in soils above stipulated guidelines of WHO (2004) are considered ecological and health risks (Marcus *et al*., 2017; Olayiwola *et al*., 2017). For examples the permissible limit set for Cr in farm soil by FAO/WHO (2011) is 80 - 100 mg/kg. In excess amount, it promotes specific diseases. An extensive distribution of Cr in environmental media (soil, water and air) is unfavourable for humans and animals. Chromium toxicity also depends on its oxidation status. Cr is basically found in two stable states in soils.

The oxyanion chromate CrO_4^{2-}, is highly mobile and more toxic in soils and groundwater. In the opposite, the reduced ion Cr (III) forms either a weakly soluble hydroxide or stable compounds with soil minerals in a range of ecosystems (Vodyanitskii, 2012). Zinc is one of the trace elements that play a vital role in the physiological and metabolic processes of numerous living organisms (Ruqia *et al.,* 2015; Yabe *et al*., 2010). It plays an important role in protein synthesis. The metal also displays fairly low concentration in surface water due to its limited mobility from the place of rock weathering or from natural sources. The permissible limit set by FAO/WHO (2011) is 100 - 500 mg/kg. Higher concentrations of zinc can be toxic and fatal to plant and animals (Manivasagaperumal *et al.,* 2011). Copper can accumulate in liver and brain. Its toxicity is a fundamental cause of Wilson's disease. Copper particulates are discharged into the atmosphere by windblown dust, volcanic eruptions and anthropogenic activities. Others are tanning industries, copper smelters, ore processing facilities, pig manure and sewage sludge (Panagos *et al.,* 2018). The maximum permissible limit set by FAO/WHO (2011) is 45-100 mg/kg. The high concentrations of total and available Mn metal in the environment could be from the soil parent constituents (basalt, trachytes and rhyolite). These are natural sources of Mn in the soil or from anthropogenic sources. If the soils are very acidic, Mn solubility will highly be favoured leading to high available concentrations. The maximum permissible limit set by FAO/WHO (2011) is 250-850 mg/kg (Shah *et al*., 2013).

Very high levels of total and available Fe in the soil are likely to be from the soil parent materials (basalt, trachytes and rhyolite).They are considered natural sources of Fe in soil. Strong Fe concentrations can be observed depending on distribution along the soil horizons. In very acidic soils, Fe solubility is highly favoured leading to high available concentrations. Surplus amount of Fe in the human body (more than 10 mg/kg) causes rapid increase in pulse rate, coagulation of blood in blood vessels, hypertension and drowsiness (Al-Fartusie *et al*., 2017). The maximum permissible limit set by FAO/WHO (2011) is 1000- 47000 mg/kg.

There has been a lot of attention directed to Pb levels in soil in recent years. This is because, it is recognised to cause adverse health effects. It is also widely distributed as a result of its past use in many commercial products, from gasoline to paint and dyes. It accumulates with advance in years in bones, aorta, kidney, liver and spleen. It can enter the human body through uptake of food (65%), water (20%) and air (15%) (Nakayama *et al.,* 2019). The permissible limit set by FAO/WHO (2011) is 20- 56 mg/kg. Nickel is an essential trace element for human and animal health (Poonkothai and Vijayavathi, 2012). The maximum permissible limit set by FAO/WHO (2011) is 50 - 68 mg/kg (Marcus *et al*., 2017; Olayiwola *et al*., 2017). Lastly, intake of inorganic arsenic for a long time can lead to chronic arsenic poisoning (*Arsenicosis*). It is also considered to cause skin lesions, peripheral neuropathy, gastrointestinal symptoms, diabetes, renal system effects, cardiovascular disease and cancer. Some can take years to progress depending on the level of exposure (WHO, 2010). The maximum permissible limit set by FAO/WHO (2011) is 0.5-50 mg/kg (Olayiwola *et al.,* 2017: Ramirez- Andreotta *et al*., 2012).

The overall content of heavy metals in soil comprises even inert (usually silicate) form of heavy metals. Some do not have toxic effects on plants, human, other animals and soil biota (Vodyanitskii, 2016). Therefore, in environmental studies more attention are paid to only potentially toxic compounds of heavy metals with toxic reference concentrations (Suresh *et al*., 2011). Variation of heavy metals in soils also relies on weather conditions and above all, the amount of rainfall and soil moisture content of a given area. The same soil can have significant differences in the concentrations of mobile forms of heavy metals in a year. Such strong differences in the contents of mobile forms of heavy metals could be ascribed to actions of soil micro-organisms. Others can be rhythmic changes in chemical elements uptake by plants and other environmental factors (Hamel *et al.,* 2010). Thus, to use mobile forms of heavy metals for soil pollution assessment, Vodyanitskii (2016) advances an argument that; it is important to standardise the procedure for selection and collection of a soil sample. That is coming to an agreement that, in what period of the year should soil sampling be done?

Vodyanitskii (2016) sums up the argument that, it is best to select and collect a soil sample in the rainy season when soil moisture is maximal rather than in the hot and dry season when the moisture content is low (Zhang *et al*., 2011). Thus, the need to further understand, the relationship between physico-chemical parameters and metal occurrence in soils at tannery dumpsites.

A number of reported studies on physico-chemical properties of most analysed soils have shown that correlations ($p < 0.05$) can exist between determined parameters (Tsozue *et al.,* 2016). For example, pH-H_2O and pH-KCl was found to correlate negatively with exchangeable acidity. This result showed that an increase in soil pH decreases acidic cations (H^+ and Al^{3+}) on the soil colloid. This is by favouring the presence of metal elements. Organic matter was shown to correlate negatively with electrical conductivity. This suggests that soils high in organic matter tend to favour the immobilisation of metal elements (Angelova *et al.,* 2013; Osobamiro and Adewuyi, 2015). Total nitrogen and pH-H_2O have been observed to correlate positively with one another. Such relationship suggested that when soil pH was increased, it increased the mobilisation of soil nitrogen, thus making it available in soil. Cation exchange capacity correlated positively with the sum of exchangeable bases. Clay was shown to correlate negatively with pH-H_2O and CEC. All these correlation results demonstrated that relationships that exist between soil physico-chemical properties interfered with metal elements availability in soils (Nannoni and Protano, 2016). Studies on metals analysed in soils have also showed significantly strong positive and negative correlations with one another ($p < 0.05$). The positive correlation between these metals at any dumpsite shows that there can be an interaction among these metals in any studied composite dumpsites. They also point at a possibly similar origin. A strong correlation between two variables or metals may be an occurrence of strong dependence of both variables on the same causal factor probably due to their common derivation from the waste complexes (Dai *et al*., 2019).

Syed *et al.* (2012) reiterated that, adverse pollution in the soils at tannery dumpsites could be connected with indiscriminate dumping of large quantities of Cr and other solid wastes from tanning processes. For instance, Cr is used in tanning skin into leather. Since the Cr metal is not degradable, their accumulation in the soil above their toxic levels due to poor disposal could be poisonous for crops and other biota (Das *et al.*, 2020). Excess Cr not only bring toxic effect on human and animals but also decreases the productivity of the soils at such dumpsites. Soil pollution lowers soil fertility, reduces nitrogen fixation and contributes to larger loss of soil and nutrients. It can also reduce crop yield and increase runoff into rivers and thus kill fish. It may poison workers in the area and cause unevenness in soil fauna and flora (Toth

et al., 2016). This poses ecological risks worth monitoring and assessing from time to time in soil and plants.

2.7: Ecological risk assessment due to Cr and other heavy metals contaminate at tannery dumpsites

Soil pollution with Cr and other metals contaminants are normally attributed to anthropogenic sources (Chen *et al*., 2015). Soil can be classified as contaminated when Cr and other metals concentrations in their bulk horizons surpass baseline values. It is normally taken as contamination threshold for non-contaminated soils (Santos-Frances *et al*., 2017). Soil pollution by Cr has undesirable effects on food safety and can result in amplified health risks (Suresh and Nagesh, 2015). Soil pollution with Cr and other heavy metals is a grave environmental concern because of their harmfulness and ability to accumulate in the biota (Ali *et al.,* 2019). Syed *et al*. (2012) explained that, there is always a need to assess the extent of Cr and other heavy metals pollution of dumpsite soils. In leather industries, monitoring and assessment of soil in the neighbourhood of tannery dumpsites has become necessary. It can be done in both dry and wet periods using diverse indices. It is reported in literature and observed that tannery workers grow edible food crops near such sites. It is therefore important to investigate bioaccumulation of heavy metals from the tannery wastes sites by these edible food crops.

Pollution indices can be used to evaluate ecological risk of Cr and other heavy metals contamination of the environment (Salman *et al.,* 2019). Pollution indices are tools for quality assessment. The two frequently used pollution indices for heavy metals in soils are single and integrated pollution indices (Ma *et al*., 2018). One of the first single pollution index is the geo-accumulation index (Igeo). This index actually helps in the assessment of pollution by comparing the present and pre- industrial levels. Initially it was used with bottom sediment deposits. Nonetheless it can similarly be modified and applied for the assessment of soil pollution in dumpsites. The technique assesses the degree of metal pollution based on seven enrichment classes (i.e. uncontaminated, moderately uncontaminated; contaminated; moderately contaminated; strongly contaminated, strongly/extremely contaminated and extremely contaminated). They are dependent on the increasing numerical values of the index. It was developed by Muller in 1969.

Enrichment factor (EF) of heavy metal concentration is another valuable tool to evaluate natural and anthropogenic sources of soil heavy metal pollution of the environment. The EF values are classified into five groups; deficiency to minimal (EF < 2), moderate (2 < EF < 5),

significant (5 < EF < 20), very high (20 < EF < 40), and extremely high enrichment (EF > 40) (Ololade, 2014; Chandrasekaran *et al.*, 2015). Contamination Factor (CF) developed by Hakanson in 1980 is also a single element index used for the assessment of soil contamination. It has four classes (Low contamination, Moderate contamination, Considerable contamination and Very high contamination).

Contamination degree (CD) is an integral index. It is the sum of the CF for the pollutant species according to Hakanson (1980). The CD is aimed at providing the degree of total contamination in surface layers in a specific sampling site. It is divided into four groups (low degree of contamination, moderate degree of contamination, considerable degree of contamination and very high degree of contamination). Pollution load index (PLI) is another integrated index conceptualised by Tomlinson *et al*. (1980) for detecting pollution. The tool makes it possible to compare pollution levels between sites and at different temporal scales. The PLI is a concentration factor of individual heavy metal with respect to the background value in the soil. In some studies, different authors use the global mean levels of the metals studied for shale as the background for heavy metals.

The PLI can be used to give an approximate index of the metal pollution status and also provide the required action that should be taken in an area (Sam *et al.,* 2015). A PLI value of ≥ 100 indicates an immediate intervention to mitigate pollution. A PLI value of ≥ 50 indicates a more detailed study is needed to monitor the site. Lastly a value of < 50 indicates that drastic rectification measures may not be necessary (Ihedioha *et al.,* 2017). Hakanson, (1980) developed the potential ecological risk index (E^ir) tool. It is extensively used in ecological risk assessments of heavy metals in sediments and soils. The subsequent tiers are used for the E^ir value: i) $E^ir \leq 40$ (low risk); ii) $40 \leq E^ir \leq 80$ (moderate risk); iii) $80 \leq E^ir \leq 160$ (considerable risk); iv) $160 \leq E^ir \leq 320$ (high risk); v) $320 < E^ir$ (very high risk) (Liu *et al*., 2018; Tian *et al.,* 2017; Hakanson, 1980).

In order to get comprehensive and accurate evaluation results of contaminated sites, a number of studies have combined the above risk methods to evaluate the potential ecological risk (PRI) of heavy metal pollution in soil. According to Devanesan *et al.* (2017), PRI can also be used to measure the overall characteristics and environmental behaviour of heavy metals contaminants in the dumpsite's soils. The main function of this index is that it can be used with the toxic-response factor (T^ir) to indicate the contaminant agents and where contamination studies need prioritisation. The PRI is normally applied to assess the degree of heavy metal pollution in soils (Suresh *et al*., 2012). It determines hazardous nature of heavy metals and

the response of the environment. Thus, PRI is calculated as the sum of all risk factors for heavy metals in soils. $E^i r$ is the monomial potential ecological risk factor. The C_f is taken as the contamination factor, and $T^i r$ is the toxic response factor representing the potential hazard of heavy metal contamination. This indicates the hazardous nature of specific heavy metal and the environmental sensitivity to pollution (Soliman *et al.,* 2015). Jiang *et al.* (2014); Soliman *et al.* (2015) and Devanesan *et al*. (2017) proposed that the relationship among PRI, $E^i r$ and pollution levels can further be expressed using the adjusted grading standards (Table 2.1).

Table 2.1: The adjusted grading standards of potential ecological risk of heavy metals in soils

$E^i r$	Pollution degree	PRI	Risk level	Risk degree
$E^i r \leq 30$	Slight	RI < 40	A	Slight
$30 \leq E^i r \leq 60$	Medium	40 ≤ RI≤ 80	B	Medium
$60 \leq E^i r \leq 120$	Strong	80 ≤ RI ≤ 160	C	Strong
$120 \leq E^i r \leq 240$	Very strong	160 ≤ RI ≤ 320	D	Very strong
$E^i r \geq 240$	Extremely strong	RI > 320	-	-

Jiang *et al*. (2014).

When PRI shows sites having any slight to very strong risk degree, then there is a need to study effects of contaminants on plants that can grow in those sites. For example, a high level of chromium and other heavy metals in the soil at dumpsites have been found to injure plant life (Tahar and Keltoum, 2011). This is because, they reduce the protein contents, inhibits the enzymes activity and cause chlorosis and necrosis in plants (Maleki *et al.,* 2017; Tchounwou *et al.,* 2012; Stambulsk *et al*., 2018). Chromium toxicity inhibit several metabolic processes in plant. They cause reduced seed germination or timeous seedling growth (Asati *et al*., 2016). Its occurrence in surplus quantity inside the plant can cause stunted growth (Mathur *et al.,* 2016). The presence of chromium in soil also disrupts the pattern of nutrient uptake in plant because of nutrient metal interaction (Sharma *et al*., 2020). The quantification of germination and growth of plants in such conditions can be done using germination percentage and stress tolerance index for plant height (TIPH). Akinci and Akinci (2010) explain that, the germination percentage is the proportion, expressed as percentage of germinated seeds to the total number of viable seeds. Stress tolerance index is also a useful tool for determining the high yield, height and stress tolerance potential of growing plants (Wilkins, 1978).

The risk of such ecologically unclean soils is contamination of the food chain (Amin *et al.,* 2013). When plants especially, edible vegetables grow on such polluted soils, they become likely threats to human and animal health. Plants may also have their growth abruptly reduced by high levels of noxious elements in their tissues. This causes a decline in crop yields and economic loss to farmers (Eze *et al.,* 2018). The uptake and circulation mechanisms of Cr in vegetative and reproductive parts of different plants are not yet entirely understood. This is because, the mechanism of Cr and other metals uptake and translocation in plants varies with passage of time (Hayat *et al*., 2012). Plants with potential to take up metals are known as phytoremediators (Andreotti *et al*., 2015). In phytoremediation, a plant can be an accumulator, excluder or an indicator according to the concentrations of metals found in its tissue (Badr *et al*., 2012). Harnessing the phytoremediation potentials of plants is presently being explored, however, the move has been criticised due to the concern that this might lead to health challenges and food shortage. In the developing countries with most plants serving as either edible vegetable or medicinal plants, this is a major concern (Olowoyo *et al.,* 2012).

Phytoremediation potential is considered, when the plant under study is able to bioaccumulate and translocate the pollutants into its different tissues at quantifiable proportions (Malik *et al*., 2010; Natasa *et al*., 2015). The abilities of plants to accumulate metals are referred to as the bioconcentration factor (BCF) and translocation factor (TF), respectively. The BCF is the ratio of metal concentration in the roots to that in the soil or water. TF is the ratio of metal concentration in the shoots to that of the roots (Mirecki *et al*., 2015). Plants are categorised as phytoextractor when TF > 1 and as phytostabiliser when BCF > 1 and TF < 1, respectively (Egendorf *et al.,* 2020). The chances of ecological risk causing health risk at tannery dumpsites needs further evaluation and explanation of risk assessment tools for long term monitoring.

2.8: Environmental and health impacts of tannery chromium wastes disposal in Africa

Tannery chromium waste enters the environment through human activities. Each ton of processed leather produces more than 0.12 kg of Cr pollution to the ecosystems (Fei and Liu, 2016). Leather tannery effluents and open dumpsites are choked with many tanning chemicals like chromium. The chemicals have direct effect on the upstream (discharge point) and downstream (receiving point) treatment processes (Zinabu *et al.*, 2018; Wosnie and Wondie, 2014). The sub-standard quality and poor management efficiency of many tanning equipment increase the risk that makes chromium waste reach terrestrial and aquatic ecosystems. Chromium can affect the air quality through buffing process, which in the long run can lead to water or soil contamination. These damages both biotic and abiotic components of

ecosystems. Studies have demonstrated the pollution of terrestrial aquifers such as lakes, wetlands, rivers, and streams started by tannery chromium effluents (Burbridge *et al*., 2012; Montalvão *et al.*, 2018). Those effects of tannery chromium effluents on aquatic organisms have shown teratogenicity in *Paracentrotus lividus* and *Sphaerechinus granularis* (sea urchin species). They have decreased growth in green microalgae *Selenastrum capricornutum* (Ke *et al.*, 2010). Other consequences of tannery chromium effluent pollution effects comprise genotoxicity in onions (*Allium cepa*). Hazardous effects were in *Daphnia magna, Ceriodaphnia dubi*a and *Hyalella azteca* and mutagenic activity in *Salmonella*/microsome (Bakshi and Panigrahi, 2018).

In vertebrates, detrimental effects from exposure to water containing tannery chromium effluents have been detected in fish (Aich *et al*., 2015; Nagpure *et al*., 2015), birds (de Souza *et al.*, 2017) and mammals (da Silva *et al.*, 2016; Guimarães *et al.*, 2017; Rabelo *et al*., 2016; Siqueira *et al.*, 2011). Recent study by Montalvão *et al*. (2018) established that tannery chromium effluents had a negative effect on amphibians in both pre- or post-metamorphosis life cycle. At this point, hexavalent chromium is considered carcinogenic only to animals in certain conditions. However, total chromium is presently not classified as a carcinogen and is fairly unregulated. According to World Health Organisation (WHO), the typical chromium levels in most fresh foods should be low. But, in recent years, chromium compounds have been found in vegetables, fruits, grains, cereals, eggs, cheese, brewer's yeast, organ meats, molasses, nuts, certain spices and fish. The levels have been in the range of 20 and 520 µg/kg (Edlira *et al*., 2019).

Consequently, these concentrations are above the average daily dietary intake requirements for human beings. It is common practice in Africa, where planting food crops such as kales, spinach, banana, sugarcane and maize are done next to internal dumpsites of tanneries. Likewise, during the dry period, farmers who practice urban dairy farming access such places to secure grass for their livestock. A study carried out by Oruko *et al.* (2014) in one of the dumpsites in African tannery, documented total Cr levels of 2633.38 mg/kg in the soil. This exceeded the WHO guideline value of 0.1 mg/kg. Such levels have the potential to be of profound environmental and health risk, particularly if Cr (VI) occurs at such sites, though the presence had not been confirmed. Moreover, if Cr (VI) does occur in such soils, it is unknown if biomagnification along the food chain takes place from these dumpsites and possibly cause health effects. The health impacts of chromium tannery waste on the human population is now a worldwide worry (Santa Mitra, 2016).

Humans are exposed to Cr species in the environment through two routes; non-occupational exposure *via* ingestion of chromium-containing food and water and occupational exposure *via* inhalation (Nigam and Shukla, 2015). In general, chromium species enters into the eukaryotic system and induce spontaneous reactions with the intracellular reductants such as ascorbate and glutathione. This generates the short-lived intermediate Cr (V) and/or Cr (IV), free radicals and Cr (III) end-product (Focardi *et al*., 2013). Within the cytoplasm, the short-lived intermediates are oxidised to Cr (VI). This simply combines with DNA–protein complexes and alters their normal physiological functions (Ateeq *et al*., 2016; Sun *et al*., 2015) and damaging their DNA (Fang *et al*., 2014). This results into genotoxic and mutagenic effects. They also block vital functional groups, dislocating other metal ions, or altering the active conformation of biological molecules (Nigam *et al*., 2015).This leads to liver damage and pulmonary congestion. Sometimes it causes skin irritation and gastrointestinal problems ending into ulcer formation.

Furthermore, Cr (VI) species can be able to amass in the placenta and harm the development of foetus resulting into birth defects and a reduction in reproductive health (Banu *et al*., 2017)**.** According to a senior officer at Leather division in Kenya, in 2014, two tannery workers died during the cleaning process of chromium-blocked tunnel in one of the tanneries in the country and such information is hardly ever documented. Therefore, there are few known cases of health effects due to poor management of chromium tanning agent and its waste disposal. Thus, countries like Ethiopia, Kenya, Namibia, Tanzania, Nigeria, Ghana, Zambia, Zimbabwe and others in sub-Saharan Africa grieve silently from environmental pollution problems of liquid and solid chromium tannery wastes. This issue appears to be a subject, which has not yet received adequate attention during the development of tannery industries. It looks certain that very little investment has been directed towards the application of modern substitutes for chromium tanning methods. This is in contrast to conventional tanning techniques in sub-Saharan Africa (Birhanie *et al.*, 2017). Thus, the need to assess health risks for consumers exposed to food crops from heavy metals contaminated sites.

2.9: Assessment of potential health risk from consumption of Cr and other heavy metals contaminated plants

Excessive Cr and other specific heavy metals (HMs) which enter into the soil might cause risks to human health. This can happen through consumption of food crops grown in these contaminated soils (Sharma *et al*., 2018). Ingestion of polluted food is one of the probable routes through which Cr and other heavy metals are reported to enter the human body (Li *et al*., 2017)**.** Cr and other specific HMs are important for the biological systems as structural and

catalytic parts of proteins and enzymes in low concentrations. But they become toxic in high concentrations**.** This is because, they can induce neutrophils, endocrine-disruption, genomic instability and neurotoxic with the vulnerable being the children (Jaishankar *et al*., 2014). Thus, Food and Agriculture Organization (FAO) and World Health Organization (WHO), United States Environment Protection Agency (US EPA) and other regulatory bodies of several other states all over the world strictly regulate the permissible concentrations of toxic Cr and other specific HMs in food stuffs (FAO/WHO, 2011; 2013; USEPA, 2011).

Potential health risk owing to Cr and specific HMs can therefore be evaluated by carcinogenic or non-carcinogenic assessment techniques. Non-cancer risk assessment approaches are established out by United States Environmental Protection Agency (USEPA, 2007). These techniques have shown to be valid and valuable tools for quantification of health risk (Nkpaa *et al*., 2018: Durowoju *et al*., 2020). For instance, edible vegetable is practically the commonly ingested food crop in most people's everyday diet over the globe. They are also the type of food crop, which many people all over the world consume in all their entire lifetime (Kearney, 2010). However, levels of toxicity of heavy metals/trace elements (HMs/TEs) in humans depend on their everyday intake of various types of vegetables and animal proteins through consumption (USEPA, 2007). Various indices and parameters have been designed and used for evaluating the human health risks posed via specific HMs/TEs through vegetable feeding. Non-carcinogenic health impacts from the soils and food by heavy metals contents can be analysed using indices like the chronic daily intake of metal (CDI). This one looks at the element level in studied medium (mg/kg), the exposure frequency, amount of days/year in the soil (350), the exposure duration (30 years for adult and 6 years for children), the ingestion rate (100 mg/day for adults and 200 mg/day for children, average time (AT = 365 x ED), and average body weight (BW, kg) (70 kg for adults and 15 kg in the case of children) (Tepanosyan *et al*., 2018; Risk Assessment Information System (RAIS), 2017; USEPA, 2007).

The hazard quotient (HQ) index is used for health risk assessment of HMs/TEs. It provides a strong understanding of the content of metals in soils and vegetables (Harmanescu *et al*., 2011). If HQ > 1, the exposed people will likely experience a harmful effect (Alam *et al*., 2019). Hazard Risk Index (HRI) evaluates the total potential health risk posed by more than one metal. It is calculated by the sum of the target hazard quotients of each metal. HQ and HRI values are calculated on the basis of Reference Dose (RfD) which represents a toxicity index of daily exposure to the people in comparison to a safe level of exposure orally over a lifetime (Gupta *et al.,* 2019). A lot of investigation has been carried out on uptake, accumulation and detoxification of HMs/TEs by a number of researchers (Dhankher *et al*., 2011; Hossain *et al*.,

2012; Mosa *et al*., 2016) at laboratory levels. But very limited studies have been conducted in the field. Gupta *et al.* (2019) reiterates that, there is a necessity to explore the gaps related to the effectiveness of measures for decrease in HMs/TEs in foods consumed every day and in high priority food products. This is for the deterrence and eradication of metal poisoning. When ecological and health risks from Cr and other heavy metals in the effluents and soils continue to exceed the permissible limits set by FAO/WHO (2011), then intervention strategies become the best options.

2.10: Current technologies used in tannery wastes management in SSA and their limitations

Several technologies have been reportedly adopted in the management of tannary waste in SSA. Our recent study revealed that, 70% of respondents dealing with tannery waste in the region dominantly use physical and chemical treatment approaches and only 0.1% of them are aware about bioremediation as an alternative method of treating chromium wastes. However, the physical and chemical treatments results are not encouraging because they are applied rudimentarily ending up polluting the ecosystems. In addition to that, the knowledge of bioremediation as eco-friendly technique is still very limited amongst the tanning industries players in the region (Oruko *et al*., 2014; Dutta and Das, 2010; Minas *et al.*, 2017).

According to Tadesse and Seyoum, (2015), chemical precipitation is the mostly widely used technique for treating chromium wastes in Africa. For instance, primary treatment of chrome effluent by the addition of lime to increase the pH of the effluent from acidic to alkaline, precipitate Cr (III) suspended in the effluent which then settle as sludge. The subsequent chromium sludge is pumped into drying beds which later form chromium oxide (cake), which regularly end up reducing the level of chromium in the final effluent. This method of chemical precipitation has been used successfully in lowering chrome content in tannery effluent. It is done prior to discharge into other ponds, sewer systems or directly to the environments (Mottalib *et al*., 2015; Tadesse and Seyoum, 2015). The management of the subsequent sludge after airing in the drying beds creates additional management challenge. Observation made in some African tanneries found that these dry sludges are reserved inside the tannery as a mound of waste. Sometimes they were unlawfully transported with other wastes to the communal municipal dumping sites. Majority of the tanneries in Africa discharge their effluent after neutralisation into municipal authorities' sewer lines for additional treatment (Oruko *et al*., 2014). Others release them into water bodies like rivers and wetlands unlawfully (Mwinyihija and Mwinyihija, 2010).

In the early 1990s, Leather Industry Research Institute (LIRI) developed biological filtration procedure for chromium wastewater. Nevertheless, it has not received wide acceptance in most local tanneries in sub Saharan Africa (Swartz *et al.,* 2017). This was confirmed by response from the questionnaire survey administered to Africa leather and Leather Products Institute secretariat staff based at Adis ababa. The institute is mandated to promote training, research, transfer of technology and resource mobilisation for the tanning industries in Common Market for Eastern and Southern African (COMESA) countries in partnership with United nations industrial development organisation (UNIDO) (ALLPI, 2020). Thus, for biological filtration to be effective, pre-treatment (screening) and primary treatment procedures (settling of lime/chrome sludge and fat oil/grease removal) are needed (Swartz *et al.,* 2017). This helps to eliminate problematic solids and fats which can block the filter bed when discharged into the equalisation ponds.

One of the trial studies carried out by LIRI, on tannery effluents, showed that primary treatment should be elevated for the probable use of biological technique in the secondary treatment method. In regard to the above technique, nothing has been reported in current years in the leather industries operating in the continent. This was supported by extensive literature review and corroborated by report from the ALLPI. Likewise, some research has been focused on the use of microfiltration to start the feasibility of its use together with the reverse osmosis procedure (Swartz *et al.,* 2017). At the close of investigation trial, it was concluded that tannery effluent required a very effective secondary biological treatment.

Further to that, tertiary treatment is necessary to remove the suspended solids and soluble fats, oils and grease for microfiltration technique (Itankar and Patil, 2014; Patil *et al*., 2016; Sundar *et al*., 2011). Until now, there is only one report of microfiltration method for tannery wastewater treatment from a leather industry in Fez-Morocco as secondary and tertiary treatment stage in African leather industries (Khalfaouy *et al.,* 2017). This confirms slow adoption of such sophisticated technique and their unsustainable high cost of maintenance by regional tanneries. Therefore, microfiltration and advance techniques that have not been fully adopted in African tanneries points towards the need to further explore, exploit, test and apply low cost-effective techniques.

The current method of solid waste disposal is indiscriminate dumping of chromium wastes and it is regularly practiced in a number of African countries. This is because it is the cheapest solution of disposing solid wastes from the leather processing industry. However, it is also extensively used in some developed nations (Ayobami and Dokumo, 2020; Pati *et al*., 2014).

The challenge encountered with these tannery-based solid waste dump sites is the buildup and later leaching of chromium salts by surface runoff into the drinking water sources. There is also the possibility for the oxidation of Cr (III) to Cr (VI) during disinfection of water for human drinking (Sallam *et al*., 2015). Such a solution is not environmentally appropriate and proper disposal of chromium-contaminated harmful sludge has also become a major environmental concern to many tanning industries (Srinivasa Gowd *et al*., 2010; Tariq *et al*., 2010). Large parts of land around such dumpsites are rendered unfit for human living and other related activities. Therefore, the present system of rudimentary dumping of chromium wastes, which is common practice in the African continent is unacceptable and needs to be addressed.

In some cases, uncontrolled combustion is also usually used to dispose off solid tannery wastes in Africa. This method totally eliminates the organic content of chrome waste. However, during the combustion process the likelihood of total oxidation of trivalent chromium into hexavalent chromium which is considered a cancer-causing compound is very high (Wells *et al*., 2014). Therefore, perfect separation of burning gases and ash is essential as well as safe disposal of the ashes. Unfortunately, the existing practices, do not address these inadequacies. More consideration should also be given to increased content of nitrogen oxides which is released during the burning of collagen proteins (Fang *et al.*, 2018). The upgraded incineration method using wet air oxidation and separation of the hexavalent chromium from the oxidised liquor should be considered as an alternative to uncontrolled combustion (Sundar *et al.*, 2011). This will also help the management of gases and the collection of raw materials for re-use in making other useful goods as opposed to uncontrolled combustion presently being practiced.

Compositing waste as a low cost and low sophisticated know-how has been found viable in managing organic wastes but it has not been found attractive in most tanning industries in Africa and other regions of the world (Chen *et al*., 2020). Compost processed from thermal fleshing's are reported to demonstrate poor plant tolerance or even plant intolerance. The fertilizing properties of the latter can be enhanced by adding animal manure. Moreover, in general, hair remnants can be composed together with thermal fleshing's. This can result into a compost product of acceptable value (Basheer and Umesh, 2018; Zuriaga-Agustí *et al*., 2015). The disadvantage of the compost method is the associated pungent oduors and the occurrence of insects in the area of compost. The other drawback is the requirement of large piece of land which is lacking in most tanneries built in the congested urban centres in Africa. Disposal of an increased volume of wastes due to the accumulation of bulking agents is another challenge. Therefore, the technology should be implemented in a vessel where

compositing with a proper waste gas treatment (bio-filter) could help to prevent these problems. Furthermore, compositing can be explored in a pit with the combination of plants residue and animals' manure for a period of one year. In this process, the final product is suggested to be safe for use as manure to grow food crops (Gupta *et al*., 2018).

In many countries in Africa, appropriately designed, constructed and/or properly maintained landfills are not existing (Kinobe *et al.,* 2015). Instead, tannery wastes are frequently, indiscriminately dumped without any control in prohibited dumpsites ending up polluting the ecosystems (soils, water, and air). This threatens human health by becoming bioavailable along the food chain (Ahamed and Kashif, 2014). The above problem is heightened by lack of properly installed effluent treatment plants in many tanneries in sub-Saharan Africa. This has led to the shutting down of tannery operations in some countries due to public complaint and environmental concerns, thus the continued search for more environmentally compatible techniques. Currently, bioremediation techniques are favoured over conventional techniques (physico-chemical) because of their eco-friendly by-products (Aniefiok *et al*., 2019**;** Mohanty and Patra, 2011).

2.11: The potential of bioremediation techniques as alternative treatment for tannery chromium wastes

Bioremediation is a procedure in which micro-organisms, plants or animals, either living or their dead biomass, are used to reduce or eliminate an unwanted chemical contaminant (Saha and Orvig, 2010). Presently, in many countries both physico-chemical and biological wastewater treatment plants are operated for the purpose of rendering the effluent from the tanning industries harmless (De Voogt, 2015; Kuppusamy *et al*., 2016). However, bioremediation is showing promising results as an alternative to these conventional environmental clean-up technologies. This is because of its eco-friendly by-products. For that reason, the number of vendors selling bioremediation technique has increased and continues to do so dramatically in recent years (Kumar *et al.,* 2011). But, in spite of that, the use of bioremediation is not understood or trusted by those who must authorise its use in Africa. The application and success of bioremediation are still a hotly debated issue in the continent (Xia *et al*., 2019). Another reason for the lack of understanding and doubt on bioremediation is that the technology requires knowledge not only of such specialties as environmental engineering and hydrology but the working mechanisms of microbes. This knowledge is important in conventional clean-up methods and the complex workings of micro-organisms (Bharagava and Mishra, 2018; Xia *et al*., 2019).

Wood *et al*. (2016) explain further that bioremediation technique, offers a unique technological challenge to scientists. This is because of the combination of the intricacies of microbial functions in the ecosystems and the physical challenge of monitoring both micro-organisms and contaminants. This is because, in the subsurface bioremediation is hard to comprehend and operate. This has made some regulators and clients cautious to trust it as a suitable clean-up strategy. In spite of those limitations, Kumar and Saxena (2020) states that the utilisation of naturally occurring prokaryotic and eurokaryotic organisms in soil (around 4,600 distinct genomes in one gram of soil) is worth applying for cleansing and rehabilitating polluted soils. They provide an effective, economical, versatile and eco-compatible means of recovering polluted land in future. Al-Battashi *et al*. (2016), sums up that, microbial conversion of toxic Cr (VI) offers the tools for green technologies which are more cost-effective. Their methods limit the toxicity of Cr (VI) by selectively enriching those naturally occurring micro-organisms to bioremediate specific toxic wastes.

Many biochemical methods have been developed, which have integral advantages but are still in their primary stages of development in most developing countries of SSA (Jacob *et al.,* 2018). They include bioaccumulation, bio-stimulation, biosorption, bioventing, biostablisation, constructed wetland, landfarming, composting, biopile, cometabolism, monitored natural attenuation, biosparging, bioslurping, biomining, biosurfactants, anaerobic bioreactor, containment, and phytoremediation. These techniques have been established to possess good potential to replace conventional physico-chemical methods (Arivalagan. *et al.,* 2014; Sharma, 2012). But the choice of which bioremediation option to use is still determined by numerous economic and environmental factors (Okoh *et al*., 2020). However, the following bioremediation and phytoremediation treatment processes are currently under investigation in African tanneries; phytoremediation, bio stimulation, bioaugmentation, adsorption of Cr (VI) onto organic matter, microbial cells (that is biosorption). The other one is the reduction of Cr (VI) to Cr (III) by enzymatic reaction or indirectly by dropping compounds produced by micro-organisms (that is biotransformation) (Carlos *et al*., 2016). The biological reduction of Cr (VI) has attracted increased attention in leather industries. This is because, the process not only relieves the harmfulness of Cr (VI) that affect living organisms. They also help in the precipitation of chromium at near-neutral pH (mainly as $Cr(OH)_3$) for easy physical removal and disposal (Liu *et al.,* 2020).

New bioremediation methods are also emerging, founded on advances in molecular biology and process engineering in the globe and in the continent of Africa (Dangi *et al.,* 2019). Newly-developed rapid-screening assays can now help identify organisms capable of degrading

definite wastes. The new gene-probe methods will help discover their richness at specific sites. These new tools and techniques to use for bioremediation in situ, in biofilters and in bioreactors are now contributing to the rapid growth of this field globally and should be embraced in the SSA (Sharma *et al.,* 2018; Sengupta and Dick, 2017). However, before their application there should be understanding of the microbial interactions in any given ecosystems.

2.12: Bacterial interspecific interactions

In the wild, most bacteria will exist in diverse communities with fungi and other microorganisms. This interaction normally leads to competition for nutrient and territory (Ijoma and Tekere, 2016)**.** Bacteria involved in such competition develop diverse mechanisms to survive. Competition in natural environments plays a key part in controlling the dispersal and richness of bacterial species (Holt and Bonsall, 2017; Díaz-Muñoz and Koskella, 2014). These responses to competition can be harnessed for biocontrol of pollutants in the environment. It is suggested that the competition for territory and nutrient by bacterial species can lead to increased degradation of pollutants in elevated levels at different ecosystems (McGenity *et al.,* 2012; Liu *et al*., 2019). Different microorganisms in nature utilise a combination of metabolic pathways for bio-transformations of pollutants. Thus, their safe co-existence in real nature have been detected in forest soils, compost piles, insect gut and mammalian intestines (Aleklett *et al*., 2018). However, in artificial set up like bioreactors; the safe co-existence of bacteria in treatment of tannery waste is less documented.

Any industrial development that would in future exploit the use of bacteria to treat waste must go for more economical method and the release of safe by-products (Lee and Pandey, 2012). For that reason, most of the objectives continue to shift on bacteria waste remediation research as years advance (Liu *et al*., 2017). However, the application of bacterial consortium appears to be gaining considerable attention than the use of single micro-organism cultures. The aim can be connected to the potential to utilise synergisms and antagonisms between the metabolic pathways of the strains involved in the consortium (Camacho-Pérez *et al*., 2012; Bader *et al*., 2010; Dwivedi *et al*., 2011; Pandya and Albert, 2014). But a clarification of the kind of interactions and mechanism of interaction shown during bacteria-bacteria interaction is important towards any forthcoming application.

A range of bacteria show broad spectrum of interaction types with different effects like neutralistic, mutualistic and combative/antagonistic with the ecosystems (Kuzyakov and Xu, 2013). However, a massive majority of these interactions are competitive. Competition is defined as ‘the negative effects of one organism upon another by either consumption or

directing admission to a scarce resource that is in short supply for both organisms in the environment (Ghoul and Mitri, 2016; Niehus *et al.,* 2015). Cornforth and Foster (2013) explained exploitation and interference as the major types of competition.

Exploitation competition takes place where organism exhausts the resources without stopping access of other organisms to the same resources in the environment. Characteristics that are favourable for exploitative competition comprise rapid germination of cells, rapid growth rate and an ability to rapidly eat the available nutrients (Bashey *et al.,* 2012). On the other hand, interference is a state where one organism prevents the proliferation of another. For instance, a bacterium may be directly attacked by parasitic or predatory organisms. Such interference could encourage conditions like bacteriophage interference or allelopathy/invasive effect (Díaz-Muñoz and Koskella, 2014; Holdridge *et al.,* 2016).

In a given bacterial community like the case with fungal, combative antagonistic interactions normally terminates with either deadlock or replacement in various ecosystems (El Ariebi *et al*., 2016; Ijoma and Tekere, 2016). Deadlock happens where neither of the two species in the given ecosystem gains headway over the other in the utilisation of the scarce resource. It is probable for both species to develop on the organic decaying matter until they meet and cell from one population touches the other with no looser or winner at the end (El Ariebi *et al*., 2016). Another deadlock is the development of barrages at the point of interaction, to stop additional invasion into their respective territories (Maynard *et al*., 2017; Jade, 2018). The last type of deadlock is when a clear zone of inhibition is established between the two combating organisms referred to as deadlock at a distance (Ijoma *et al*., 2019).

Replacement in the natural environment takes place when an existing bacteria population decrease in a substrate as a result of nutrient depletion. Besides that, the substrate can also become toxic in content for bacterial growth contributing to the disappearance of the species. Lastly, replacement can take place when the late arriving bacteria are capable of utilising the remaining toxic organic compound as well as capable of tolerating its high prevailing conditions (Hibbing *et al*., 2010; Llorens *et al*., 2010). Partial replacement of bacterial population lead to a stalemate with no outright winner in the ecosystems (Brun, 2019; Ujor, 2010). Mutual replacement happens when one species takes some of the region formerly occupied by the other and vice versa (Brun, 2019). In later stages of replacement, the entire substrate becomes territory for one bacterial population leading to the demise of the other population (Buchkowski *et al.,* 2017). Thus, the bacteria must have the adaption mechanisms to cope with such conditions and situations in varying nature.

2.13: Bacterial population adaptations

In natural environment, bacteria can exist as a varied, mixed population (Kester and Fortune, 2014). Cases of population heterogeneity comprise the formation of phenotypic variants such as persister cells and small colony variants (SCV). Both phenotypes share many resemblances like metabolism and heightened tolerance to a range of pollutants in the ecosystems (Alreshidi *et al*., 2013; Dal *et al.*, 2019). Persister cells can be defined as a sub population of a genetically same culture. They show phenotypic variations which help them to cope and withstand toxicity that other associates of the population are vulnerable to (Fisher *et al.,* 2017). In soil, water or air, these specialised survivor cells are thought to be pre-existing through random development. Some through evolutionary process to attain the uppermost survival fitness of the population under a wide variety of pollutants (Day, 2016).

Persister cell formation can also be attributed to as a way bacterium responds to environmental indicators by quantitatively and qualitatively curbing the frequency at which their associates undergo phenotypic changes (Harms *et al.,* 2016). The devices that help phenotypic heterogeneity are epigenetic in nature. They do not involve changes in the DNA. Therefore, they allow phenotypic reversibility in pollution free subculture. Understanding the fundamental processes that permit phenotypic persistence is important to help advance bioremediation strategies and identify resistance development nature of bacteria (Onyango and Alreshidi, 2018). The other adaption mechanism is small colony variant (SCV).

In bacteria, SCVs are taken as the manufacturing points of haemin, menadione, and thymidine. Their deficiencies change the electron transport chain (ETC) combined with deprived uptake of carbon sources. Eventually, they affect adenosine triphosphate (ATP) production. The end result of these constraints is measured growth rates, changed cell division, cell wall biosynthesis, amino acid transport and protein synthesis. Other constraints are diminished membrane potential, cationic and peptide transport, and carotenoid synthesis (Malone *et al.,* 2010; Proctor *et al*., 2014). The presence of SCVs in the population led to substantial reduction in biosynthesis of proteins. These are proteins associated with tricarboxylic acid (TCA) cycle, purine/ pyrimidine, arginine and proline synthesis, folate metabolism and a slowdown regulation of the activity of their citric acid cycle (Kriegeskorte *et al*., 2011; 2014). This alters bacteria population morphology and biochemistry and enables them to tolerate significantly higher levels of many pollutants in adverse environments (Onyango and Alreshidi, 2018).

The SCV phenotype have several features that can enable them to adapt to dormancy state in toxic conditions. They achieve this by producing less antigenic fragments, thus masking them from effects of toxic pollutants. This inhibits their quick clearance and allowing their circulation from high to low concentrations and safeguards a prolonged niche for their persistence (Meyla *et al*., 2018). SCV can express increased fibronectin-binding proteins that help them withstand high pollution by regulating protective mechanisms highly effective in preserving viability (Séguin *et al*., 2010). An up regulated arginine-deiminase pathway, for instance, compensates for shortcomings in ATP production and counter acts the intracellular acidic environment (Proctor, 2019). SCVs are also connected with biofilm structures.

The combination of a SCV phenotype and hyper biofilm formation results in a bacterial mass that is capable of recurring in adverse polluted environments when they are thought to be totally cleared off. The switch between phenotypes (Wild Type - SCV) may contribute to resurgence of bacteria after initial reaction in anaerobic bioreactors. In general, the SCV phenotype is a cost-effective strategy that increases bacterial persistence in a variety of polluted conditions. This could be highly advantageous for bacteria used in bioremediation techniques (Schleimer *et al.,* 2019; Onyango and Alreshidi, 2018). However, at individual levels, bacteria species have different resistance mechanisms.

2.14: Bacterial chromate resistance

There are bacterial species capable of surviving in Cr (VI) contaminated environment as either tolerant or resistant bacteria (Bharagava and Mishra, 2018). The resistance is defined as the capacity of a bacteria to withstand poisonous effects of Cr exposure. This is by means of a detoxification mechanism formed to respond to the Cr species in the environment (Wani *et al.,* 2018). Different bacteria have developed numerous mechanisms of chromate resistance such as, ion transport, reduction, DNA repair and other mechanisms (Saxena *et al.,* 2020; Viti *et al*., 2014). When a bacterium uses transport mechanism, a transporter protein (ChrA) plays a prominent part in efflux of cytoplasmic chromate (Pradhan *et al.,* 2016). Another reported chromate resistance mechanism is the DNA repair (Nickens *et al*., 2010). Bacteria like other microbes can also fight chromate toxicity by eliminating various metallic and metalloid species from the environment or waste streams by reducing them to a lesser oxidation state (Malaviya and Singh, 2016).

Bacterial reduction of Cr (VI) to Cr (III) is among the chromate detoxification mechanisms which are not linked to plasmid (Thatoi *et al.*, 2014). When Cr (VI) enters inside the cell, it is readily reduced to Cr (III) by several enzymatic or non-enzymatic activities. The release of Cr (III) may

then create varied toxic effects in the cytoplasm (Zhu *et al*., 2019). Cr (VI) can be reduced to Cr (III) in the presence of diverse chemical compounds formed in the bacterial metabolic process in the case of non-enzymatic process. For example, hexavalent chromium can be reduced by Fe (II) and HS as the anaerobic metabolic end products of iron- and sulfate-reducing bacteria. Poljsak *et al*. (2010) reports that ascorbic acid, glutathione (GSH), cysteine, hydrogen peroxide (H_2O_2) could be the powerful non- enzymatic chromate reductants for microbial cells while ascorbate could be for advance organisms. Dhal *et al*. (2010) further reports that Cr (VI) reduction may also take place by chemical reactions linked to compounds existing in intra- or extracellular compounds. These are amino acids, nucleotides, sugars, vitamins, organic acids, or glutathione.

Thatoi *et al*. (2014) explain in their review, that among chromate reducing bacteria (CRB), gram- positive CRB are significantly tolerant to Cr (VI) harmfulness at fairly high concentration, while gram- negative CRB are more sensitive to Cr (VI). The *Bacillus sp*. (Das *et al.,* 2014; Dhal *et al.,* 2010); *Arthrobacter sp*, *Agrobacterium sp*, *Vigribacillus sp*. and *Microbacterium sp*. (Gutierrez *et al.,* 2010) isolated from diverse environmental settings resisted/tolerated Cr (VI) toxicity and they belong to different taxa. Thus, the need to understand the enzymatic chromate reduction strategies inside the bacteria cell.

2.15: Enzymatic chromate reduction

Thatoi *et al*. (2014) and Zhu *et al*. (2019) reports that bacteria have different strategies to reduce Cr (VI) in aerobic or anaerobic settings or both. Cr (VI) reduction occurs in the aerobic bacteria by soluble fraction (protein) while in anaerobic bacteria it is carried out by membrane bound proteins. Soluble reductases can take part either in extracellular or intracellular reduction of Cr (VI) (Das *et al*., 2014; Elangovan *et al.,* 2010). Membrane bound reductases like flavin reductases, cytochromes and hydrogenases could be associated with electron transport system. They use chromate as the terminal electron acceptor in anaerobic reduction by bacteria (Luo *et al*., 2019). The soluble proteins require nicotinamide adenine dinucleotide phosphate (NADPH) which is one form of universal coenzymes functioning as hydride(H^-) carrier at a standard reduction potential of $^-$320Mv and as an electron donor for aerobic Cr (VI) reduction to occur (Mala and Rose, 2015). These soluble proteins are placed as cytosolic proteins (Soni *et al*., 2013).

The enzymes used in reduction of Cr (VI) have been isolated from bacteria to mammals and they include aldehyde oxidases, cytochrome p450, and Dt-diaphorase. However, microbial enzymes seem to be extra active for Cr (VI) reduction in bioremediation method. The enzyme

chromate reductase from different bacterial species capable of reducing Cr (VI) have been reported by several researchers (Rath *et al*., 2014; Soni *et al*., 2013). These reductases enzymes are not limited to chromate reduction, but they contain multiple activities and substrates range from organic compounds to inorganic metal ions. To understand their structure-function relationship are of fundamental interest as they are associated with transfer of electrons from NADP and flavin mononucleotide (FMN) (Li *et al.,* 2020).

To reduce Cr (VI) to Cr (III) by enzymes, three electrons are needed, and proteins must also be involved for the transfer process to be complete (Pradhan *et al.,* 2016). The intracellular proteins involved in reduction of Cr (VI) to Cr (III) can be classified as either one-electron reducers or two-electron reducers grounded on the extent of oxidative stress released. In the case of one electron reduction, Cr (VI) gets reduced to form the highly unstable Cr (V) intermediate that displays a semi tight mechanism of chromate reduction. The reduction of Cr (VI) to Cr (V) is associated with the reduction of molecular oxygen to peroxide, which again produces hydrogen peroxide. Hydroxyl radicals are generated when Cr (V) reacts with hydrogen peroxide (Liu *et al*., 2011). In the process, reactive oxidative substances (ROS) are created during the successive reduction of Cr (VI) to the final Cr (V) (Green*,* 2012). Lipoyl dehydrogenase (LpDH) which is flavin-dependent enzymes, cytochrome c, glutathione reductase, ferridoxin-NAD and the physiological roles of these enzymes are there to catalyse energetic or biosynthetic reactions (Kay and Jewett, 2015*;* Thatoi *et al*., 2014; Pradhan *et al.,* 2016)

When the two electron reducers take place, reduction from Cr (VI) to Cr (III) can continue without creating Cr (V) intermediate that show tight mechanism. Due to that, a much reduced amount of ROS is created during this procedure than that of one electron reduction (Green*,* 2012). Two electron reducers are characterised by the transfer of hydride ion (H^-, the equal of a proton and two electrons) because of being NAD (P)-dependent enzymes. Chromate reducing enzymes acting as two electron reducers include ChrR from *P. putida*; YieF and NfsA from *E. coli* (Pradhan *et al.,* 2019). These chromate reductions techniques are worth exploring and exploiting in bioreactors reactions using animal organic substrates materials (dung) in bioremediation of tannery chromium wastes challenges in sub-Saharan Africa. Therefore, the outcomes of this extensive literature search are reported in chapters six and Appendix A1.0 of this thesis (solutions to address the identified gaps).

2.16: The use of animal dung in tannery chromium waste management and existing knowledge gap

Tannery industrial waste is one of the main sources of heavy metals pollution of the environment in SSA. It has been found that its wastes are involved in the elimination of flora and fauna of water and land ecosystems (Saha *et al*., 2017; Babanyara *et al*., 2010). Ahamed and Kashif (2014); Mohan and Gupta (2014) and Belay (2010) reported that for a long time, treatment of chromium in the environment has been done by electrolytic deposition, electrodialysis, electrochemical, evaporation, precipitation, ion exchange, reduction, reverse osmosis, filtration, adsorption, chemical precipitation and distillation. However, all these procedures are expensive and not environmentally friendly. Thus, the need to explore a cheap, cleaner and greener method using abundant animal wastes**.**

Cow dung and its microorganisms have lately been selected for the remediation of heavy metals like chromium (Gupta *et al*., 2016). This can be attributed to its higher biosorbent property of various functional groups present in the undigested plant materials such as lignin, cellulose, tannins, hemicellulose and others responsible for hexavalent chromium biosorption (Saha and Orgiv, 2010). Dry cow dung powder was of late used as a source of adsorption for the elimination of chromium from aqueous solution and realised 73.8 % elimination of chromium (Mohan and Gupta, 2014). Thus, dry cow dung powder might be favoured over other synthetic adsorbents for their low production cost, less time and energy requirements. Cow dung has also been found to contain various group of microorganisms such as *Acinetobacter, Micrococcus, Bacillus, Pseudomonas, Serratia* and *Alcaligenes spp*. which makes them suitable candidates for microbial decay of pollutants (Neethu *et al.*, 2019; Mukhuba *et al*., 2019; Umanu *et al.,* 2013). Randhawa and Kullar (2011) have reported the application of cow dung microorganisms in the remediation of biomedical and pharmaceutical waste. This was supported by research work of Pandey and Gundevia (2008) using *Periconiella* fungus*,* that presented comprehensive biodegradation of biomedical waste placed in culture medium of a cow dung*.*

Other literature reports the use of Ilala, Carmel, goat and poultry to treat different pollutants in the environment as well. All these research results show that cow and other dungs can adsorb contaminants and also provide nutrients and energy essential for microbial growth thereby resulting in the bioremediation of hazardous pollutants in the environment. Cow dung like other animal waste is an economically worthwhile renewable resource which is easily obtainable. According to the above-explanation, cow dung and other animal wastes can be used with or without pre- or post-treatment as an outstanding measure to bioremediate non-biodegradable

and actually toxic pollutants like chromium. Using cow dung and other dungs for bioremediation is a simple and eco-friendly technique as it does not yield any damaging by products (Randhawa and Kullar, 2011; Neethu *et al.*, 2019). However, much more wide-ranging studies are still required to be done in this field worldwide.

In Africa, biosorption technique for the removal of tannery chromium has been applied by Olubunm and Ejechi (2016). The study complemented chromium biosorption capacity of micro fungi with biosorption ability of brown-rot wood decay products in a sequential biosorption arrangement. Cr (VI) levels of 139.2 mg/kg was significantly reduced to *33.2, 96.9* and 99.9% after treatments with *Pseudomonas aeruginosa, Penicillium chrysogenum and Aspergillus niger,* respectively. This was accomplished in the presence of cow dung as electron donor. The salvage of 65.4% - 87.7% of the Cr (VI) removed by treatment with micro fungi by elution showed that adsorption was the main mechanism for Cr (VI) reduction. In 2014, the initial up-scaled demonstration of an actual biological Cr (VI) bioremediation system using bioreactor was erected in South Africa. It effectively led to 99% reduction of contaminated effluent (Williams *et al.*, 2014). But this was not followed up in other African countries due to several issues. The issues identified include inadequate finances, lack of required skills and poor knowledge in remediation technology.

Currently, the world is shifting to eco-friendly techniques for remediation of wastes. Techniques such as biosorption, bio stimulation, bioaugmentation and control bioreactors technologies offer potential for significant developments in African and other worldwide tannery sectors. However, biosorption of Cr effluents from the tanning industry using other raw animals dungs without the incorporation of micro-organisms are yet to be reported. Further still, it is yet to be exploited extensively in this sub-continent. On another front, controlled bioreactor systems are another substitute technique that has not appealed to tanners who want to use animal waste in the Cr removal (Varjani *et al*., 2018). This is because, the choice of the suitable bioreactor should be connected with the diverse operational conditions required for Cr removal. They include, hydrodynamics, mass transfer and growth conditions (Fernández *et al*., 2018). Furthermore, in SSA, there is still scarcity of data on the use of animal wastes and micro-organisms to remedy tannery wastes, particularly tannery chromium, as supported by Olubunm and Ejechi (2016). The latter authors and others, reported in literature, advance the argument that animal waste or compost which are abundant in most countries in the continent, have potential for bioremediation. They can be used as fertilizers, source of inoculum or as adsorbent materials.

The animal dung contains as reported for cow dung high nutrient composition. They can provide the polluted soils or wastewater with nutrient elements required by both the endogenous microbes and those provided by the diverse animal dung for their bioremediation activities (Gupta *et al*., 2016; Angela *et al*., 2012). These nutrients may help the diverse microbial species resident in the dung to multiply for subsequent utilisation of the pollutants (Angela *et al.,* 2012). On the other hand, numerous remediation approaches have also been studied. This is for the advance of effective and competent technology for the elimination of the heavy metals especially Cr (VI) from the wastewater (Bhavsar and Patel, 2012). However, among them adsorption technique has proved to be a versatile method for removing Cr and other heavy metals. This is because, the traditional approaches for removing Cr (VI) ions from industrial effluent are expensive and involving due to the use of sophisticated equipment (Ibrahim and Jimoh, 2011; Thangavel and Gopal, 2017).

The availability of cow dung, goat pellets and poultry waste in great amount has made their choice of developing a low-cost adsorbent possible as reported in several literature (Gupta *et al*., 2016; Angela *et al*., 2012; Olubunm and Ejechi, 2016). However, the use of donkey dung either in raw form, activated carbon, in char form for adsorption or as a bio stimulant in anaerobic bioreactor seems to have received less attention so far. Furthermore, detailed information on the biosorption mechanisms of this biomass resources on environmental pollutants is lacking. This study was set out to fill that knowledge gap. The study initially used donkey dung as a bio stimulant in a set-up of bioreactors with possibility of setting it up in field environment in future (Thangavel and Gopal, 2017). It also supplemented the anaerobic bioreactor study by carrying out preliminary exploratory characterisation and test of donkey dung as a biosorbent material for real and synthetic Cr. These results provided preliminary insights on donkey dung potential as an eco-friendly biosorbent for tannery Cr effluent bioremediation. The choice of donkey was also found necessary by other factors as well. In the SSA, especially South Africa and Kenya, donkeys have been found to produce much dung wastes that are causing environmental pollution to tourists along the Lamu Island beach of Amu in Kenya and small towns like Paulshoek in parts of Eastern Cape of South Africa (Karisa *et al*., 2014; Tshangela, 2016). This implies that they can be abundant as raw material and their negative effects can be put in pollution control in leather tanning industries in the region.

Fermentation of donkey dung has also been reported to produce higher methane gas content (50-60%) as compared to other wastes, such as cow, goat, chicken and sheep manure (Tshangela, 2016). This is attributed to the poor digestion system of donkeys, compared to other animals, which chew their cud. The donkey digestion takes place in caecum and colon

guts, not in the rumen like other animals (Liu *et al*., 2020). This means that their dung might be having a large proportion of undigested cellulose material and a lot of bacteria species. It was speculated that, undigested cellulose when used as raw material after simple grinding without turning them into char could make good material for adsorption of Cr effluent (Gupta *et al*., 2016). On the other hand, the anaerobic bacteria resident in the gut and excreted with dungs were speculated to posess potential that could be used to reduce chromium effluents levels. This was done so, because they normally assist in digestion of lignin cellulose in the anaerobic gut of the donkey. These potentials were worth exploring by mimicking their natural conditions in the gut, thus setting up anaerobic batch bioreactors.

The potential of donkey dung as a cheap adsorbent for Cr effluent and the occurrence of endemic bacteria with potential to reduce Cr levels in anaerobic bioreactors were still unknown and were yet to be reported in literature. These unknown potentials were worth researching upon in relation to donkey dung bioremediation potential for Cr (VI) wastes in SSA. Donkey dung material presented a knowledge gap which was exploited in the bioremediation of tannery chromium effluents from selected tanneries in the sub region. To our knowledge, no work has been reported in the literature on the application of donkey dung on the bioremediation of tannery-based chromium effluent especially from *Equus africanus asinus species* in the SSA. Hence, a study on the use of donkey waste to mitigate the accumulation of Cr (VI) on tannery effluents in Kenya and South Africa, is an eco-friendly innovation.

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CHAPTER THREE

METHODOLOGY

3.1 Preamble

This chapter presents the details of methods and materials deployed in this research. The methods/materials covered mail based survey technique, field sampling, experimental materials, sample preparation techniques, analytical methods, quality control and Ethical statement.

3.2: Sampling

3.2.1: Wastewater sampling

The chromium wastewater samples were collected once from BO and DB, according to APHA (1989). The samples for physico-chemical, selected metal contents, anaerobic bioreactors and adsorption study were collected in clean polyethylene plastic bottles. In order to minimise metal precipitation, ion exchange as well as altering the sample's metal composition, concentrated nitric acid was added to the samples for metal content analysis only. The polyethylene plastic bottles were rinsed properly with the wastewater to avoid cross contamination. The samples were collected in 1 L plastic containers before transporting them to the laboratory for sample pre-treatment. The wastewater samples were kept in the fridge in the laboratory before the analysis. The wastewater sample codes were BO untreated chrome liquor, BO Re-used liquor, DB untreated chrome liquor and DB treated chrome liquor.

3.2.2: Donkey dung sampling

The donkey dung used in bioreactor and adsorption study was obtained from Vuwani village of Vhembe district, Limpopo province, following the modified methods of Oyeyiola *et al*. (2017); Ntui *et al.* (2014). The dung was collected from *Equus africanus asinus* species. The sampling was done in South Africa only based on the assumption of the study stated in chapter one, section 1.7 of this thesis. They were then placed in polyethylene bags which were first washed with nitric acid (0.1 M) and, rinsed with distilled water to remove any adhering dust particles to avoid contamination. The samples were immediately packed into the bags and put into the portable cooler box. The box was covered with frozen ice packs and transported to University of Venda Hydrology and Water Resources laboratory for temporary storage in the fridge at 4^{o}C. The samples were later transferred in the same conditions to UNISA laboratory in Johannesburg for experimental analysis and testing.

3.2.3: Survey technique

The mail based questionaire survey technique was used in the literature review study as a convenient tool to the key informants. It was conducted in 2018 among selected key informants and the technical secretariat experts at African leather and leather products institute (ALLPI) based in Adis-ababa. The key informants were purposely selected from fourteen countries in the sub Saharan Africa after identification with the support of ALLPI secretariat staff. They were from South Africa, Malawi, Mozambique, Zambia, Zimbabwe, Botswana, Namibia, Kenya, Uganda, Tanzania, Ethiopia, Cameroon, Nigeria and Democratic Republic of Congo.

The key informants were tannery owners, tannery managers/supervisors, leather experts employed by the government, environmental impact assessment practioners, waste and sewerage managers employed by the municipality and lead researchers in leather field. The survey aimed to establish types of chromium tanning, techniques of treating chromium wastes, disposal procedures, challenges faced, government policies on chromium, environmental/health risk and awareness on application of bioremediation techniques in the region (Appendix Figure 3.1). The data was analysed through descriptive statistics in Excel to get the most common techniques used to treat chromium wastes, level of awareness about bioremediation techniques and challenges of applying these eco-friendly techniques in the sub region, among others. Descriptive statistics were derived and used in the synthesis of existing literature.

3.3: Methods applied, sample pre-treatment and analysis

3.3.1: Pre-treatment of soil samples and physico-chemical analysis

A mass of 20 g air-dried soil was weighed and sieved to 600 µm mess size and then 50 mL distilled water added. The soil-water suspension was shaken for 24 h. The Cation exchange capacity (CEC) was determined by extracting the cations with 1 M ammonium acetate buffered at pH 7. Another volume of 30 mL of 1 M CH_3COONH_4 was added to 5 g of soil. The suspension was shaken for 2 h and then centrifuged at 6000 rpm for 15 min. After centrifugation and filtration, the filtrate was transferred into a 100 mL flask and two other volumes of 30 mL ammonium acetate were added successively after 30 min of agitation and centrifugation. The final filtrates were completed to 100 mL with ammonium acetate solution. Calcium (Ca) and magnesium (Mg) were then determined by EDTA titration while potassium (K) and sodium (Na) were determined by flame photometry following procedures of Hinga *et al.* (1980) and Page *et al.* (1982). The 900F flame atomic absorption spectrophotometer

(FAAS) (PerkinElmer, Massachusetts, USA) was used for the analysis of exchangeable bases (K and Na). Calcium and magnesium ions were estimated by complexometric titration. Cation Exchange Capacity (CEC) was estimated by distillation and titration. The analyses were done in both KALRO and University of Venda soil laboratories (see Appendix Table 3.1).

Soil texture was analysed by taking 50 g soil sample which was sieved to less than 2 mm mesh size. After that, it was then placed in an oven to dry at 40^{o}C. Later on, it was weighed and transferred into a 500 mL plastic shaking bottle. Then 300 mL distilled water and 50 mL of dispersion agent (calgon) was added and shaken overnight. The soil suspension was transferred into a sedimentation cylinder and topped up to the 1 L mark where it was mixed thoroughly to bring the soil particles into suspension. A hydrometer was lowered into the solution and reading taken at various time intervals (Bouyoucos, 1936). Soil hydrometer, ASTM 152H from Gilson Company Inc (Lewis Center, Orange Township, OH, USA) was used for soil texture. A portion of the soil sample (from the corer) was put into an oven and dried at 105^{o}C for 24 hr. The dried soil was removed from the oven and put in a desiccator with silica gel to cool for 30 - 40 min. The samples were weighed again to get the dry weight. The percentage moisture content was derived using Soil Survey Laboratory Methods Manual, (2014).

Total organic carbon (TOC) analysis was achieved by taking 1 ± 0.001 g ground soil sample (< 0.15 mm) into a labelled 100 mL digestion tube with the addition of 10 mL of 0.5 M potassium dichromate solution. Slowly 5 mL H_2SO_4 was added from a slow burette and gently, the mixture was swirled and then digested at 150^{o}C for 30 min. After cooling, 50 mL of 0.4% barium chloride was added, mixed thoroughly and allowed to stand overnight. This was done so as to leave a clear supernatant solution and was subsequently analysed using Anderson and Ingram, (1993) method. TOC was measured using a spectrophotometer SPEKOL 1500 from Analytik Jena AG, (Jena, Germany). The percent organic matter (% OM) was calculated from the percent total organic carbon (TOC %) measured.

3.3.2: Pre-treatment of chromium effluent and selected heavy metals analysis

Chromium effluent samples were prepared using standard methods established by APHA (1998). An amount of 2.5 mL of concentrated HNO_3 was added to 25 mL of chromium effluent samples in a clean Teflon beaker. The mixture was heated on a hot plate to concentrate the sample to about 10 mL. Heating of the sample continued with interval addition of 1 mL portion of concentrated HNO_3 until a clear solution was obtained. This was allowed to cool and then transferred into 25 mL standard flask and made up to the mark with distilled water. Blank

samples were also prepared using the same method. The solutions were filtered with filter paper and then syringe filtered.

A volume of 1 mL of the filtrate was taken and diluted to 100 mL with deionised water. A volume of 10 mL of the diluted sample was put into 15 mL centrifuge tubes and analysed for heavy metals using Perkin-Elmer Optima 5300 DV Inductively coupled plasma optical emission spectroscopy, ICP-OES (Perkin-Elmer, Massachusetts, USA). A calibration blank was analysed together with all samples to confirm the calibration status of the ICP-OES. The calibration curves with $r^2 > 0.999$ were accepted for concentration calculation. The instrumental parameters used for the ICP-OES lines were power (1.5kW), plasma flow (15 L/min^{-1}), auxiliary flow (1.5 L/min^{-1}), nebulizer flow (0.75 L/min^{-1}), pump rate (15 rpm). The metals were analysed using the following lines (Λ in nm) Al (396.152), Cd (228.802), Co (238.892), Cr (267.716), Cu (324.754), Fe (238), Mn (261.815), Ni (231.604), Pb (220.535) and Zn (213.857) (see Appendix Table 3.2 for raw data).

3.3.3: Pre-treatment of soil and plant samples for chromium and selected heavy metals analysis

Soil samples were digested and analysed for selected heavy metals and total chromium concentrations by modifying the EPA method 3052 (Kington and Haswell, 1997). The samples were dried, ground, sieved to homogenise and digested in triplicates by taking 1 g of soil sample and 9 mL of nitric acid. This was allowed to react before adding 2 mL of hydrogen peroxide. The solution was given time to stabilise before loading into the microwave vessel, model Defy DMO350 microwave from DEFY appliances (Pty) Jacobs (Durban, South Africa). The samples were heated at 180^{o}C for 1 h and allowed to cool for 30 min. The digested solutions were filtered using Whatman filter paper (0.45 µm). Then 1 mL of the filtrate was taken and diluted to 10 mL. The solution was analysed for total chromium and other selected heavy metals using ICP-OES.

Plant samples were first cut at root-stem-leaf junctions, respectively. The fresh weight of root, stem and leaf samples were measured using an electronic balance (Mettler Toledo, Model AG 204, Switzerland) with accuracy of 0.0001g and recorded in gram per plant. Then plant parts were dried in an oven at 60^{o}C for 24 h to get constant dry weight for root, stem and leaf**.** Plant materials were lyophilised and homogenised by grinding in milling machine Knifetec 1095 sample mill Adendorff machinery mart (Krugerdrop Johannesburg, South Africa). The samples were digested in microwave following the same procedure adapted for soil as modified from

method 3052 EPA (1996). Instrument parameters and calibration remained the same as those reported for the soil.

3.3.4: Pre-treatment of chromium effluent for Cr (VI) analysis

Homa *et al.* (2016) method was modified for the determination of Cr (VI) in chromium effluent samples. The effluent samples were filtered through a Whatman filter paper (0.45 µm). An aliquot of 5 mL was taken from each sample and then acidified with 4 mL of 0.2 M sulfuric acid, topped up to a mark of 25 mL with deionised water. The samples were analysed for chromium (VI) by a Lambda 650 Perkin-Elmer Ultra Violet -visible spectrophotometer (UV-Vis spectrophotometer) (Perkin Elmer, Inc., Waltham, MA, USA). The spectrophotometer was first scanned for lambda mark peaks between 400 and 800 nm with blanks and samples. Then they were calibrated with standards ranging from 0.1 µL to 7 mg/L. The above were done to exclude possible interferences/contaminations to Cr (VI) from oxidising or reducing agents in the samples. Samples were quantified using a UV winlab- run- wavelength quant lambda 650S-Perkin Elmer at 540 nm, with 2.00 nm slit aperture and curvete of 10,000 mm. This was done after adding 1,5 diphenylcarbazide reagent that was prepared by taking 0.25 g 1,5-diphenylcarbazide and dissolving in 50 mL of acetone as complexing agent. The complex reacted with Cr (VI), forming a pink coloured complex that absorbed Cr (VI) at the wavelength of 540 nm. The calibration curves with $r^2 > 0.999$ were accepted for concentration calculation. Concentrations were reported as the averages of triple measurements (see Appendix Table 3.3 for raw data).

3.3.5: Pre-treatment of soil and plant samples for Cr (VI) analysis

An amount of Na_2CO_3 weighing 1.07 g was dissolved in 100 mL deionised water. This was to make enough stock solution of 0.1 M Na_2CO_3 for digestion of soil and plant samples for Cr (VI) analysis by modifying the method of Lesniewska *et al*. (2017). About 0.25 g of soil/plant samples from both dumpsites and their controls were digested with 25 mL Na_2CO_3. The digested solution was acidified with 4 mL of 0.2 M sulphuric acid and complexed with 1 mL of 1,5-diphenylcarbazide (DPC), topped up to 25 mL and analysed for Cr (VI) levels. This was done using UV Vis spectrophotometer method as stated above for chromium effluent. A calibration blank and certified standard were analysed together with all samples to confirm the calibration status of the UV Vis spectrophotometer and accuracy of the method. This was done to help reduce sources of interferences to Cr (VI) in the samples. The calibration curves with $r^2 > 0.999$ were accepted for concentration calculation. Concentrations were reported as the averages of triple measurements.

3.3.6: Preparation of chromium stock and standard solution

Stock solution of chromium was prepared by dissolving 2.28 g of potassium dichromate in 1000 mL of deionised water following the modified procedure of Amin *et al.* (2014). The stock solution was then serially diluted to get the test solutions of desired chromium concentrations. All working solutions of varying concentrations for chromium were prepared daily, through the appropriate dilutions. The working standards solutions for calibrating UV-Vis spectrophotometer for the analysis of Cr (VI) were prepared by diluting 10 mL of the prepared standard solution to 100 mL. From this, a series of standards were prepared, acidified and complexed with DPC and made up to 25 mL with deionised water before use for calibration as modified from Onchoke and Sasu (2016). The working standards solutions for calibrating ICP-OES for the analysis of heavy metals were prepared from bought standards for a range of selected metals.

3.3.7: Preparation of sodium bicarbonate stock solution

An appropriate amount of 0.1 mol L Na_2CO_3 (pH ~10.0) was dissolved in 100 ml of ultra-pure water to prepare sodium carbonate solutions as modified from Elcia *et al*. (2010).Then 25 mL of the solution was taken and used for leaching of soil as the proposed extraction is good for soluble and insoluble Cr (VI) forms. It shortens the procedure of their extraction at a high temperature, i.e. 90–95°C, for only 10 to 15 min.

3.3.8: Quality assurance and control

All solutions were prepared using ultrapure quality water. All the plastic and glasswares were cleaned by soaking in dilute HNO_3 and rinsed with ultrapure water, dried prior to use. Samples were kept in polythene bags and glass that were free from heavy metals and organics. They were well covered with ice while transporting from field to the laboratory to avoid contamination and changes from the external environment. Reagent blanks were used in all analyses to check reagent impurities and other environmental contaminations/interferences during analysis. All the reagents were standardised against primary standards to determine their actual concentrations. These standards were run before, midway through and after each sample batch to calibrate the instrument. Triplicate samples and their controls were analysed to check precision and consistency of the analytical methods.

Calibration curve was constructed for Cr (VI), CrT and selected heavy metals analyses and the correlation coefficients were determined. The calibration curves with $r^2 > 0.999$ were accepted for concentration calculation. Concentrations were reported as the averages of triple

measurements for both samples and controls. The initial and final concentration of all triplicate control/blank samples (without donkey dung or unspiked soil) run along with real/treated samples were measured in every analysis carried out. The closeness of their average or percentage was used as quality assurance, control and reproducibility of the procedure.

The certified Cr (VI) standard solution was used for recovery test on soil and plant samples to confirm reliability and validity of the method. The recovery falling within the range of 75 – 120% was accepted and adopted as further assurance for the validity of the method. This reference material was processed one time in every tenth soil/plant sample. Edible vegetables were grown in simulated and none simulated soil for quality assurance and control. Plants analysed in this study were first identified on the basis of morphological similarities to existing plants on the data base at the East Africa herbarium in Museum of Kenya and University of South Africa, herbarium departments. The donkey dung used in this study were handled with care to avoid contamination. They were also characterised using various standardised spectroscopic and solid state techniques.

3.3.10: Ethics statement

This study required ethical clearance and authority to access the research locations. Therefore, before its commencement, ethical clearances and consent agreements (permissions) from the following institutions and owners of the tanneries were acquired. The ethical clearances were to allow us to officially undertake the research as per the regulations governing research work in Kenya and South Africa, respectively. The ethical clearance for South Africa was issued by University of Venda, Research ethics committee (Appendix Figure 3.2). The ethical clearance of Kenya was issued by National Commission for Science, Technology and Innovation (NACOSTI) (Appendix Figure 3.3). Informed consent forms enabled access to conduct research in Dogbone and Beit Ore, which are private tanneries. The consent agreement for Dog Bone is attached as Appendix figure 3.4 while the one for Beit Ore is attached as Appendix Figure 3.5.

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CHAPTER FOUR

Investigating chromium status, heavy metals contamination and ecological risk assessment via tannery waste disposal in Kenya and South Africa

Abstract

This thesis chapter reports on the investigation of chromium status, heavy metals contamination and ecological risk assessment of tannery waste disposal in Kenya and South Africa. The sub-Saharan African countries have instituted several environmental regulations and policies within the last two decades, to mitigate the negative environmental and public health concerns associated with rapid industrial growth and their increased discharges. However, a paradox of good environmental policies but low enforcement exists in most of these countries, with information on the pollution level unknown due to poor monitoring resources. In this study, potentially toxic heavy metals (HMs) contamination and physicochemical characteristics in effluents and dumpsite soils of two tanneries, one in Kenya (Dongo Bonde, DB) and one in South Africa (Bath Ore, BO) were evaluated during the dry and rainy seasons of 2018. Pollution levels and ecological risk in the dumpsite soils were assessed by adopting geoaccumulation index (Igeo), contamination factor (CF), pollution load index (PLI), and potential ecological risk index (PRI). The results showed that the mean final effluent concentrations of, total dissolved solids (TDS), chemical oxygen demand (COD), Cr, Cu, Fe, Ni, Zn and Cd for BO were (2127, 890, 1.82, 1.38, 1.96, 0.60, 1.21 and 1.16 mg/L), respectively and (8157, 1369, 7.90, 0.69, 1.05, 0.60, 0.72 and 0.53 mg/L), respectively for DB tannery and were above the limits of discharge guidelines. The mean total Cr and Cr (VI) concentrations in tannery dumpsite soils were (204.9 ± 29.1; 0.31 ± 0.01 and 943 ± 29.8; 0.4 ± 0.07 mg/kg for BO and DB), respectively and Fe (2498 ± 62 mg/kg in DB) exceeded acceptable thresholds of the World Health Organization (WHO), Food and Agriculture Organisation (FAO) and local background levels. Positive strong correlations were observed between Cr and organic matter, OM ($r>0.7$, $p<0.001$), electrical conductivity, EC ($r=0.99$, $p<0.05$) and As ($r=0.62$; $p<0.05$), suggesting a common anthropogenic point source. The mean PLI values of 5.3 and 1.6 for DB and BO dumpsites indicated significant pollution of the soils with HMs, specifically Cr (Igeo = 18 and 2.4 for DB and BO, respectively). Similarly, PRI values of 174.8 and 57.4 indicated moderate and low potential ecological risks for DB and BO tannery dumpsites, respectively, with several plants sampled within the two sites exhibiting elevated levels of Cr contamination. In summary, these results provide scientific insights on the need for both improved effluent management and treatment technologies of tannery

wastes, coupled with the strengthening of continuous monitoring and enforcement for compliance of industrial discharges in sub-Saharan African countries, in particular Kenya and South Africa.

Keywords: Anthropogenic contamination, Chromium effluent, Cr (VI), Ecological risk, Integrated pollution indices, Policy enforcement, Tannery dumpsites.

4.1: Introduction

In recent years, sub-Saharan Africa has seen tremendous growth in its leather industry as countries shift from exporting raw unprocessed hides and skins to semi-processed and finished leather goods to take advantage of the economy of scale provided by value addition. However, the tannery industry is considered one of the greatest contributors to global pollution, capable of generating approximately 6 million tons of solid wastes per annum and 50000 L of wastewater per ton of leather produced (Kolomaznik *et al.,* 2008; Teklay *et al.*, 2018). Solid and liquid wastes from tanneries contain large amounts of toxic HMs and metalloids such as Cr, Cd, Pb, and As which are responsible for the contamination of water bodies, agricultural soils, recreational lands and plants in urban and rural areas globally (Mwinyihija and Mwinyihija, 2010; Srinivasa *et al*., 2010; Dixit *et al*., 2015; Shi *et al.*, 2020). As a consequence, the tanning industry worldwide has been under sharp scrutiny relating to its negative image concerning pollution potential.

The great ecological significance of these HMs is their toxicity at certain concentrations, translocation through food chains, and non-biodegradability accounting for their accumulation and persistence in the environment. For example, it is estimated that waste from tanneries can generate high Cr-laden (containing up to 5000 mg Cr/kg) solid and liquid wastes (Hashem *et al.*, 2015). Cr (III) and the highly mobile and toxic species of Cr (VI) generally leach from tannery wastes into the soil, where they are either absorbed by vegetation, retained by the soil for a long period of time or washed out into the environment, contaminating terrestrial ecosystem, surface and groundwater sources (Bolan *et al.,* 2014; Shi *et al*., 2020). Although Cr (III) and some HMs in trace levels are considered essential elements because they help regulate metabolism and other activities in the human body, excess amounts of these elements in the environment are hazardous and are potentially related to their teratogenicity, genotoxicity, carcinogenicity and reduced growth in plants and animals (Dayan and Paine, 2001; Shanker *et al.,* 2005; Okereafor *et al.*, 2020). The negative consequences of environmental impact and potential public health concern of tannery waste pollution are,

consequently, a hard reality that sub-Saharan African countries must address to develop sustainable industrial economies.

One of the oldest but crude methods in Africa of disposing of industrial wastes is direct discharge into water bodies and open dumping grounds. Often than not, these wastes rarely do not meet the recommended regulations and standard guidelines *(*Akele *et al.,* 2016*;* Ndimele *et al.,* 2017*;* Zinabu *et al*., 2018). The bulk of tannery solids wastes constituting chromium sludge, chrome-tanned leather shavings (CTLSs), buffing dust, chrome leather trimmings, and other associated wastes are disposed-off in open dumpsite or landfills. Pertinently, the dumpsites for these wastes are often unlined shallow hollow excavations arising from abandoned borrow-pits and quarry-sites and often, open ground spaces, that can potentially spread into other ecosystems. Moreover, the re-use of these land spaces for agricultural purposes more often than not, are, without any environmental impact assessment studies done, thus guaranteeing future wastes compaction releases of leachates that spread from these sites into other ecosystems (Oketola and Akpotu, 2015; Hammed *et al.*, 2017). Likewise, untreated tannery effluents are indiscriminately released into municipal sewer lines and waterways such as rivers (Ndimele *et al.,* 2017; Zinabu *et al.*, 2018).

In the last decade, there has been enormous pressure on sub-Saharan African countries from the local public and various pollution control bodies to regulate and minimise discharges from different industries. As these countries are signatory to United Nation's vision for Sustainable Development Goals (SDGs) (UN, 2016), they are also under pressure to address detailed targets for pollution control and enhance regulation of pollution sources as they pursue their agro-based industrial expansion. Consequently, many countries have enacted and are enforcing environmental regulations and policies to control the disposal of industrial wastes within standard/regulatory limits that are in tandem with WHO and EPA standards. Stringent enforcement of these environmental regulations has forced some of the tanneries in sub-Saharan African countries to shut down permanently due to their inefficiency or failure to meet the strict guidelines set for chromium and other wastes disposal (Oruko *et al.*, 2014).

Currently, there are initiatives of tanneries investing in eco-friendly tanning technologies to minimise Cr waste production and tannery waste treatment and management (Solidaridad, 2018). Unfortunately, investments in these technologies have been slow due to low profit margins of the leather tanning industry, and the management of the solid wastes generated is still a big challenge. An emerging waste disposal practice by tanneries is the packing of solid and semi-solid tannery sludge wastes into plastic gunny sacks that are illegally dumped within

open spaces or public dumpsites or unused spaces within their premises. However, an increase in unplanned settlements and urban agriculture around tanneries is becoming a worrying trend. Due to the possible potential ecological risk in the ecosystems and human population around such dumpsites, it is essential to research these sites to understand pollution status and characteristics of HMs and other pollutants. So far, only limited studies have been carried out to assess the status of chromium oxidation states and selected HMs in tannery dumpsites in sub-Saharan Africa (Oketola and Akpotu, 2015; Hammed *et al*., 2017).

In the context of the prevailing environmental legislation, this study focused on the characterisation of industrial wastes of two leather processing plants, one in Kenya and the other one in South Africa, to gain insight on the current progress made in the management of the tannery wastes. In this chapter, the general characteristics of chromium effluents and dumpsites soils including heavy metals contents (As, Ni, Cr, Cu, Mn, Fe, Zn, and Pb) of two tanneries were evaluated. Further, single and integrated pollution indices like geo-accumulation (I_{geo}), individual potential ecological risk coefficient (E^i_R), contamination degree (CD), pollution load index (PLI) and potential ecological risk factor index (PRI) were used to evaluate current pollution status and the potential risks of Cr and other toxic HMs related to dumping of tannery wastes in the two sites. These results are important, considering a mismatch of good environmental policies but poor enforcement against overriding interests to attract industrial investments still existing in most of these nations, and the potential impact of environmental degradation risking future sustainable industrialisation growth.

4.2: Materials and Methods

4.2.1: Chemicals and materials

Analytical grade acids (55% HNO_3, 36% HCl and 99.9% H_2SO_4) and chemicals (99.0% anhydrous $K_2Cr_2O_7$, 30% H_2O_2, 99.7% Na_2CO_3, 0.05 mg/L Cr (VI) certified standard, 98% diphenylcarbazide, 99% ammonium acetate, 99.4% ethylenediaminetetraacetic acid (EDTA), 99% barium chloride, 99.0% potassium sulphate, 99.0% potassium chloride and acetone 99.5%) were purchased from Merck Chemicals (Pty) (Johannesburg, South Africa), and were used without any further purification. Double deionised water (Milli-QR04 system, Millipore Ltd, MA, USA) was used for the preparation of all solutions. All glassware used were thoroughly cleaned by first soaking in dilute nitric acid for at least 24 h and rinsed abundantly in deionised water before use.

4.2.2: Study area

These study sites are described in details in chapter one, section 1.8, sub section 1.8.1 to 1.8.3 of this thesis. In the published paper, the real identity and locations of the tanneries are withheld because of legal implications (pseudo names used are DB-Dongo Bonde and BO-Bath Ore).

4.2.3: Chromium waste water sample collection

The chromium wastewater samples were collected once from BO and DB sites and coded as stated in chapter three, subsection 3.1 of this thesis. Chromium treated tan liquor from DB was collected from the oxidation pond before discharge into the sewer line, whereas the untreated tan liquor was sampled directly from holding pond before treatment. In BO tannery, chrome untreated tan and re-tan liquor were sampled from the drum and vat, respectively, before discharge to sewer lines.

4.2.4: Soil sample collection

Sampling for soils was done twice to accommodate the wet and dry seasons in these study areas. Soil samples and their controls for physico-chemical and selected metals contents analyse from (DB) Kenya were taken from the topsoil profile. This was done using a spade and an improvised hand trowel to open and sample soil using a modified method reported by Mandal *et al*. (2011). Six soil samples (200 g each) were taken from a depth of between 10 - 20 cm and 20 cm wide at different spots around the dumpsites (Appendix Figure 4.1). Control soil samples were collected from different locations 100 m away from outside the tannery. This was to help determine background heavy metal content. The equipment used were cleanse

in between sampling points with distilled water. The top layer was removed and the soil in depth from 10 cm were put on a clean wide heavy paper spread under shade to avoid any potential contamination occurring where the soil was exposed. Then the soil was thoroughly mixed to make a homogenous sample. After that, approximately 1 kg of sub-sample was packed into three clean plastic bags and sealed.

Another soil sub-sample was collected using corer with cover and sealed with cello tape to prevent the loss of moisture. They were both placed into a portable cooler box with frozen ice packs and transported within 1 hour to Kenya Agriculture Livestock Research Organisation Laboratory (KALRO) and University of Nairobi in Westland, Nairobi. The sub-samples were submitted in duplicate for the analyses of the following parameters: moisture content, electric conductivity, pH, cation exchange capacity, total organic carbon, exchangeable cation, soil texture and organic matter. These parameters were analysed in Kenya to get prompt data on them to pre-empt their conditional changes and interferences during shipment to South Africa (Appendix Figure 3.1). The other soils were rapidly sealed in the polyethylene bags (Martin-Gomez *et al.,* 2015). The samples were then placed in cooler box with enough dry ice in the field before transporting and shipping them to the laboratory for further analysis in SA.

In the case of BO in SA the above procedure was also followed. A sub sample of soil of 1 kg was submitted to University of Venda Soil Science Laboratory for immediate analyses of physico-chemical parameters. The remaining soil samples were kept together safely with those from Kenya in the fridge at 4^{o}C before they were taken to University of South Africa (UNISA) laboratory for selected metal content analysis. The soil sample codes were BO dumpsite, BO control, DB dumpsite and DB control soils. Seasonal variations of the soil's composition were observed; 'W' stands for wet season, ''D' dry season in South Africa and Kenya. In this study, soil samples for DB were collected during dry and rainy periods while BO were collected in winter and summer seasons of 2018.

4.2.5: Vegetation sample collection

The vegetations were sampled once to establish association between bioavailability and chromium contamination of the soils from the two locations. They included *Spinacae oleracea, Dactyloctenium aegyptenium, Cynodon dactylon, Alternanthera caracasana, Flaveria bidentis* and *Corchorus tridens* from BO. *Amaranthus dubius Thell, Cynodon nemfluensis vanderst and Dactyloctenium aegyptenium* from DB. These plant samples were purposively collected using procedure modified by Chizoruo *et al.* (2017). The dominant plant

species of interest were collected by uprooting the plants with all their components from the root to leaves (whole plant) from the dumpsites. The plants were shaken to remove soil debris in roots and then put in an airtight plastic bag, tied and labelled with a marker, then conveyed to the laboratory in South Africa. Plant samples were marked DB and BO plants to represent the samples locations. The plants were identified and named scientifically at the East African museum of Kenya and the University of South Africa, herbarium section of Horticulture department in the college of Agriculture and Environmental Science, Florida campus.

Vegetable and other plants sampled for chemical analyses were taken from the surrounding of the dumpsites in the study areas. The whole plants were sampled owing to their ability to selectively germinate and grow in such contaminated sites and possibly accumulate trace/heavy metals from the soils (Pyle *et al.,* 1996). The whole plants were handpicked manually and a representative sample was achieved by taking several sufficient quantities randomly. They were combined to form a composite sample. All plant samples were stored in polypropylene bags and transported to the laboratory.

4.2.6: Pre-treatment of chromium effluents and physico-chemical analysis

A volume of 100 mL chromium effluent samples was taken and filtered using fine Whatman filter paper (0.45 µm,). This was to remove the suspended materials before the analysis as modified from APHA (1989). Portable Hanna Multi-parameter Meter HI98194 from Hanna Instruments (Pty) (Johannesburg, South Africa) was first calibrated using the appropriate standards. It was then inserted into the chromium effluents to measure physico-chemical parameters such as pH, salinity, electrical-conductivity (EC), temperature and total dissolved solids (TDS). The readings were taken in triplicate. The BOD and COD analyses were carried out using kits purchased from Sigma-Aldrich (Missouri, USA) and Merck (Darmstadt, German). Their analyses were done following the manufacturers instruction in the manual. The same procedure was applied to samples analysed in KALRO Kenya.

4.2.7: Pre-treatment of soil samples and physico-chemical analysis

Total nitrogen was determined by semi micro Kjeldahl digestion method where soil sample of 100 mg was finely grounded to less than 100 mesh. Then 0.5 g potassium sulphate (catalyst) and 1 mL of sulfuric acid were added. Then 5 mL of the aliquot was put on Tecator block digester and heated at 375°C for 3 h, cooled, diluted to 100 mL. Then 40 mL was taken for determination of the nitrogen content by steam distillation following Kjeldahl method APHA, (1992). All analyses were done in triplicate. In addition to the above, moisture content, electric conductivity, pH, cation exchange capacity, exchangeable cation and soil texture were also

analyse as described in detail in chapter 3, subsection 3.3.1 of this thesis (see Appendix 3.1 for more details).

4.2.4: Total chromium and other trace/heavy metals analyses

The total chromium and other HMs concentrations of the digested chromium effluents, sample soils and plants were analysed using the detailed methodologies described in chapter three subsection 3.3.2 - 3.3.3. The equipment used for analyse are also detailed in the same subsections of this thesis while the chemicals used for the analyse are mentioned in subsection 4.2.1 of this thesis.

4.2.5: Chromium (VI) analyses

To analyse for Cr (VI) concentrations in effluents and soils/plants, the detailed methodologies are described in subsection 3.3.4 - 3.3.5. The chemicals used are stated above in subsection 4.2.1, while the equipment are described in subsection 3.3.1 and 3.3.2 of this thesis. Cr (III) was calculated by subtracting Cr (VI) from the total Cr content. Reference standards of $K_2Cr_2O_7$ solution were analysed as indicated in chapter three sub section 3.3.8 of this thesis for method validation.

4.2.5: Assessment of chromium and selected heavy metals pollution levels of the two tannery dumpsites

The single indices, such as geo-accumulation (I_{geo}), contamination factor (CF) and ecological risk factor ($E^i r$), as well as the integrated indices like; contamination degree (DC), pollution load index (PLI), and potential ecological risk index (PRI) were used to assess the pollution levels. Integrated pollution indices for the studied samples were determined to build an overall view of the extent of contamination of the dumpsite by the Cr and selected heavy metals according to Salman *et al*. (2019); Marcus *et al.* (2017); Sallam *et al*. (2015); Agyarko *et al.* (2010)**;** Hakanson (1980) and Muller (1979).

Geo-accumulation index was expressed by equation (4.1) as proposed by Muller (1979).

$$I_{geo} = log_2 \left(\frac{Cn}{1.5} \; X \; B_n\right) \quad (4.1)$$

Where: C_n – measured concentration of metal in the refuse dump soil (mg/g); B_n – background value of heavy metal (mg/g); and 1.5 – background matrix correction factor.

Contamination factor (CF) was determined from equation (4.2) proposed by Hakanson (1980).

$$CF = \frac{[C1]}{[C2]} \quad (4.2)$$

Where C_1 is heavy metal at dumpsite of study. C_2 is heavy metal at non-dumpsite (control site).

Contamination degree (CD) was expressed by equation (4.3) of Hakanson (1980), as sum of CF.

$$CD = \Sigma CF \quad (4.3)$$

Pollution load index (PLI) was determined as the n^{th} root of the product of the n CF as expressed in equation (4.4), developed by Tomilson *et al.* (1980).

$$PLI = (CF_1 \, x \, CF_2 \, x \, CF_3 \ldots\ldots\ldots\ldots x \, CF_n)\frac{1}{n} \quad (4.4)$$

Where CF is the single contamination factor and is the count of the elements present, where n is the number of metals studied.

Bioaccumulation factor (BF) is computed according to the following formula (4.5) adapted from Rashed (2010); Carbonell *et al*. (2011); Bose & Bhattacharyya (2008)

$$BF = C_p/C_s \quad (4.5)$$

Where C_p and C_s are average concentrations of metal in plant and soil, respectively.

Ecological risk factor E_r^i was mathematically calculated using equation (4.6) proposed by Hakanson (1980); Marcus *et al*. (2017).

$$E_r^i = T_r^i \, x \, Cf \quad (4.6)$$

Where T_r^i = the toxic-response factor for a given element and Cf = the contamination factor of the element.

Potential ecological risk (PRI) was used to sum up the multiple effect of metals pollution in equation (4.7) according to Hakanson (1980); Marcus *et al*. (2017).

$$PRI = \sum E_r^i \quad (4.7)$$

The ecological risk factor could also be represented further as shown in Table 2.1 in chapter two of this thesis**.**

4.2.6: Statistical analysis

The data generated were analysed as described in methodology chapter three, subsection 3.9 of this thesis.

4.3: Results and Discussion

4.3.1: Physico-chemical properties of wastewater in Dongo bonde and Bath Ore dumpsites

The variation in the physicochemical parameters of chromium effluent streams from untreated, treated and recycling/reuse processes from the two tanneries is presented in Table 4.1. The pH of the study sites ranging from 3.42 ± 0.15 to 5.82 ± 0.58 at BO and DB, respectively, were below the WHO regulatory limit of 6.0 - 9.0 (WHO, 2011). But, the DB range fell within South African Green drop standard pH limit of 5.5 to 9.5 possibly due to partial neutralisation in the oxidation pond before discharge (DWAF 1996; Adewumi *et al.,* 2011). However, the pH values of untreated tan liquor in BO was significantly different ($p < 0.05$) than the final chromium liquor waste. This indicates that, the recycling/reuse of chrome tan liquor waste influences the final wastewater physicochemical characteristics. Consistent with findings of this study, Hashem *et al.* (2015) also reported that chromium effluents of tanneries in Bangladesh were generally acidic in nature. However, their study reported lower pH (2.5-3.0) compared to the current study, with the authors attributing the low pH to lack of neutralisation of the effluents before discharge.

Before the tanning process, pelts are normally pickled at pH 2.5-3.0. This is to prepare the pelt for better penetration of Cr (III) into the hide collagen fibres before fixing it at pH 4.0 (Onem *et al*., 2017). Comparatively, BO final chromium tan waste had lower pH than DB lime treated effluents from oxidation ponds. This implied that the residual acid in the pelts from the pickling and buffering process before tanning and recycling/reuse of chromium liquor and other tannins greatly contributed to BO effluent's low pH, and thus requires further neutralisation step before discharge into municipal sewer lines.

The chromium effluent streams from the two tanneries also exhibited variability in BOD, COD, TDS, and salinity levels. Overall, both chrome liquor recycling process and oxidation pond treatment resulted in a marked reduction in the BOD, COD, TDS, and salinity levels of the final effluents of BO and DB tanneries (Table 4.1). However, the final effluent BOD, COD, salinity and TDS levels in BO (447±7.9, 890 ± 6.1, 1025±7.4 and 2127±8.1 mg/L, respectively) were comparatively lower than DB effluent (1051±258, 1369±301, 14903±5796 and 8157±748 mg/L, respectively). Despite the final tannery effluent BOD, COD, TDS, and salinity levels being lower than in untreated samples, the recorded levels were higher than South African Green drop standard and permissible effluent discharge limits (DWAF 1996; Adewumi *et al*., 2011). These results are in agreement with several studies around the world that show BOD,

COD, TDS and salinity values in the tannery effluents are generally higher than the tolerance discharge limits for inland surface water (Vasudevan *et al., 2012;* Chowdhury *et al., 2015;* Boujelben *et al.*, 2019; Sawalha *et al.*, 2019).

Tannery wastewater is characterised by high organic, inorganic and nitrogenous compounds, chromium, suspended solids, and dissolved solids, that contribute significantly to the BOD, COD, and TDS that have a negative effect on aquatic life. The high BOD levels could cause anaerobic fermentation processes and the release of ammonia and hydrogen sulphide gases resulting in poor smell as well as affecting aquatic organisms in receiving water bodies due to low dissolved oxygen (Sahu *et al*., 2007). On the other hand, the high levels of salinity in the tannery effluent could be linked to the inorganic compound used for wet salting, deliming, and pickling processes for skin and hide preservation, removal of lime and buffer effects during the pickling process. Similarly, the high salinity and TDS from tannery effluent into the freshwater environment has been reported to have a detrimental effect on freshwater organisms, especially osmotic imbalance, impairment of homeostasis process in aquatic lives, increase in plasma that affects the heart rate and nerve excitability resulting into death and total loss of aquatic organisms (Calheiros *et al.*, 2012).

In addition to physicochemical parameters, tannery effluent samples were analysed for the identification and determination of heavy metals concentrations. In total, 8 elements were detected at different concentrations (Table 4.1). Cr levels were highest among all the metals analysed, with untreated chrome liquor recording 42±2.16 and 24±6.57 mg/L Cr in BO and DB samples, respectively. However, the recorded level of Cr in the final effluents was significantly above the WHO permissible limit of 0.1 mg/L (WHO, 2011). This is consistent with similar observations from other developing and Sub-Saharan African countries, where high values (3.5-5; 420 mg/L Cr) have been reported in tannery waste effluents (Mwinyihija *et al*., 2006; Oguttu *et al*., 2008; Hashem *et al*., 2015; Zinabu *et al*., 2018; Sawalha *et al*., 2019). These high Cr concentrations may be attributed to the amount of unspent chromium sulphate applied as a tanning agent to convert pelts into leathers. Comparatively, the chrome liquor recycling step used by BO resulted in the approximately 20-fold reduction in the Cr levels in the final effluent, whereas oxidation pond treatment of DB effluents only gave a 3-fold reduction in total Cr levels (Table 4.1).

It is estimated that under conventional leather processing, practiced by DB tannery in Kenya, chromium tanning produces large quantities of chromium-based waste (up to 3.5 – 4.5 % w/w chromium as Cr_2O_3) both in liquid and solid form, which has to be treated before disposal

*(*Kanagaraj *et al.,* 2015). Thus, the oxidation pond treatments (sedimentation) coupled with chemical precipitation practiced by DB do not effectively reduce the Cr levels (7.9±3.14 mg/L) to meet the regulatory limits before discharge. In contrast, recycling/reuse process is one of the eco-friendly technologies used to minimise the production and management of chromium wastes. It is a process, in which the spent chrome liquor is recycled and reused for tanning to maximise exhaustion of Cr in the tan liqour before discharge (Sundar *et al*., 2002).

Similar to Cr levels, all other heavy metals contents of the two tanneries were evidently above the discharge limits of 0.01 mg/L Cu, 0.3 mg/L Fe, 0.1 mg/L Zn and 0.005 mg/L Cd for industrial wastewater in South Africa (DWAF, 1996). Findings of this research concur with the observations from several studies around the world that tanneries are part of the main contributors of Cr and other heavy metals pollutants into the environment (Chowdhury *et al*., 2015; Hashem *et al*., 2015; Ramamurthy *et al*., 2015). Overall, it is estimated that a medium-sized tannery practicing conventional tanning process discharge daily over 300 million cubic meters of waste liquor and tanning sludge with Cr levels up to 5.2 mg/L (Sundar *et al.*, 2011; Hashem *et al*., 2015; Kanagaraj *et al*., 2015). The non-biogenic HMs (such as Cd and Cr) are highly hazardous and can have potentially toxic effects on living organisms. Further, these HMs are generally persistent in the wastewater treatment system because of their non-biodegradable and recalcitrant nature. Thus, their presence in tannery wastewater and subsequent release to the environment may result in detrimental effects to both human health and aquatic ecosystems.

Table 4.1: The mean (± SE) values for physico-chemical parameters and heavy metals concentrations in the different chromium waste streams from tanning operations of DB and BO tanneries

Parameter [a]	BO			DB			Permissible limits (mg/L)[c]
	Untreated chrome liquor	Recycle liquor	p-value[b]	Untreated chrome liquor	Treated chrome liquor	p-value[b]	DWAF 1996
BOD (mg/L)	2181 ± 649	447 ± 7.9	0.1105	1479 ± 296	1051 ± 258	0.3021	-
COD (mg/L)	3342 ± 761	890 ± 6.1	0.0637	2940 ± 775	1369 ± 301	0.0881	75
Salinity (mg/L)	*25082 ± 3653*	*1025 ± 7.4*	*0.0028*	22611 ± 4034	14903 ± 5796	0.3007	-
TDS (mg/L)	78945 ± 4448	2127 ± 8.1	0.2767	*24424 ± 943*	*8157 ± 748*	*<0.0001*	25
pH	*3.42 ± 0.15*	*4.67 ± 0.00*	*0.0006*	4.93 ± 0.58	5.82 ± 0.58	0.3792	5.5-9.5
Cr (mg/L)	*42 ± 2.16*	*1.82 ± 0.01*	*<0.0001*	*24 ± 6.57*	*7.9 ± 3.14*	*0.0449*	0.05
Cu (mg/L)	0.70 ± 0.30	1.38 ± 0.00	0.1631	0.71 ± 0.30	0.69 ± 0.30	0.9681	0.01
Fe (mg/L)	1.76 ± 0.84	1.96 ± 0.00	0.8749	1.06 ± 0.45	1.05 ± 0.45	0.9915	0.3
Ni (mg/L)	0.62 ± 0.28	0.60 ± 0.00	0.2253	0.61 ± 0.26	0.60 ± 025	0.9932	-
Zn (mg/L)	1.10 ± 0.10	1.21 ± 0.00	0.4684	*1.33 ± 0.06*	*0.72 ± 0.22*	*0.0232*	0.1
Cd (mg/L)	0.58 ±0.26	1.16 ± 0.00	0.1725	0.58 ± 0.26	0.53 ± 0.26	0.9992	0.005
Ag (mg/L)	0.59 ± 0.26	1.18 ± 0.00	0.1664	0.59 ± 0.26	0.92 ± 0.32	0.444	-
Co (mg/L)	0.61 ± 0.28	1.17 ± 0.01	0.2028	0.58 ± 0.26	0.59 ± 0.26	0.9914	-

[a] BOD – biological oxygen demand; COD – chemical oxygen demand and TDS – total dissolved solids

[b] Based on Welch two sample t-test values, where the t-test values were significant has been italicized for clarity

c Legal permissible limits for industrial wastewater set by South African Government Gazette No.36820, 201

4.3.2: Physico-chemical properties of soils in Dongo bonde and Bath Ore dumpsites

In sub-Saharan Africa, many tanneries dispose of the bulk of their chromium-based solids wastes such as chromium sludge, chrome-tanned leather shavings (CTLSs), and chrome leather trimmings in landfills and sometimes into illegal dumpsites (Oketola and Akpotu, 2015; Hammed *et al*., 2017). Due to the potential of these wastes to contaminate the surrounding ecosystems, this study analysed the soil physicochemical properties and heavy metal concentrations of the two tanneries dumpsites vis-à-vis surrounding environment to delineate the impact of tannery wastes disposal on soil quality and health. As shown in Table 4.2, the two tanneries dumpsites exhibited unique soil physicochemical profiles with elevated levels of several parameters than the control sites.

The concentrations of the metals in the BO dumpsite soil were found to be in the order Cr>Fe>Mn>Cu>Zn>Ni>Pb>Cr(VI)>As with samples having significantly higher Cr (*Welch's two-sample t-test,* t = -6.214, p < 0.01), total organic carbon, TOC (*Welch's two-sample t-test,* t=--4.180, p <0.01) organic matter, OM (*Welch's two-sample t-test,* t=-4.548, p <0.01) and total nitrogen TN (*Welch's two-sample t-test,* t=-30.74, p < 0.001) than surrounding background soil levels (control samples). In contrast, Fe, Cr and Mn were the major HMs in the DB dumpsite (Table 4.2), with their concentrations in the order Fe > Cr > Mn > Pb > Ni = Cu > Zn >Cr (VI) > As. However, the Welch two-sample t-test revealed significantly elevated levels of Cr (p < 0.001), Fe (p < 0.05), Mn (p < 0.01), As (p < 0.05), EC (p < 0.001), pH (p< 0.01), TOC (p < 0.001), OM (p < 0.01) and TN (p < 0.01) in the DB dumpsite than control sites (Table 4.2). Consistent with this study, higher values of EC, Cr, and Mn in tannery dumpsite soil samples than control samples have been reported in Saudi Arabia *(*Sallam *et al*., 2015), India (Paul *et al.*, 2015), Brazil (Augustin and Viero, 2012) and Bangladesh (Alam *et al*., 2012; Islam *et al.*, 2017).

The high levels of Mn and Fe point to DB tannery using either $MnSO_4$ and $FeSO_4.7H_2O$ or $FeCl_3.H_2O$, as coagulants in the oxidation ponds to treat limeyard tannery effluents (Buljan *et al.*, 2011) as confirmed by the DB effluent treatment attendant. One curious observation in DB dumpsite was the dumping of broken-down plant equipment and scrap metals together with chromium wastes. Furthermore, some waste dyes and vegetable tannins used in post leather tanning and finishing are rich in Fe and may have contributed to contamination of the dumpsite soil.

Among the HMs analysed, Cr levels in both tannery dumpsites (204.9 ± 29.1 and 943 ± 29.8 mg/kg for BO and DB, respectively) and Fe (2498 ± 626 mg/kg in DB) exceeded the permissible limits of 100 mg/kg Cr and 500 mg/kg Fe in soils (WHO, 1996; Van Lynden *et al.*, 2004). Of great public health concern and ecological risk, was the significant (p <0.001)

elevated levels (15 to 40-fold increase) of the Cr (VI) status in the dumpsite site than control site (Table 4.2), which exceeded the permitted levels of 0.05 mg/kg (DWAF 1996; WHO 1996). In the analysis, spiking uncontaminated soils using 0.05 mg/L certified Cr (VI) standard gave recovery percentage of 98.6%, falling within the recommended range of 75 – 120% according to USEPA method 3060A (Vitale *et al*., 1997). Therefore, the elevated Cr (VI) levels point to the potential transformation of the thermodynamically stable and less toxic residual Cr (III) in the solid tannery wastes to the highly toxic and mobile Cr (VI) species under the dumpsite environment (Kim and Dixon, 2002).

There are reports that the transformation of Cr (III) to Cr (VI) within the environment can be influenced by soil physicochemical parameters mobile ligands such as citric acid, diethylene triamine pentaacetic acid (DTPA), and fulvic acid-mediated oxidation, decomposition of organic matter by microbes, the mixing of dissolved reduced sulphates, abiotic factors and other composites in the effluents/wastes (Kim and Dixon, 2002; Namieśnik and Raba Jczyk, 2012). For example, Apte *et al*. (2006) reported up to 17% conversion of Cr (III) to Cr (VI) occurred in sludge under natural environmental aerobic conditions in 30 days. The oxidation of chromium oxide (Cr_2O_3) by oxygen and oxidation of chromium hydroxide ($Cr(OH)_3$) by manganese dioxide (MnO_2) to Cr (VI) species are thermodynamically feasible in both aerobic and mildly anoxic environments (Apte *et al*., 2006; Namieśnik and Raba Jczyk, 2012). pH may also play a critical role in the interconversion and mobility of chromium status and other HMs.

In most terrestrial ecosystems, inorganic Cr (III) occurs mainly in the stable insoluble $Cr(OH)_3$ form. However, Cr (III) exceeding the background value continues to be observed in dumpsites soils at depths beyond even 40 cm, indicating their mobility within soil strata (Reijonen and Hartikainen, 2016). Under landfill/dumpsite environments, Cr (III) can form Cr (III)-organic complexes with dissolved organic matter and other oxidising ligands in the leachate that enhances their solubility, mobility and conversion in the soils. On the other hand, Cr (VI) exhibits high redox potential under the acidic conditions which favour the reduction of Cr (VI) to Cr (III), while at the alkaline pH the oxidation character of Cr (VI) is less effective, thus it tends to persist as Cr (VI) status rather than be reduced to Cr (III) status (Markiewicz *et al*., 2015). Moreover, the high levels of OM and TN may have stimulated the proliferation of niche microbial groups capable of degrading chromium wastes by either aerobic or anaerobic reduction process (Zeng *et al.*, 2016). In the presence of Fe (II) or sulphide, Cr (VI) can readily be reduced to Cr (III) by microbial activities (Park *et al*., 2018; Liu *et al.*, 2019). Conversely, microbial oxidation of Cr (VI) may also occur in the presence of high organic matter, MnO_2, Fe (III), and reduced sulphur as an electron donor (Dhal *et al.*, 2013). Therefore, it is plausible that a combination of factors such as high Fe (III), Mn, OM, and TN levels coupled with pH contributed to the transformation of Cr state and the reported Cr (VI) levels in the dumpsites.

Table 4.2: Comparison of physicochemical and heavy metals concentrations parameters in the control site and dumpsite soils from two tanneries

Parameter[a]	BO			DB		
	Dumpsite	Control site	t-value[c]	Dumpsite	Control site	t-value[c]
Cr (mg/kg)	*204.9 ± 29.12*	*17.1 ± 2.75*	*-6.214***	*943 ± 29.8*	*10.7 ± 2.28*	*-17.734****
Cr (VI) (mg/kg)	*0.31 ± 0.01*	*0.02 ± 0.01*	*-19.52****	*0.4 ± 0.04*	*0.01 ± 0.00*	*-26.83****
Cu (mg/kg)	10.9 ± 2.04	13.4 ± 3.07	0.497	12.3 ± 2.41	20.6 ± 4.31	-1.111
Fe (mg/kg)	59.2 ± 1.48	46.0 ± 10.8	-0.666	2498 ± 626	*15.4 ± 3.8*	*-2.796**
Ni (mg/kg)	7.0 ± 0.75	5.9 ± 0.03	-0.26	12.3 ± 1.98	12.6 ± 1.88	0.078
Pb (mg/kg)	6.1 ± 0.68	8.7 ± 0.94	0.864	15.4 ± 1.84	9.7 ± 1.98	-0.995
Zn (mg/kg)	8.1 ± 0.82	8.3 ± 1.68	0.055	10.1 ± 3.32	11.9 ± 2.45	0.410
Mn (mg/kg)	12.0 ± 0.73	7.8 ± 1.39	-2.271	*240 ± 60.6*	*10.6 ± 1.70*	*-2.510***
As (mg/kg)	0.20 ± 0.02	0.08 ± 0.01	-2.219	*0.28 ± 0.02*	*0.11 ± 0.02*	*-3.142**
EC (µS/cm)	545 ± 36	430 ± 36	-1.366	*7000 ± 81*	*353 ± 56*	*-17.951****
pH	6.50 ± 0.32	6.60 ± 0.27	0.244	*9.80 ± 0.35*	*7.80 ± 0.56*	*-4.542***
TOC (%)	*1.68 ± 0.22*	*0.32 ± 0.10*	*-4.180***	*4.95 ± 0.23*	*1.85 ± 0.12*	*-7.020****
OM (%)	*2.69 ± 0.32*	*0.44 ± 0.11*	*-4.548***	*8.78 ± 1.16*	*3.17 ± 0.37*	*-3.459***
CEC (cmol/kg)	26.7 ± 4.93	30 ± 5.38	0.4434	19.7 ± 3.84	25.3 ± 4.50	0.828
TN (%)	*1.06 ± 0.02*	*0.14 ± 0.03*	*-30.74****	*3.47 ± 0.52*	*1.09 ± 0.12*	*-4.314***

[a] EC – electrical conductivity; TOC – total organic carbon; OM – organic matter; CEC - cation exchange capacity and TN - total nitrogen and pH-hydrogen ion concentration

[b] Results are given as means ± standard error of the mean for physicochemical parameters and for heavy metal concentrations

[c] Welch two sample t-test values. Significance difference is denoted by *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

4.3.3: Correlation coefficients of soils in DB and BO dumpsites

The analysis of the interrelationship between physical parameters and HMs also gave an insight into the influence of the tannery wastes on the dumpsite soil quality. The results of bivariate two-tailed Pearson correlation analysis are presented in Table 4.3. Overall, results showed that significant and positive strong correlations ($r > 0.7$, $p < 0.001$) existed between Cr, OM and TN, indicating that they may have a common point source and distribution in the dumpsite soils (related to chrome sludge, chrome-tanned leather shavings (CTLSs), and chrome leather trimmings wastes). Additionally, the two dumpsites exhibited moderate significant positive correlation between Cr and As ($r=0.62$; $p<0.05$ and $r=0.39$; $p<0.05$, for BO and DB dumpsites, respectively). A strong and significant positive correlation was also observed between OM and As ($r=0.79$, $p < 0.01$) for BO dumpsite.

These results point to the use of As as antiseptic/fungicide for either raw skin preservation or wet blue leather, with the residual As contaminating the soil via tannery wastes dumping (Nasr *et al.*, 2011). However, a strong positive correlation between Cr and EC ($r=0.99$, $p < 0.001$) was also observed in DB, providing evidence of the significant contribution of chromium sludge cakes to the pollution of dumpsite soils. Other subtle variations observed in DB dumpsite included significant ($p < 0.05$) positive Cu-Ni ($r = 0.83$, $p < 0.001$), Cu-Zn ($r= 0.71$, $p < 0.05$) and Zn-Ni ($r= 0.58$, $p < 0.05$), OM-Cr ($r= 0.77$, $p < 0.01$) (Table 4.3). In contrast, EC levels in BO dumpsite was negatively correlated to Cu, Ni, Pb and Zn, with HMs (Ni and Cu, Zn and Pb) showing moderate and strong correlations in BO dumpsite at significant levels of $p < 0.05$ and $p < 0.01$, respectively.

Table 4.3: Two-tailed Pearson's correlation coefficients for physico-chemical parameters in the soil samples of BO (lower unshaded panel) and DB (upper shaded panel) chromium waste dumpsites

Parameter	EC	OM	TN	Cr	Cu	Ni	Pb	Zn	As
EC		0.80**	0.84***	0.99***	0.45	0.10	0.43	-0.07	-0.20
OM	0.25		0.78**	0.77**	0.36	0.07	0.69*	-0.13	0.24
TN	0.44	0.83***		0.80**	0.47	0.05	0.37	0.07	0.71
Cr	0.41	0.94***	0.89***		0.41	0.11	0.41	-0.12	0.39*
Cu	-0.61*	0.13	-0.21	0.04		0.83***	0.46	0.71*	0.58
Ni	-0.64*	0.34	0.07	0.20	0.65*		0.35	0.58*	0.10
Pb	-0.69*	0.13	0.29	-0.10	0.77**	0.75**		-0.08	0.31
Zn	-0.67*	0.28	-0.04	0.14	0.89***	0.84***	0.78**		0.32
As	-0.11	0.79**	0.56	0.62*	0.36	0.39	0.50	0.43	

Correlations are defined as weak ($0 < |r| < 0.3$), moderate ($0.3 < |r| < 0.7$) or strong ($|r| > 0.7$). Significant correlations (at $p < 0.001$ '***', $p < 0.01$ '**', $p < 0.05$ '*'). EC, (electrical conductivity), OM, (organic matter) and TN, (total nitrogen)

4.3.4: Seasonal effect on selected heavy metals in the two dumpsites

The variability in heavy metals contents (Cr, Cu, Pb, Ni and As) of tannery dumpsite soils for wet and dry seasons are summarised in Figure 4.1. While the DB dumpsite metal levels were higher in rainy (wet) than the dry season, BO dumpsite had higher levels during winter (dry) than the summer season (wet). However, it is worth noting that the concentrations of total Cr and Cr (VI), generally persisted above the permissible limits across the two seasons. The differential temporal variations in HMs, especially Cr state, could be ascribed to the differences in waste profiles and management practices within the two tannery dump sites. In BO, the major dumpsites solid chromium wastes are chrome-tanned leather shavings (CTLSs), and chrome leather trimmings while the chromium tannery effluents (liquid) after recycling/reuse during tanning stage are directly discharged into municipal sewer lines for further treatment. In contrast, about 50% of the DB solid chromium wastes are dried chromium sludge cakes precipitated from the tanning effluent discharged into oxidation treatment ponds. The sludge are packed in sacks including chrome-tanned leather shavings (CTLSs), and chrome leather trimmings wastes and dump in open spaces inside the tannery.

During the wet season, the intervening rainfall, surface runoff, and percolation are expected to dilute the HMs within the soil environment especially for BO, where acidic pH favours solubilisation and leaching of Cr in the soils (Mondol *et al.*, 2014). In the dry season, however, reduced dilution will lead to concentration of pollutants and thus more serious pollution of the soil components. The high total Cr in DB dumpsite during the rainy season may also be due to the partial dissolution of dried chromium sludge cakes in the plastic bags/sacks and their leachates into the soil. It is plausible that under the slight alkaline soil pH in DB, the soluble Cr precipitates in the soil matrix and further increases the soil total Cr level, while the high mobile Cr (VI) percolates into the deeper soil substratum. Similar findings have previously been reported in literature (Kim and Dixon, 2002; Reijonen and Hartikainen, 2016). Overall, leaching of the highly toxic and mobile Cr (VI) and other HMs can be a potential ecological risk and public health concern due to the ability of the landfill/dumpsites leachates to contaminate the ground and surface water sources.

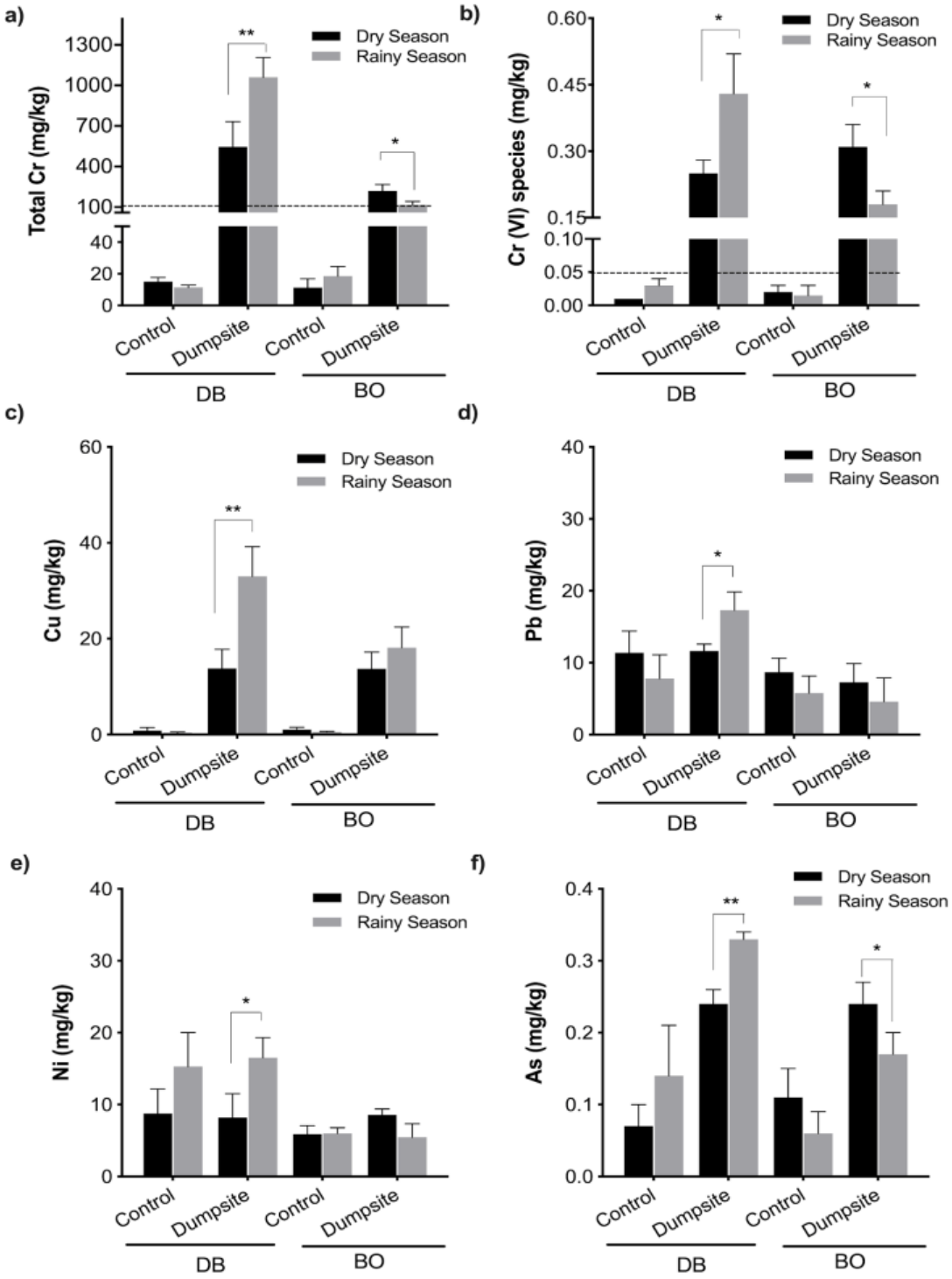

Figure 4.1: Mean Seasonal variations of toxic HMs in soil samples from DB and BO tanneries dumpsites (n = 3, RSD). WHO permissible limits for total Cr, Cr (VI), Cu, Pb, Ni, and As in agricultural soils are 100, 0.05, 100, 56, 50, and 0.5 mg/kg, respectively (WHO 1996; Van Lynden *et al*., 2004). Samples which exhibited significant two-sample t-test differences are denoted by *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. The breakpoint in the Y-axis has been used to bring clarity in data presentation in Figures 4.1a and b due to very large scale differences in analysed chromium oxidation states

4.3.5: Environmental quality evaluation and ecological risk assessment of the dumpsite soils

Based on monitoring data of soil quality in the study areas, quantitative analysis of chromium and other HMs pollution in soil from the tannery waste dumpsites was conducted by calculating geo-accumulation (I_{geo}), contamination factor (CF), contamination degree (CD), pollution load index (PLI), ecological risk assessment (E_R^i) and potential ecological risk index (PRI) (Table 4.3, Figure 4.2). Further, the Cr content of the plants sampled within the vicinity of the tannery dumpsites was also quantified (Figure 4.3).

As shown in Table 4.3, the mean I_{geo} and CF values of the HMs in DB tannery dumpsite were ranked in the order of Fe > Cr > Mn > As > Pb > Ni = Zn > Cu and Fe >Cr >Mn >As >Pb >Ni >Zn >Cu, respectively. Among the metals, only Cr, Fe and Mn had mean I_{geo} values greater than one with CF values of 88.1, 162.2 and 22.6, respectively. These results indicated that they are the major HMs contaminating DB dumpsite. In contrast, I_{geo} values for BO dumpsite soil exhibited heavy metal contamination in decreasing order of Cr > As > Mn = Fe > Ni = Zn = Cu > Pb. However, only Cr had mean I_{geo} value greater than one. The typical classifications of contamination level based on I_{geo} are: $I_{geo} \leq 0$ is no contamination (grade I), $0 < I_{geo} \leq 1$ is light to moderate (grade II), $1 < I_{geo} \leq 2$ is moderate (grade III), $2 < I_{geo} \leq 3$ is moderate to heavy (grade IV), $3 < I_{geo} \leq 4$ is heavy (grade V), $4 < I_{geo} \leq 5$ is heavy to extremely serious (grade VI) and $I_{geo} \geq 5$ is extremely serious (VII), respectively (Kowalska *et al.*, 2018; Kuerban *et al.*, 2020).

Hence, Cr and Fe contamination of the DB dumpsite can be categorised to be extremely serious, with heavy to extremely serious contamination with Mn, while Cr contamination of BO dumpsite was moderate to heavy. The individual contamination factor (CF) of elements may be used to estimate the degree of contamination and relative retention time of HMs in the soils. Specifically, higher CF values have been reported to demonstrate low retention time and high risk to the environment *(*Sakan *et al.,* 2019) and thus, the high values reported for Cr in both sites and Fe and Mn in DB site are of great ecological concern. Consistent with these results is the PLI for DB and BO sites which were 5.3 and 1.6, respectively, indicating that both dumpsites soils were polluted by HMs albeit at different levels.

Table 4.3: Indices of pollution (geo-accumulation factor, contamination factor, pollution load index for DB and BO dumpsite soils)

Metal	Geo-accumulation factor (I_{geo})		Contamination factor (CF)		PLI	
	DB	BO	DB	BO	DB	BO
Cr	18	2.4	88.1	11.9		
Cu	0.1	0.2	0.6	0.8		
Fe	33	0.3	162.2	1.3		
Ni	0.2	0.2	1	1.2		
Pb	0.3	0.1	1.6	0.7		
Zn	0.2	0.2	0.8	1		
Mn	4.5	0.3	22.6	1.5		
As	0.5	0.5	2.5	2.5		
All					5.3	1.6

In contrast to I_{geo} and PLI values that focus on the comparative evaluation of heavy metal content in the environment, potential ecological risk indices E_R^i and PRI calculate the toxic differences of heavy metal contamination, and are thus, better indices to delineate potential risk of toxic heavy metal even under a situation of low pollution (Kuerban *et al.*, 2020). As illustrated in Figure 4.2(a), the E_R^i of the toxic heavy metal in DB dumpsite ranked in the order Cr > As > Pb > Ni > Cu > Zn with corresponding values of 133, 25, 8, 5, 3 and 0.8, respectively. These results show that there is a considerable ecological risk ($80 \leq E_R^i < 160$) due to Cr contamination, and low potential ecological risk ($E_R^i < 40$) of As, Cu, Pb, Ni, and Zn at DB dumpsite.

In contrast, all E_R^i for the same toxic metals were <40 at BO site (Figure 4.2(b). Figure 4.2 shows PRI values of 174.8 and 57.4 (yellow bars graphs) indicating moderate ($110 \leq PRI < 220$) and light ($PRI < 110$) potential ecological risks for DB and BO tannery dumpsites, respectively. Zhang *et al*. (2018) reported that PRI can characterise the sensitivity of a local ecosystem to the toxic metals and represents the ecological risk that results from the overall contamination. In this study, E_R^i contributed by Cr metal was higher than other HMs. Collectively, these findings highlight the potential ecological risks in Cr pollution,that may contaminate other environmental ecosystems such as ground and surface water sources due to possible surface runoff from rainfall and percolation from tannery dump sites.

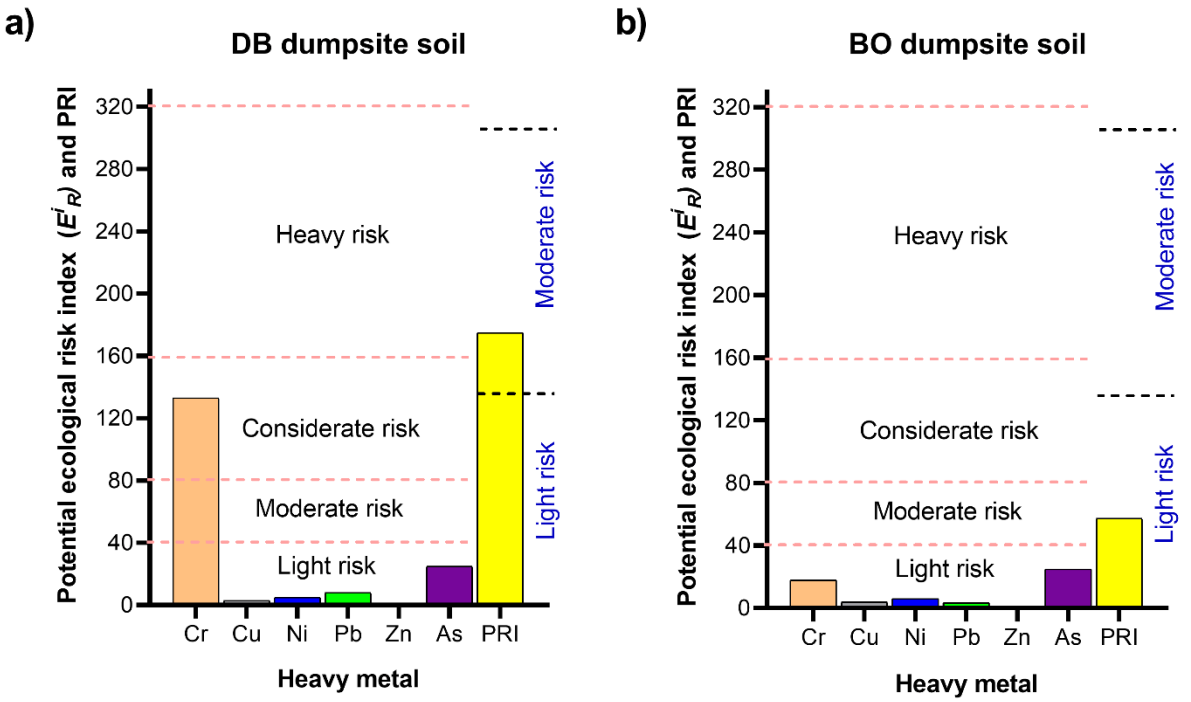

Figure 4.2. Ecological risk assessment of the tannery dumpsite soils. Results show individual metal potential ecological risk coefficients (E_R^i) and overall heavy potential ecological risk coefficients (PRI) for DB (a) and BO dumpsite (b) in different colours. The different classification conditions of the E_R^i (red dashed lines) and PRI (black dashed lines) as described by Kuerban *et al*. (2020) are indicated

To evaluate the impact of the tannery waste pollution on the environment (plants), Cr levels in common and widespread plants spontaneously growing or grown within or on the edges of the dumpsites were analysed. These included *Spinacae oleracea, Dactyloctenium aegyptenium, Cynodon dactylon, Alternanthera caracasana, Flaveria bidentis* and *Corchorus tridens* at BO site while *Amaranthus dubius Thell, Cynodon nemfluensis vanderst* and *Dactyloctenium aegyptenium* were sampled from DB dumpsite. As shown in Figure 4.3, in DB dumpsite, *Amaranthus dubius Thell* and *Cynodon nemfluensis vanderst* showed the highest Cr content (13.0±1.2 and 11.3±0.8 mg/kg, respectively), while in BO dumpsite, *S. oleracea* showed the highest chromium content value (12.08 ±2.4 mg/kg). Other plants showed moderate to low accumulation of Cr. These results are consistent with several studies reporting that vegetable crops such as spinach and *Amaranthus* can germinate and grow in soils with high Cr concentration (Gupta *et al*., 2019). Other studies have generally shown that heavy metals including Cr are bioaccumulated in vegetable leaves from the soil from a variety of sources (Akinsanya *et al*., 2019).

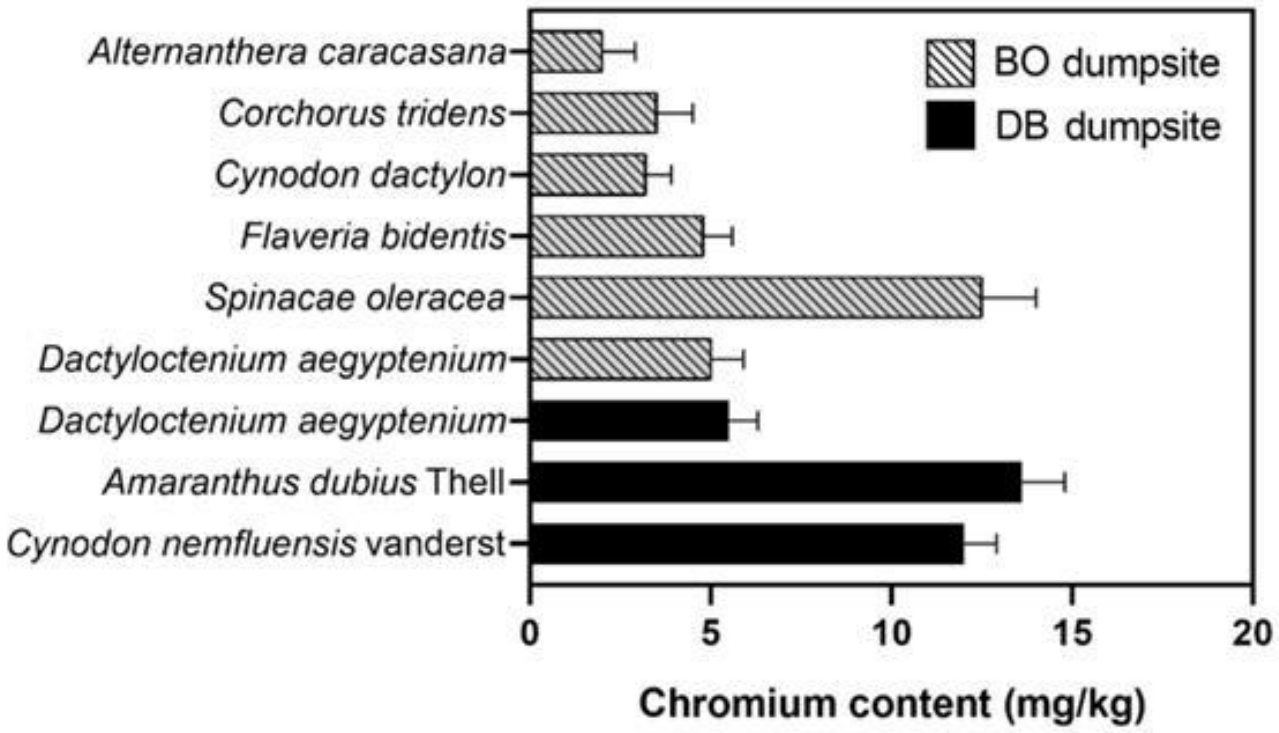

Figure 4.3 :The mean dry weight of chromium content of the different plant species samples found growing in the dumpsites of the two tanneries (n = 3, RSD)

Generally, plants growing in 'normal' soils are usually expected to contain less than 1 mg/kg Cr (Zayed and Terry, 2003). Consequently, the higher levels recorded in the plants are a pointer to anthropogenic pollution of the soils by the tannery wastes disposal in the dumpsites. The *S. oleracea* and *A. dubius Thell* form part of everyday meals of the many Kenyans, South Africans, and other African countries, while *D. aegyptenium and Cynodon* species are common pasture grasses for cattle feeding in the region. Interestingly, Spinach (*S. oleraceae*) found in the BO dumpsite was grown for consumption by caretakers living close to the tannery oblivious of the risks. Harvesting of the grasses for zero-grazing of cattle by urban dairy farmers within the DB dumpsite during the dry season was another common observation made. The use of soils from highly contaminated dumpsites as soil improver to grow edible crops is a common practice in many developing countries. Thus, the contamination of edible parts of vegetables and other plants with high levels of total Cr represents a direct pathway for their incorporation into the human food chain, constituting a public health problem around tannery dump sites in sub-Saharan Africa.

4.4: Conclusion

The objective of this chapter was to investigate chromium status, other metals contamination and ecological risks assessments of waste disposal of selected tannery waste dumpsites in sub Saharan Africa (objective one). The study aimed at determining the physico-chemical characteristics, oxidation status of chromium and other selected heavy metals contamination

levels in chromium effluents, soils and plants from the two selected tanneries, one in South Africa and one in Kenya. From the study it was noted that, recent introduction of strict environmental laws and regulations in sub-Saharan Africa has made tanning industries to put in place mechanisms to reduce the amount of chromium pollutants in their discharged effluents. However tannery industry effluent discharge and solid wastes disposal remains a common point source of chromium and other HMs contamination in the region. The objective of the study was achieved by specifically finding out that;-

(1) Despite the improved in-house processes and/or waste treatment of the two tannery effluents resulting in significant reduction in Cr, BOD, COD, TDS and salinity levels, the final effluents concentrations including Cu, Fe, Ni, Zn and Cd remain above the national guidelines for industrial discharge, and the WHO standards.

(2) Open-space dumping of tannery solid wastes contributed significantly ($p < 0.05$, $p < 0.01$ and $p < 0.001$) to elevated levels of Cr, Cr (VI), Fe, Mn, TOC and OM above the local background levels and exceeded the acceptable thresholds of the World Health Organization (WHO) and the Food and Agriculture Organisation (FAO).

(3) Assessment of pollution indices, indicated that the two tanneries are moderately to heavily polluted in the case of Cr for BO site to seriously polluted in the case of Cr, Fe and Mn for DB site. Thus, the overall potential ecological risk grouped DB and BO dumpsites to be moderate to light risk in relation to HMs contamination, respectively.

The elevated Cr levels observed in plants growing within the dumpsites such as grass species (*C. nemfluensis*) and vegetable crops such as *S. oleracea* and *A. dubius,* suggest potential biomagnification of Cr along the human food chain. Collectively, these findings provide evidence that the practice of long-term poor disposal of the solid waste in open spaces by tanneries operators within their private premises to avoid penalties by enforcement authorities, need urgent attention. The lack of proper containment infrastructure for solid wastes and their exposure to climatic elements amplifies ecological contamination and public health risks. The findings proved our hypothesis that “chromium and heavy metal wastes contamination increases potential ecological risks on soils and plants from selected tanneries waste dumpsites”.

As a risk reduction measure, tanneries are recommended to regularly assess potential risk posed to their workers consuming edible vegetables grown around dumpsites as well as adopt emerging eco-friendly tanning technologies such as chromium minimisation, recycling, and/or re-use of chromium and bio-remediation of their wastes. Additionally, there is need for strengthening operational capacity of environmental institutions charged with monitoring compliance and enforcement in the region. These results are important in considering

management plans to control the aggravation of heavy metals pollution and ultimately to protect environmental resources and the human population in this region.

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CHAPTER FIVE

The Potential Health Risk Associated with Edible Vegetables Grown on Cr (VI) Polluted Soils

Abstract

This thesis chapter reports on the assessment of the growth potential of five edible vegetables, which were grown in Cr (VI) spiked soils. The vegetable plants that were used in this study were *Vigna angularis, Cicer arietinum, Spinacea oleracea, Amaranthus dubius Thell* and *Phaseolus vulgaris*. Dried ground samples from roots, stems and leaves were analysed for various oxidation states of Cr. The daily intake of chromium, hazard quotient (HQ) and hazard index (HI) methods were employed to assess the potential human health risks posed by these Cr oxidation states through vegetable consumption. The results showed that Vigna angularis was the only vegetable that germinated in highly concentrated Cr (VI) in the simulated soil (456 mg/kg). The highest total chromium (ChT) bioaccumulated in the roots was found in *Phaseolus vulgaris* at 0.8. The highest ChT translocation factor in the stem was that of *Cicer arietinum* and *Vigna angularis* at 0.30. The same plants translocated the highest ChT to the leaf at 0.7. A child or an adult consuming such contaminated *Cicer arietinum* vegetable was likely to take in between 508 and 785 mg/day of ChT, which are above the World Health Organisation guidelines of 220 and 340 mg/day, respectively. The highest HQ was found in *Cicer arietinum* at 8.7 and 13.4 for adults and children, respectively. The same species of plants also had high HI at 17.4 and 27.2 for adults and children, respectively. This indicated that consumers of the edible vegetables grown in Cr (VI) rich soils may be exposed to health risks, and the children were more likely to be vulnerable to these adverse effects than the adults.

Keywords: Bioaccumulation; Edible vegetables; Hazard quotient; Health index; Speciation

5.1: Introduction

Generally, industrial activities tend to generate a wide range of wastes, which have the potential to impact the ecosystems negatively and also lead to high-costs of treatment when such wastes are discharged to unprotected environments (De Andrade *et al*., 2019). In developing countries, environmental laws on waste management and waste disposal are either non-existent or ineffective where they do exist (Ullah *et al.,* 2016). The use of soils from highly contaminated dumpsites for soil improvement to grow edible crops is a common practice in many developing countries. However, such a practice tends to cause bioaccumulation of toxic heavy metals in them. High concentrations of these metal pollutants in edible crops is known to be associated with potential health risks to consumers (Olafisoye *et al*., 2013; Faber *et al*., 2010). The contamination of foods is a problem that has the potential to affect populations far away from the place where such crops are grown through trade pacts or food aid (Islam *et al*., 2014).

Tannery effluents and solid wastes generated from the tanning process are known to pollute the environment with heavy metals and acids (Islam *et al*., 2014). In developing countries, most of these wastes find their way into the environment through poor open dumping. Open dumping of solid tannery wastes containing chromium has been found to be unsanitary and unaesthetic because they pollute the soils around dumpsites. This is due to the fact that waste dump leachates are transported and distributed to the surroundings by a variety of environmental factors, such as rainfall or spread into the adjacent river system by groundwater flow. Other factors include; chemical reactions such as biodegradation, adsorption, hydrolysis, dissolution, dilution, partitioning and precipitation (Magaji, 2012; Xaypanya *et al*., 2018). Edible vegetable crops grown in chromium contaminated sites take up and accumulate chromium at concentrations that are potentially toxic to human health (Gangwar, 2009).

Chromium has been reported to be the second most common heavy-metal contaminant in groundwater, soil and plants (Singh *et al.*, 2013). In addition, chromium (III) ions are known to be partially soluble in the soils after complexing with organic matters. At high concentrations, it creates potentially toxic environments for plant growth, causing stunted shoots, poor root development and leads to leaf chlorosis, tissue necrosis, decreased enzyme activity, membrane damage, diminished photosynthesis and changing of chloroplasts (Jun *et al*., 2009). The hexavalent form of Cr is one that is biologically toxic and to date, there are no signs indicating any potential biological role it plays in plants. Its complex electronic chemistry has been a major hurdle in disentangling its toxicity mechanism in plants. Therefore, the hazardous effects of Cr are primarily dependent on metal speciation, which

determines its uptake, translocation and accumulation in roots and edible portions (Hayat *et al.,* 2012).

The presence of Cr contamination in soil is toxic to edible crops as reported by Soundari and Sundaramoorthy (2017). The increasing chromium concentrations interfered with the biochemical parameters, such as protein, sugars and amino acid contents. This was significant in the plant, as they decreased in all different concentrations of Cr treated soil as compared to control (Amin *et al*., 2014). Cr affects the plants indirectly by replacing essential nutrients at cation exchange sites. The high concentration of Cr in soils causes several adverse effects on vegetables and, in the long run, affects human health (Bahira *et al*., 2018). For example, Gupta *et al*. (2019) reported that Cr contamination in marketed vegetables in Hong Kong was found at 0.56%.

This study thus, aimed at evaluating the levels of Cr species that can be accumulated by *Spinacia oleracea, Amaranthus dubius Thell, Phaseolus vulgaris, Cicer arietinum* and *Vigna angularis* as they germinated and grew in Cr (VI) spiked soils. The study also aimed at assessing which vegetables were consistently able to germinate in the highest concentration of Cr (VI). It again determined how much Cr oxidation states these edible vegetables bioaccumulate and translocate in different parts of their tissues (root, stem and leaf). Lastly, it estimated human health risk indices through edible vegetables consumption. The increase in unplanned settlements and urban agriculture around tannery dump sites could increase the risk of cancer associated with the consumption of edible vegetables from such contaminated sites (Oruko *et al*., 2014). Assessing health risks could contribute to proactive mitigations.

5.2: Methods and materials

5.2.1: Equipment

Various equipment was used for analysis of total chromium and Cr (VI) concentrations in soils and plants samples as described in detailed methodology chapter three, subsection 3.3.3 and 3.3.5 of this thesis. The spectrophotometers were calibrated as explained in subsection 3.3.6. The soil-water suspension was homogenised by vigorous shaking using a mechanical shaker from Lab connections (St. Augustine, FL, USA). The Accsen multi-parameter probe from XS instruments (Carpi, Modena, Italy) was used for measurements of pH/EC. A model Defy DMO 350 oven from Defy Appliances (Pty) Jacobs (Durban, South Africa) was used for drying soil/plant samples and their controls. The dried plant materials were ground with a milling machine Knifetec 1095 sample mill, from Adendorff machinery mart, (Krugerdrop Johannesburg, South Africa). Total organic carbon (TOC) and soil particle size analysis was achieved as stated in chapter three, subsection 3.3.1 of this thesis.

5.2.2: Sampling experimental Soil

Bulk soil samples were collected to a depth of 30 cm for the simulation experiments from the University of South Africa (UNISA) horticulture experimental greenhouse station. This was done for control and quality assurance. The greenhouse is situated at *Latitude*: S 26°9.501. *Longitude*: E 27° 54.113. The soil collected from the UNISA greenhouse was classified as silt loam with 60% clay as described by Bouyous (1936). The silt loam soil prior to collection at UNISA horticulture laboratory site was drawn from virgin land in the forest which was free from contamination. It was used due to its mild acidic pH. The soil samples were air-dried, passed through a 2-mm sieve for physicochemical analysis and for a pot experiment as modified from Fernandes *et al*. (2002).

5.2.3: Sampling of plants seeds

The seeds of *Amaranthus dubius Thell* and *Cicer arietinum* used in this study were sampled randomly within Dogbone tannery dumpsite in Kenya by using a modified method reported by Ensconet (2009). Sampling from across the seed head was done to avoid sampling very immature or very old seeds. To compensate for possible losses during transfer, extra 20% of the seeds were collected. Seeds of *Spinacea oleracea*, *Vigna angularis* and *Phaseoulus vulgaris* were purchased directly from Agricol, a licenced seeds dealer in South Africa. These seeds were identified, verified and named scientifically together with their plants materials at herbarium section of East Africa as stated earlier. The seeds of *Cicer arietinum* and *Amaranthus dubius Thell* from the field were packed into sterile polythene bags. *Spinacea oleracea*, *Vigna angularis* and *Phaseoulus vulgaris* seeds were obtained in sealed packages.

5.2.4: Preparation of seeds for germination

The germination and growth potential of edible vegetable seeds in polluted soil was tested in soils simulated with different concentrations of Cr (VI) solutions. Before planting seeds in soil packed plastic containers in the greenhouse, the seeds surfaces were sterilised to prevent fungal and bacteria contaminations. This was done by first soaking them in 30% bleach for 30 minutes. The seeds were then rinsed with Millique (MQ) water four times. They were again soaked in MQ water for 30 minutes. Seed imbibition (hydration) was done with MQ water overnight as modified from Taye *et al*. (2013).

5.2.5: Preparation of Stock, standard, spiking solutions, quality assurance and control

A 1000 mg/L stock solution of chromium was prepared using 2.9583 g of potassium dichromate in deionised water according to the procedure reported by Soundari and Sundaramoorthy (2017) and Sundaramoorthy *et al*. (2015). From this stock, solutions measuring 10, 50, 100 and 200 mL of Cr VI was added to triplicates of all 0.5 kg of soil used in the germination and growth experiment of edible vegetables. A portion of the same stock solution was then serially diluted to get the daily working standard solutions of desired Cr (VI) as described in subsections 3.3.6 - 3.3.8 of the methodology to provide quality assurance, control, and reliability of the experiment.

5.2.6: Experimental design in the greenhouse

The experiment was arranged in a complete randomised block design with three replicates for each treatment as modified from Amin *et al*. (2014) and Fernandes *et al.* (2002) .The air-dried soil artificially spiked with different volumes of Cr (VI) solutions i.e. 10, 50, 100 and 200 mL respectively, along with an untreated control were adapted. The 10 mL and 50 mL solutions were mixed with 100 mL of deionised water to get enough solution before mixing with the experimental soil (0.5 kg). Thereafter, chromium solutions were uniformly mixed with air dried soil and kept for two weeks to stabilise. Three seeds of each of the edible vegetables were sowed into the spiked soil and their controls in triplicates.

A total of 675 seeds were planted in the 15 containers. The planted seeds were irrigated with 80% deionised water equally three times a week. The amount of water added was sufficient and kept the plants growing without any effect seen. Before and at the end of the experiments, the soil and plants samples were subjected to various analyses. Details are given in the results and discussion of this chapter five.

5.2.7: Estimation/observation of germination and growth pattern

The seed germination was monitored after every 24 h until the germination percentage and growth height was constant. For the evaluation of seedling growth, all germinated seedlings of similar morphology were allowed to grow within concentrations of 23, 114, 228 and 456 mg/kg chromium in the soils. During their growth period, the seeds were monitored for the germination trend, growth rate/pattern and increase in their heights. Physiological changes on their stems and leaves were also observed. Insects and pests' attacks were monitored as well. The plants were harvested carefully after 56 days, washed with distilled water to remove soil particles and analysed for growth attributes such as germination percentage and growth height**.**

5.2.8: Germination percentage, growth height and quantification of health risk

The germination percentage (%G) is the proportion, expressed as percentage of germinated seeds to the total number of viable seeds that were tested by using the formula of Akinci & Akinci (2010), equation (5.1). The plant growth height was measured periodically. At the end of the experiment the mean height was taken for each plant under different concentration i.e. 0, 23, 114, 228 and 456 mg/kg and then stress tolerance index for plant height (TIPH) calculated using the formula of Wilkins (1978), equation (5.2).

$$\%G = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ planted\ seeds} \times 100 \quad (5.1)$$

$$(TI_{PH}) = \frac{Height\ of\ treated\ plant}{Height\ of\ control\ plant} \times 100 \quad (5.2)$$

Shahid *et al.* (2017); Chaturvedi *et al*. (2019); de Sousa *et al.* (2018); Carbonell *et al*. (2011); Lesniewska and Gontarska (2017) and Ciu *et al*. (2015) proposed that the quantification of health risk potential due to Cr ingestion, like for other heavy metals may be assessed or estimated using bioaccumulation factor (BF), translocation factor (TF), daily intake of chromium (DIC), hazard quotient (HQ) and Hazard Index (HI). Other than bioaccumulation factor, they were all calculated using modified equations (5.3) to (5.6) (see Appendix Table 5.3 for raw data).

Translocation factor (TF) was calculated by equation (5.3) modified from de Sousa *et al.* (2018) and Carbonell *et al*. (2011).

$$TF = \frac{Cr\ content\ in\ the\ leaf\ (mg/kg)}{Cr\ \ content\ in\ the\ roots\ (mg/kg)} \quad (5.3)$$

Daily intake of chromium (DIC) was estimated by the modified equation (5.4) of Ciu *et al*. (2015)

DIC= $DIV \times Cr\ content\ of\ vegetable,$ (5.4)

Where DIV - daily intake of vegetable; Cr - total Cr/Cr (VI) content of vegetable

Hazard quotient (HQ) an estimate of potential hazard was determined by equation (5.5) modified from Bose and Bhattacharyya (2008).

HQ = $\frac{DIV \times C_{metal}}{RfD \times BO}$ (5.5)

Where- Div is the daily intake of vegetable leaves (mg/kg/day), (C_{metal}) - is the concentration of total Cr/Cr (VI) in the vegetable (mg/kg). RfD- is the oral reference dose (RFD value for Cr is 1.5 mg/kg of body weight/day). Bo- is the human body weight (60 kg for adults and 25 kg for children).

Hazard index (HI) is calculated as an arithmetic sum of the hazard quotient for each pollutant as shown in the following modified equation (5.6) of Chaturvedi *et al*. (2019).

HI = $\sum_{i=0}^{n} HQ$ (5.6)

Where- HQ was the sum total of total Cr, Cr (VI) and Cr (III) in vegetable species leaves. n is the number of Cr oxidation states studied. Further analysis was made through principal component analysis (PCA), which is one of the multivariate statistical methods used in environmental studies. The technique was applied in this study to establish major variations and relationships among the different edible parts of vegetable species and ChT, Cr (VI) and Cr (III) oxidation states (Chandrasekaran *et al.,* 2015).

5.2.9: Statistical analysis

Data were statistically analysed as described in methodology chapter three, subsection 3.13 of this thesis. Multivariate statistics in terms of principal component analysis (PCA) was performed using Xlstart statistical software (Edokpayi *et al.,* 2018). The PCA was used to establish major variations and relationships among the different edible parts of vegetable species and ChT, Cr (VI) and Cr (III) oxidation states. It was used to specifically identify similar active and observable variables to provide a visual summary of the results based on the dimensionality of the original data.

5.3: Results and Discussion

5.3.1: General Properties of Soil

Various physico-chemical properties of the homogenised soil used in this study were measured and are depicted in Table 5.1. The textural particle size of experimental soil had 25% sand, 15% silt and 60% clay loam suggesting the soil to be predominantly clay.

Clay soils are the most balanced and support the greatest diversity of plants life. The types of plants that grow well in clay soil include grasses, bamboo, wetland and aquatic plants, vegetables and fruit trees (Banks *et al*., 2015). The mean environmental data for the greenhouse are shown in Table 5.2.;

Table 5.1: Physico-chemical properties of experimental soil

Soil Properties	Mean Values
pH	6.2 ± 0.05
EC (µS/cm)	42.8 ± 0.01
Total organic carbon (%)	0.98 ± 0.01
Moisture (%)	10.9 ± 0.15
Sand (%)	25
Silt (%)	15
Clay loam (%)	60
Texture Class	Clay
Cr_T in soil (mg/kg)	1.2 ± 0.03

Table 5. 2: Environmental data in the greenhouse

Temperature (^oC)	Relative humidity(g^3)	Humidity (g/m^3)	Atmospheric humidity(g/m^3)	Dewpoint temperature (^oC)	Radiation (J/cm^2)
17.2± 4.3	74.8± 62.9	4.7± 1.5	10.6± 1.8	5.4± 3.0	35274± 433

5.3.2: Effect of Chromium Concentration on Seed Germination and Growth

The observed results of the present study show that higher chromium concentration adversely influences the germination process of various vegetable seeds (Figure 5.1). Chromium treatment in soils at the level of 23 to 456 mg/kg had different effects. It was observed that 24% germination took place in the control soils but decreased to 16% in soils with concentrations of 23 mg/kg. The 10% germination correspondes to soils with 114 mg/kg of chromium concentration, 7% germinated in 228 mg/kg Cr levels and 1% germinated in 456 mg/kg Cr levels. Significant variations in Cr tolerance and sensitivity in terms of seed germination have been recorded in literature (Shahid *et al.,* 2017). This study has established that the germination of different plant seeds is affected differently with different Cr (VI) concentrations.

The germination time in this study was prolonged to (8±1days), while the control germination was observed after (6±1days). The prolonged germination period was observed as the levels of chromium increased from 23 mg/kg to 456 mg/kg. This suggests that the seeds may have undergone secondary induced dormancy in Cr conditions before germination, and this lengthened their germination period. Eze *et al*. (2018) stated that at a treatment level of 400 mg/kg of chromium in soil, a prolonged germination time (9±1 days) was observed unlike the control (4±1 days) and this implied that germination time increased with increase in chromium

dosage. In relation to height, the Cr transported to the aerial parts or retained at the roots might have affected the physiological processes of plant growth. It contributed to their various reductions in height, as shown in Figure 5.2. Significant differences ($p < 0.05$) were found in plants heights with increased concentrations of chromium from 0 mg/kg (control) to 456 mg/kg. There were significant differences between the heights of plants in the control and those grown in soils containing varying levels of Cr (23–456 kg).

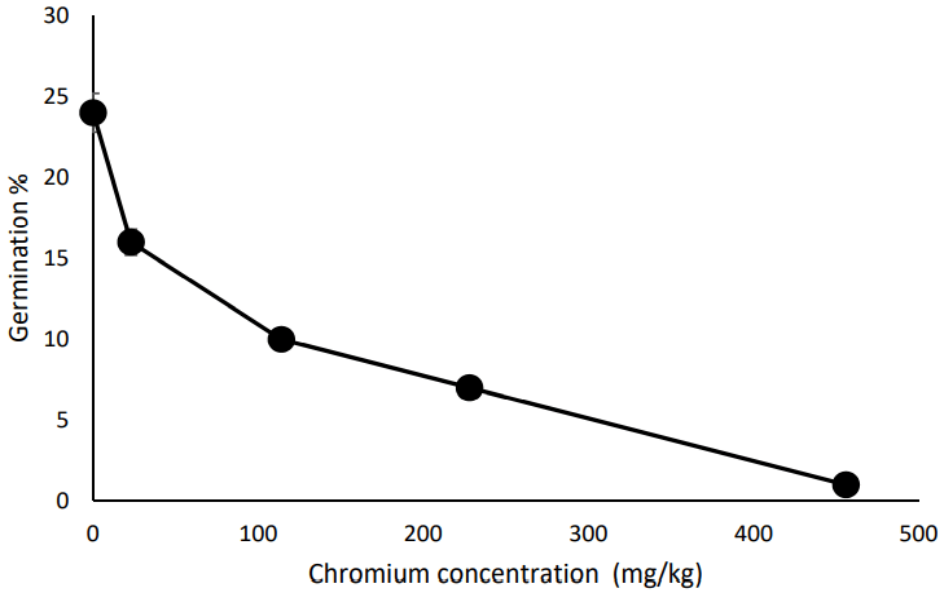

Figure 5.1: Germination percentage of plants in different concentrations of Cr (VI) along the y-axis (n = 3, SD); SD- standard deviation

There were also significant differences observed between *Cicer arietinum and Spinacea oleracea, Phaseolus vulgaris and Spinacea oleracea, Cicer arietinum* and *Amaranthus dubius Thell, Phaseolus vulgaris and Amaranthus dubius Thell.* The significant decrease in height could be attributed to increase in Cr (VI) concentrations. In the 114 mg/kg Cr levels, the highest height recorded was that of *Cicer arietinum* while the lowest were *Spinacea oleracea* and *Amaranthus dubius Thell*. This possibly imply that *Spinacea oleracea* and *Amaranthus dubius Thell* seeds growth are sensitive to high increase in Cr (VI) concentrations at 114 mg/kg in comparison to other plants. However, there was an exceptional increase in height for *Cicer arietinum* from Cr levels of 23 mg/kg to 114 mg/kg which need further study to find out the cause of increase.

In 228 mg/kg Cr levels, the maximum height recorded was that of *Phaseoulus vulgaris* and the least was *Vigna angularis*. The *Spinacea oleracea* and *Amarantha dubius Thell* seeds never germinated in 228 mg/kg Cr levels simulated soil, depicting total inhibition of growth hormones. Lastly, *Vigna angularis* was the only plant that germinated and grew (up-to 1 cm) in 456 mg/kg Cr levels. This suggests that *Vigna angularis* may be having unique adaption

mechanism that enables it to absorb nutrients in high chromium contaminated environment. *Vigna angularis* and other plants in this study may have used sulphate transporters mechanism to actively transport Cr (VI) into their body cells. This is because, Cr (VI) is an anion which is known to be very mobile because of its negative ions, which compete with sulphate ions in the uptake due to their similarities in structure. In addition to that, these plants may have also used the passive sorption mechanism after reducing Cr (VI) absorbed in their tissues to Cr (III). This mechanism may have involved the diffusion of Cr (III) ions across the cell wall and plasma membranes into the plants body for translocation to other parts. These uptake mechanisms as suggested in this study are also supported by literatures reports (Bluskov *et al*., 2005; Kotas and Stasicka, 2000; Kaszycki *et al*., 2005; Appenroth *et al.*, 2008).

Cr transported to the aerial parts of these plants directly impacted cellular metabolism of shoots contributing to the reduction in their height as seen in Figure 5.2 of this study. Sundarmoorthy *et al.* (2015) and Bahira *et al*. (2018) agree and report that high Cr (VI) concentrations (500 mg/kg) in soil affected shoot growth of wheat and oat. It also led to decrease in plant height as reduced root growth was observed, and consequently decreased nutrients and water transport to the higher parts of the plant.

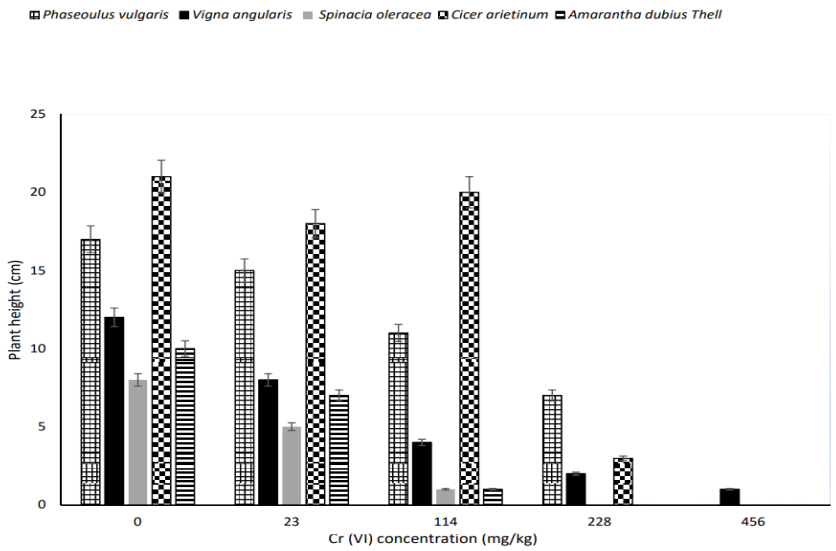

Figure 5.2: Effect of Cr (VI) concentration on different plant heights in simulated soil, (n = 3, SD). SD: standard deviation

The current study observed significant variations in the germination and growth of edible vegetable seeds in Cr (VI) polluted soils (Figure 5.3c). Simulated studies using Cr (VI) as the spiking agent to investigate different crop reactions and growth have been reported by several authors (Amin *et al*., 2013; Akinci and Akinci, 2010; Augustynowicz *et al*., 2014). Cr is considered strongly toxic because Cr (III) compounds in the soil are more or less insoluble

and their ions are tightly bound to humus and clay particles while Cr (VI) is very soluble and easily passes through the plant cells into vacuoles where they combine with cations and form stable compounds which either accelerate or retard plants growth (Augustynowicz *et al*., 2014).

Amaranthus dubuis Thell and Spinacea oleracea germinated in low concentrations only while *Cicer arietinum, Phaseoulus vulgaris and Vigna angularis* germinated in both low and high concentrations of Cr (VI). It may be possible to state here that seed coats of different plants' impermeability and embryos' selectivity could have affected the tolerance of chromium impacts. Those events naturally could have helped in the selection of high and low tolerant species or chromium varieties during the germination, early seedling stages and growth height in this study. These findings are in agreement with those reported by Kidd and Mart (2004), who reported that higher concentrations of heavy metal significantly reduced the strength of germination as compared to the lower concentrations, which had the least harmful effect on germination. Peralta *et al*. (2001) also found that, there was no germination of spinach with applied Cr level at 320 mg/kg in the soil.

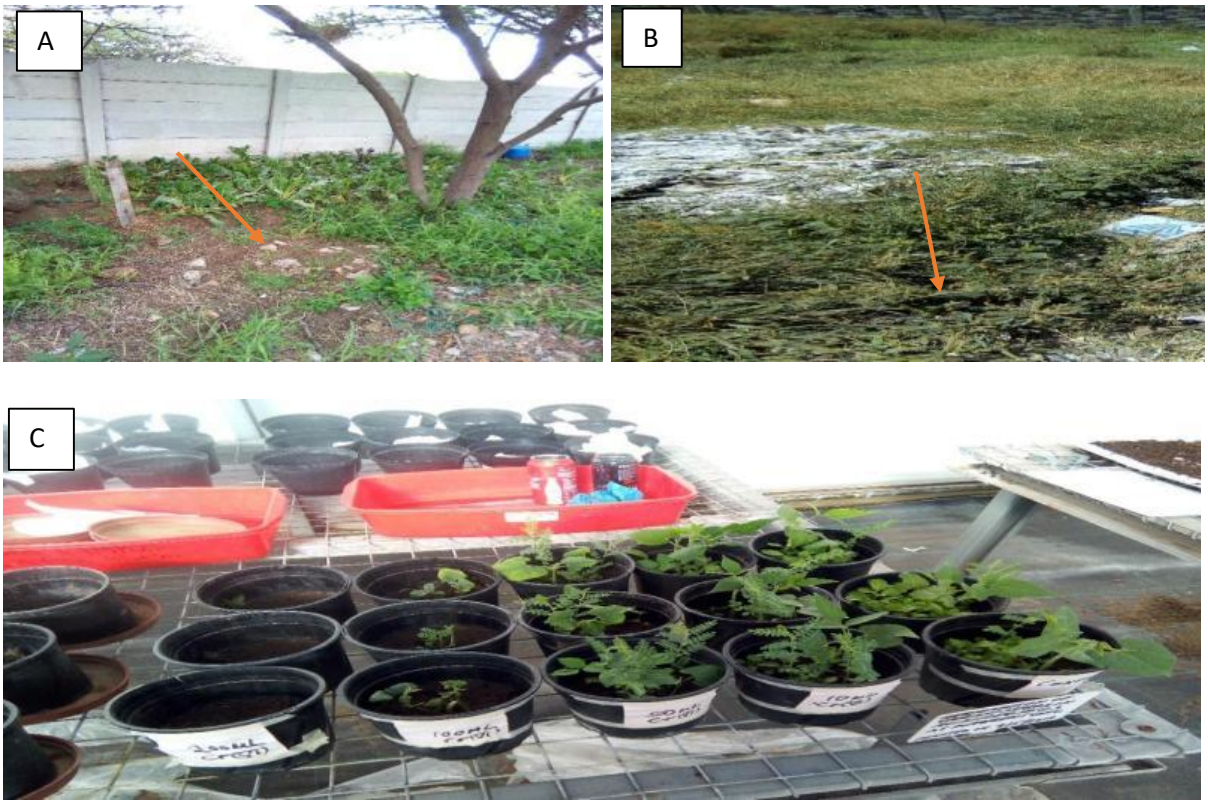

Figure 5.3: (a). *Spinacea oleracea* grown by tannery workers near tannery chromium wastes dumpsite in South Africa. (b) *Amaranthus dubuis Thell* growing wildly in a tannery chromium wastes dumpsite in Kenya. (c) Experimental set up and germination of edible vegetables in Cr (VI) polluted soil in the University of South Africa (UNISA) greenhouse number 6

The consistent germination and significant growth of *Phaseoulus vulgaris*, *Cicer arietinum* and *Vigna angularis* in chromium contaminated soil observed in this study seem to suggest that these plants might be tolerant to chromium. Alternatively, they have mechanisms that allow them to germinate and grow in Cr toxic environment. It could be observed that the roots of *Vigna angularis* at the highest concentration was modified with fewer hair roots (Appendix Figure 5.1) as compared to those from low concentrations and control. This could be the part of mechanism this plant applied in the roots to exclude excess Cr (VI) to its aerial parts, which made it possible for it to grow in such high concentration. Sharma *et al*. (2005) stated that toxic properties of Cr (VI) could be reduced by oxidising agents as well as from the formation of free radicals during the reduction of Cr (VI) to Cr (III) inside the root cells of plants.

To understand practically how these plants may have applied these strategies and mechanisms to survive in Cr polluted soil, the sampled plants in this study were divided into root, stem and leaf. Then measurements involving the assessment of different levels of Cr oxidation states; ChT, Cr (VI) and Cr (III) in the vegetables grown in the polluted soil were undertaken, and their occurrence is given in Table 5.3. Different vegetable cultivars were found to differ in their ability to take up Cr oxidation states as the occurrence varied in this study, as depicted in Table 5.3 (Appendix Table 5.1 for raw data). This is in agreement with Fitz and Wenzel (2002), who reported that different plants exhibit diverse strategies to high concentrations of Cr, such as indicators, excluders and accumulators.

The mean values of chromium oxidation states in the sampled parts of edible vegetables and their controls were then subjected to Tukey's test at confidence level ($p < 0.05$). The values were found to have statistically significant differences in their control and parts ($p = 0.000$), indicating strong significant difference between the measured variables. This could be attributed to the Cr (VI) spiked in the experimental soils which could have been accumulated by these plants differentially through their tolerance mechanisms. In the roots, *Cicer arietinum* had the highest ChT while *Spinacea oleracea* had the lowest. *Vigna angularis* had the highest Cr (VI) and *Spinacea oleracea* registered the lowest level in the roots. The *Cicer arietinum* had the highest Cr (III) in the roots while the lowest level was also detected in *Amaranthus dubuis Thell*. This depicted that *Cicer arietinum* and *Vigna angularis* tolerance mechanisms for different Cr oxidation states were superior to *Spinacea oleracea* and *Amaranthus dubuis Thell* in their roots. This was probably from the modification of their root hairs.

The level of total Cr translocated to the stem was high in *Cicer arietinum* and *Vigna angularis* and least in *Spinacea oleracea*. *Vigna angularis* accumulated Cr (VI), in the stem as *Phaseoulus vulgaris, Spinacea oleracea, Cicer arietinum* and *Amaranthus dubuis Thell* registered nil in the stem. *Phaseoulus vulgaris* had a high concentration of Cr (III) in the stem,

and nil was recorded in *Spinacea oleracea*. *Spinacea oleracea* and *Amaranthus dubuis Thell* maintained their low uptake implying either efficient exclusion or poor translocation mechanism. *Vigna angularis* seems efficient in the uptake of Cr (VI), suggesting that it has a unique mechanism of transporting it in the root and stem which requires further study. The level of ChT translocated to the leaf parts was highest in *Cicer arietinum* and *Spinacea oleracea* but least *in Phaseoulus vulgaris*. *Cicer arietinum, Vigna angularis* and *Amaranthus dubuis Thell* accumulated maximum Cr (VI) in the leaves and a very low undetectable concentration in *Spinacea oleracea*.

However, *Spinacea oleracea* had the highest level of Cr (III) in the leaf, while the least concentration was found in *Phaseoulus vulgaris* and Amaranthus *dubuis Thell*. *Spinacea oleracea* had the same amount of CrT and Cr (III) in the leaf followed very closely by *Cicer arietinum*. These plants may have accumulated high levels of Fe from the soil used in the experiment into their root tissues as they grow, which probably interacted positively with Cr (VI) resulting in translocation of Cr ions to aerial tissues (leaf) (Singh *et al.,* 2013). Alternatively, some of these plants may have also applied phytovolatirisation of the excess Cr (VI) pollutants through their leaves as coping mechanisms in such highly polluted soils. Residual chromium concentrations (ChT, Cr (VI) and Cr (III)) were still detected in simulated soil at the end of the experiment, despite the effects of soil natural matrices and uptake by plants.

Table 5.3: The occurrence of ChT, Cr (VI) and Cr (III) in root, stem and leaf of different plant species in the simulated soil. Experimental errors lower than 0.01 mg/kg have been omitted

Name of Plant	Portion of Plant	Chromium Oxidation States (mg/kg)						P>|t| (tukey effect)
		Cr_T	Control	Cr (VI)	Control	Cr(III)	Control	
Phaseoulus vulgaris	Root	2.80 ± 0.31	0.2	0.7 ± 0.03	ND	2.10 ± 0.30	0.2	0.000
	Stem	0.10 ± 0.07	0.2 ± 0.01	ND	ND	1.20 ± 0.08	0.20 ± 0.01	
	Leaf	1.0	0.2	0.10 ± 0.03	ND	1.00 ± 0.03	0.2	
Vigna angularis	Root	3.40 ± 0.61	0.2	0.90 ± 0.04	ND	2.50 ±0.60	0.2	0.000
	Stem	1.0	0.2	0 .10 ± 0.02	ND	1.10 ± 0.02	0.2	
	Leaf	1.80 ± 0.30	0.2	0 .20 ± 0.03	ND	1.60 ± 0.30	0.2	
Spinacea oleracea	Root	1.10 ± 0.03	0.1	0 .10 ± 0.03	ND	1.00 ± 0.03	0.1	0.000
	Stem	ND	ND	ND	ND	ND	ND	
	Leaf	2.1	ND	ND	ND	2.1	ND	
Cicer arietinum	Root	3.50 ± 0.51	0.30 ± 0.01	0.8	ND	2.90 ± 0.05	0.30 ± 0.01	0.000
	Stem	1.0	0.20 ± 0.03	ND	ND	1.10 ± 0.01	0.20 ± 0.03	
	Leaf	2.10 ± 0.21	0.30 ± 0.03	0 .20 ± 0.12	ND	2.00 ± 0.01	0.30 ± 0.03	
Amaranthus dubuis Thell	Root	1.20 ± 0.01	ND	0.30 ± 0.03	ND	0.90 ± 0.03	ND	0.000
	Stem	0.11 ± 0.02	ND	ND	ND	0.1	ND	
	Leaf	1.20 ± 0.03	0.09 ± 0.06	0.20 ± 0.01	ND	1.00 ± 0.02	0.10 ± 0.07	
Chrome simulated soil	Soil	4.9	1.20 ± 0.03	1.80 ± 0.07	ND	3.00 ± 0.13	1.20 ± 0.03	0.000

ND means not detected

This indicated that Cr is persistent in soil environments, and this is a source of concern if levels are above set guidelines. de Andrade *et al*. (2019) stated that the risk that could be associated with Cr (VI) amendment in soils may involve the chance of Cr persistence in soil and accumulation in plants that may eventually enter into the food chain. The concentrations derived were used to assess the transfer of Cr pollutants from soil to plants' portions using pollution indices, such as BF and TF, as monitoring tools for pollution effect on edible vegetables grown in Cr contaminated sites.

5.3.3: Bioaccumulation/Bioconcentration Factor (BF/BCF)

In this study, Cr oxidation states levels revealed significant variations of BF/BCF in the five studied vegetables, as shown in Table 5.4. Significant differences ($p < 0.05$) was found in ChT, and Cr (VI) levels between BF in the plants' roots. ChT concentrations between *Amaranthus dubuis Thell* and *Vigna angularis*, *Spinacea oleracea* and *Vigna angularis*, *Spinacea oleracea* and *Cicer arietinum* were significant. Cr (VI) significant levels were found between *Spinacea oleracea* and *Vigna angularis* and *Spinacea oleracea* and *Cicer arietinum*. However no significant differences was observed with Cr (III). This suggested that these edible vegetables may have the potential to bioaccumulate and transfer ChT and Cr (VI) into their tissues. BF/BCF is an index that measures the ability of the plant to accumulate a particular metal with respect to its concentration in the soil and the root of the plant (Korzeniowska and Stanislawska-Glubiak, 2015). Under normal conditions, the concentration of Cr in plants is supposed to be less than 0.001 mg/kg (Oliveira, 2012).

Thus in terms of accumulation, *Cicer arietinum* could be grouped as Cr moderate accumulator plant while *Vigna angularis*, *Spinacea oleracea*, *Phaseoulus vulgaris* and *Amaranthus dubuis Thell* were low Cr accumulator plants based on the Malayeri *et al.* (2008) and Korzeniowska and Stanislawska-Glubiak (2015) categorisation system. In this categorisation, the plant described as high accumulators are characterised by BF>1(1-10), moderate accumulator BF<1 (0.1-1) and low accumulator or excluders by BF< 0.1(0.1- 0.01). Alternatively, plants with high bioaccumulation factor (BF>1) and enough high biomass yield can be used for phytoextraction while plants having high bioconcentration factor (BCF > 1) and translocation factor (TF < 1) are considered appropriate for phytostabilisation. However, there were non-accumulator and hyper-accumulator plants in this study. Instead, these plants could be grouped as phytoextractants or phytostabilisers. Since they are edible crops, this disqualifies them for phytoremediation of Cr contaminated sites.

The high values of BF/BCF confirmed that the roots are the main bio-accumulators of Cr in all its oxidation states. The highest Cr accumulation in the roots occurred due to their direct contact with Cr oxidation states in the soil. Cr accumulation in the roots could be apportioned

to that fraction of Cr ions physically adsorbed to the cell walls of the root and another fraction absorbed by the cells that were possibly immobilised in the root vacuoles. Since there was an increase in the shoot biomass, these mechanisms were suspected of having played a key role in the BF/BCF of the plants under toxic conditions in the soil. *Amaranthus dubuis Thell* and *Spinacea oleracea* vegetables were found to have limited potential for the bioaccumulation of higher Cr concentration in their roots. That is why they were unable to germinate and grow in 228 and 456 mg/kg levels (Figure 5.2) in this study.

Oliveira (2012) and Chandra *et al*. (2010) explained that enhanced accumulation of chromium in the roots of *angularis* and *arietinum* species may have been due to the presence of organic acids (carboxylic acid and amino acids) in the root exudates which form complexes with chromium, thereby making them available for the uptake by roots while *Amaranthus* and *Spinacea* had low potential for accumulation of Cr. Rashed (2010) reiterated that the concentration of Cr is always higher in the roots than in the shoots. These differences in Cr accumulation in different parts of the plants suggested that different cellular mechanisms of bioaccumulation of Cr took place, and this influenced Cr bioaccumulation and partitioning in these plants, thus the need to find out the quantity that was translocated from the root to the shoot.

Table 5.4: The Bioaccumulation Factor (BF) and Translocation Factor (TF) of total chromium (ChT), Cr (VI) and Cr (III) in the different parts of vegetable plants at the harvesting stage found between Cr oxidation states and plant species ($p < 0.05$)

Name of plant	Treatment	BF	TF		Name of plant	P < |t|(0.05)
		Root	Stem	Leaf		
Phaseoulus vulgaris	Cr_T	0.8	0.05	0.3	Other plants	P > (0.05)
	Cr (VI)	0.4	0.01	0.2	Other plants	P > (0.05)
	Cr (III)	0.4	0.04	0.1	Other plants	P > (0.05)
Vigna angularis	Cr_T	1.0	0.3	0.7	*Amarantha dubuis Thell*	0.01*
	Cr (VI)	0.5	0.2	0.3	Other plants	P > (0.05)
	Cr (III)	0.5	0.1	0.4	Other plants	P > (0.05)
Spinacia oleracea	Cr_T	0.3	0.02	0.2	*Vigna angularis*	0.01*
	Cr (VI)	0.1	-	0.01	*Vigna angularis*	0.01*
	Cr (III)	0.2	0.01	0.2	Other plants	P > (0.05)
Cicer Arietinum	Cr_T	1.0	0.3	0.7	*Spinacia oleracea*	0.03*
	Cr (VI)	0.4	0.01	0.4	*Spinacia oleracea*	0.02*
	Cr (III)	0.6	0.02	0.3	Other plants	P > (0.05)
Amarantha dubuis Thell	Cr_T	0.3	0.04	0.4	*Vigna angularis*	0.01*
	Cr (VI)	0.1	0.02	0.3	Other plants	P > (0.05)
	Cr (III)	0.2	0.02	0.1	Other plants	P > (0.05)

5.3.4: Translocation Factors

In this study, significant differences ($p < 0.05$) were found in ChT, Cr (VI) and Cr (III) levels between TFs in the plants stems and leaves. The significant differences trend between ChT and Cr (VI) concentrations shown in BF were also observed in TF. To confirm the distance moved by ChT, Cr (VI) and Cr (III) between these plant parts, further statistical analysis was done using principal component analysis (PCA). The relationship between the concentration of ChT, Cr (VI) and Cr (III) (active variables) in the stems (A) and leaves (C) of the edible vegetables species, such as *P. vulgaris, A. dubuis*, *S. oleracea, V.angularis* and *C.arietinum* (active observations), for the first two principal components obtained (PC1 and PC2, which account for the 74.87% and the 25.10% of the total variance, respectively) are shown in Figure 5.4. The active observations show that *Cicer arietinum* and *Vigna angularis* had a closer positive upper relationship than *Spinacea oleracea* that had a lower positive relationship but far distant from the two. *Amaranthus dubuis Thell* and *Phaseoulus vulgaris* had a negative relationship with *Phaseoulus vulgaris* appearing in the upper region and *Amaranthus dubuis Thell* in the lower area. The active variables showed that Cr (VI) was active in the upper positive region, while ChT and Cr (III) were active in the lower positive region.

This depicted Cr (VI) to be more mobile than ChT and Cr (III). *Cicer arietinum* and *Vigna angularis* were observed to be likely related in their accumulation and transfer of chromium species. Other reported studies found out that Cr is absorbed by roots from nutrient solution as Cr (III) or Cr (VI) and translocated to aerial portions and roots, but it is largely retained in the roots (Kacholi, 2018). Given that these plants have shown bioaccumulation and translocation potential for Cr states in edible parts, they are of major health risk concern to the human population in Kenya and South Africa. Some of these plants have also been surveyed at tannery chromium dumpsites either growing wildly or grown near/at abandoned dumpsites by tannery workers in both countries, as shown in Figure 5.3a, b. The tendency to retain more Cr in the root > leaf > stem was seen to be common in most of the plant species, but there was also quantitative translocation among the studied plant species (Table 5.4). This may also mean that plants with high levels of Cr in their leaves may be trying to phytovolatirise the metal into the atmosphere through their leaves, as seen by yellowing from the edge towards the petal and eventually dropping off the plant after possibly translocating it and starting a new leaf growth.

The above is in agreement with Khan *et al*. (2013) who reported that the maximum amount of Cr is accumulated in the roots followed by leaves and then fruits, which is close to findings in this study. According to Rashed (2010) dicotyledon species, such as *Vigna angularis*, uptake and transport more Cr to shoots than monocotyledons plants, such as maize.This is due to

differences in the rooting patterns, transpiration rates and metabolism between these two groups of plants. Cui *et al.* (2015) in the contrary found that *Amaranthus dubius* tolerated high Cr (VI) concentrations by accumulating and transferring them to aerial parts. The outcome of this study also compares to the reported findings by Oliveira *et al*. (2015), which found that an increase in Cr concentration in the leaves may be related to higher soil Cr concentration from the spiked soil and therefore, the metal was bioaccumulated from the roots to the leaves. Lower accumulation of Cr in leaves than in roots can be related to the conservation of photosynthesis processes from toxic levels of trace elements as well (Zojaji *et al*., 2014). Thus, the levels of Cr (VI) transferred and detected in *Cicer arietinum, Phaseoulus vulgaris, Amaranthus dubuis Thell* and *Vigna angularis* in the leaf portions of the plants signify potential high health risk to consumers.

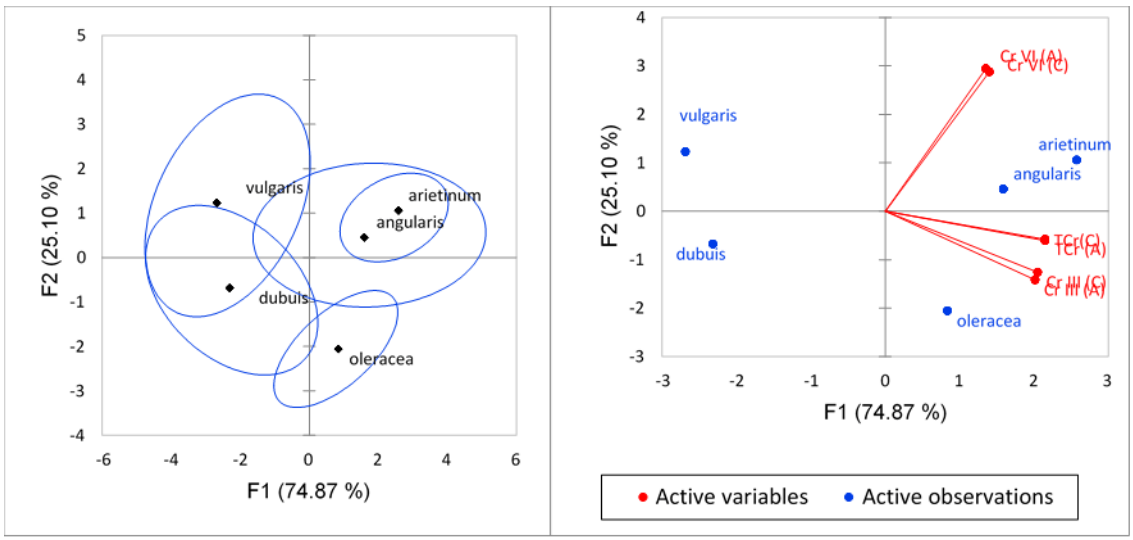

Figure 5.4: Principal component analysis (PCA) plot of the relationship between the sampled plants species (vulgaris, dubuis, oleracea, angularis and arietinum) and the relationship between concentration of ChT, Cr(VI) and Cr(III) (active variables) in the stems (A) and leaves (C) of the edible vegetables (active observations) for the first two principal components obtained (PC1 and PC2)

5.3.5: Daily intake of chromium (ChT, Cr (VI), Cr (III)) through edible vegetables grown on Cr (VI) spiked soils

This study focused on the cultivation of vegetables on Cr (VI) simulated soils and then estimated the average daily intake of Cr by adult and children consumers of the vegetables. A significant difference ($p < 0.05$) was observed between the child and adult consuming edible vegetables with ChT and Cr (III). This shows that edible vegetables in this study may have accumulated more Cr (VI) by converting it either to ChT or Cr (III) in various concentrations. But the uptake became greater than the maximum permissible limits of Food Agriculture Organisation (FAO)/World Health Organisation (WHO) (1999) for plant (0.10 mg/kg) in the

spiked soil. In principle, the required amount of vegetables in people's daily diet is supposed to be between 300 and 350g per person (adult) and 200–230 g per child, as suggested by guidelines of WHO (Ogwu *et al.,* 2016). Figure 5.5 depicts the possible daily intake of high ChT, Cr (VI) and Cr (III) by humans from the edible vegetables grown in spiked soil in this study. The intake values were calculated by taking the average value of Cr oxidation states to assess the level of exposure in all the five varieties of the vegetables (Table 5.4) and taking into account that each adult and child in Kenya and South Africa (assuming 60 and 25 kg of body weight for adult and child, respectively) consume approximately 340 and 220 g, respectively, of vegetables per day according to WHO (1989). However, the amounts in Figure 5.5 give a picture showing that daily intake of vegetable species was far above recommended intake levels.

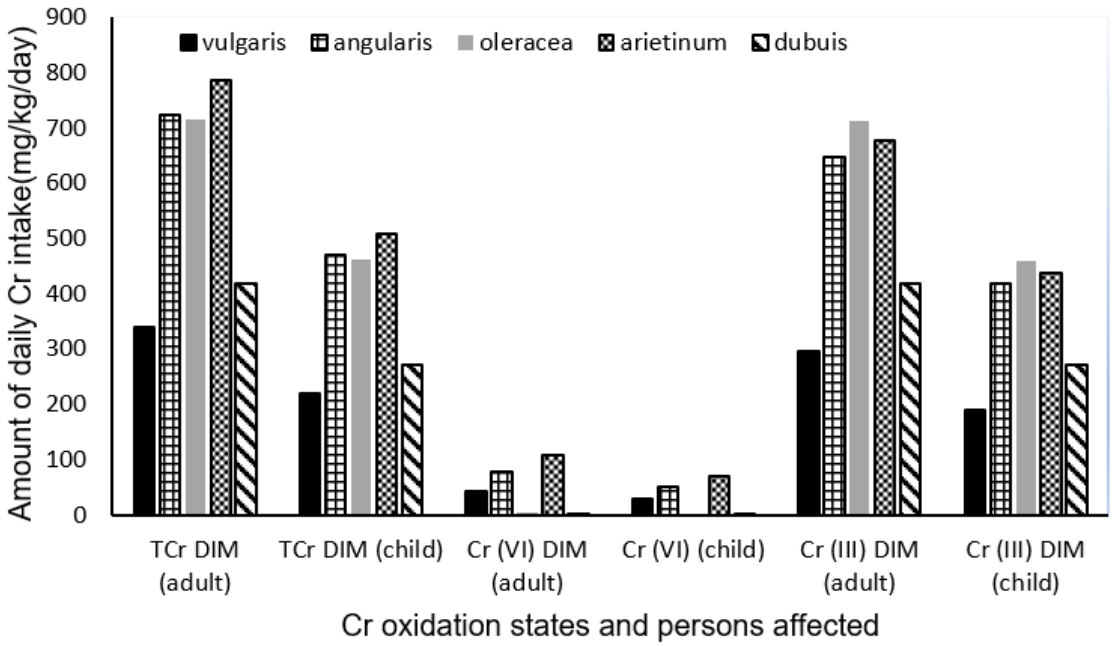

Figure 5.5: Daily intake of Chromium (DIC), ChT, Cr (VI) and Cr (III) from the leaves of different vegetables from soil spiked with Cr (VI)

In the consumption habits of local residents of Kenya and South Africa, *Spinacea oleracea, Cicer arietinum, Phaseoulus vulgaris, Amaranthus dubuis Thell, and Vigna angularis* are consumed as leafy vegetables, which accounts for 90% of total consumption of vegetables within the region with the remaining percentage being taken as seeds or pods (Cui *et al*., 2015; Faber *et al.,* 2010). This means that a very large population from these two countries are potentially at health risk due to exposure to Ch_T, Cr (VI) and Cr (III). Therefore daily intake of chromium by human consumers of these vegetables are likely to expose them to these types of clinical disorders i.e. respiratory, carcinogenic, renal, hepatic, gastrointestinal,

cardiovascular, haematological, reproductive developmental, genotoxic and mutagenic effects (Cui *et al*., 2015).

5.3.6: Hazard Quotient

The results of HQ for Cr oxidation states in this study are shown in Table 5.5. The HQ analysis showed a significant difference ($p < 0.05$) between a child and an adult exposed to edible vegetables with CrT and Cr (III) from this study. For different exposure population, HQ of ChT and Cr (III) oxidation states were all above one. This suggested that the daily intake of Cr oxidation states through the consumption of *Spinacea oleracea*, *Cicer arietinum*, *Phaseoulus vulgaris, Amaranthus dubuis Thell,* and *Vigna angularis* may likely cause adverse health effects for residents of South Africa and Kenya. Indeed, the potential non-cancer risk for ChT, Cr (VI) and Cr (III) is expressed as hazard quotient (HQ). When HQ > 1, it implies that there may be a concern for potential non-cancer effects when the chronic daily intake exceeds the threshold (Huang *et al.,* 2008). The levels of Cr (VI) were found to be insignificantly different, implying that consumers may be safe when they get exposed to vegetables containing it. However, according to FAO/WHO (1999) guidelines for plant, the maximum permissible limits of chromium (0.10 mg/kg) was exceeded. Thus, the consumers were still not safe. The safe levels were only found in *Spinacea oleracea* and *Amaranthus dubuis Thell* for both adults and children. It can also be shown from Table 5.5 that HQ of ChT, Cr (VI) and Cr (III) for children were higher than those for adults in all the plants analysed, which agrees with previous studies of Bose and Bhattacharyya (2008).

Table 5.5: Hazard quotients of CrT, Cr (VI) and Cr (III) to consumers of vegetables grown on soil spiked with Cr (VI)

Plant Name	Total Cr		Cr(VI)		Cr(III)	
	Adult	Child	Adult	Child	Adult	Child
P. vulgaris	3.8	5.8	0.5	0.8	3.3	5.1
V.angularis	8.0	12.3	0.9	1.3	7.1	11
S. oleracea	7.9	12.2	0.04	0.06	7.9	12.1
C. arietinum	8.7	13.4	1.2	1.8	7.5	12
A. dubuis (T)	4.6	7.1	0.007	0.01	4.6	7.1

5.3.7: Hazard Index

The HI method was used to assess the total of all potential health risks of ChT, Cr (VI) and Cr (III) accumulation through leafy vegetable consumption for adults and children. The risk is considered unacceptable at HI > 1. The results of the five leaf vegetables were all found to be

above one, which may present a risk to adults and children in terms of ChT, Cr (VI) and Cr (III) exposure. HI values were observed in this decreasing order for adults and children as *Cicer arietinum* > *Vigna angularis* > *Spinacea oleracea* > *Amaranthus dubuis Thell* > *Phaseoulus vulgaris,* as shown in Table 5.6. This suggested that the potential HI of Cr oxidation states through vegetable consumption were higher in *Cicer arietinum*, being as high as 17.2 and 27.2 for adults and children, respectively. These results implied that the potential health risk of Cr oxidation states through the consumption of leafy vegetables was high for all vegetable types studied. The estimation of HI, which takes care of the chemical mixtures, is very important in assessing multiple effects of heavy metals, such as Cr. In nature, chronic low-level intake of toxic metal elements can have a negative effect on human health, and the detrimental impact only becomes known after several years of exposure (Zhou *et al*., 2016).

Table 5.6: Hazard index (HI) of CrT, Cr (VI) and Cr (III) in vegetables grown in soil spiked with Cr (VI) for adults and children

HI	*Phaseoulus vulgaris*	*Vigna angularis*	*Spinacea oleracea*	*Cicer arietinum*	*Amarantha dubuis Thell*
Adult	7.6	16	15.8	17.4	9.2
Child	11.6	24.6	24.4	27.2	14

The HI values of Cr oxidation states through vegetable consumption for children were higher than the values for adults in all vegetables. Therefore, Cr oxidation states are likely to contribute to the potential health risks of vegetable consumption for residents living and accessing sites contaminated with tannery Cr wastes. The HI findings in this study were lower than that of Chaturvedi *et al.* (2019), who found HI for Cr in children's toys at 91.9. Huang *et al.* (2008) stated in their work that Cr speciation was of concern. This was because, according to them, health risk from Cr exposure may be overestimated if Cr (III) co-exists with Cr (VI). Cr (III) is considered essential in the metabolism of carbohydrates in animals, but high levels are equally risky under HI assessment, as seen in this study. Therefore, this study considered Cr speciation in vegetables from polluted sites and found out that their HI is a potential risk to both adults and children consuming them.

5.4: Conclusions

This chapter's objective was to evaluate the potential health risk associated with edible vegetables grown on Cr (VI) polluted soils (objective two). The study aimed at testing how several vegetables among them *Vigna angularis*, *Cicer arietinum*, *Spinacea oleracea*, *Amaranthus dubius Thell* and *Phaseolus vulgaris* were able to germinate and grow in different concentrations of Cr (VI) polluted soils. Besides that, the study analysed Cr (VI), Cr (III) and ChT bioaccumulation and translocation factors in these plants, daily intake of chromium,

hazard quotient and hazard index on consumers. The findings of the study, established that *Vigna angularis* was the only vegetable that germinated at the highest Cr concentration of 456 mg/kg. *Phaseoulus vulgaris, Vigna angularis* and *Cicer arietinum* germinated at Cr concentration of up to 228 mg/kg, while *Spinacea oleracea* and *Amaranthus dubuis Thell* in addition to the vegetables above germinated at higher concentrations and grew at concentrations of upto 114 mg/kg. Bioaccumulations/ bioconcentrations factor of *Vigna angularis* and *Cicer arietinum* were higher than those of *Phaseoulus vulgaris*, *Spinacea oleracea* and *Amaranthus dubuis Thell*. These plants can be grouped as moderate and low accumulators but cannot be used for phytoremediation because they are edible vegetables.

An adult and a child who consume these vegetables, especially leaf parts of *Cicer arietinum* and *Spinacea oleracea*, are likely to consume high levels of chromium above the recommended values of WHO. The HQ and HI of ChT and Cr (III) oxidation states were all above one, while that of Cr (VI) was mostly below one. This portends non-carcinogenic risks to consumers, with children being more vulnerable to such risks. Therefore, the findings of this thesis support the hypothesis that “selected edible vegetables that germinate and grow in high concentration of Cr (VI) polluted soils have the potential to bioaccumulate and translocate toxic chromium compounds to edible parts, which pose public health risk to their consumers”. Tannery workers usually cultivate these edible vegetables at or near these dumpsites and consume them without adequate knowledge of the health risk associated with such food crops, thus there is need to create awareness and remediate sites polluted with tannery-based Cr wastes.

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CHAPTER SIX

Investigating the bacterial profile in donkey dung-assisted anaerobic bioreactor remediation of tannery chromium wastes

This thesis chapter reports the investigation of the bacterial profile in donkey dung assisted anaerobic bioreactor remediation of tannery chromium wastes. Bioprospecting for organic materials that are eco-friendly to remediate tannery chromium effluents has been going on for some time. This study investigated the use of *Equus africanus asinus* dung in anaerobic bioreactor set up to remediate tannery chromium wastewater from Dogbone and Beit Ore tanneries in Kenya and South Africa, respectively. The aim was to explore the potential of cellulose degrading bacteria species that naturally originate from donkey colon and reside in the dung for the removal of chromium (Cr) concentrations. The experiment was optimised by taking 100 mL of chromium effluents mixed with 300 mL of deionised water and supplemented with 15 and 30 g of pulverised, unsteriled dung for a period of 30 days. Monitoring involved taking samples for analysis at day 0, 7, 14, 21 and 30. The removal percentages of Cr (VI) and total Cr were determined with Ultraviolet visible spectrophotometer and Inductively coupled plasma-optical emission spectrophotometer, respectively. The profile of bacterial DNA library was sequenced on an Illumina MiSeq System. The results revealed bacterial activity and Cr concentrations removal efficiency in both bioreactors under various optimised conditions. Higher and faster removal of total chromium levels were observed in supplemented bioreactors (99%) than in the control (60%). The most dominant bacterial communities in BO bioreactors were *Firmicutes phylum* (48.28%), Bacilli class (40.29%) and *Streptococcus genus* (27.31%). DB bioreactors had *Proteobacteria phylum* (51.70%), *Gammaproteobacteria* (20.44%) and genus of *Selenviibrio* (13.87%). These bacterial consortium tolerated the chromium stress conditions. Possibly they reduced it by either chromosol, plasmid, enzymatic, assimilatory, dissimilatory, persister cell or small colony variant, which are diverse metabolic resistance mechanisms.

Key words: Bacteria consortia, Cellulose degrading consortium, Donkey dung, Fermentation, Methanogenesis, Tannery chromium effluent.

6.1: Introduction

Worldwide, leather manufacturing is considered one of the most polluting industrial activities due to the high production of hazardous diverse wastes (Polizzia *et al.*, 2018). In particular, the increasing presence of toxic chromium compounds in tannery wastewaters is a great environmental and public health concern as mentioned earlier in chapter two section 2.7 and 2.8 of this thesis. This is because of the expensive treatment involved before their discharge into ecosystems. Currently, the known and commonly practised conventional treatment methods for chromium remediation are explained in chapter two section 2.11 and 2.16 of this thesis (Gupta *et al.,* 2014). However, these approaches are not very effective and efficient because they only succeed in the partial removal of chromium in effluent. In a number of cases, they produce very harmful residual products that require additional treatment process before release to the environment. The extensive reviewed literature report in chapter two of this thesis and the published review paper (See outputs link in chapter one) show that, there is a renewed research interest on simple innovative safe disposal and treatment technologies for prevalent indiscriminate chromium waste disposal problem in sub Saharan Africa into an ecologically acceptable manner.

The traditional treatment procedures for chromium waste are known to be expensive, unsafe and disruptive to the natural environment in comparison to the application of emerging bioremediation strategies. These new procedures are considered more ecologically friendly. These technologies use the existing *in situ* and/or *ex situ* microbes like bacteria, fungi, algae, yeast and plants that are either tolerant to chromium biosorption or having bioreduction capabilities (Igiri *et al.,* 2018 ; Gupta *et al.*, 2014; Saranraj and Sujitha, 2013). Direct or indirect microbial biological metabolic mechanisms known as assimilatory or dissimilatory and chemical processes reduces the oxidation/reduction state of the toxic Cr (VI) to less toxic Cr (III). Besides that, they may also facilitate the precipitation of chromium at a neutral pH for further physical removal as alternative routes in bioremediation processes (Prasad *et al.*, 2012; Dhal *et al.*, 2013).

In the last decade, there are numerous reports on isolation and characterisation of various chromium reducing microbial strains of bacteria (*Pseudomonas* spp., *Bacillus* spp., *Enterobacter* spp., *Acinetobacter* spp., *Burkholderia* spp.), fungi (*Paecilomyces* spp., *Aspergillus* spp., *Phanerochaete* spp., *Penicillium* spp., *Rhizopus* spp.), Yeast (*Candida* spp., *Saccharomyces* spp.) and algae. These details can be found in two comprehensive review articles by Mala *et al.* (2020) and Bhattacharya *et al.* (2019). Engineered bioremediation strategies, involving anaerobic treatment of different tannery wastes has also been demonstrated (Basak *et al.,* 2014; Priebe *et al*., 2016; Zupančič & Jemec, 2010).

At present, there are numerous reports on the involvement of diverse and complex assemblages of *in situ* bacteria and archaea in anaerobic digestion for nutrient recycling processes and biogas production from various organic wastes and sewage sludge (Lozano *et al*., 2019; Sun *et al*., 2015; Lee *et al.,* 2016; Zheng *et al.,* 2014; Zigashina *et al.*, 2013). The key roles played by niche-specific guilds of known hydrolytic and acetogenic bacteria (*Acetivibrio, Clostridium, Bacteroides, Ruminococcus* and *Thermotoga*), non-hydrolytic acidogens (*Bifidobacterium, Lactobacillus* and *Anaerolineaceae*), syntrophic acetogens (*Smithllela, Syntrophobacter, Pelotomaculum Syntrophus* and *Syntrophomonas*) and methanogenic archeae (*Methanoculleus, Methanobacterium, Methanobrevibacter, Methanospirillum, Methanothermobacter Methanosaeta,* among others) have been highlighted (Lim *et al.*, 2020). Nevertheless, due to the complexity of anaerobic digestor (AD) ecosystems, the community of microbes are yet to be precisely and fully characterised and the mechanisms under which they act is still regarded as unexploited areas of study (Vanwonterghem *et al*., 2014; Lim *et al,* 2020).

In tannery wastes, the extreme conditions provided by the intrinsic toxicity and recalcitrance nature of the Cr (VI) not common in typical AD systems, may impair the growth and catabolic activity of indigenous microorganisms, thus limiting bioremediation efficiency. Unfortunately, there is a general paucity in the knowledge on microbial community structure and inter-relationships involved in anaerobic reduction of tannery chromium effluent. As this information is key in identifying the microbial changes, functions/processes and roles on organic and inorganic biodegradation for optimisation of AD bioreactor performance, there is need for exhaustive study. Coupled with microbial community structure, studies on environmental factors such as pH, temperature, oxygen, and nutrients availability, that must be optimised to promote microbial growth and bioremediation efficiency are also warranted.

To gain a better insight into the nature of *in situ* microbial community within tannery waste and evaluate the prospect for *in situ* bioremediation of Cr by biostimulation, we adopted the *in situ* bioremediation technique in the present study. We used microcosm-based culture-independent metagenomic approach. We have also investigated the effect of biostimulation (addition of donkey dung) on biodegradation potential as well as change in microbial community structure of wastewater from Dogbone and Beit Ore.

Biostimulation, mainly linked to the amendment of contaminated site with specific nutrients (fertilizers, growth supplements, trace minerals or biosurfactants), is one of the promising strategies that have found wider use to stimulate and speed up the growth and metabolism rate of indigenous microorganisms, and thus, improve bioremediation efficiency (Adams *et al.,*

2015 ; Kumar *et al.*, 2011). In this study, we hypothesised that the introduction of organic matter will improve the nutrient availability, in addition to introducing *ex situ* microbes that may improve Cr bioremediation efficiency. The overall study attempted to evaluate: (i) the composition of autochthonous microbial community within tannery sludge, (ii) the impact of donkey dung on the overall microbial community structure dynamics under anaerobic co-digestion of tannery wastes and (iii) the effect of specific biostimulation strategy on the indigenous microbial community composition and the associated improvements in the intrinsic Cr bioremediation.

6.2: Methods and materials

6.2.1: Materials

Tannery chromium wastewater effluent samples for this study were collected from the sites described in chapter one, section 1.8, subsection 1.8.1 and attached appendix Figure 1.5. The dried dung was sampled as described in methodology chapter three, section 3.2 and subsection 3.2.2 of this thesis. Both tannery effluent and donkey dung were stored at 4°C before use to prevent biodegradation by microorganisms and environmental factors from the atmosphere. In the laboratory, donkey dung was pulverised to ~ 2 mm prior to use as a co-substrate in anaerobic digestion (AD) experiments.

6.2.2: Bench scale bio-stimulated anaerobic bioreactor experiments using donkey dung and chromium effluents

The experiment was carried in a batch set-up or closed system model using 500 mL bio stimulated anaerobic digestion glass bioreactors incubated in thermostatic water baths at 37^{o}C with modifications based on the method descriptions of Priebe *et al.* (2016) and Basak *et al*. (2014). The bioreactors were made of glass in conical shape and the reactors had a funnel which consisted of three openings (Figure 6.1). One opening for liquid sample withdrawal, another for gas sample withdrawal and finally one for measuring the volume of gases produced. As a self-sustaining system where biological process proceeded dynamically, the bioreactors were fed once separately with 0 g, 15 g and 30 g donkey dung, respectively (as seed inoculum/substrate). It was in co-digestion with chromium wastewater solution of 100 mL diluted with 300 mL deionised water for each tannery batch (BO and DB). The mixture was maintained at 30°C for 24 h, in order to acclimatise the sludge to the experimental conditions.

The experiment was carried out in three separate bioreactors to optimise donkey dung as a bio- substrate/inoculum ratio and digestion time as hydraulic retention time (between 0 - 30 days). This was done to find out favourable conditions for anaerobic toxicity assay for total Cr and Cr (VI) reduction. The possible biochemical methane potential was also observed as

re-defined from Owen *et al*. (1979). The donkey dung was used without prior isolation and pre-treatment. Temperature was maintained in a thermostatic water bath at 37^{o}C throughout the experiments period. This was to enable mesophilic temperature ranges of bacteria to grow. The pH was not controlled but was found to vary during reactions in BO and DB samples. The initial concentration of chromium in control and bioreactor samples were measured. The high volume of water was used for dilution because the initial volume of 150 mL (desired volume) was not enough and the slurry (mixture of water and substrate) was thick and the dung could not dissolve. According to Sacks and Buckley (2004), the function of the control sample in this study was to help determine the volume of gas produced due to the microbial degradation of residual organic molecules in the inoculum cum substrates and quality assurance of the procedure.

Daily increment in the headspace gas pressure (though not the aim of this study) in the bioreactor was measured using manometer. The gas volume was quantified by subtracting the volume of gas produced in the control blanks. The experimental bioreactors were labelled according to the source of chromium effluents added and the donkey dung amount applied e.g. BOc (0-control), BO_{15} (15 gm of dung), BO_{30} (30 gm of dung), DBc (0-control), DB_{15} (15 gm of dung) and DB_{30} (30 gm of dung). Before the headspace was sealed with a metallic ring cap fitted with a rubber septum, the dissolved oxygen was removed from the bioreactors to strictly create anaerobic environment. This was done with pure nitrogen gas which was bubbled for 5 minutes into the bioreactor before sealing the bioreactor openings.

The bioreactors were shaken manually once before each sampling to facilitate contact between the micro-organisms and the substrate and to release trapped gases along the tubes. This was to enable their correct measurements at the fall of day 0, 7, 14, 21 and 30 in all the 6 bottles. This was done using clean sterilised syringes, bottles and vials. The samples were taken for the analysis of pH, total Cr, Cr (VI) and the extraction of DNA for bacteria identification. The biogas compositions were determined by gas chromatography on the 30th day (Appendix Table 6.1). A total of fifteen samples were collected in the bioreactors from day 0 to day 30. Samples for physico-chemical analysis were analysed immediately while those for microbial analysis were stored at −20^{o}C until further processing and analysis.

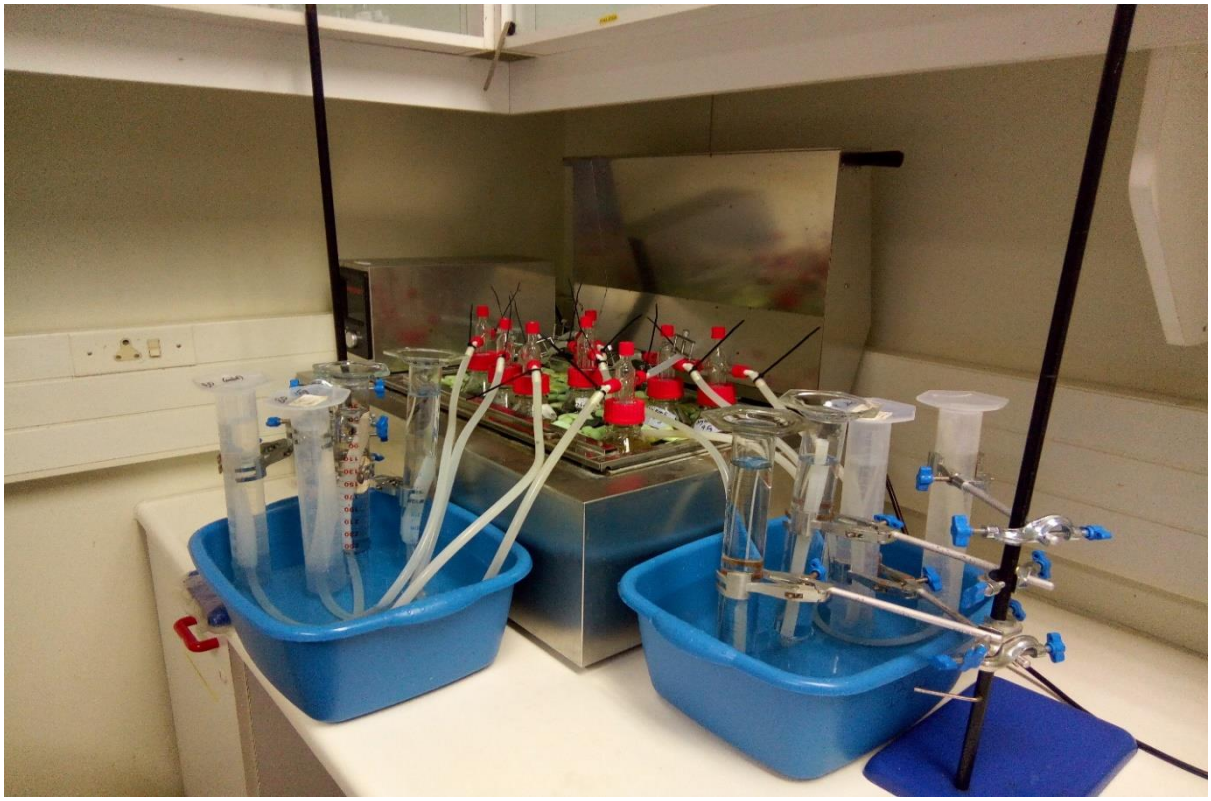

Figure 6.1: The bioreactor set up for BO and DB chromium effluents reduction with donkey dung reaction at the Institute for Development of Energy for African Sustainability, UNISA laboratory

6.2.3: Proximate analysis of crude nutritional values of raw donkey dung

To characterise the proximate nutritional values of raw donkey dung, one gm was weighed in crucibles in triplicates. The moisture content was determined by heating at 105^{o}C in oven for 5 h. Crude fibre was extracted using 98% H_2SO_4, KOH, acetone and n-octanol while fat content was analysed by heating with petroleum ether at 79^{o}C for 2 h. The samples were then placed in a furnace for 5 hr to determine the ash content. Protein content was analysed by total combustion using Dumus analyser Leco Trumac model from LECO Corporation (Saint Joseph Michigan, USA). Total carbohydrate was derived by getting the difference between 100% and the sum total (%) (Protein, fat, fibre, moisture and ash) in adherence to AOAC (1995) procedures.

6.2.4: Physico-chemical parameters analysis

pH was measured using Adwa AD11 pH meter (Adwa Instruments, Szeged, Hungary). Cr (VI) species content was measured using UV/Vis spectrophotometry at 540 nm following upon complexation with 1,5-diphenylcarbazide reagent as described by Homa *et al.* (2016). Total Cr content was analysed, following acid microwave digestion, on Agilent 700 Series Inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Agilent

Technologies, Santa Clara, CA, United States) as previously described by Mondol *et al.*, (2014). The percent removal efficiency (%) for total Cr was determined by the equation (6.1):

$$\mathrm{RE} = \frac{(\mathrm{C_{In}} - \mathrm{C_f})}{\mathrm{C_{In}}} \times 100 \qquad (6.1)$$

Where C_{In} is initial Cr concentration and C_f the final Cr concentration (mg/L).

The biogas compositions at day 30 was determined (depended on the column and calibration gas available) by gas chromatography (Agilent 7890B GC system, Agilent Technologies, Santa Clara, CA, United States) according to manufacturer's instructions.

6.2.5: DNA extraction, polymerase chain reaction (PCR) and high throughput sequencing (HTS)

The random sequencing of genomic DNA of bacteria isolated from the donkey dung sampled from the anaerobic bioreactors were extracted for total environmental DNA using Faecal/Soil Total DNA™ extraction kit (Zymo Research Corporation, CA, USA). This was done according to manufacturer's instructions with slight modifications. Briefly, about 2 g of dung sample was initially mixed with 5 mL phosphate buffered saline (PBS, pH 7.4). The mixture was agitated by vortexing and allowed to stand for a few minutes at room temperature to dislodge bacterial cells from their adhesion to solid waste. An aliquot of 400 µL of the supernatant was used for DNA extraction using Faecal/Soil Total DNA™ extraction kit. Libraries of bacterial 16S rRNA gene fragments were amplified from each DNA extract using PCR with 27F and 1492R primers under the following conditions: initial denaturation at 95°C for 5 min, 32 cycles consisting of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C, and a final extension of 7 min at 72°C followed by cooling at 4°C. This was followed by a second nested PCR using 27F and 518R primer pairs having overhang adapter sequences compatible with Illumina indexes and sequencing adapters as described by Ramganesh *et al*. (2018).

6.2.6: MiSeq library Preparation and Sequencing

The amplified 16S rRNA gene fragments in each library were purified using AMPure XP beads (Beckman Coulter, Agencourt Bioscience Corporation, Massachusetts, USA) following manufacturer's protocol. Upon purification, an eight cycle PCR (95°C for 30s, 55°C for 30s, and 72°C for 30s including initial denaturation at 95°C for 3 min and final extension at 72°C for 5 min) was used to add Illumina sequencing adapters and dual-index barcodes to each amplicon library using full complement of Nextera XT indices (Illumina, Inc. San Diego, CA, USA). The resultant barcorded PCR products were purified using AMPure XP beads. The fragment size (~630 bp) was validated using Bioanalyzer DNA 1000 chip (Agilent, Santa Clara, CA, USA), and then quantified using QuBit HS assay (Life Technologies, Carlsbad, CA, USA). This was prior to pooling into equimolar amounts in the final DNA library.

The pooled final DNA library (4 nM) was denatured and sequenced on an Illumina MiSeq System (Illumina, San Diego, CA, USA) (Figure 6.2) using paired 300-bp reads to generate high-quality, full-length reads of the V3 and V4 region. The raw fastq files obtained after trimming the adapters and primer sequences was used for further bioinformatics analysis.

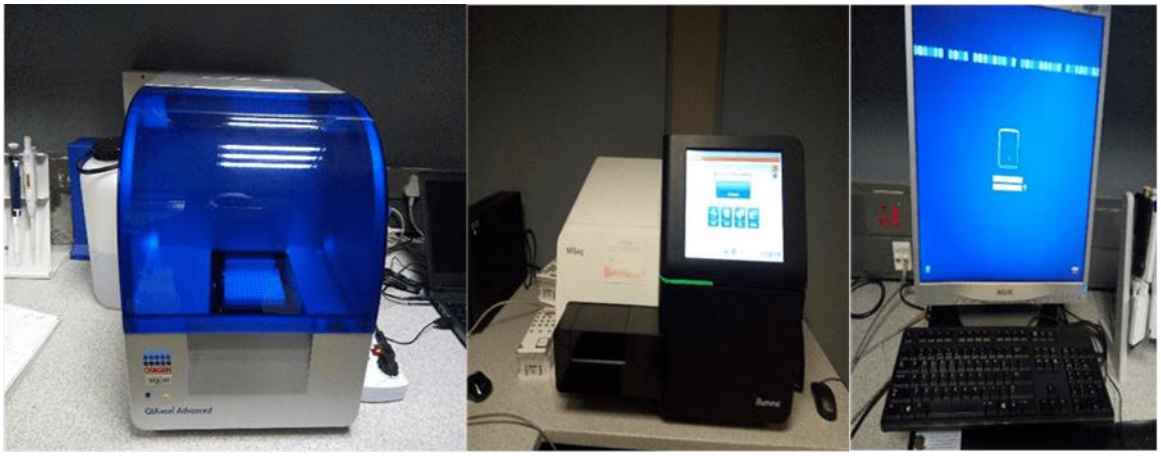

Figure 6.2: The complete Illumina MiSeq System used in sequencing extracted DNA (Laboratory analysis at UNISA)

6.2.7: Bioinformatic data analyses

Pre-processing of the raw fastq datasets was performed to remove PCR artefacts and low-quality reads (reads with >50% bases having a quality score < 2) using ngsShoRT trimmer as described by Chen *et al.* (2014), before being subjected to Mothur v.1.40.0 pipeline (Schloss *et al*., 2009). During the analysis, sequence reads containing low nucleotides (<50 nts), ambiguities (>2%) and homopolymers (7%) were excluded, including sequences of mitochondrial and chloroplast origins and chimeric sequences (removed using UCHIME algorithm) (Edgar *et al*., 2011). Naïve Bayesian classifier algorithm (Wang *et al*., 2007) was used to classify non-chimeric sequence reads against the SILVA database version 132 (Quast *et al*., 2013), assigning taxonomic identity of bacteria at a confidence threshold of 80%. A pairwise distance matrix (Euclidean distance matrix) was grouped to the aligned sequences into operational taxonomic units (OTUs) at a sequence similarity of 97%.

The dominant OTUs at different taxonomic levels were used to generate stacked bar charts and heatmap using *ggplot2* (Wickham, 2016) and *heatmap.2* packages (Warnes *et al*., 2019) in R version 3.6.1 (R Core Team, 2019), respectively, to visualise the variations and distributions of present bacterial communities. Alpha diversity indices (Shannon-Weaver, Simpson) and microbial community richness index (Chao 1, ACE) were calculated at the genetic distance of 0.03 using the plot_richness function of *phyloseq* (McMurdie and Holmes, 2013). β-diversity based Bray-Curtis dissimilarity distance and canonical correspondence

analysis (CCA) to visualise the community relationships between and within each AD treatment with explanatory environmental variables was also performed using *phyloseq* (McMurdie and Holmes, 2013).

6.3: Results

6.3.1: Donkey dung nutritional characteristics

The proximate composition of the donkey dung used was: 24.1% fibre, 1.83% total lipids, 5.08% protein, 5.70% moisture, 19.2% ash and 44.2% carbohydrate content.

6.3.2: Chromium removal efficiency during AD experiments

Results on the changes in the total chromium (suspected to be dominated by Cr III used in tanning leather) and Cr (VI) concentrations in two tannery chrome wastes at different supplementation of donkey dung are illustrated in Figure 6.1. Overall, the removal efficiency of up to 99% CrT was achieved by the 21st day in both DB and BO chromium effluents co-digested with donkey dung. This was in comparison to two control samples that had less than 60% removal after 30 days (Figure 6.3a & c). However, changes in Cr (VI) exhibited different trends dependent on the chromium wastes. For BO bioreactors, Cr (VI) in all the treatments dropped from the initial average levels of 0.9 mg/L to 0 on the 7th Day (Figure 6.3b). But, the Cr (VI) level in BO control started increasing after day 14, peaking at 0.2 mg/L at day 21, before dropping to 0 mg/L on day 30. The levels of Cr (VI) in BO bioreactors co-digested with 15 g donkey dung remained at 0 up to 21 days but only increased to around 0.3 mg/L on day 30. In contrast, the levels of Cr (VI) at 30 g supplementation increased exponentially from day 14 to peak around 0.3 mg/L at day 30. All DB samples had initial levels of Cr (VI) species below 0.25 mg/L, that was observed up to day 7 (Fig 6.3d). Increase in the initial levels was, however, detected at day 14. This depended on the donkey dung supplementation mass. The Cr (VI) levels in the DB control decreased to zero levels by day 7 and remained at zero until day 21, before increasing to the initial levels by day 30. However, both 15 and 30 g donkey dung supplementation exhibited exponential increases in Cr (VI) species by day 30 of AD treatment.

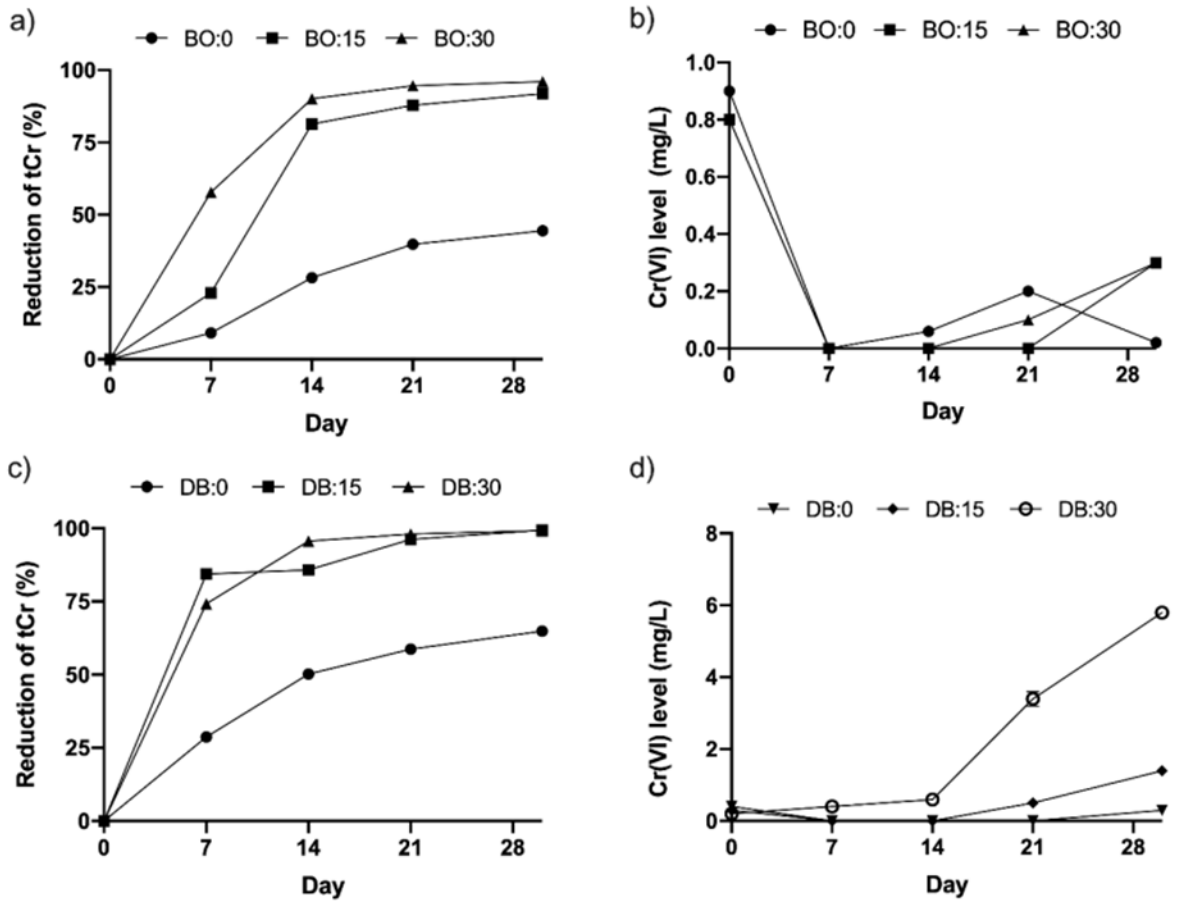

Figure 6.3: Total chromium removal efficiency and the generation of Cr (VI) in the anaerobic bioreactors co-digested with chromium effluent from BO and DB with donkey dung

6.3.3: pH changes, CO_2 and CH_4 production

The study also monitored the pH, CO_2 and CH_4 production as indicators of the bacterial metabolic activities occurring during AD experiments. Both BO and DB chrome waste were acidic in nature initially (pH 3.8 and 4.0, respectively). But pH changes in the two chromes wastes were affected differently on donkey dung supplementation during AD (Figure 6.4a and b). Whereas subtle changes were observed in all BO samples, the pH remained largely acidic (pH < 5.0) regardless of the donkey dung supplementation levels (Fig 6.4a). Furthermore, BO supplementation with donkey dung was only associated with CO_2 production (Fig 6.4c). No methane gas production was detected in 30 days. In DB, supplemented samples were associated with shift of pH from acidic (~pH 5.0) to near neutral to mild alkaline conditions (pH 7.5-8.0) (Fig 6.4b). These treatments were associated with production of both CO_2 and CH_4. However, higher CH_4:CO_2 ratio was observed at higher donkey dung supplementation, while lower CH_4:CO_2 ratio was observed at lower supplementation (Figure 6.4d). Another notable occurrence (not indicated in the graph) but observed was the strong smell of rotten egg suspected to be H_2S gas. This was sense in DB supplementation from day 21 to 30. However,

it was not quantified due to lack of column and calibration gas in the GC. Like BO control, DB control samples were consistently acidic during the AD experiments, with no CO_2, CH_4 and H_2S production (Fig 6.4c and d and Appendix Figure 6.1).

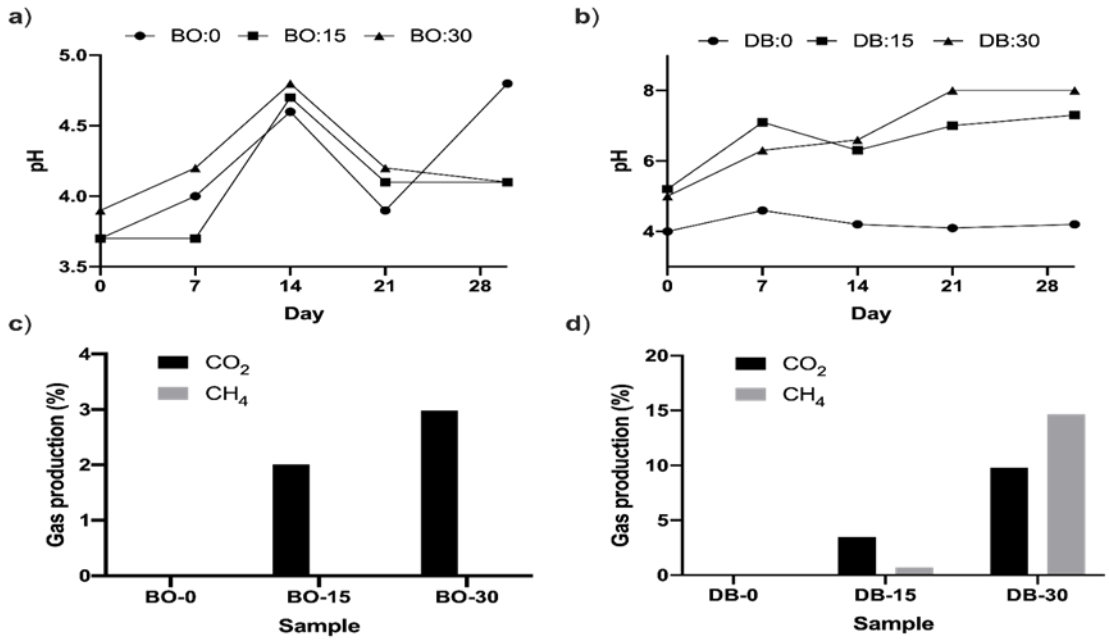

Figure 6.4: Fluctuations in pH, carbon dioxide (CO_2) and methane (CH_4) during anaerobic co-digestion of BO and DB chrome waste liquor with different donkey dung supplementation

6.4: Bacterial community diversity and distribution under different donkey dung supplementation

6.4.1: Ecological indices of bacterial community profile

In this study, bacterial community diversity from six different treatments were profiled using the Illumina high throughput sequencing of 16S rRNA gene. This was done to identify the variations of bacterial groups and Cr reduction capacity. Observation was made, on the different dosages of donkey dung under anaerobic digestion process over a retention period of 30 days.

Bacterial diversity analysis based on 16S rDNA gene resulted in quality reads ranging from 3,753 to 29,900 sequences and 670 OTUs across 24 samples. For comparisons, the dataset was subsampled and rarefied to an even depth of 3,753 sequences (minimum amount of sequences recorded). Summary of the alpha diversity indices is given in Appendix Table 6.1. Good coverage of all the samples were >98.5%, indicating that the sampling depth was enough to estimate bacterial diversity. The rarefaction curves were also asymptote approaching a plateau (Appendix Figure 6.2), indicating the sampling depth accurately reflected bacterial community in the samples.

Overall, Wilcoxon rank-sum test showed DB samples had significantly ($p<0.05$) higher species richness (ACE, Chao1 and Jacknife) than BO samples. However, no significant ($p>0.05$) difference was observed on species diversity indices (Appendix Figure 6.3). Fluctuation in the bacterial diversity (Shannon) and richness (Chao1) for all treatments during the course of 30-days AD experiments is illustrated in Figure 6.5. Although subtle variations in Shannon diversity index was observed, no clear trend was discernible within the different sample treatments over 30 days period. In contrast, a progressive reduction in Chao1 index from day 0 to 30 was observed for all DB samples, but not with BO samples (Figure 6.5b).

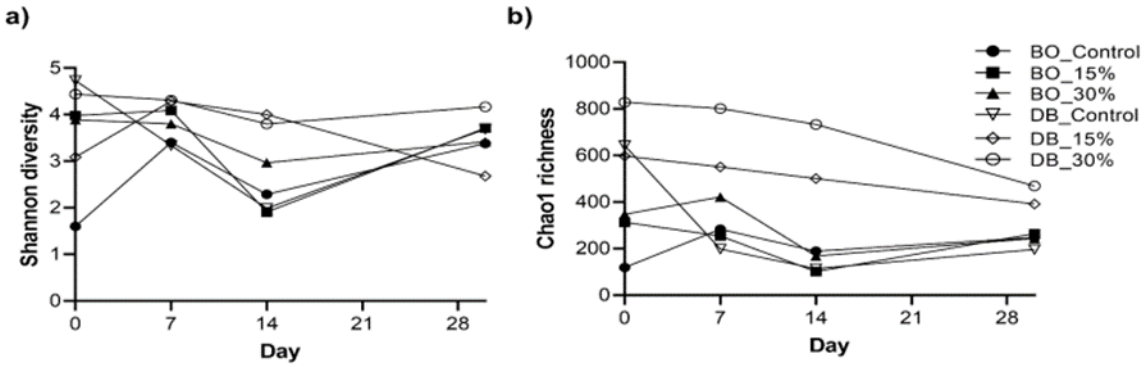

Figure 6.5: Plot of the changes in Chao1 richness and Shannon diversity indices during anaerobic digestion of chrome liquor samples supplemented with different levels of donkey dung based on 16S rDNA targeted amplicon sequencing

Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity was used to assess the differences of the bacterial community composition among the samples and sample treatments (Appendix Figure 6.4). Principal components 1 and 2 described 35.9% and 21.7% of the bacterial community composition variations, respectively. Most of the DB samples clustered together in the positive side showing a closely strong relationship, implying distinct microbial community profiles. Similarly, BO samples clustered in the negative side, however, the observed sparse scattering implied a weak relationship.

6.4.2: Microbial Community Composition and Shift during anaerobic co-digestion

The total bacterial community consisted of 24 phyla, 57 classes, 104 orders, 230 families and 670 genera. Overall, *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteriodetes* and *Deferribacteres* were the most dominant phyla, accounting for >99% total abundance. However, subtle differences were discernible dependent on chrome liquor waste, donkey dung supplementation level and days of anaerobic co-digestion (Figure 6.6 and Appendix Table 6.2). For example, the most abundant bacteria phyla in BO samples were *Firmicutes* (48.28%), followed by *Proteobacteria* (32.57%), *Actinobacteria* (16.30%) and *Bacteroidetes* (1.58%). On the other hand, *Proteobacteria* (51.70%) were the most abundant phyla in DB samples

followed by *Firmicutes* (24.56%), *Actinobacteria* and *Deferribacteres* (8.70%) and *Bacteroidetes* (5.47%).

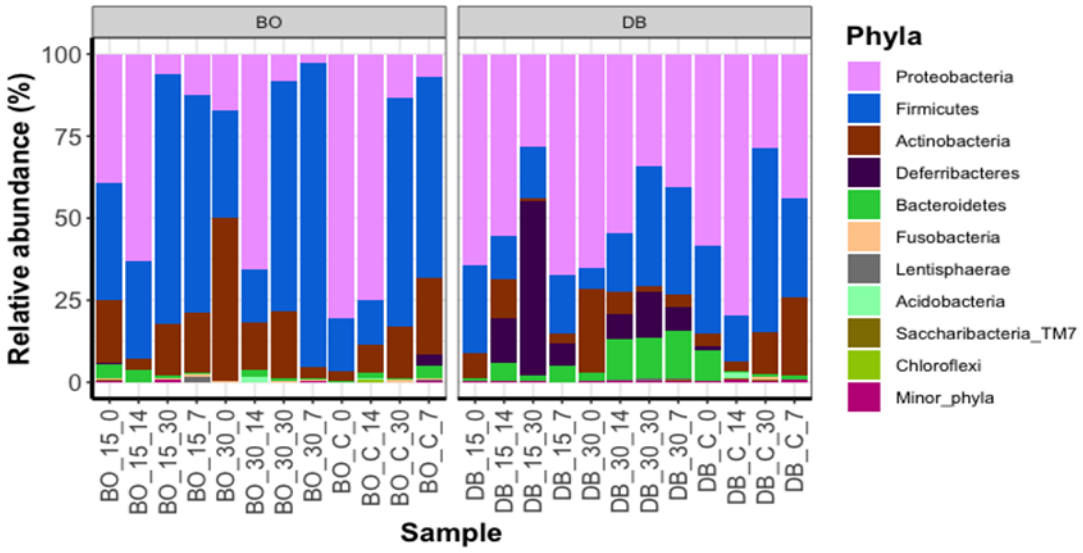

Figure 6.6: Phylum level identification of all the sequences and overall comparative composition of top bacteria phyla during anaerobic digestion of BO and DB chrome liquor effluents supplemented with donkey dung

Similar trend was observed at class level, where BO samples were dominated by *Bacilli* (40.29%) followed by *Betaproteobacteria* (24.81%), *Actinobacteria* (16.01%), *Gammaproteobacteria* (3.96%), Alphaproteobacteria (3.70%), *Clostridia* (3.58%), *Tissierellia* (3.32%) and *Negativicutes* (1.04%). However, DB samples had abundance of *Gammaproteobacteria* (20.44%), followed by *Betaproteobacteria* (18.40%), *Alphaproteobacteria* (12.72%), *Clostridia* (11.36%), *Bacilli* (10.22%), *Deferribacteres_c* (8.67%), unclassified *Actinobacteria* (8.59%), *Bacteroidia* (4.82%) and *Tissierellia* (2.06%) as shown in Figure 6.7 and Appendix Table 6.3

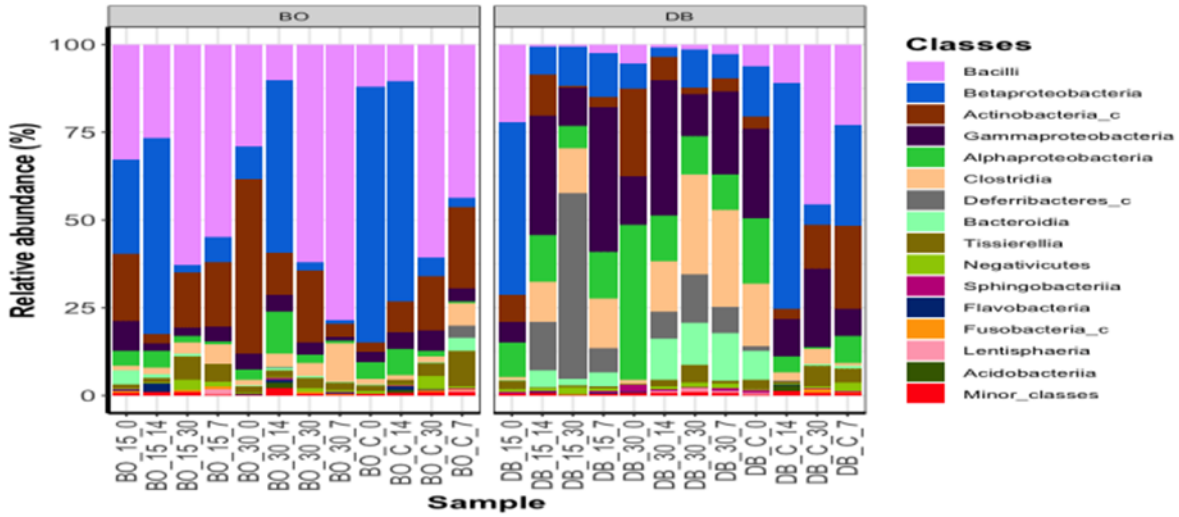

Figure 6.7: Class level identification of all the sequences and overall comparative composition of top bacteria classes during anaerobic digestion of BO and DB chrome liquor effluents supplemented with donkey dung

A heatmap analysis of the top 40 OTUs (genera) was also used to investigate the differences in the bacterial community structural shifts during anaerobic co-digestion of chrome liquor waste supplemented with different levels of donkey dung (Figure 6.8 and Appendix Table 6.3). In DB samples, the dominant autochthonous genera (presented by DB control at day 0 (DB_C_0)) included *Enterobacter* (8.20%), *Parabacteroides* (7.72%) and *Brucella* (7.55%). They were succeeded by *Delftia* (23.85%), *Cutibacterium* (14.46%) and *Streptococcus* (11.22%) from day 7, with *Delftia* peaking at 64.0% total abundance on day 14 followed by *Pseudomonas* (4.79%) and *Streptococcus* (3.89%). However, *Streptococcus* (18.86%), *Pantoea* (16.16%) and *Staphylococcus* (15.65%) were the highly enriched genera at day 30, with *Delftia* detected at <0.01% total abundance. In contrast, *Delftia* (72.36%), *Streptococcus* (6.43%) and *Staphylococcus* (4.26%) were the dominant *in situ* bacterial genera in BO samples (BO_C_0) but exhibited different bacterial succession profile from DB control during AD process (Figure 6.6 and Appendix Table 6.3). In the initial stages of AD process (day 7), *Streptococcus* (22.99%), *Cutibacterium* (20.62%) and *Staphylococcus* (14.90%) dominated, but *Delftia* (59.47%) succeeded at day 14, before repeat dominance of *Streptococcus* (43.05%) followed by *Staphylococcus* (14.84%) and *Cutibacterium* (10.75%) at day 30.

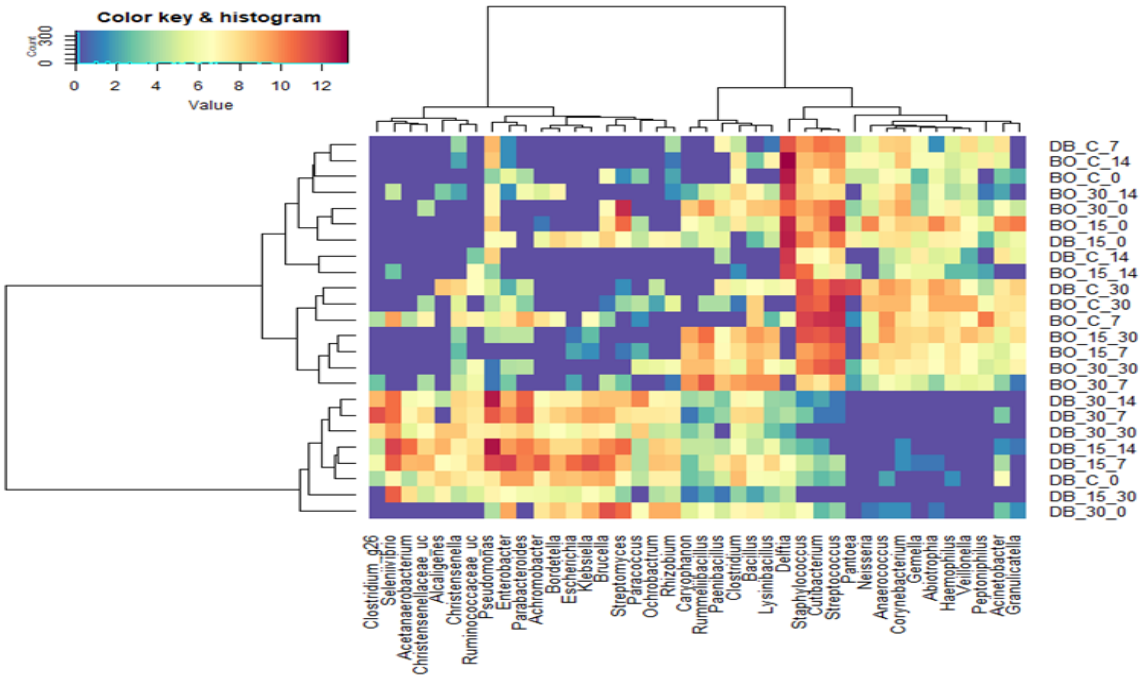

Figure 6.8: Heatmap of the relative abundance of top forty bacteria genera during anaerobic digestion of BO and DB chrome liquor effluents supplemented with donkey dung. Heatmap was based on the $\log^2$ normalised counts of the 40 most abundant genera. The dendrogram shows complete-linkage agglomerative clustering based on a Euclidean distance

Donkey dung supplementation resulted in the plasticity of the bacterial succession profiles during anaerobic co-digestion, a feature that was dependent on the chrome waste liquor and level of supplementation. Whereas the addition of donkey dung to DB chrome waste was associated with dominance of genus *Delftia*, *Streptococcus*, *Staphylococcus*, *Enterobacter*, *Brucella*, and *Streptomyces,* different bacterial community succession profiles were observed at 15 and 30 g supplementation. At 15 g donkey dung (DB_15) supplementation, *Enterobacter* (12.84%), *Pseudomonas* (10.42%) and *Klebsiella* (8.74%) dominated the initial stages (day 7) of anaerobic co-digestion. In contrast, *Clostridium*_g26 (13.21%) followed by *Pseudomonas* (10.60%) and *Parabacteroides* (10.25%) dominated in DB_30 samples by day 7.

At day 14, *Pseudomonas* (27.00%), *Seleniivibrio* (13.77%) and *Streptomyces* (6.36%) were highly enriched in DB_15, while in the DB_30, *Pseudomonas* (32.47%) was the mostly dominant followed by *Parabacteroides* (8.57%) and *Seleniivibrio* (7.67%). Finally, *Seleniivibrio* (53.03%) was the key genera in DB_15 at day 30, followed by *Acetanaerobacterium* (6.10%) and *Alcaligenes* (3.78%), compared to *Seleniivibrio* (13.87%) *Christensenella* (6.40%) and *Alcaligenes* (6.37%) in DB_30. In BO chrome waste samples, donkey dung supplementation was characterised by increased abundance of Streptococcus (10.57-19.24%), Delftia (8.13-21.17%), *and Streptomyces (6.29*-28.60%), with *Cutibacterium, Rummeliibacillus, Lysinibacillus* and *Bacillus* playing an important role in the early stages of anaerobic digestion (day 0-7). However, resurgence of genus *Delftia* and *Streptococcus*, including emergence of groups such as *Staphylococcus, Corynebacterium and Acidocella* was observed at day 14, before dominance of *Streptococcus, Cutibacterium* and *Staphylococcus* in the final stages (day 30) (Appendix Table 6.3).

6.4.3: Identification of taxonomic biomarkers based on LEfSe analysis

In this study, LEfSe analysis was performed to identify differential abundant taxonomic biomarkers in the anaerobic co-digestion of chrome liquor wastes at class and genus level. Eight classes and 126 genera were differentially enriched between DB and BO samples (Figure 6.9, Appendix Figure 6.5). While *Bacilli* was identified as major class linked to BO samples (LDA effect size = 5.18, p(FDR) =0.001), *Gammaproteobacteria* (LDA effect size = 4.92, p(FDR) <0.0001), *Alphaproteobacteria* (LDA effect size = 4.65, p(FDR) =0.003) and unclassified *Deferribacteres* (LDA effect size = 4.62, p(FDR) =0.026) were enriched in DB samples. In total, 6 and 49 genera were identified to be unique to BO and DB samples, respectively. Among the genera, *Seleniivibro*, *Pseudomonas*, and *Novosphingobium* had significantly highest LDA scores (>3.6, p<0.05) in DB samples compared to *Streptococcus*, *Parvimonas*, *Neisseria*, *Peptoniphilus* and *Haemophilus* in BO samples.

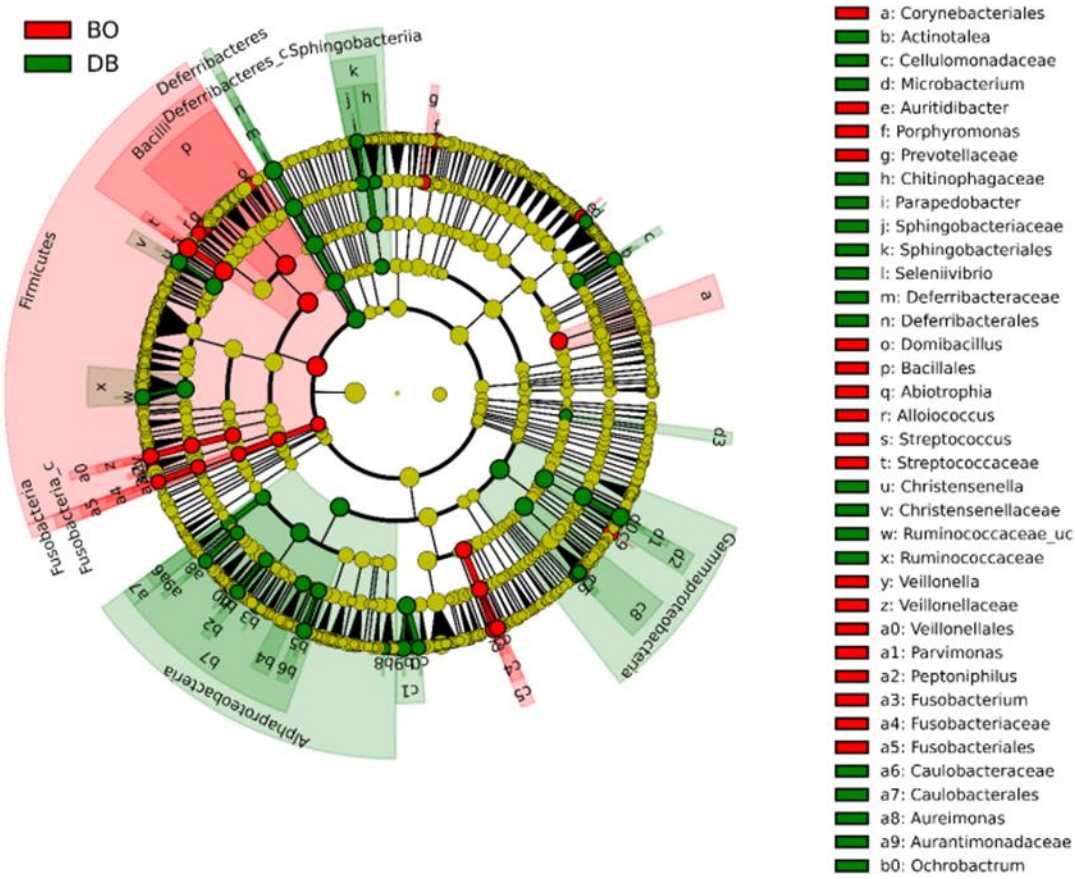

Figure 6.9. LEfSe plot for clades of bacteria enriched within BO and DB samples. The cladogram reports the taxa (highlighted by small circles and shading (BO = red; DB = green) that are enriched within corresponding treatments. LEfSe utilizes Kruskal-Wallis to determine significantly different taxonomic features ($p < 0.01$) between experimental groups, a pairwise Wilcoxon rank sum statistic to test biological consistency across subgroups ($p < 0.01$), and finally a linear discriminant analysis (LDA score >2.0) to determine the effect size, or magnitude of variation of the features between groups

6.5: Relationship between bacterial community and environmental variables

The relationship between measured parameters (pH, total Cr, Cr (VI), digestion period (day) and donkey dung supplementation) and the relative abundances within bacterial classes in BO and DB treatments was investigated using the canonical correspondence analysis (CCA). As shown in Figure 6.10A, class *Alphaproteobacteria*, *Betaproteobacteria* and *Flavobacteria* were greatly influenced by pH, while the distribution of *Gammaproteobacteria* was influenced more by Cr (VI) level. In contrast, time of anaerobic digestion (day) greatly influenced the distribution of members class *Bacilli*, *Clostridia*, unclassified *Deferribacteres*, *Negativicutes* and *Tissierellia*. Only members of unclassified *Actinobacteria* and *Bacteriodia* were greatly affected by level of donkey dung supplementation. In DB samples, *Bacteroidia* and *Clostridia* were positively correlated with dung supplementation, pH and Cr (VI), whereas *Alphaproteobacteria and Sphingobacteriia* were closely related with changes in total Cr levels (Figure 6.10B).

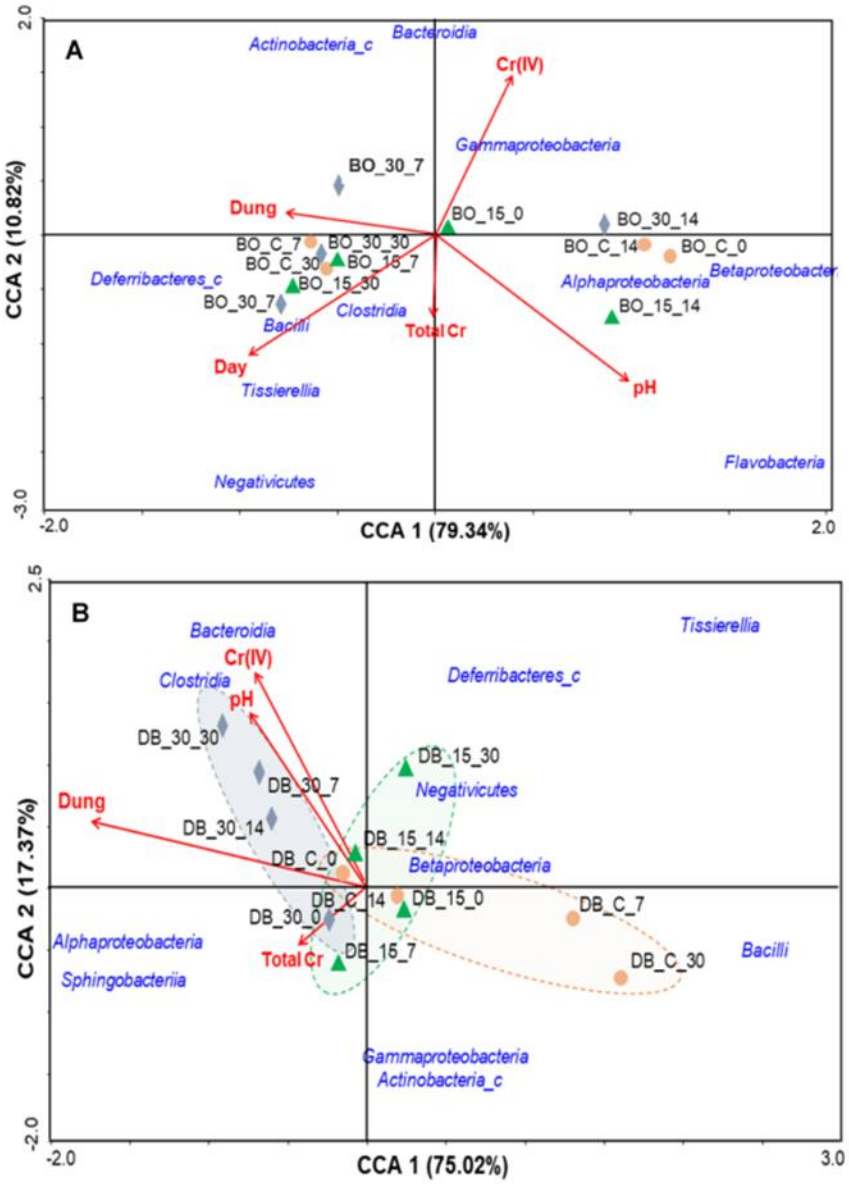

Figure 6.10: Canonical correspondence analysis (CCA). Triplot graph of CCA showing ordination of top ten (10) bacterial classes, samples and environmental variables during anaerobic digestion of chrome liquor from (A) BO and (B) DB tannery wastes at different donkey dung supplementation in the first two axes. Orange circles, green triangles and gray diamond are samples supplemented with 0, 15 and 30% donkey dung, respectively

6.6: Discussion

Bioremediation programmes succeed when microbial communities present in an environment, increase in biomass strength. This is necessary for the efficient interaction, through either absorption or mineralisation of persistent compounds. Thus, leading to the overarching objective of the removal of these pollutants from the environment (Mateo *et al.*, 2001). Often, this process is hampered by the absence of rate limiting nutrients in the environment needing treatment. Tannery chromium wastes present such a situation. Although, there are autochthonous micro-organisms that have adapted to the harsh conditions found in these wastes, i.e. low pH and high chromium concentrations. These organisms are usually not found

in large biomass, because of the absence of rate-limiting nutrients required for increased biomass (Figures 6.3). It is this understanding by scientists that has led to the incorporation of biostimulation strategies in addressing chromium wasteswater with low nutrient contents (Sawatdeenarunat *et al*., 2015; Tyagi *et al.,* 2011; North *et al*., 2004; Kanissery and Sim, 2011).

Moreover, this study incorporated non-sterile donkey dung which provided an array of its own diverse microbial communities that remarkably interacted with the tannery chromium wastewater. This was observed with the removal of total chromium (dominated with Cr (III) and production of biogas (methane) in some of the samples (Figures 6.3 and 6.4). The result introduces a further perspective of bioaugmentation, as these organisms present in the donkey dung are by no means normal to these tannery wastes. But, they were observed to be metabolically active in some of the bioreacting vessels. Further to these findings, was the change in pH conditions. The initial pH analysed in all bioreactors samples were in acidic ranges; with BO at 3.8 and DB at 4.0 respectively. But they tended towards weak acidic and mild alkaline with DB bioreactors reaching a maximum pH of 8. This tendency is commonly observed during anaerobic digestions leading to the production of methane gas, thus demonstrating bacterial activity (Malematja *et al.,* 2019). The possibility of integrating bioremediation and methane production in leather industries waste management will however require further studies. However, it seems promising as an alternative source of energy for the industry as shown in the results of Figure 6.4 and Appendix Table 6.1.

This study provides proximate analysis of crude nutritional values of donkey dung essentially the digestate. They contain organic matter similar to that of monogastric farm livestock and sufficiently provides for anaerobic bacterial growth (Gupta *et al.,* 2016). Therefore, inclusion of donkey dung in this experimental set-up provided for such nutrients (carbon, nitrogen, phosphorous, sulphur) needed for biomass increase and subsequent removal of chromium. Although, anaerobic conditions were promoted in this study to encourage the growth of anaerobic micro-organisms' resident in the donkey dung, methane gas production was not possible in BO reactors. However, there was consistence removal in total chromium levels in both bioreactors (BO and DB). There is a possibility that the tanning process used at the BO tannery may have contributed inhibitors to the wastewater samples that affected the introduced micro-organisms present in the donkey dung. This implied that, the degradation (fermentation but no methanogenesis) occurring was mostly achieved by the autochthonous micro-organisms present in the actual tannery effluent.

Afterall, the pH remained acidic indicating that organisms that thrived inside, were those that continued to maintain the acidic conditions that is normal to the tannery chromium

wastewaters. It also indicated that the absence of biogas production provided no influential change in the pH that could have changed conditions in bioreactors to alkaline, as was observed in the DB bioreactors (Chen *et al.,* 2008). Although, under those conditions (time, pH and organic load), the active adsorption sites and microbes in the BO reactors supplemented with dung were able to remove total chromium to almost above 90% along with the concurrent decrease in Cr (VI) upto day 14. After day 14, a slight increase in Cr (VI) was observed. This could be attributed to continous fermentation by the active microbes (Figure 6.4). In contrast for the case of DB, there could have been fermentation and methanogensis taking place at the same time in the bioreactors. But, the increase in Cr (VI) from day 14 onwards, correlated with methane gas formation which was unique. This could be associated with breakdown of organic carbon molecules in the dung and hydrogen in wastewater molecules (biohydrogenenation) in the bioreactors by active microbes to form methane gas. Their activities may have been accompanied with concurrent removal of CrT (Cr (III) and reduction of Cr (VI). At day 30, the CrT adsorbed into the bacterial cell during the exponential growth phase, may have been released back into the solution upon the cell death or as a survival technique by microbes under such stressful conditions. This resulted into appreciable amount of adsorbed CrT being converted back into Cr (VI) state by redox reactions (Ijoma and Tekere, 2016; Ijoma *et al.*, 2019).

It is, however, important to state that high methane production was observed in DB bioreactors containing 30 g donkey dung compared to the 15 g organic load. This suggested that optimisation of high organic load substrates ratio in anaerobic bio-stimulated bioreactor is also important in methane gas generation (Deublein and Steinhauser, 2008). This study found, that the best optimum parameters for chromium removal in terms of hydraulic time, pH and organic load were between 7 - 14 days, acidic pH (3.8- 5.0) and organic load of 15 g. These optimium conditions helped to remove total chromium and suppressed the formation of Cr (VI) and other obnoxious gases, as donkey dung provided more adsorption sites. In addition to that, the anaerobic microbes also proliferated and competed for the scarce substrates contributing to Cr removal (Siddique *et al*., 2005; Ijoma and Tekere, 2016; Stahl and Christensen, 1992). Thus, for this anaerobic process to succeed in removing CrT as well as regeneration of Cr (VI) in tannery effluent; there is a need to balance between the two by optimising the appropriate conditions. These optimum conditions still requires further research.

Microbial diversity is often altered by stress conditions, particularly nutrient depletion and toxicity both of which characterised the tannery chromium waste samples. Nutrient depletion may lead to successional growth patterns and prevalence in microbial populations. The ACE, Chao, Shannon's and Simpson diversity indices tests on samples confirmed the richness and diversity of samples, whilst the PCoA depicted the clustered closeness of interacting

organisms. These are all normal attributes in microbial population dynamics in any given environment (Li *et al*., 2008). In this study, there were three groups of PCoA cluster as depicted in Appendix Figure 6.5. Most of the DB samples were clustered closely. A few were widely and sparsely clustered in BO samples. This was an indication of the replacement,that microbial profile populations shifted through from the onset community, in response to the operations and conditions in the anaerobic bioreactors.

At the beginning of degradation in the bioreactors the most abundant phyla in BO, was *Firmicutes* while DB was dominated by *Proteobacteria* (Figure 6.6). *Firmicutes* phyla comprises species like *Streptococcus, Staphylococcus* and others that normally grow in animals body especially the skin and other wide ranges of environments. Their presence in this study, is readily attributed to the mixture of organics derived from animals' dung and dissolved skin proteins present in chromium effluents (Gomez-Montano *et al*., 2013). It is important to point out that bacterial persistence in such toxic environments may be attributed to the transfer of plasmid genes that encode resistance mechanisms on membrane transporters as described in studies done on *Streptococcus* genus in the bioreactors, by Wani *et al*. (2015). Moreover, Onyango and Alreshidi (2018) postulate that *Staphylococcus* resistance to toxic chromium levels may be possible through formation of persister cell or small colony variant. This is a resistance mechanism of producing low molecular weight binding proteins in their cell walls. There are other authors that support the plausibility of cell wall binding of chromium by *Staphylococcus* (Gaupp *et al*., 2015; Begic *et al*., 2009).

The relative abundance of *Proteobacteria* (i.e *Delftia, Pseudomonas, Alcacigens*) in DB bioreactors may be attributed to their capacity to adapt to harsh conditions of high (mesophilic) temperatures and pH in anaerobic bio-stimulated bioreactors. Being opportunistic saprophytes, the presence of organic material like donkey dung and soluble protein from tanned skin fibres in the effluent, possibly made their growth enhanced (Jardine *et al.,* 2017). Several researchers have alluded to members of the phyla *Bacteriodetes* involvement in skin fibres degradation (Kim *et al*., 2011; Danielsson *et al*., 2012; Kampmann *et al*., 2012; Sundberg *et al*., 2013; St-Pierre and Wright, 2014; Robert *et al*., 2007; Hatamoto *et al*., 2014; Naas *et al*., 2014). This possibly accounted for their prevalence in the bioreactors.

There is a need to explain *Seleniivibrio genus* activity in DB bioreactors. This gram negative bacteria belongs to *Deferribacteres* phylum and it dominated DB supplemented bioreactors as from day 21 to 30 as a replacement bacteria. Rauschenbach *et al*. (2013), suggested that, it may have originated from the chromium effluents, as it has been isolated from activated sludge of wastewater treatment plants. It is reported in literature by Mishra and Häggblom (2013) that, *Seleniivibrio* species has the potential to utilise metals or metalloids for respiration.

They also have the ability to reduce elemental sulphur and oxidise organic substrates at mesophilic temperature. In the process, they play an important role in the carbon cycle by accepting acetate as electron donor. This may enables them to reduce iron (III), manganese (IV), nitrate or sulphate whose oxides are known to oxidise Cr and reduce sulphide (Tamazawa *et al.,* 2017; Rauschenbach *et al*., 2013).

In the process, they may have caused dissimilatory metabolic mechanisms that probably changed chromium states and generated the gases. For instance, CH_4 and CO_2 occurrence were quantified as shown in Figure 6.4 and Appendix Table 6.1. However, the strong smell of a rotten egg sense during sampling in DB bioreactors on day 21 and 30 suggests the generation of H_2S gas along with CH_4 and other gases. Since, the gas was not quantified due to lack of hydrogen sulphide column and calibration gas in the GC, their simultaneous occurrence requires further study. However, the occurrence of the H_2S gas could be linked to the residual quantities of sodium sulphide in the used effluent associated with the hair removal process in leather tanning in DB tannery. Their residue in the DB effluent may have contributed to the generation of H_2S under anaerobic conditions and as the *Seleniivibrio* bacteria became the dominant replacement species in the AD (Midha and Dey, 2008).

Bacilli was found dominant in BO while *Gammaproteobacteria* was prominent in DB bioreactors (Figure 6.7). *Bacilli* are extremely stress-resistant and endospore-forming bacteria capable of surviving in extreme conditions such as high Cr concentration (Upadhya *et al.,* 2017). Similarly, *Gammaproteobacteria* are known to degrade sulphur containing compounds and survive in harsh conditions as characterised by their increased presence in DB than BO (Goni-Urriza *et al.*, 2000; Weil *et al.*, 2017; Wu *et al*., 2010; Felfoeldi *et al*., 2010; Desai *et al*., 2009; Joynt *et al*., 2006; Campbell, 2014). In the DB bioreactors, another representative of *Gammaproteobacteria,* observed was *Enterobacter*. This enteric bacteria likely originated from both tannery effluent and donkey dung.

Previous studies have indicated that their resistance mechanism for chromium could be linked to the presence of the chromosol genes (*ars,* arsC operon and arr) (Jain and Häggblom, 2016; Rahman *et al*., 2015; Wang *et al.,* 1989). It is again important to note the prevalence of *Cutibacterium* in the BO bioreactor. They are bacteria associated with soil, but the proximity of soil to animal's body during foraging and bedding has made it prevalence on animal skin. This is typical, where their interspecific interactions is commensal. Previous studies have also isolated these organisms from chromium effluents and tannery wastewaters (Elahia *et al.,* 2019). Several studies, have also demonstrated the ability of *Cutibacterium* to produce immunomodulatory molecules as stress responses in toxic environments (Bharagava and Mishra, 2017; Kuehnast *et al.,* 2018; O'Neill and Gallo, 2018; Elahia *et al.,* 2019).

Several other classes of bacteria including *Alphaproteobacteria, Actinobacteria, Clostridia, Acidobacteria, Bacteroidia, Tissierellia, Flavobacteria, Sphingobacteria, Lentisphaeria, Negativicutes and Fusobacteria* were present in varying percentages in the two samples.Their percentages were particularly high in bio-stimulated bioreactors. Most ecological environments tend to typically contain diverse, heterogeneous population of microorganisms (Kester and Fortune, 2014). This study demonstrated that consortia population in bio-stimulated bioreactors were metabolically superior to the controls that had no nutrients supplements (Figures 6.4 and 6.8). There is a possibility that they contributed to degradation of the Cr, using a variety of mechanisms. This could have included complexation, adsorption, active transport, enzymes, specific metal binding proteins, metallothioneins, reduction and active efflux.

Others probably used sequestration of heavy metals ions to a less toxic state in plasmid, chromosomal resistance, inter/intraspecies electron transfers and formation of either persister cells or small colony variants (Mutiat *et al*., 2018; Onyango and Alreshidi, 2018; Klapper *et al*., 2007; Rands *et al*., 2017; Willis,1977; Sun *et al*., 2013; Lebuhn *et al*., 2014; Lu *et al*., 2013; Rana *et al*., 2014; Kashyap *et al*., 2003; Cervantes *et al.*, 1990; Nies *et al*., 1990). The bacteria with diverse occurrence in both bioreactors in this study was *Delftia* species which are gram negative. They are usually acidovorans, metabolically diverse *β-proteobacteria.* Previous reports have isolated *Delftia sp.* from waste sludge and sites polluted with chromium (Morel *et al*., 2009; Hernández *et al.* 2012). Remarkably, in this study, their prevalence was observed even at wide ranges of pH (3.8 - 4.8 and 4.0-8.0) in BO and DB bioreactors respectively. Some authors have alluded to *Delftia sp*. using certain enzymes and membrane bound proteins (reductases) in the reduction of Chromium (Morel *et al*. 2016; Pradhan *et al.* 2016). Specifically, Morel *et al*. (2011; 2016) reported that *Delftia sp*. JD2 was a chromium-resistant bacterium that reduced chromium and they linked this to reductase activity within dominant gene cluster known as *chrBACF*.

Several other bacteria that have been speculated in previous studies to be possibly linked to chromium removal/reduction and were also found in this study, includes *Alcaligens* and *Pseudomonas.* The previous findings postulated the possible involvement of plasmid mediated mechanisms for the reduced accumulation of Cr in their cell wall. Thus conferring them with chromium resistance (Pradhan *et al.*, 2016; Gargi *et al*., 2015; Poornima *et al*., 2010; Cervantes *et al.*, 1990; Nies *et al*., 1990). Ramirez-Diaz *et al*. (2008); Gadd (2010) and Alvarez *et al*. (1999) report, that microorganisms like bacteria have cells made up of chromosome and plasmids genes that play resistant roles to many toxic heavy metals and metalloids. The mechanisms they involve may be an encoded systems, dedicated to shield bacterial cells from the oxidative stress caused by excess harmful chromate ions. In some cases, bacterial

resistance systems linked to plasmid genes might be encoded with membrane transporters, which facilitate the efflux of toxic chromate ions across the cytoplasmic membrane.

Further analysis were done using LEfSe and CCA to identify the biomarkers and observe the relationship of bacteria to environmental factors in the bioreactors. LEfSe found that, at genus level, *Seleniivibro*, *Pseudomonas*, *Novosphingobium, Streptococcus*, *Parvimonas*, *Neisseria*, *Peptoniphilus* and *Haemophilus* were differentiated by both distribution and abundance. However *Seleniivibro*, *Pseudomonas*, *Novosphingobium* genus were detected by LEfSe to have a very high LDA score (more than three orders of magnitude) (Figure, 6.9). This showed a marked abundance in DB effluent and consistently low abundance in BO effluent (Segata *et al.,* 2011). Lastly the application of CCA on pH, total Cr, Cr (VI), digestion period (day) and donkey dung supplementation established strong and weak collinearity with various classes of bacteria in BO and DB as shown in Figure 6.10. These environmental factors either strongly favoured or weakly disfavoured the bacterial growth or community structure distribution in the bioreactors (Gleeson *et al*., 2006).

6.7: Conclusion

The objective of this chapter was to investigate the bacterial profile in donkey dung assisted anaerobic bioreactor remediation of tannery chromium wastes (objective three). The study aimed at identifying the dominant bacteria species that occurred in the donkey dung with potential to remediate different amount of tannery chromium wastewater, their profile shift in the anaerobic bioreactors with addition of dung as source of nutrient and changes over time. This study established that non-sterile donkey dung provides nutrients and an array of metabolically active and diverse bacterial communities in anaerobic environment. The introduced bacteria from the donkey dung interacted with those resident in tannery chromium effluents and caused 99% removal in the levels of Cr effluents compared to 60% of the control in anaerobic digestion. The optimum conditions for total Cr and Cr (VI) safe removal were found to be in the range of 3.8- 5.0 which is acidic pH, 7-14 days, organic load of 15 g and mesophilic temperature (37^{o}C). When the effluent supplemented with donkey dung in the DB bioreactors were in alkaline pH, as from days 21-30, under the same temperature and organic load of 30 g, it was found that the conditions favoured methane and other gases generation. Further studies is required to find out why there was a concurrence formatiion of Cr (VI) (Figure 6.1d) in removed Cr solution and release of H_2S gas (strongly sense by smell but not measured during sampling in the DB bioreactors). The ACE, Chao 1, Shannon's, Simpson diversity indexes, LEfSe and CCA analysis confirmed species richness, diversity, biomarkers and collinearity in the two bioreactors.

DB was predominated with *Proteobacteria, Gammaproteobacteria* and *Seleniivibro,* while BO had prevalence of *Firmicutes, Bacilli* and *Streptococcus at* the end of bioreactions. pH, total Cr, Cr (VI), digestion period (days) and donkey dung supplementation had strong and weak collinearity with various classes of bacteria which is in support of our hypothesis that, the introduction of organic matter improves nutrient availability and introduces *ex situ* microbes that improve Cr bioremediation efficiency. Lastly, bacterial diversity and profile were altered by chromium stress conditions. But they applied different adsorption and resistance mechanisms which possibly included chromosol, plasmid, enzymatic, assimilatory, dissimilatory, persister cell or small colony metabolic resistance mechanisms which still requires further exploration and exploitation.

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CHAPTER SEVEN

GENERAL CONCLUSIONS AND RECOMMENDATION

In this chapter, general conclusions based on experimental findings are discussed. The work carried out in this research and a discussion of the achieved objectives and proved hypothesis are also presented. The recommended future work is presented at the end of this section.

7.1: CONCLUSION

Tannery chromium wastes dumpsites (Dogbone and Beit Ore) were studied comprehensively as shown in Figure 7.1. This was done to elucidate on the impact of chromium wastes dumped inside the tanneries on the soils and vegetations around such sites and their possible effects on human health. This study first demonstrated the occurrence of chromium oxidation states and ecological risks at these selected tannery dumpsites due to poor disposal of chromium wastes. The ineffective management of these tannery chromium wastes and other wastes containing other selected metals have resulted into contamination of soils and vegetations at the study areas. The application of pollution indices tools on the analytical data generated from sampled soils and plants depicted BO as ecologically moderately risk site, while DB was found to be ecologically high risk site. The findings help prove the first hypothesis that stated that: “Chromium and other heavy metals wastes contamination increases potential ecological risk on soils and plants from selected tannery waste dumpsites in Kenya and South Africa”.

The study went further to simulate how edible vegetables can germinate and grow in chromium polluted soils. The selected edible vegetables were evaluated for bioaccumulation and translocation of chromium oxidation states from polluted soils into the roots and then edible leaves. The analysis detected quantifiable levels of chromium oxidation states in the edible leaves. When the data was subjected to DIC, HQ and HI indices model tools, they confirmed that consumers exposed to those selected edible vegetables were potentially at high health risk, with children being more vulnerable than adults. This outcome also proves the second hypothesis that states that: “Selected edible vegetables that germinate and grow in high concentration of Cr (VI) polluted soils pose high health risk to their consumers”.

Three eco-friendly, innovative bioremediation approaches were conceptualised, applied and observed. They showed the possibility of effectively remediating chromium wastes at tannery dumpsites. The first remediation potential observed was phytoremediation technique by selected edible vegetable plants. These plants showed phytostablisation and phytoextraction

potential for chromium oxidation states in the simulated study. This technique was not pursued further because of FAO and WHO scientific research guidelines and regulations. They restrict the use of food crops for remediation of pollutants in the environment. The second technique was the use of donkey dung as a bio-stimulant and source of inoculum bacteria for reduction of real tannery effluent concentrations in a bench set up of anaerobic bioreactors. In the bioreactor set up, the bio-stimulated anaerobic resident bacteria in donkey dung co-digested with chromium effluent removed 99% chromium levels in real tannery effluents between 7 to 14 days with mass loading of 15 g as the best optimised conditions. The study established that profile of anaerobic bacteria communities' composition and shifts existed in the donkey dung and chromium effluents. In the anaerobic bioreactors, they contributed to removal of chromium levels and even generated methane gas under optimised conditions. These results prove the third hypothesis that states that: "The introduction of organic matter improves nutrient availability and introduces Ex-situ microbes that improve Cr bioremediation efficiency.

The third approach (as attached in Appendix A 1.0) was the preliminary application of raw *Equus africanus asinus* species dung to supplement the remediation of tannery chromium wastes for the first time. The third technique used the raw donkey dung, in the batch adsorption experiment using synthetic Cr (VI) and real tannery chromium effluent under optimised conditions. The raw *Equus africanus asinus* dung as an innovative adsorbent material removed 93.3% of synthetic chromium from Cr (VI) rich wastewater. When it was applied to real tannery chromium effluents from DB and BO tanneries it removed 83.4% and 85.4% of total chromium respectively. These preliminary results of the supplemented study, show that *Equus africanus asinus* raw dung can adsorb and remove synthetic hexavalent chromium and real tannery effluents from waste water.

From the above findings, this study has for the first time advance and enhance existing knowledge on the eco-friendly bioremediation technique of tannery chromium effluent using raw organic donkey dung as a bio-stimulant source of naturally occurring microbes and an adsorbent to remove high concentrations before discharge into the ecosystems.

7.2: Novelty of this research and its addition to knowledge gaps existing in leather waste management in SSA

The novelty of this research and contribution to knowledge includes:-

(a). This is the first time when donkey dung was fully characterised up-to the species level and its dung used for the bioremediation of tannery based total chromium and Cr (VI). Thus contributing to new set of data.

(b). This is the first study to characterise the anaerobic bacteria resident in donkey dung and tannery effluent using metagenomic approach in SSA.

(c). This is the first study to demonstrate the potential of anaerobic bacteria resident in donkey dung and tannery effluent to remove the high levels of total tannery chromium effluents and generate biogas in SSA.

(d). This is the first study to show that edible vegetables can grow in Cr contaminated tannery dumpsite in SSA.

Due to the novelty of part of this thesis, a proposed patent is at an advance stage of consideration.

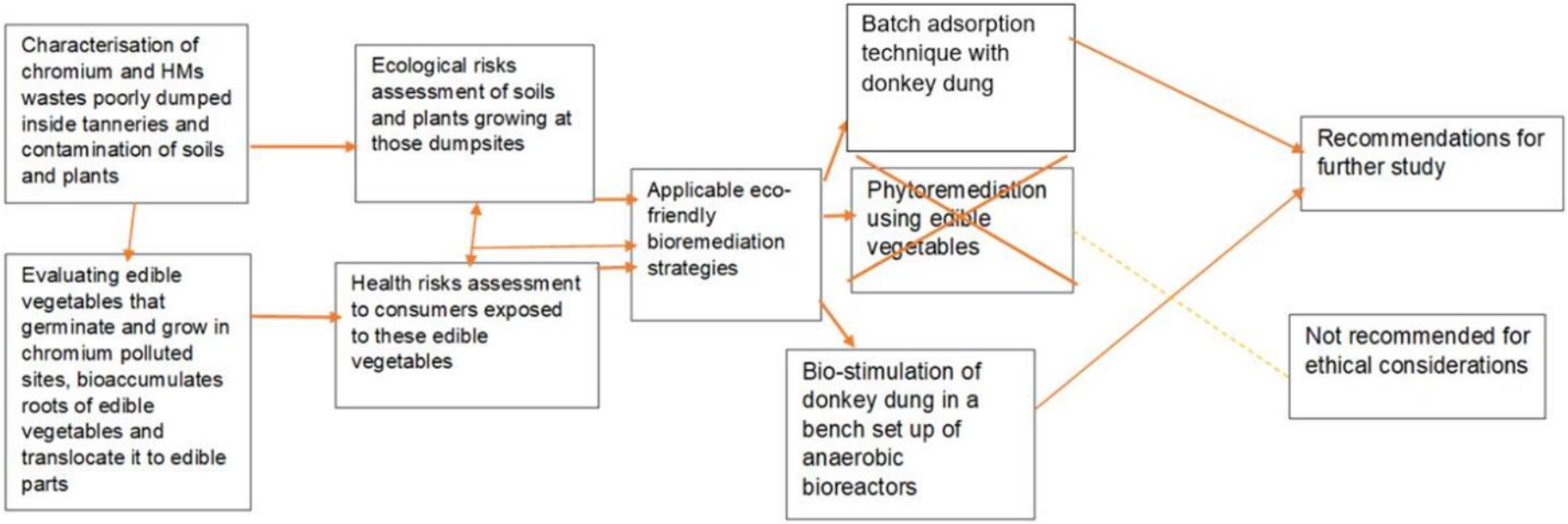


Figure 7.1: Schematic conclusion and synthesis of information flow describing the main highlights of the thesis

7.3: Recommendations

Though this research on bioremediation of tannery based chromium wastes from Beit Ore and Dogbone tanneries in South Africa and Kenya respectively using *Equus africanus asinus* dung contributed to the body of knowledge of bioremediation, it also gave room for future research. The following are recommendations for future research:

- To perform more research on chromium and other heavy metals hyper accumulator plants which are not edible to help phytoremediate chromium contaminated dumpsites in sub–Saharan African tanneries.
- The health effects of eating vegetables (bioaccumulated with Cr) on tannery workers in sub–Saharan African should be investigated.
- The potential of endemic archea and fungus occurring in donkey dung to remediate tannery chromium effluent concentrations for comparison with that of bacteria to help establish which microbes reduce chromium faster under anaerobic and aerobic conditions should be explored.
- To carry out a more comprehensive research on the Cr and other heavy metal adsorption using donkey dung, optimise conditions for its application and carry out further study on parameters such as zeta charge, thermodynamics and desorption of tannery chromium effluents using the same material.
- To find out the specific bacteria that contributes to fermentation and reduction of total chromium, generation of Cr (VI), methane and hydrogen sulphide gases in anaerobic bioreactors using donkey dung.

APPENDICES

Appendix A 1.0: Characterisation and application of a novel green adsorbent prepared from Equus *africanus asinus* dung for the removal of hexavalent chromium from tannery effluents

Abstract

This thesis Appendix chapter reports the preliminary performance of *Equus africanus asinus* dung on adsorption of synthetic Cr (VI) and tannery effluents from Dogbone and Beit Ore tanneries. Various spectroscopic and solid-state techniques such as FTIR, TGA, SEM, FEEM and BET were employed to identify and characterise the bio-sorbent prepared from donkey dung as well as its Cr (VI) adsorption mechanisms. Parameters such as contact time, adsorbent dose, concentration and pH, were optimised. Adsorption was found to be mainly due to the exchange of ligands between the carboxylate functional groups on the adsorbent which formed complexes with Cr (VI). The results of the adsorption isotherms showed that the synthetic chromium wastewater treated with donkey dung powder had a removal efficiency of 93.3% for Cr (VI). When applied to real tannery chromium effluent, the removal efficiency was found to be 83.6%. The adsorption data fitted better to the pseudo-second order kinetic model (R^2 = 1). Thus, the interaction of the cationic species with the donkey dung powder was predominantly via chemisorption. Since no other chemicals were added to the donkey dung in the experimental make up, this approach can be regarded as a novel and green approach and can provide environmental protection and profitability to the tanner.

Keywords. Adsorption, *Equus africanus asinus* dung, chemisorption, Cr (VI), tannery effluents, wastewaters.

Appendix A1.1: Introduction

The processing of raw hides and skins of animals by tanning operations transforms them into durable material, preventing their decay and making them persistent and flexible (Beghetto *et al*., 2013). The transformation process involves a series of chemical and mechanical operations which generate substantial quantities of solid, liquid, and gaseous wastes (Pati *et al*., 2014). Chromium based tanning process is practised globally in leather industries due to versatility of chromium tanned leathers. This has resulted into large chromium containing wastes such as chromium sludge, chrome tanned leather shavings, chrome leather trimmings and chrome dusts (Pati *et al*., 2014). Kanagaraj *et al*. (2015) reported that from a medium sized tannery, over 300 million m^3 of waste liquor containing thousands of tons of chemicals and solid waste are released daily by the tanning industry. A mass of 1000 kg of raw skin/hide tanned with chrome yields 150 kg of leather. The remaining 850 kg contributes to the chromium solid wastes, out of which 450 kg is chromium collagen wastes and 400 kg are chromium fleshing wastes with 30 m^3 of chromium effluents (Kanagaraj *et al.,* 2010). Birhanie *et al*. (2017) added that tanneries generate chromium wastewaters in the range of 30 – 35 mgL^{-1} of skin or hide.

The generated wastewater requires adequate treatment and disposal in order to comply with the statutory requirements and regulations (Kanagaraj *et al*., 2015). This is because, these wastewaters can accumulate in the environment leading to sludge pollution problem as well as chocking of treatment pipes, which results in reduction of the efficiency of treatment plants. Disposal of these chromium wastes is also an economic burden to many tanners. Those tanneries which are unable to meet the set limits are sometimes forced to close down due to wastes disposal problems and environmental concern from the affected population and government agencies (Kanagaraj *et al*., 2015).

In sub-Saharan Africa (SSA), effluents containing chromium wastes are treated with lime to cause precipitation of sludge which is dried in beds. The dried cakes are later packed in sacks and dumped in open spaces inside these tanneries (Oruko *et al.,* 2014). This practise is normally observed in many tanneries in Africa. This is happening because most of the tanneries in the region have not found better ways or technology of utilising generated chromium wastes. Many countries in SSA, have restricted the disposal of leather industry chromium wastes to municipal landfills, illegal dumpsites and their incineration inside these tanneries. This is due to their increased generation and hazardous nature of the discharged chromium wastes from the tanning process (Manimita *et al*., 2015).

In the treatment plants, Cr (III) wastes which is the dominant contaminant mixes with other composite wastes and gets oxidised to Cr (VI) under favourable biotic and abiotic factors

(Bolan *et al., 2014*; Al- Battashi *et al*., 2016). Oxidation of Cr (III) to Cr (VI) tends to enhance its mobilisation and bioavailability (Ahameda and Mohammed, 2014). Cr (VI) easily diffuses away from the native site of contamination as soil pH increases resulting into leaching effects (Focardi *et al*., 2013). This makes Cr (VI) more bioavailable in the higher trophic level, especially food crops consumed by humans. Its exposure in humans induces several health disorders such as irritations, eczema, ulceration, nasal and skin irritations, perforation of eardrum, respiratory track disorders and lung carcinoma (Rahman and Rizwan, 2015; Focardi *et al*., 2013).

In SSA, the current methods of treating chromium wastewaters are costly due to their energy requirements, as well as their ineffectiveness in removing metal ions at low concentration (Birhanie *et al*., 2017). Some of the methods often lead to the generation of toxic sludge while others are unaffordable for large- scale treatment of wastewater (Kebede *et al*., 2018; Birhanie *et al*., 2017). The desired technique, therefore, need to be more ecologically-friendly, low-cost and effective. Adsorption technology has been used for the sequestration of metals from aqueous solution even at low concentration. Therefore, it is a potential technique with a novel adsorbent that can be used for the treatment of tannery effluents. It can also be implemented at any stage of a tannery technological advancement due to simplicity of design, easy operational conditions, and low harmful secondary products (Kebede *et al*., 2018).

Different adsorbent materials from plants and animal organic wastes have been tested for metal ions removal (Batool *et al*., 2019). The following materials have been reported; zeolites (Saltali *et al*., 2007), clays (Auta *et al*., 2012), nano-magnetic particles (Gupta *et al.,* 2009), plants wastes (Bhattacharya *et al.*, 2008), among others. But a number of them suffer from low adsorption capacities and selectivity due to poor porosity, low surface area, lack of functional groups and surface deactivation. Thus, the need to explore and apply adsorbent materials with large surface area to volume ratio, high porosity and high functionalities (Kebede *et al*., 2018; Batool *et al*., 2019). Raut *et al*. (2015) recommended that more studies should be carried out to increase the efficiency of chromium removal using adsorption and biosorption methods.

Equus africanus asinus dung which is available locally as a natural bio-adsorbent displayed attractive property which conforms to the requirements of a suitable adsorbent material towards removal of Cr ions. To our knowledge, no work has been reported thus far on the potential use of the dung of *Equus africanus asinus* species powder to remove Cr ions from tannery wastewater. Thus, the aim of this study was to characterise dung from *Equus africanus asinus* species and to evaluate its potential for the removal of Cr ions from tan liquors. Later on, propose the possibility of using it as a cheaper and greener yet effective

adsorbent to tanning industries for the removal of Cr ions from tannery effluents.

Appendix A 2.2: Materials and methods

Appendix A 2.2.1: Analytical reagents

All primary chemicals used were of analytical reagent grade. $K_2Cr_2O_7$, (99.0%), NaOH (99.4%), HCl (37%), H_2SO_4 (98%) and Diphenyl carbazide (98%). All were purchased from Merck (Johannesburg, South Africa).

Appendix A 2.2.2: Sampling and partial preparation of donkey dung

Dried donkey dung for adsorption study was sampled as explained in methodology chapter three, subsection 3.2.2 of this thesis. In the laboratory, dungs were pulverised into powder and dried in the sun using very clean containers and safeguarded from external contamination.

Appendix A 2.2.3: Tannery wastewater sampling and treatment

Tannery chromium wastewater effluent samples for real application test were collected as described in methodology chapter three, subsection 3.2.1 of this thesis. In the laboratory, all the sample solutions were mixed and homogenised using a mechanical orbit shaker for 3 min. The homogenised sample was vacuum filtered immediately through the Whatman No. 90 filter paper. The samples were then preserved at < 4°C for later use.

Appendix A 2.2.4: Samples preparatory instruments

An oven, model Defy DMO 350 (Zhengzhou China) was used for drying samples. An analytical balance from Mettler Toledo, Model AG 204 (Schwerzenbach, Switzerland) with accuracy of 0.0001 g was used for weighing. General purpose water bath shaker machine from thermo Scientific model Precision GP 15D (Waltham, MA USA) was used for temperature control.

Appendix A 2.2.5: Determination of physico-chemical parameters, total chromium and Cr (VI)

Total organic carbon and dissolved organic compounds like protein and organic acids were determined by modified methods reported by Peiris *et al*. (2011); Hansen *et al*. (2018). Kjeldahl- total nitrogen was determined following the modified APHA (1992) procedure. pH was determined with thermo scientific pH/ISE meter Orion star A214 multi-parameter probe (Johannesburg, South Africa). Total chromium analysis was performed by adopting the modified EPA (1996) method 3052 as their concentration were quantified with ICP-OES Model Agilent ICP-OES 700 series using expert II varian ICP expert software, (Waltham, MA USA). Lambda 650 Perkin-Elmer UV-visible spectrophotometer from (Perkin-Elmer, Inc.,

Waltham, MA, USA) was employed for the determination of residual concentrations of Cr (VI) in the sample by complexing with 1,5-diphenylcarbazide in acid medium.

Appendix A 2.2.6: Characterisation of raw and treated donkey dung using FEEM

Fluorescence excitation-emission matrix (FEEM) spectroscopy is widely used in dissolve natural organic material characterisation. The technique is capable of identifying both humic and protein-like dissolved organic material (Peiris *et al.,* 2010; Hansen *et al.,* 2018). In this study, the methods of Croft *et al*. (2012); Hansen *et al*. (2018) were modified as donkey dung weighing 10 g was prepared for analysis. One portion was dissolved in 50 mL of deionised water for control analysis. The other portion of the same quantity was dissolved in 50 mL of chromium solution for analysis of humic and protein-like dissolved organic material that reacted with chromium. The two solutions were kept overnight. The solutions were then filtered twice with pore size syringe (0.45 um). An aliquot of 5 mL was taken and diluted to 15 mL. The solutions were analysed in the wastewater mode (samples that are too concentrated that they mimick wastewater) with Fluorescence excitation emission matrix from Aqualog Horiba scientific model (Kyoto, Japan).

Total organic carbon in the same solutions were determined by TOC Torch model from Teledyne tekmak (Mason, OH 45040, USA) with a furnace lamp of 700^{o}C and 3.5 quartz clear cell and beam light set at 90^{o}. The beam light was set at that angle to help identify six protein-like dissolved organic materials of interest. This test was based on excitation wavelength and emission range. The protein-like dissolved organic materials were then quantified.

Appendix A 2.2.7: Fourier transform infrared (FT-IR) characterisation of raw and treated donkey dung

Two milligrams of milled dried raw donkey dung and dung treated with chromium were analysed after homogenisation following the modified method of Carballo *et al*. (2008). Infrared (FTIR) spectra were recorded by direct application of the powder using Bruker Fourier transform infrared spectrophotometer (Vertex 70 model) from Bruker optic GmbH (Hamburg, Germany). It was equipped with a diamond ATR fitting and was used for analysis of the samples for functional groups. The spectra were obtained in transmittance mode with 50 scans at a resolution of 2 cm^{-1} in the spectral region of 4000– 400 cm^{-1}. The data was processed using Opus 7.3.139.1294 software.

Appendix A 2.2.8: Scanning Electron Microscope (SEM) characterisation of raw and treated donkey dung

The surface morphology of the finely powdered samples of raw donkey dung and those contaminated with chromium were studied following the modified protocol of Li *et al*. (2018). The samples were coated with gold using Quorum Q15OR Es machine from Advance laboratory solutions. The samples with 3 mm thickness were ran for QT 5 nm gold. They were then scanned using JOEL scanning electron microscope (JSM-300 PLUS /LA, Rikagu, Japan) equipped with the oxford x-ray dispersive spectroscope (EDS) which was used to detect the distribution, pre-and post-sorption of elemental ratios in the samples.

Appendix A 2.2.9: Brunau-Emmett-Teller (BET) characterisation of raw donkey dung

The specific surface area, total pore volume and average pore size of nanofibers parameters of donkey dung treated and raw samples were obtained from nitrogen adsorption-desorption isotherm determined by micromeritics TriStar II 3020 instrument used for Brunau-Emmett-Teller (BET) analysis. The specific surface area was calculated according to Brunaue-Emmett-Teller (BET), most common Appendix equation A (2.1).

$$\text{BET} = \frac{1}{\frac{W((P_0}{P)-1)}} = \frac{1}{W_m C} + \frac{C-1}{W_m C}\left(\frac{P}{P_0}\right) \quad (2.1)$$

Where W= weight of gas adsorbed, P/P_0 = relative pressure, Wm = weight of adsorbate as monolayer and C = BET constant.

Appendix A 2.2.10: Thermogravimetric analysis (TGA) of raw donkey dung

Thermogravimetric analysis (TGA) of the powdered donkey dung (PDD) was conducted using a TA Instruments TGA Q500 thermogravimetric analyser (TA Instruments, New Castle, DE, USA). The heating procedure for the samples was 10°C per minute from 20 to 900°C with a flow rate of 100 mL/min of synthetic air (composition 21 ± 1% O_2 and 79 ± 1% N_2; purity > 99.9994%). The manometric pressure was maintained at 101 kP_a and the sample weight was approximately 10 mg. Each raw samples were analysed and mean values of the three replicates were estimated for each raw donkey dung (Carballo *et al*., 2008).

Appendix A 2.2.11: Preparation of stock, standard solutions, batch adsorption solution, quality assurance and control

A stock solution of chromium (VI) with a concentration of 20 mg/L was prepared by dissolving 1.767g of $K_2Cr_2O_7$ in 500 mL distilled water. From this stock, triplicates measuring 25 mL of the synthetic solution of Cr (VI) and their controls were put in 100 mL conical flasks and used for batch adsorption study. The working standard solution for calibration was prepared by

diluting to appropriate concentration of the stock solution with distilled water daily. The standards were run before, midway through and after each sample batch to calibrate the UV instrument. Calibration curve was constructed for Cr (VI) of known concentrations and obtained correlation coefficients. Depending on the sample analysis, the calibration curves with $r^2 > 0.999$ were set as default value for the calculation of the unknown concentrations through interpolation and extrapolation. Concentrations were reported as the averages of triple measurements for both samples and blanks. The closeness of initial and final mean concentrations of the blank samples (without donkey dung) run under the same conditions was used for quality assurance, control and reliability of the procedure. Donkey dung applied in this study was sampled and pre treated according to quality standards.

Appendix A 2.2.12: Preparation of donkey dung for adsorption of chromium effluent

In the laboratory, dried dung was milled into submicron particle sizes for 90 min. The milling machine from MCL model Retsch MM 200 (Haan, Germany) was used. This was done to increase surface area and adsorption sites. The homogenised samples were put into clean small plastic containers, sealed tightly and kept in clean dry place for future use in batch adsorption studies.

Appendix A 2.2.13: Batch adsorption study

Batch adsorption studies using donkey dung was carried out after modification of Li *et al*. (2018) procedure. It was done by weighing 25 –100 mg of dried and milled powdered donkey dung. They were put into a clean 100-mL conical flasks containing 25 mL of the synthetic Cr (VI) solution along with their blanks at the desired initial chromium concentration (20 ppm) and pH (2 to 9). The flask contents were shaken at 100 rpm for the required contact time (60 min) at 25^{o}C in a warm water bath shaker until equilibrium was reached. The solution of 0.10 M HCl and 0.10 M NaOH were used to prepare samples for pH (2 - 9) optimisation studies. The effect of concentration was evaluated at temperatures of 25 and 30^{o}C. The contents of the flask were filtered through filter paper, centrifuged and the supernatant was analysed for final chromium concentrations using UV Vis spectrophotometer.

The experiments were carried out in triplicates and with controls. The closeness of initial and final mean concentrations of the blank/control samples (without donkey dung) run under the same parameters was used as quality assurance control and reliability of the procedure. The mean values were taken for calculation. The percentage removal and adsorption capacity at equilibrium of synthetic chromium were calculated as in Appendix equation (2.2) and (2.3).

$$\% \text{ Removal} = \left(\frac{C_0 - C_f}{C_o}\right) x\ 100 \qquad (2.2)$$

$$q_e = (C_o \quad - C_e \ )x \ \frac{V}{W} \tag{2.3}$$

where C_o is the initial concentration (mg L^{-1}); C_f is the solution concentration at the end of the sorption process (mg L^{-1}); qe (mg g^{-1}) is adsorption capacity at equilibrium; Co and Ce (mg L^{-1}) are the liquid-phase concentrations of initial Cr ions at time zero and equilibrium, respectively; V is the volume (L) of the sample solution and W is the weight (g) of the dry sorbent.

Appendix A 2.2.14: Adsorption isotherms

In the current study the two most common Isotherm models (Langmuir and Freundlich) were applied. This was to investigate the adsorption capacity and the nature of surface of the donkey dung whether heterogenous or homogenous. The adsorption capacity of the donkey dung was first evaluated by fitting the obtained experimental results of optimised concentrations to the linear Langmuir isotherm, expressed by Appendix equation A (2.4) (Langmuir, 1918) and the linear form of Freundlich adsorption isotherm expressed by Appendix equation A (2.5) (Freundlich, 1907).

$$\frac{C_e}{q_e} = \frac{1}{q_{max*b}} + \frac{C_e}{q_{max}} \tag{2.4}$$

Where C_e (mol/L) is the equilibrium concentration of adsorbate, q_e (mg/g) is the amount of adsorbates adsorbed at the equilibrium concentration, q_{max} (mg/g) represents the maximum monolayer adsorption capacity and *b* (L/mol) is the Langmuir constant related to energy of adsorption and the affinity of the binding sites.

$$log(q_e) = logK_f + \frac{1}{n} log \ (C_e) \tag{2.5}$$

Where Ce is liquid-phase equilibrium concentration (mg/ L), qe is solid phase equilibrium concentration (mg/g), K_f and n are the Freundlich constants. The value of n gives an indication of the favourability with which the adsorption process takes place. The Freundlich isotherm constant K_f (mg/g or L/mg) is the adsorption capacity of the adsorbent and represents the quantity of Cr (VI) adsorbed onto the surface of ground donkey dung per unit of equilibrium concentration (Zhang *et al.*, 2010).

Appendix A 2.2.15: Kinetic modelling

The kinetic parameters for the adsorption process were studied on the batch adsorption of 20 ppm of Cr (VI) at 25°C at pH 2 to 9. The contact time was varied between 10 to 180 min and the percentage removal of Cr (VI) was monitored. The data were fitted to the Lagergren (1898) equation for the pseudo-first order kinetic model presented in Appendix Equation A (2.5) and pseudo-second-order kinetic model in Appendix Equation A (2.6)

$$log\ qe - qt\ = log\ qe - \frac{K_1 t}{2.303} \quad (2.5)$$

$$\frac{t}{q_t} = \frac{1}{k_2 \quad qe^2} + \frac{1}{q_e} t \quad (2.6)$$

Where q_e and q_t (mg g^{-1}) are the amount of Cr ions adsorbed per unit mass of the adsorbent at equilibrium and time t, respectively, and K_1 is the first order adsorption rate constant and K_2 is the second order kinetic model rate constant (mg g^{-1} min^{-1}).

Appendix A 2.2.16: Pre-treated chromium effluent and application of donkey dung for its adsorption

Effluent samples were collected from Dogbone (Kenya) and Beit Ore (South Africa) tanneries with pH of 3.63 and 3.10, respectively. An amount of 30 mL of effluent was measured in triplicate and added into 50 mL conical flasks. Then 100 mg of donkey dung powder as adsorbent was added to the solution in each flask while their blank samples (without donkey dung) in the same volume and concentration was also run as control. The flasks were then shaken in a thermostatic water bath shaker (Appendix figure A 1.0) at the speed of 100 rpm at temperature of 25°C for 60 min. The solutions were then filtered for analysis. The closeness of initial and final mean concentrations of the blanks samples (without donkey dung) ran under the same parameters was used as quality assurance, control and reliability of the procedure.

Appendix Figure A 1.0: Thermostatic water bath stirrer used for batch adsorption studies

Appendix A 2.2.17: Data analyses

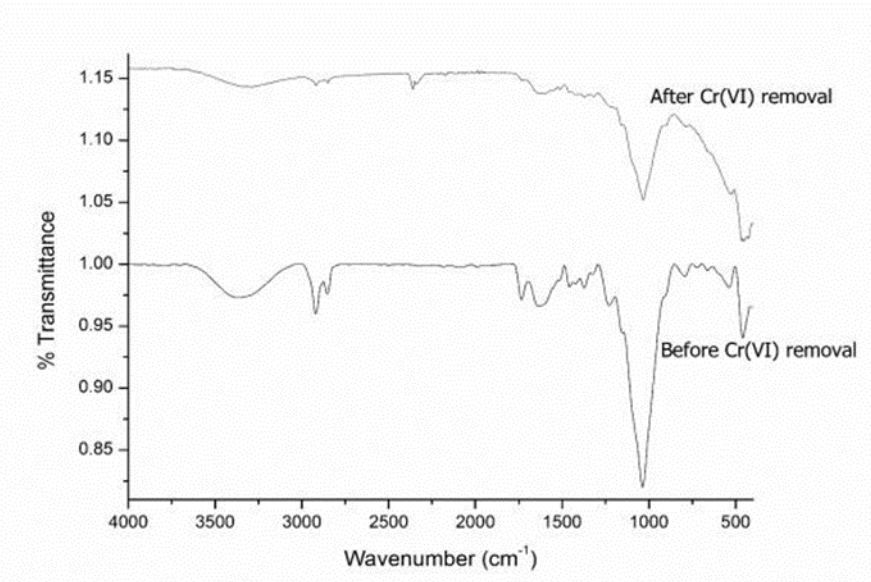
Averages of triplicate analysis were applied to plot adsorption equilibrium and kinetics models as linearised regression fittings were used for empirical and kinetic model equations. Slope and intercept values were used to calculate different models parameters.

Appendix A .3.0: Results and Discussion

Appendix A 3.1: FT-IR of donkey dung powder

FT-IR measurements were carried out to identify the functional groups of raw donkey dung and to understand the reaction mechanism/changes with chromium ions. The functional groups of raw donkey dung before and after adsorption of Cr (VI) are shown in Appendix Figure A 3.1. It is characterised by shifting in the position of bonds, disappearing of peaks and appearing of new peaks. This indicated that reaction may have taken place between the functional groups in donkey dung and Cr (VI) as depicted in the same Figure. A broad shallow wide band was observed around 3500 cm^{-1} in donkey dung mixed with Cr (VI) and may be ascribed to O-H stretching vibrations of H bonded to OH groups while bonded N-H/C-H/O-H stretching amine and amide occurred at 3308.18 cm^{-1} (Kebede *et al*., 2018; Bhusari *et al*., 2016; Zakaria *et al.,* 2009; Batool *et al.,* 2019). The presence of O-H functional group appears predominantly due to the presence of protein and fatty acid in structures of donkey dung. The presence of these proteins in the dung, may have contributed to the NH stretching of amide groups (Kebede *et al*., 2018; Zakaria *et al.,* 2009; Barot and Bagla, 2012; Sivakumar and Amutha, 2012).

Band in the region of 2918 – 2851 cm^{-1} could correspond to asymmetric deformation of methylene and methyl ester groups indicating the presence of aliphatic chains or C-H stretching and O-H from carboxylic acid present in fatty acids as also explained by Sivakumar and Amutha, (2012); Barot and Bagla (2012) and Zakaria *et al.* (2009). The 1638 -1461 cm^{-1} bands may correspond to the asymmetric deformation of carboxylate ions, ketones, N-H bending or C=O amide due to presence of fatty acid and protein. The peak at 1319-1158 cm^{-1} could correspond to symmetric axial deformation of carboxylate ions COO^- or CH_3 bending (Bhusari *et al*., 2016; Batool *et al*., 2019). According to Bhusari *et al*. (2016), peak bands observed in the region 1638 -1033 cm^{-1} may have acted as the sites for metal bonding. Therefore, bonding sites for chromium ions with donkey dung could be associated with those regions. The protonation of groups such as COO^- to COOH and NH_2 to NH^+ may have promoted their interaction with the negatively charged Cr (VI) ions (Zakaria *et al.,* 2009; Batool *et al.*, 2019). Thus, Cr (VI) was likely to be complexed by the organic functional groups that were implicated in its reduction (Bartlett, 1991).



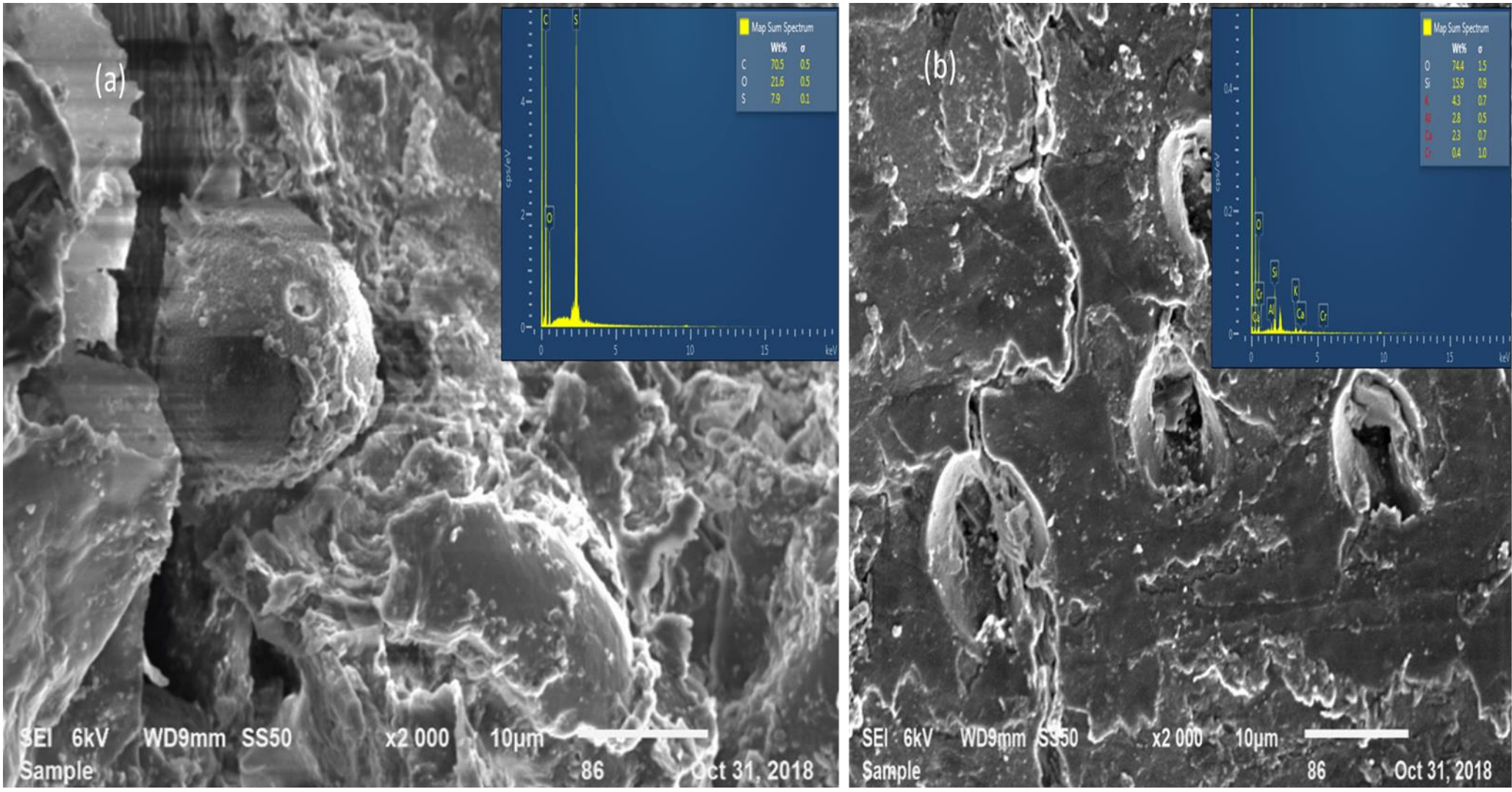
Appendix figure A 3.1: FT-IR spectra for donkey dung without and with Cr (VI)

Appendix A 3.2: Surface morphology and elemental composition of donkey dung powder

The surface morphology of donkey dung powder was studied to understand the surface characteristic and chemical composition of raw dung and its reaction mechanism with Cr (VI) ions. It was done using scanning electron microscopy (SEM) and X-ray energy dispersive spectroscope (EDS). The surface morphological changes observed are shown in Appendix Figure A 3.2. Appendix Figure A 3.2 (a) shows the SEM images of the dung powder without Cr (VI) and with Cr (VI) after adsorption. The surface morphology of the dung powder confirm the heterogeneous and porous nature of the material under study. Some sections of the material look like irregular and rough surfaces with some short rod and angular shaped objects attached to each other. The appearance of open porous holes with flaps were observed throughout the surface of the image. Some of the observed pores appeared like cross-linked chains.

The identified structures could be the sites that may have facilitated the processes of chromium ions adsorption. Appendix Figure A 3.2(b) on morphology of dung with Cr (VI) showed changes in the appearance, with opening porous holes looking like folded large plant leaves. Based on these observed characteristics, it could be concluded that this material might have an adequate morphological profile for retaining and complexing with chromium ions as observed by Batool *et al*. (2019), while studying the poultry manure-derived biochar for adsorption of Cr (III) ions. Therefore, the role of donkey dung particle sizes, realignment and porosity in relation to reaction with Cr (VI) could be attributed as the possible crucial routes for adsorption.

The EDS elemental analysis found that the most abundant element in raw donkey dung was carbon, followed by oxygen and sulphur indicating that donkey dung is more of a carbonaceous material. In reaction with Cr (VI) it was observed that there was an addition of Cr along with inorganic micro and macro nutrients elements like K, Si, Al and Ca.

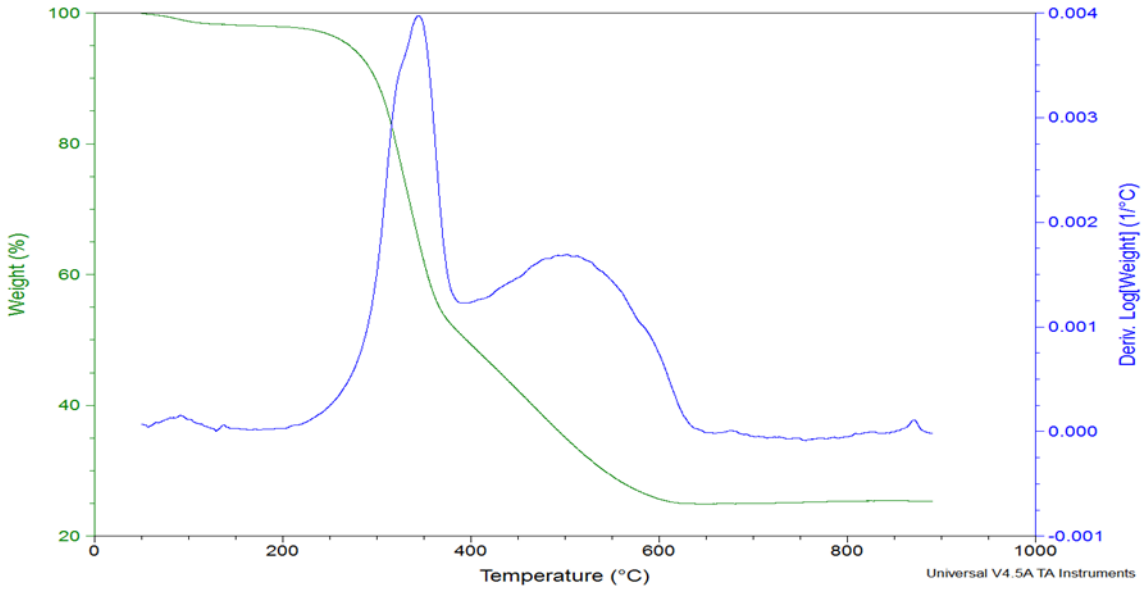


Appendix Figure A 3.2: Scanning electron micrographs (SEM) morphology of dung (a) without Cr (VI) and (b) with Cr (VI) (The respective EDS are inserted)

Appendix A 3.3: Thermal properties of donkey dung powder

The thermal stability of the donkey dung was studied in order to determine the decomposition temperature of the dung constituents using thermogravimetric analysis and differential scan calorimetry (TGA-DSC). As shown in Appendix Figure A 3.3, the small weight loses observed at temperatures below 100^{o}C (as depicted by green line in the graph) was attributed to the loss of bound water and confirmed by the small exothermic peak in the DSC plot (blue line in the graph). At temperature of 288^{o}C, (5%) of weight loss was observed, which could be attributed to the loss of low molecular gases, such as CO_2 and NH_3. The weight loss of 37.5% was observed at a temperature of 348°C, which is ascribed to the decomposition of aromatic groups of humic acid (HA). The aromatic structures can be both, present in the original composition of the HA or can be generated by cyclisation of aliphatic chains, upon heating. This is also confirmed by strong exothermic peak at the DSC curve appearing at 348^{o}C.

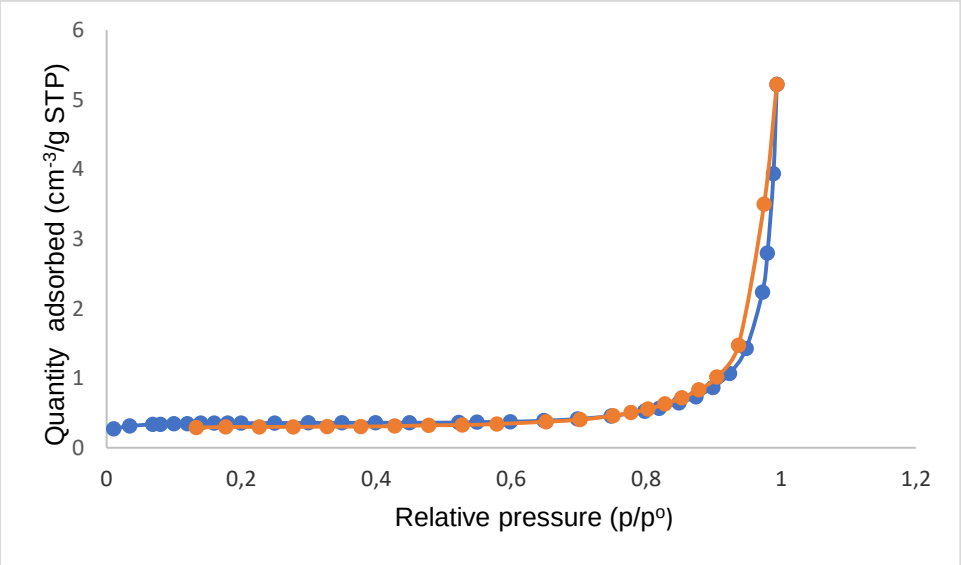
The 75% mass loss after 600°C, is associated to the permanent decomposition of carbon residues (organic matters) in the donkey dung, which is also confirmed by the broad exothermic peak at the DSC curve appearing at the temperature of 550°C. The remaining 25% could be assigned to inorganic materials which could not be degraded. The findings in this study differ with the one of Idrees *et al*. (2018) which had a mass loss of 85% at temperature of 650 - 750°C. They used cattle derived manure biochar and guinea fowl manure-derived biochar respectively. But in this study we used dry raw donkey dung which were only milled into nano particles.



Appendix Figure A 3.3: TGA and DSC degradation of *Equus africanus asinus* dung powder. Greenline is weight loses observed against temperature. Blue line is DSC plot

Appendix A 3.4: Brunauer-Emmett-Teller analysis

The N_2 adsorption-desorption isotherm of powdered donkey dung is shown in Appendix Figure A 3.4. The powdered donkey dung showed a reversible Type II isotherm which is given by the physio- sorption of most of the adsorbates on macroporous surfaces of the adsorbents. The surface area, the Barrett Joyner and Halenda (BJH) cumulative volume of pores and BJH average pore diameter were determined as 2.4 ± 0.62 m^2/g^{-1}, 0.078 cm^3/g^{-1} and 226.81Å, respectively. The surface area obtained is very understandable for the donkey dung powder, due to the compact nature of the material. However, the presence of active functional groups with the surface area mentioned above, makes the donkey dung powder effective for the targeted application.



Appendix Figure A 3.4: BET curve for the *Equus africanus asinus* dung powder. Blue dotted lines and red dotted lines represent adsorption and desorption respectively

Appendix A 3.5: Total nitrogen, total Organic Carbon and pH analysis in donkey dung solution

The study recorded that raw donkey dung contains organic matter with high percentage of total organic carbon (TOC) and total nitrogen levels as shown in Appendix Table A 1.0

Appendix Table A 1.0: Physico-chemical parameters of donkey dung

Parameter	Value
Total nitrogen (%)	6.40
pH of dung without Cr (VI)	6.90
pH of dung with Cr (VI)	6.30
TOC of dung without Cr (VI)(%)	19.9
TOC of dung with Cr (VI) (%)	104

The pH of donkey dung in this study compares well with those of horse (6.8) and elephant (6.9) dung in the study reported by Abdulyekeen *et al*. (2016). The pH falls within the range of 6.0- 7.0 and can favour electron donating capacity or electron accepting capacity for the dung. This is natural as different species of animals cannot necessarily have the same pH in their dung. The variation is attributed to the digestion system and occurrence of microorganisms in the ruminant of these animals with potential for biohydrogen production from lignocellulosic hydrolysate and hemicellulosic fraction of lignocellulose which controls pH (Fangkum and Reungsang, 2011). The total nitrogen content in donkey dung was higher (6.4%) than elephant dung (1.96%) and horse dung (0.98%). This could be due to feeds consumed.

Different feeds do not have the same elemental compositions (C, O and N) while they are determinant factors in the concentration of those elements in the dung. Gomgnimbou *et al*. (2016) reported that donkey digestion system may have a slow speed of mineralisation of organic nitrogen and biohydrogenisation of lignocellulosic hydrolysate and hemicellulosic fraction. This differs from the findings of this study. Here it may be suggested that donkey dung mineralisation during digestion process contributed to high total nitrogen content. Nitrogen in the undigested protein is excreted in the solid dung by the animal. In the digested proteins, it is absorbed and later excreted in the urine except for the portion that is used to build flesh in the animal (Adesanya *et al.,* 2016).

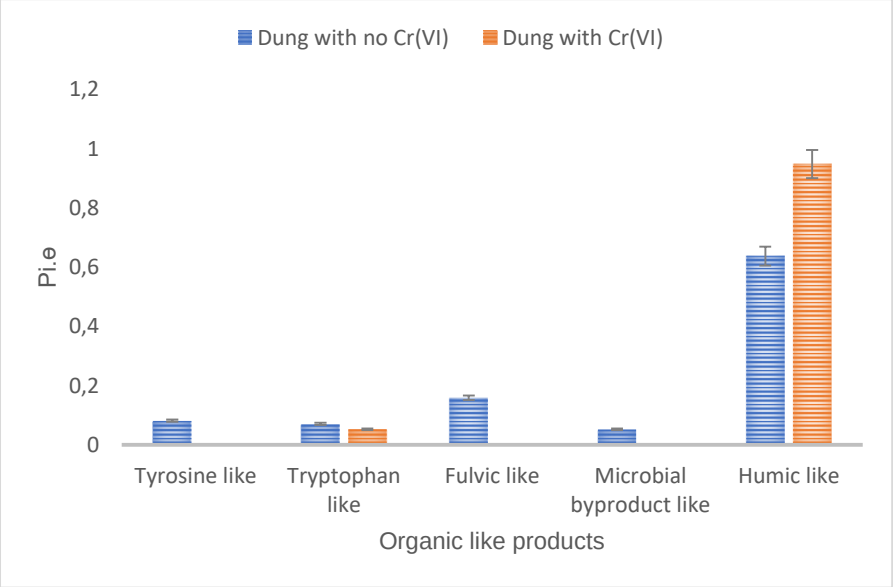
The total organic carbon (TOC) of the donkey dung samples gave different values as shown in Appendix Table A 1.0. The lowest TOC (19.9%) was that of raw donkey dung without Cr (VI) solution. Donkey dung mixed with Cr (VI) suddenly showed high value of TOC (104%). This is above normal percentage and thus required further investigation. The TOC reported by Abdulyekeen *et al*. (2016) were lower (4.79 %) for elephant dung and (10.51%) for horse dung when compared to raw donkey dung (19.9%) in this study. Wani *et al*. (2013) also reported low TOC on cow dung (18.4%). This variation is normal because of species morphological diversity. Donkeys do not complete their digestion in rumen like other animals (do not chew the cud). This may be the source of incomplete digestion of the feed. However, the abrupt increment of TOC in dung with Cr (VI) was abnormal. It necessitated further investigation and analysis by fluorescence excitation emission matrix technique to identify the cause.

Appendix A 3.6: Fluorescence excitation emission matrix (FEEM) analysis

This study revealed that organic like products such as tyrosine like, fulvic acid like and microbial like products in donkey dung reacted appreciably with Cr (VI) solution, resulting into

soluble compounds that suppress their detection (Appendix Figure A 3.5). Tryptophan like and humic substances were the only substances that retained fluorescence intensity light detection after the addition of Cr (VI). However, Tryptophan like quantity was lower than humic like substances indicating that less tryptophan-like aromatics were released from the donkey dung (Fan *et al*., 2020).

Humic substances (HS) are heterogeneous, redox-active organic macromolecules (Aeschbacher *et al.*, 2012). HS contain a wide variety of moieties that are oxidised at different potentials and that, upon oxidation, release protons and undergo irreversible follow-up reactions. Phenolic moieties have been suggested as major electron donating groups in HS. The oxidative transformation of HS in the environment can result in depletion of electron donating phenolic moieties with antioxidant properties relative to the electron accepting quinone moieties (Xu *et al*., 2019; Aeschbacher *et al.*, 2012). Therefore, donkey dung as a natural organic material may be containing quinone moieties which may have reacted with Cr (VI) resulting to high amount of humic acid protons. This was easily detected while analysing for total organic carbon (TOC) in the raw dung and dung mixed with Cr (VI) as the difference was very wide. Humic acid is also insoluble in acidic solution (Peiris *et al*., 2011). This suggests the source of higher concentrations of humic acid as depicted in Appendix Figure A 3.5.



Appendix Figure A 3.5: Graphical representation of reaction between Cr (VI) and donkey dung analysed with fluorescence excitation emission matrix (n = 3, RSD)

Appendix A 3.7: Physico-chemical parameters of tannery effluent

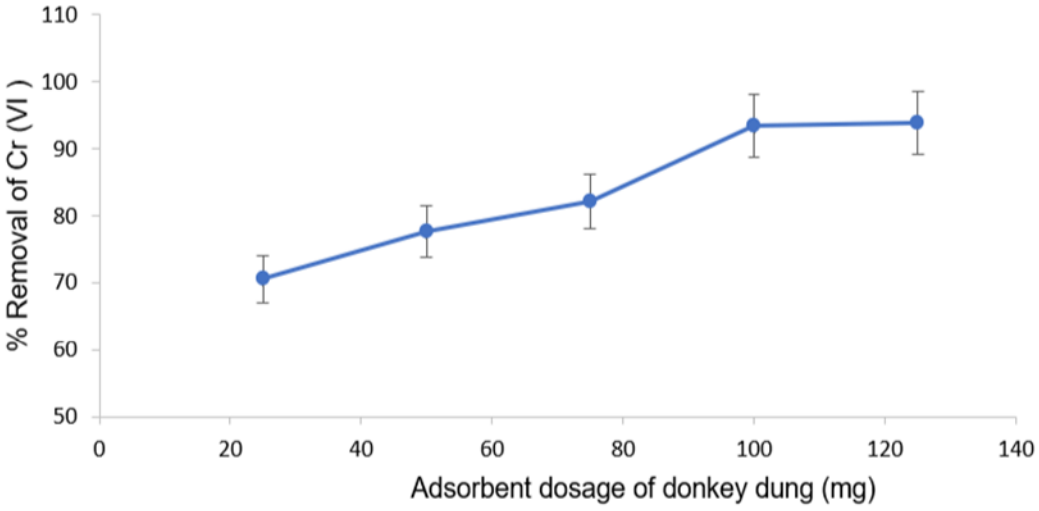
The tannery effluent from Dogbone and Beit Ore were characterised as shown in Appendix Table A 2.0. The pH of effluent was in the acidic range due to residual acid added to chromium tanning process at the pickling and tanning stages. TDS, salinity, EC and initial chromium concentration were high in both tanneries' effluents. The initial total chromium concentrations of tannery effluents from Dogbone and Beit Ore were 390 and 460 mg/L respectively, as measured using ICP-OES. This suggested that untreated effluent solutions from tanneries are high in chromium and other chemicals. Thus, the need to develop a low-cost and environmentally friendly technique to treat the chromium wastewater before discharge into the environment.

Appendix Table A 2.0: The characterisation results of tannery effluent

Parameters	Dogbone	Beit ore
pH	3.63	3.10
Total dissolved solid (mg L^{-1})	22715	32310
Salinity (ppt)	31617	33217
Electrical-conductivity (mS/cm)	51.1	55.3
Initial chromium concentration (mg L^{-1})	390	460

Appendix A 3.8: Effect of adsorbent dose on the adsorption of Cr (VI)

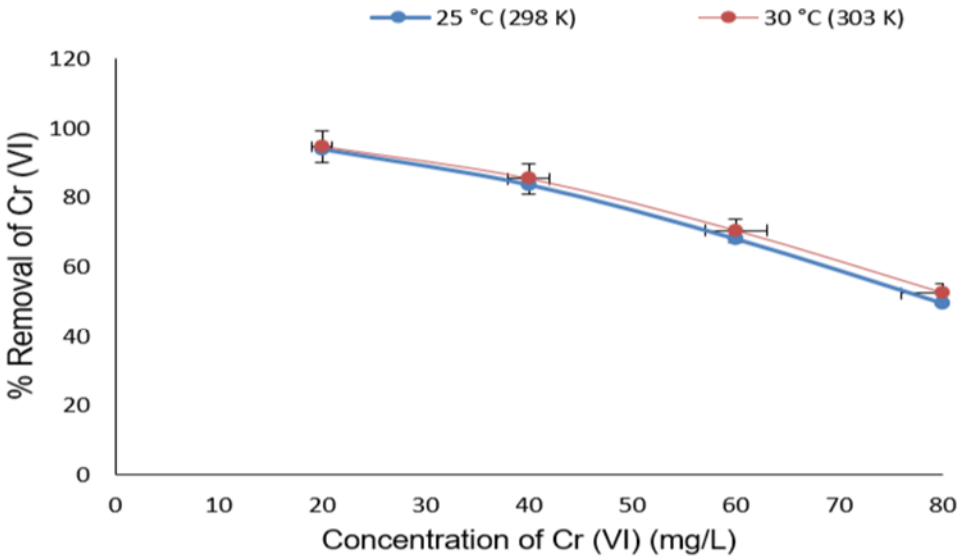
Adsorbent dose is basically the core parameter that actually depicts the extent of the removal of adsorbate and may also be used to predict the cost of the material required for treatment. The study of the effect of adsorbent dose on the removal of Cr (VI) was investigated using 25–120 mg of the donkey dung powder while other experimental parameters were kept constant. Appendix Figure A 3.6 shows that an increase in the adsorbent dose from initial dose resulted in an improved percentage removal of Cr (VI). The maximum percentage removal of Cr (VI) (93.32%) was determined at high adsorbent dose of 100 mg. Further increase in the adsorbent dosage did not bring any significant change. Therefore, 100 mg of adsorbent was selected as the optimum adsorbent dosage



Appendix Figure A 3.6: Effect of adsorbent dosage on percentage removal of Cr (VI) by donkey dung. Experimental conditions were; concentration 20 mg L^{-1}, agitation speed 100 rpm, pH 2, contact time 60 min (n = 3, RSD)

Appendix A 3.9: Effect of concentration on the adsorption of Cr (VI)

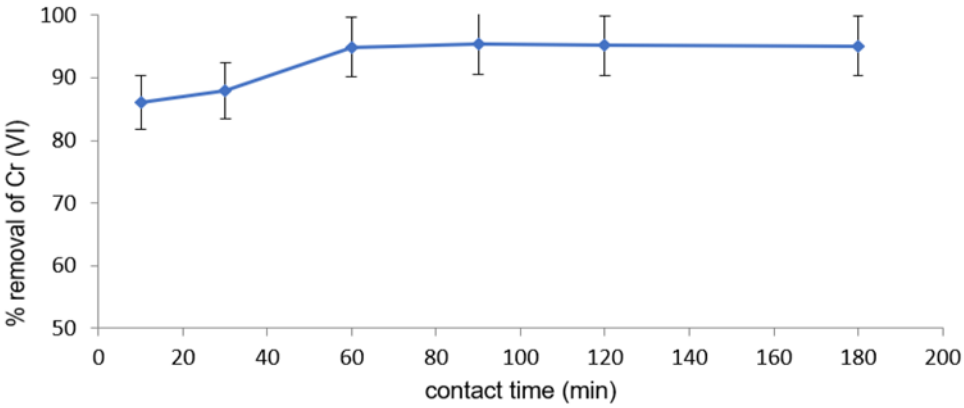
The effect of initial concentration was evaluated using the powdered donkey dung at temperatures of 25 and 30°C. The percentage removal was investigated using different concentration levels (20 – 80 mg/L) of the Cr (VI) solutions. The amount of Cr (VI) adsorbed decreased as the corresponding concentration increased from 20 to 80 mg/L as shown in Appendix Figure A 3.7. This could be due to the decrease in available active sites. From the results, it was observed that at higher temperature (30°C), there was a slight increase in removal efficiency of the dung when compared to sorption process at 25°C. This could be due to kinetic energy, which facilitated the surface diffusion of Cr (VI) to the adsorbent surface at higher temperature. The maximum percentage removal was observed at concentration of 20 mg/L for both temperature of 25°C and 30°C. However, higher percentage removal (94.6%) was observed at 30°C. From the results, the maximum adsorption occurred at a temperature of 30°C. However, the removal efficiency of the Cr (VI) by powdered donkey dung at 25°C was also satisfactory and comparable (93.9%) to that of 30°C (94.6%). Moreover, working at high temperature may cause high design and operational cost, therefore, 25°C was utilised for further optimisation.



Appendix Figure A 3.7: Effect of concentration on removal of Cr (VI) using donkey dung. Experimental conditions were; pH of 2, agitation speed 100 rpm, dosage 100 mg, contact time 90 min (n = 3, RSD)

Appendix A 3.10: Effect of contact time on the adsorption of Cr (VI)

The contact time studies are very important as they provide the minimum time required to remove maximum amount of metal ions from solution and thus help in scaling up the process. The effect of contact time of Cr (VI) on the surface of donkey dung was investigated between 10 min and 180 min. Appendix Figure A 3.8 shows that increase in contact time was accompanied by an increase in the adsorption efficiency until 90 min when the optimum condition was established. This phenomenon could be related to the instant utilisation of the most readily available adsorbing sites on the adsorbent surface (Edokpayi *et al*., 2019). After equilibrium there was no significant increase in adsorption due to filled adsorption sites. The maximum removal efficiencies observed at 90 min was 95.4% of Cr (VI).

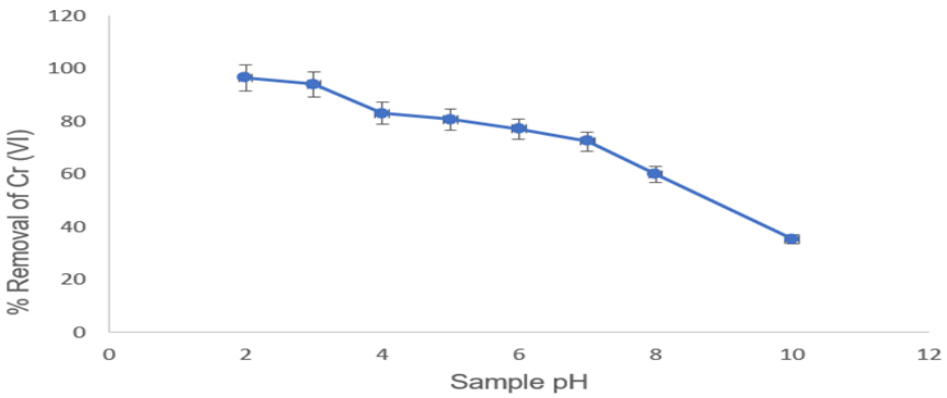


Appendix Figure A 3.8: Effect of contact time on removal of Cr (VI) by donkey dung. Experimental conditions were; concentration 20 mg L^{-1}, agitation speed 100 rpm, pH 2, dosage 100 mg (n = 3, RSD)

Appendix A 3.11: Effect of pH on the adsorption of Cr (VI)

The donkey dung application resulted in decrease in the Cr (VI) removal as the pH increases. It can be concluded that the removal of Cr (VI) was most effective in acidic condition. Xu *et al.* (2019) and Batool *et al*. (2019) elaborated that pH influences the surface charge of the adsorbent, the degree of ionisation of the material present in the solution, the dissociation of functional groups on the active sites of the adsorbent and the solution chemistry of Cr ions. At pH 2, the predominant species/forms of Cr (VI) ions are acidic (>90%) (Sitko *et al.,* 2013). Dakiky *et al.* (2002) found out that the percentage removal of Cr reached a maximum value at pH 2. At pH 2 the dominant form of Cr (VI) is $HCrO^{4-}$ but raising the pH shifted the concentration of $HCrO^{4-}$ to other species of Cr (VI) such as CrO^{2-} and $Cr_2O_7^{2-}$. However, the form of Cr (VI) that may have been adsorbed by donkey dung is $HCrO^{4-}$.

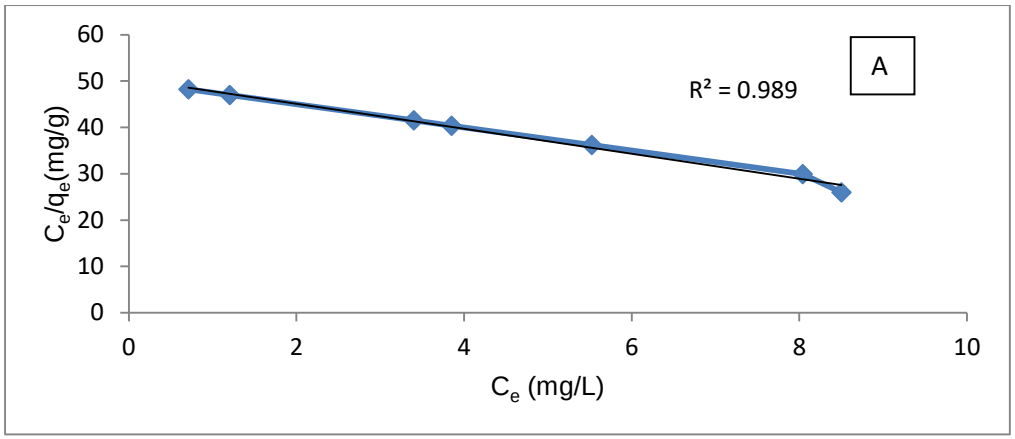
Dakiky *et al.* (2002) explained further that increase in pH with contact time from beginning to the end becomes well described by hydrolysis of adsorbent in water, which creates positively charged sites. But upon adsorption of $HCrO^{4-}$, a net production of hydroxide ions occurs. Such changes in pH are very small at low pH because the solutions are well buffered by the acids used in this pH range. Namasivayam and Yamuna (1995) had earlier confirmed this reaction by conducting similar experiments on Cr (III) under the same conditions. However, no removal of Cr (III) by any of the adsorbents was observed, due to repulsion of the positive Cr (III) ions by the positively charged active centres on the adsorbents at pH (2). Cr (VI) adsorption is highly reduced at pH> 5 due to the presence of excessive hydroxyl ions that competes with the anionic species of the designated Cr (VI) sorption sites. Thus, the process is showing selectivity for the removal of Cr (VI) from any matrix under low pH. In this study, the maximum percentage removal of 96.5% for Cr (VI) was observed at pH 2 as shown in Appendix Figure A 3.9.



Appendix Figure A 3.9: Effect of pH on removal of Cr (VI) by donkey dung. Experimental conditions were; concentration 20 mg/L^{-1}, agitation speed 100 rpm, dosage 100 mg, contact time 90 min (n = 3, RSD)

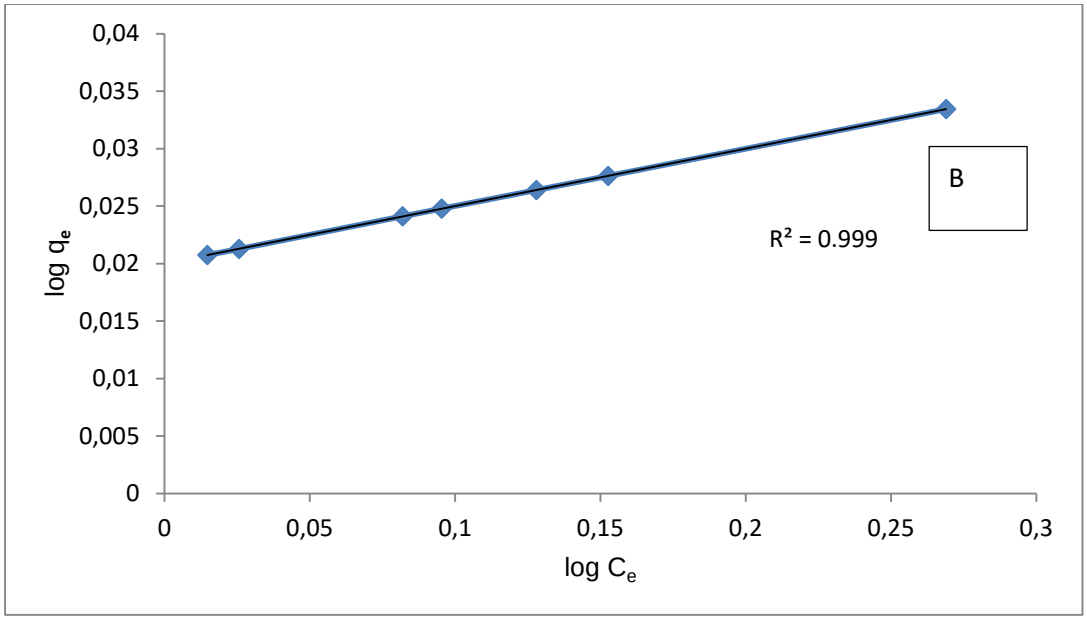
Appendix A 3.12: Adsorption Isotherms

It is important to establish the most appropriate correlation of equilibrium curves to optimise the conditions for designing an adsorption system. The Langmuir model assumes monolayer adsorption on a surface which contains a finite number of active sites in which all the active sites are identical and energetically equivalent. In the current study, the interaction between the donkey dung and Cr ions was first evaluated by fitting the obtained adsorption results to the linear Langmuir equation which is valid for monolayer surface as expressed in Appendix Eq.2.3. The plot of Ce/qe vs Ce gave a liner relationship (Appendix Fig. 3.10A). The values of q_{max}, b and R^2 are presented in Appendix Table A 3.0.



Appendix Figure A 3.10 A: Langmuir isotherms for the removal of Cr (VI)

The Freundlich isotherm model deals with interaction between adsorbate and adsorbent in multilayer heterogeneous surfaces (Acar and Malkoc, 2004; Crini and Badot, 2008; Freundlich, 1907). The plot of log (q_e) vs. log (C_e) gave a linear curve (Appendix Figure 3.10B). The values of K_f, n and R^2 are presented in Appendix Table A 3.0. A value of 1/n close to 1 represents a linear relationship, while 1/n < 1 represents a non-linear relationship. A value for 1/n below 1 indicates a normal Freundlich isotherm, while 1/n above one is indicative of cooperative adsorption (Fytianos *et al.*, 2000). Higher 1/n values indicate that the system approaches irreversible adsorption, especially when the value of 1/n > 10 (Fytianos *et al.*, 2000).



Appendix Figure A 3.10 B: *Freundlich* isotherm for the removal of Cr (VI) using the donkey dung

Appendix Table A 3.0: Langmuir and Freundlich model parameters for adsorption of Cr ions by donkey dung powder

Metal ions	Langmuir			Freundlich		
Chromium	Qo (mg/g)	b (L/mg)	R^2	Kf (L/mg)	1/n	R^2
20	2.29	0.989	10.26	1.9	0.999	

The values of *n* lie between 1 and 10, this indicates a favourable adsorption of Cr (VI) on the surface of milled donkey dung. The linear plots of the Langmuir and Freundlich isotherm models indicate that the adsorption of Cr (VI) on the donkey dung surface can be described by both models. However, the Freundlich isotherm model provides a better fit for the adsorption based on the linearised coefficient. Therefore, the adsorption of Cr (VI) on the heterogenous surface of donkey dung would be the predominant adsorption process. The maximum adsorption capacity obtained through the Langmuir model is 20 mg/g.

Appendix A 3.13: Adsorption kinetics

Adsorption kinetics is an important paramater for the study of reaction rate phenomenon, since they explain how fast the chemical reaction occurs and provide information on the factors affecting the reaction rate. In order to investigate the mechanism of adsorption and potential rate-controlling steps such as mass transport and chemical reaction processes, kinetic models are commonly used to test experimental data (Barot and Bagla, 2012; Bhattacharya *et al.,* 2008). When the linear form of pseudo first order, rate of adsorption was applied the numerical

values of q_e and K_1 calculated was found to be 0.0023 mg g^{-1} and 0.0021 min^{-1}, respectively, as shown in Appendix Table A 4.0. The q_e value were not very close to the experimental values of 0.0477 mg g^{-1}. When the linear form of pseudo second order, rate of adsorption mechanism was followed, the calculated value of q_e (0.0481 mg g^{-1}) was found to be closer to the experimental value of q_e (0.0477 mg g^{-1}). This showed a better fit than pseudo-first order. Moreover, the correlation coefficient (R^2) of pseudo-second order is 0.999, while pseudo- first order is 0.675. Therefore, the adsorption of Cr ion on the donkey dung was found to follow pseudo-second order kinetic model which depends on the assumption that chemisorption or chemical adsorption could be the rate-determining step.

Appendix Table A 4.0: Pseudo first and pseudo second order kinetic model parameters for adsorption of metal ions

		Pseudo first order	
q_e (mg g^{-1}) exp	q_e (mg g^{-1}) cal	K_1 (min^{-1})	R^2
0.0477	0.0023	0.0021	0.675
		Pseudo second order	
q_e (mg g^{-1}) exp	q_e (mg g^{-1}) cal	K_2 (min^{-1})	R^2
0.0477	0.0481	0.034	0.999

Appendix A 3.14: Comparison of Cr (VI) removal with different adsorbents

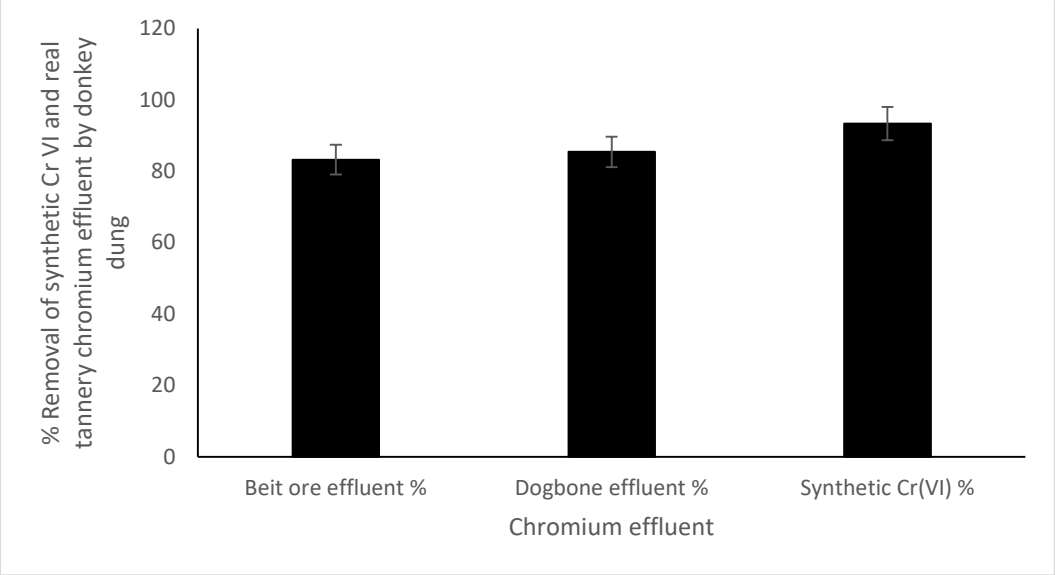
The adsorption capacities of the adsorbents for the removal of Cr (VI) have been compared with those of other adsorbents reported in literature (Appendix Table A 5.0). The raw donkey dung adsorption capacity was higher in comparison to raw cow dung in this study, but cow dung ash, showed varied adsorption capacity to raw donkey dung. However, the adsorption capacity of both manure-derived bio chars were higher than the donkey dung. The poultry and farm manure adsorbent were first converted into biochar before use while the dry donkey dung in this study was only milled into smaller fractions. Bhattacharya *et al.* (2008) stated that the adsorption capacity varies and it depends on the characteristics of the individual adsorbent, the extent of surface/surface modification and the initial concentration of the adsorbate. However, the present experiments were conducted to find out the simplest technical applicability of the low-cost adsorbents to treat Cr (VI) in tannery. Thus, the dry raw donkey dung showed superior performance compared to dry raw cow dung powder from other studies. No heat was required to convert it into another form before use. Unlike biochar which was used to remove Cr (III), raw donkey dung removed Cr (VI).

Appendix Table A 5.0: Adsorption capacities, Qo and pH of different adsorbents for Cr (VI) removal

Adsorbent	Metal ion	Qo (mgg)	pH	Reference
Dry cow dung powder	Cr (VI)	10.20	1	Barot and Bagla, 2012
Cow dung ash	Cr (VI)	4.21	1	Singh and Rattan, 2014
Bio Char-Farm Manure	Cr(III)	37.75	2.0	Batool *et al*., 2019
Bio Char-Poultry Manure	Cr(III)	34.00	2.0	Batool *et al*., 2019
Cow dung ash	Cr (VI)	29.10	1.0	Mullai *et al*., 2014
Cow dung slurry	Cr (VI)	7.80	2.5.	Namasivayam and Yamuna,1999
Activated cow dung carbon	Cr (VI)	10.10	3.5	Das *et al*.,2000
Dry donkey dung powder	Cr (VI)	20.00	2.0	Present work

Appendix A 3.15: Application to real samples

The chromium wastewater from the two tanneries were analysed. The adsorption of Cr ions in tannery effluent (from Dogbone and Beit Ore) samples was carried out with the method optimised and developed using the synthetic Cr (VI) solution prepared in the laboratory. The result showed that the donkey dung powder was effective in the removal of Cr ions from chromium wastewater effluents. The maximum Cr ions removal was obtained after analysing the samples in triplicates for reproducibility and quality assurance. The percentage removal efficiencies of the Cr ions from real chromium wastewater samples were slightly lower than those obtained for the synthetic Cr (VI) solution under the same experimental conditions as shown in Appendix Figure A 3.11. The difference detected could be attributed to the presence of different competing ions in the chromium effluent samples such as Al, Ca and Na ions originating from tanning solutions and the concentration of Cr (VI) ions in the effluent. Thus, donkey dung powder is an effective adsorbing material for the removal of chromium metal and can be applied for bioremediation of tannery effluents.



Appendix Figure A 3.11: Percentage (%) removal of chromium concentration from real tannery effluent and synthetic Cr (VI) (n=3; RSD)

Appendix A 3.4: Conclusion

The objective of this Appendix A 1.0 chapter was to preliminarily characterise and test the application of a novel green adsorbent prepared from *Equus africanus asinus* dung for the supplement removal of hexavalent chromium from tannery effluents.The characteristic properties of *Equus africanus asinus* dung powder including functional groups, surface morphology, thermal stability, surface area and organic like products of the donkey dung powder/solution were determined using techniques described in this Appendix chapter. The sorption capacity of the dung was tested by applying the donkey dung powder as a suitable adsorbent for the removal of Cr (VI) ions from synthetic and real tannery wastewater samples. The optimum conditions for the adsorption of Cr ions were, dosage 100 mg, concentration 20 mg/L, pH 2 and contact time 90 min. The findings of the study proves the hypothesis that, *"Equus africanus asinus* dung has the potential to adsorb synthetic hexavalent chromium and real tannery effluents from Dogbone and Beit Ore tanneries of Kenya and South Africa respectively. Thus, donkey dung can therefore be used preliminarly to treat tannery effluent before discharge into various environmental media. The method is simple, cost effective, environmentally friendly and easily applicable. Since this is a biosorbent, it implies that the method served as a green approach and can provide environmental protection and profitability to the tanners.

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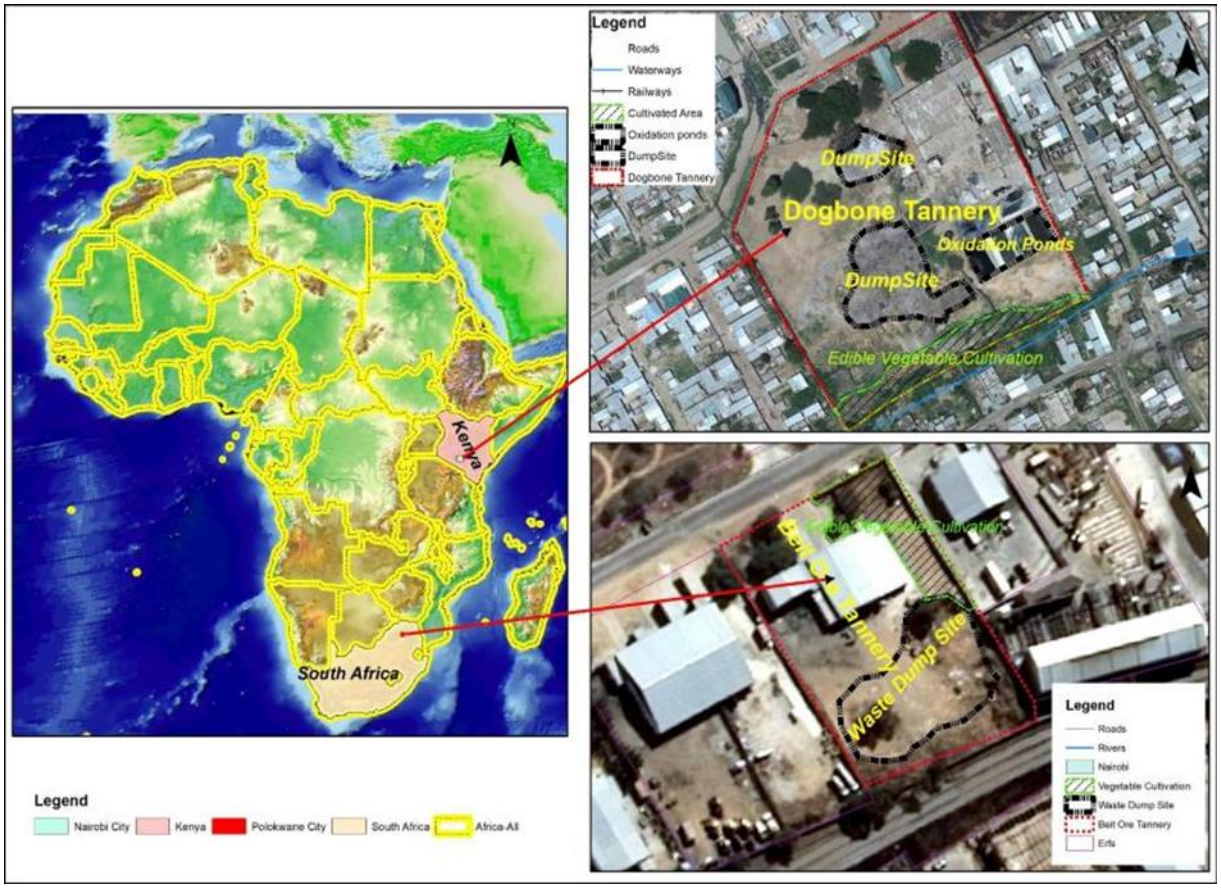
Appendix Figure 1.1: Kales and spinach grown next to a tannery wastes dumpsite in Kenya and South Africa (2017)



Appendix Figure 1.2: Maps showing locations of Dogbone and Beit ore in Kenya and South Africa, respectively

Appendix Figure 1.3: solid wastes like green trimmings, lime fleshings, wet blue trimmings, shavings and dried sludge dump inside a tannery

Kenya Agricultural Research Institu

National Agricultural Research Laboratori

P.O. Box 14733, 00800 NAIROBI

Tel:
0202464435

Email: soilabs@yahoo.co.uk

SOIL SURVEY TEST REPORT

Name	Richard Oruko
Address	
Location of farm	Mowlem
Date sample received	2018/08/12
Date sample reported	08-02-19
Reporting officer (through Director NARL)	G.N. Gachini

Sample designation					
Soil depth cm					
Lab. No/ 2013	8103	8104	8105	8106	8107
* Soil pH-H_2O (1:2.5)					
Elect. Cond. mS/cm					
* Total Org. Carbon %					
Sand %					
Silt %					
Clay %					
Texture Class					
Cat. Exch. Cap. me%	25.20	24.80	34.80	35.20	25.60
Calcium me%	40.84	3.83	30.54	5.43	3.74
Magnesium me%	1.95	0.58	3.70	0.80	4.23
Potassium me%	1.24	0.10	2.60	1.22	9.40
Sodium me%	47.66	1.07	4.67	0.92	4.77
Sum me%	284.74	5.58	44.51	8.37	22.14
Base %	100+	23	100+	24	86
ESP	189.1	4.3	119.3	2.6	18.6

*** ISO/IEC 17025 accredited**

NOTE: Test results are based on customer sampled sample(s).

University of Venda

Questionnaire

My name is Richard Oruko Ongon'g, a PhD student at the school of Environmental Sciences, Department of Ecology and Resource Management University of Venda, (Registration Number 17007722), Republic of South Africa. I am carrying out research and review paper on Bioremediation of tannery based chromium wastes within sub Saharan Africa. I humbly request for your assistance in filling in this questionnaire. This is an academic exercise in pursuit of a PhD in Environmental Science. All information provided will be treated as confidential and your identity will not be revealed, unless by your own authority. If you would want any clarification, feel free to ask.

(email-richardoruko@gmail.com).

1. Name of the institution……

2. Name of the respondent on behalf of the institution……..(optional)

3. What is your position in the institution …….?

4. What are the mandates of your institution……?

…….

5. Name the countries that have registered with your institution……

……

……

6. How many tanneries are registered/operational in this region……

……..?

7. What is their tanning capacity in the region…….

8. Which type of tanning methods are practiced in the region?

.....

……

9. Please provide an estimate of generated wastes (by type) in the region……..

Solid……

Liquid……

Gaseous……..

10. What are the various technologies used in treatment of the following wastes

Solid……

……

……

……

……

……

Liquid

……

……

……

……

……

……..

Gaseous

……

……

……

……

11. How do they specifically treat chromium wastes? Fill up the table below to answer question number 11:-

Tanning process that generate chromium wastes	**Type of chromium wastes generated**	**Approximate quantity produced per year/tannery**	**Physical treatments**	**Chemical treatments**	**Biological treatments**	**Disposal method used**
Tanning stage	Chromium sludge					
	Chromium trimmings					
	Chromium splitings					
	Chromium shavings					
Crust and finishing stage	Crust trimmings					
	Buffing dusts					
	Lindal leather					

12. Which treatment technique is common for tannery based chromium wastes from the region before disposal.....?

13. Does the tannery based chromium wastes from the region undergo any laboratory analysis before and after treatment before they are eventually disposed? Yes…….NO……

If Yes name the types of analysis they undergo

…….....

…….

14. How many tanneries have adopted cleaner technology of recycling chromium wastes in the region……..?

15.How do they recycle the chromium wastes……..

……?

16. Are there any incentives/subsidies given by member governments to tanneries in their countries to promote cleaner technologies? Yes…….NO…….

Please elaborate……

……..

……..

…….

17. What are the existing national policies and regulations guiding the disposal of chromium wastes from the tanning industries in the region ?

……

…….

……

……

……

18. How effectively are they being adhered to by the tanning industries in the region……..……

……

……

19. Name the challenges faced by these industries in achieving these set limits/guidelines/application of techniques in the region ……

……...……

……

……..

……

……..

20. Do chromium wastes generated and poorly disposed from the tanning process pose any risk to the environment and Health of workers/residents?

List Environmental risks……

……

…..……

……

……

……

List Health risks

……

……

……

……

……..

20. Are there tanneries practicing **bioremediation technique** in the the treatment of chromium wastes in the region? Yes….NO……

If Yes, which types of bioremediation techniques, do they practice……

……

……

……

……

……

…….

21. In your opinion do you believe chromium salts should be replaced by other technologies Yes……No…….

Please elaborate.....

……

……

Thank you for your time and cooperation.

Appendix Table 3.2: Raw data on heavy metals analysis in soil and plants with ICP-OES

ppm	Tube	Sample La	Al 167.019	As 197.19	Ca 317.93	Cd 228.80	Co 237.86	Cr 357.868	Cu 324.75	Fe 238.20	Mg 280.27	Mn 279.48	Na 589.59	Ni 225.385
blank	0 : 1	Blank	14.9195	29.708	433.006	423476	31.306	122932	256.42	696.718	1102.29	74.1674	2291.99	21.032
0.2	0 : 2	Standard	15.7937	24.1194	990.204	34.6274	73.5339	160517	203.366	94.0162	3956.46	90.4514	12884	31.4836
0.4	0 : 3	Standard	35.9632	185.547	31446.5	6695.91	4764.47	7254.43	11797.6	12724.7	105512	1748.16	63244.1	858.204
0.6	0 : 4	Standard	38.7025	309.577	34069.6	116023	8180.06	12567.4	20328	23642.6	175684	2910.42	99178.9	1437.49
0.8	0 : 5	Standard	57.4335	460.128	52772.9	179929	12394.4	19548.8	31459.9	36713.4	269750	4615.96	151597	2210.81
1	0 : 6	Standard	76.2636	607.759	64175.2	23258.5	16263.4	25265.4	40707.7	47932.1	345100	5775.75	188659	2806.63
2	0 : 7	Standard	139.652	725.42	294848	53832.2	38306.6	39698.1	79049.6	62887.6	811216	13810.2	441383	6562.34
3	0 : 8	Standard	172.155	1402.33	225336	57931.3	40978.2	63215.8	100969	116492	827944	14819.7	486054	7008.48
4	0 : 9	Standard	237.081	2169.57	249887	83757.4	59788.6	93514.4	147450	174360	1180050	21808.8	721449	10111
5	0 : 10	Standard	326.049	2823.38	503061	113084	79832.4	127873	198873	238979	1579520	29304.5	1009520	13707.9
1a	1 : 1	Sample 1	375.393 u	3518.96 u	407239 ur	137212 un	96562.4 u	154745 ur	240218 un	282681 un	1891500 u	33591.8 u	1231420 u	16641.0 u
1b	1 : 2	Sample 2	591.856 u	45.9376 u	2290100 u	134.868 u	460.370 u	64735.9 u	125907 un	1214650 u	6559110 u	10249.5 u	3711530 u	533.531 u
2a	1 : 3	Sample 3	439.438 u	47.9352 u	4414540 u	182.862 u	458.417 u	13202.7 u	86006.0 u	405954 un	9864220 u	54598.5 u	18530200	408.902 u
2b	1 : 4	Sample 4	734.530 u	29.4644 u	2231150 u	90.6200 u	392.508 u	511603 u	52942.2 u	1704890 u	17542700	6917.81 u	15331800	738.821 u
3a	1 : 5	Sample 5	904.561 u	40.7508 u	3372860 u	104.721 u	507.014 u	71203.7 u	82150.7 u	2474480 u	19769200	9625.09 u	20371900	635.206 u
3b	1 : 6	Sample 6	21.1446 u	17.7841 u	2955.69 u	32.4860 u	29.7692 u	184.682 u	136.709 u	2279.42 u	233603 u	50.7260 u	810230 u	27.8346 u
4a	1 : 7	Sample 7	14.2953 u	31.5791 u	5141.58 u	47.7220 u	31.0311 u	229.810 u	134.173 u	3584.21 u	26196.2 u	119.007 u	5842.45 u	24.9177 u
4b	1 : 8	Sample 8	11.3234 u	25.7707 u	273.763 u	24.7633 u	58.6995 u	135.990 u	119.071 u	197.685 u	1318.30 u	74.8382 u	3587.45 u	42.6647 u
5a	1 : 9	Sample 9	1462.90 u	46.5080 u	4581360 u	83.6021 u	765.113 u	121524 ur	5474.09 u	1213660 u	14391800	9568.26 u	1488780 u	313.336 u
5b	1 : 10	Sample 10	231.679 u	34.0358 u	8614370 u	71.8990 u	120.349 u	1280.84 u	7353.13 u	258965 un	9111760 u	6931.10 u	373080 un	73.8533 u
6a	1 : 11	Sample 11	17.8004 u	23.8245 u	766.237 u	28.2785 u	43.1553 u	140.548 u	164.559 u	74.4834 u	1054.22 u	43.2430 u	2593.18 u	38.5531 u
6b	1 : 12	Sample 12	10.2846 u	16.5597 u	87.2328 u	30.8642 u	36.5130 u	230.906 u	112.220 u	59.0273 u	183.439 u	59.8575 u	3063.26 u	40.0308 u
7a	1 : 13	Sample 13	1715.26 u	49.0877 u	560877 ur	68.4326 u	1374.24 u	538203 u	6396.33 u	4852620 u	3742710 u	5508.35 u	430883 un	610.356 u
7b	1 : 14	Sample 14	1693.14 u	30.1084 u	373966 ur	58.5951 u	1059.43 u	93328.6 u	5187.99 u	4348680 u	2681790 u	3679.19 u	282819 un	569.371 u
8a	1 : 15	Sample 15	1642.15 u	37.7173 u	201213 ur	45.5575 u	760.998 u	541905 u	3511.96 u	3019640 u	1845730 u	2888.39 u	113354 un	361.428 u
8b	1 : 16	Sample 16	1670.40 u	57.9796 u	124688 ur	28.0037 u	793.906 u	32084.0 u	322256 u	2941060 u	1572820 u	1837.94 u	87464.7 u	359.082 u
9a	1 : 17	Sample 17	1645.67 u	14.0308 u	163130 ur	41.5933 u	1050.80 u	43100.8 u	3524.97 u	3124320 u	1819880 u	3134.36 u	121126 un	366.096 u
9b	1 : 18	Sample 18	1586.77 u	34.7427 u	590623 ur	40.3979 u	609.811 u	20468.1 u	183254 u	2979820 u	2473830 u	16537.1 u	256698 un	194.951 u
10b	1 : 19	Sample 19	1787.11 u	66.8147 u	5325620 u	82.7008 u	2274.42 u	9030.62 u	5568.70 u	12051600	11918900	181080 ur	2919670 u	460.750 u
11a	1 : 20	Sample 20	31.4562 u	18.3749 u	363.457 u	14.1389 u	35.0956 u	157.333 u	91.0892 u	4719.23 u	3183.71 u	45.2669 u	122257 u	21.0631 u
11b	1 : 21	Sample 21	1747.36 u	40.6585 u	197344 ur	71.9945 u	976.237 u	40712.1 u	415201 u	3568080 u	2090490 u	3446.29 u	222320 un	414.134 u
12a	1 : 22	Sample 22	1716.07 u	30.1044 u	122517 ur	31.0414 u	731.202 u	32559.8 u	2978.70 u	2605410 u	1449260 u	1834.33 u	331283 un	337.311 u
12b	1 : 23	Sample 23	1752.17 u	32.1591 u	158225 ur	37.8898 u	967.490 u	41112.4 u	3755.08 u	3294880 u	1877720 u	2015.66 u	313407 un	379.573 u
13a	1 : 24	Sample 24	1682.40 u	37.1613 u	108458 ur	50.2073 u	591.356 u	29153.6 u	2344.33 u	2112590 u	1223300 u	1920.91 u	84956.3 u	261.714 u
13b	1 : 25	Sample 25	1423.10 u	34.2438 u	39480.7 u	36.8044 u	230.706 u	9964.20 u	920.435 u	742217 un	464365 ur	754.013 u	265120 u	96.3461 u
14a	1 : 26	Sample 26	1626.37 u	40.9308 u	232037 ur	68.2799 u	571.854 u	19608.4 u	1920.55 u	1479920 u	1559100 u	1250.04 u	82283.2 u	204.331 u
14b	1 : 27	Sample 27	683.089 u	26.7275 u	484701 ur	42.0026 u	66.7372 u	1464.72 u	1452.46 u	129615 un	2015640 u	1494.93 u	914821 u	84.4043 u
15a	1 : 28	Sample 28	21.9700 u	44.6057 u	171.042 u	49.8331 u	40.9042 u	143.630 u	131.757 u	122.031 u	435.876 u	48.3691 u	214258 u	34.2950 u
15b	1 : 29	Sample 29	13.9593 u	22.2063 u	59.3786 u	41.9231 u	47.0625 u	157.214 u	825449 u	91.5910 u	79.1891 u	66.2711 u	3766.82 u	53.7180 u
16a	1 : 30	Sample 30	1425.56 u	51.0504 u	3274430 u	91.2430 u	691.435 u	21269.2 u	7366.22 u	3682360 u	4523170 u	155800 ur	2988420 u	196.165 u
16b	1 : 31	Sample 31	18.1644 u	21.9967 u	250.779 u	41.3704 u	70.9591 u	142.152 u	145.493 u	272.083 u	339.064 u	84.2524 u	1717.31 u	30.2734 u
17a	1 : 32	Sample 32	1203.26 u	35.3021 u	7380720 u	42.8402 u	235.337 u	3441.30 u	13445.3 u	881689 un	7793950 u	12541.9 u	1979550 u	196.602 u
17b	1 : 33	Sample 33	1297.61 u	43.1583 u	6754120 u	51.1267 u	402.685 u	3102.77 u	12526.9 u	979902 un	7097280 u	11097.6 u	1733280 u	214.222 u
18a	1 : 34	Sample 34	2154.43 u	74.1477 u	320553 ur	45.4718 u	1559.51 u	68445.5 u	6299.06 u	5174190 u	3151370 u	5357.18 u	1977510 u	675.364 u
18b	1 : 35	Sample 35	2157.13 u	50.2380 u	246757 ur	56.5693 u	1643.06 u	69276.1 u	6388.21 u	5265780 u	2947810 u	3757.95 u	1688060 u	706.460 u
19a	1 : 36	Sample 36	15.0593 u	27.5953 u	148.160 u	54.2950 u	75.8503 u	160.895 u	122.618 u	686.688 u	424.093 u	45.3908 u	15229.3 u	14.4756 u
19b	1 : 37	Sample 37	9.11873 u	21.2261 u	65.9886 u	42.9108 u	51.1925 u	109.439 u	96.4616 u	59.3081 u	48.4753 u	33.5085 u	2225.31 u	48.6582 u
20a	1 : 38	Sample 38	2246.91 u	70.3253 u	753320 ur	80.9678 u	9730.05 u	63831.4 u	86707.5 u	19459300	3505260 u	129107 ur	366324 un	2372.10 u
20b	1 : 39	Sample 39	2321.83 u	89.6095 u	1495480 u	54.7967 u	10455.3 u	70480.4 u	87892.5 u	19719100	4063700 u	137959 ur	356485 un	2524.68 u
21a	1 : 40	Sample 40	799.440 u	38.4855 u	4590620 u	77.5447 u	255.193 u	28483.8 u	8656.81 u	764635 un	13616200	29424.8 u	1908220 u	165.865 u
21b	1 : 41	Sample 41	779.988 u	17.8388 u	4694170 u	108.801 u	153.780 u	29336.0 u	8782.20 u	723463 un	13778000	31509.2 u	1962810 u	154.384 u
22a	1 : 42	Sample 42	888.131 u	37.0914 u	2322530 u	95.7453 u	578.956 u	76569.6 u	121406 un	1255700 u	7289370 u	12720.9 u	4137740 u	577.927 u
22b	1 : 43	Sample 43	668.914 u	7.27667 u	3496680 u	180.343 u	378.942 u	111843 u	64029.2 u	329007 un	8158470 u	41486.2 u	15121700	324.240 u
23a	1 : 44	Sample 44	13.9008 u	23.9117 u	426.572 u	29.8518 u	55.4762 u	196.275 u	177.699 u	231.426 u	989.599 u	68.1422 u	5351.08 u	43.3332 u
23b	1 : 45	Sample 45	3.77660 u	33.2480 u	54.8771 u	25.9333 u	62.2550 u	157.795 u	103.026 u	85.8812 u	528194 u	59.7960 u	2549.59 u	37.6854 u
24a	1 : 46	Sample 46	77.4642 u	31.2330 u	353450 ur	53.1263 u	31.3688 u	15827.7 u	290.018 u	34888.6 u	51368.5 u	441.406 u	276194 un	39.9719 u
24b	1 : 47	Sample 47	9.48062 u	22.5994 u	360.497 u	25.8245 u	29.0810 u	105.010 u	92.7615 u	54.0777 u	131.876 u	29.8947 u	2497.59 u	10.1825 u
25a	1 : 48	Sample 48	2336.77 u	37.8171 u	369829 ur	46.0670 u	1440.80 u	75055.2 u	6163.49 u	4936180 u	2902950 u	4383.73 u	1009500 u	627.797 u
25b	1 : 49	Sample 49	2261.15 u	61.8280 u	225487 ur	27.9419 u	1205.79 u	44356.6 u	5006.33 u	4288060 u	2374430 u	3448.66 u	340717 un	551.602 u
26a	1 : 50	Sample 50	23.1118 u	22.7015 u	138.319 u	37.3986 u	23.7959 u	135.393 u	144.101 u	173.094 u	131.478 u	46.6835 u	2099.05 u	48.1272 u
26b	1 : 51	Sample 51	121.907 u	33.9260 u	850820 ur	48.8749 u	124.080 u	5916.20 u	358.241 u	68287.0 u	122641 ur	1123.32 u	451998 un	33.0883 u
27a	1 : 52	Sample 52	1942.37 u	87.7280 u	36674100	130.194 u	1422.07 u	1150690 u	22977.0 u	8982420 u	9751850 u	206732 ur	12091000	610.081 u
27b	1 : 53	Sample 53	2020.28 u	92.1893 u	34834700	104.165 u	1212.26 u	870134 ur	18264.2 u	7691750 u	8209520 u	158313 ur	9927450 u	562.737 u
28a	1 : 54	Sample 54	2369.88 u	48.1820 u	1542400 u	82.4733 u	1834.12 u	155328 ur	23438.0 u	9693370 u	4674450 u	18571.4 u	2781180 u	1603.80 u
28b	1 : 55	Sample 55	293.086 u	31.8971 u	20142.7 u	48.1412 u	80.8212 u	3020.23 u	448.534 u	155174 un	541203 u	3523.21 u	56857.4 u	66.3698 u
29a	1 : 56	Sample 56	2515.22 u	65.4002 u	269505 ur	41.0549 u	1465.14 u	17115.6 u	5611.99 u	4520080 u	2727480 u	4077.04 u	289214 un	608.063 u
29b	1 : 57	Sample 57	2595.54 u	50.0979 u	288227 ur	41.8635 u	1344.45 u	18468.5 u	6165.37 u	4850830 u	2982490 u	4849.43 u	226106 un	638.218 u
30a	1 : 58	Sample 58	37.0796 u	27.4523 u	211.150 u	18.8978 u	46.9814 u	217.729 u	174.056 u	2360.13 u	1114.27 u	66.3997 u	2516.94 u	45.0167 u
30b	1 : 59	Sample 59	2569.83 u	65.2297 u	325856 ur	36.1064 u	1637.91 u	75284.7 u	6447.30 u	5258800 u	3347080 u	5355.42 u	877046 un	711.616 u
31a	1 : 60	Sample 60	2612.35 u	53.8399 u	456160 ur	54.2371 u	1672.52 u	91870.7 u	6989.99 u	5338200 u	3722060 u	5846.39 u	370293 un	739.146 u
31b	2 : 1	Sample 61	2632.56 u	66.7208 u	452749 ur	35.0168 u	1484.10 u	85925.3 u	6500.53 u	5013360 u	3336550 u	4818.63 u	369891 un	701.622 u
32a	2 : 2	Sample 62	786.443 u	12.0557 u	2396380 u	60.5792 u	196.076 u	18526.5 u	12317.9 u	484036 un	9407150 u	10724.9 u	6655220 u	226.056 u
32b	2 : 3	Sample 63	725.391 u	29.1969 u	2082610 u	66.0912 u	215.744 u	16724.6 u	10748.6 u	441967 un	8480790 u	9409.57 u	5829710 u	201.547 u
33a	2 : 4	Sample 64	533.758 u	34.3717 u	783703 u	50.4625 u	108.502 u	5130.58 u	359.153 u	64746.2 u	425665 ur	347.544 u	82928.6 u	68.3282 u
33b	2 : 5	Sample 65	2403.90 u	18.4718 u	1311540 u	48.0002 u	527.470 u	62572.9 u	3779.86 u	968328 un	6712180 u	2840.52 u	894933 un	246.022 u
34a	2 : 6	Sample 66	2443.78 u	24.5547 u	1842590 u	49.2593 u	606.702 u	18962.7 u	3270.40 u	1033520 u	7235950 u	3274.44 u	717270 un	310.252 u
34b	2 : 7	Sample 67	2471.64 u	45.3380 u	2065300 u	57.2009 u	510.814 u	206425 u	3393.21 u	1098980 u	7934970 u	4024.30 u	794619 un	239.587 u
35a	2 : 8	Sample 68	1356.40 u	18.1786 u	4216370 u	141.641 u	448.692 u	102747 ur	11033.2 u	1199800 u	6892520 u	36083.0 u	6092170 u	193.372 u
35b	2 : 9	Sample 69	1320.00 u	33.5001 u	4264260 u	146.190 u	446.219 u	100841 ur	11515.6 u	1139640 u	7135630 u	35271.3 u	6431690 u	256.715 u
36a	2 : 10	Sample 70	1675.76 u	37.3746 u	1594590 u	36.3987 u	517.916 u	173616 ur	5808.81 u	1327830 u	4950300 u	10706.7 u	1742090 u	308.691 u
36b	2 : 11	Sample 71	32.3456 u	19.6000 u	11887.3 u	31.6602 u	47.2011 u	182.281 u	160.503 u	8426.34 u	46622.1 u	125.470 u	11228.1 u	34.4473 u
37a	2 : 12	Sample 72	564.412 u	28.9195 u	6908870 u	73.7124 u	164.323 u	4706.40 u	16659.8 u	267275 un	9533300 u	4298.94 u	15350700	208.078 u
37b	2 : 13	Sample 73	10.9509 u	17.3173 u	562.275 u	42.9215 u	66.8388 u	130.019 u	154.097 u	62.2089 u	886.429 u	55.5845 u	7392.28 u	42.5833 u
38a	2 : 14	Sample 74	1055.67 u	25.6154 u	8280390 u	232.321 u	120.588 u	26465.7 u	6487.09 u	769523 un	6049690 u	25764.9 u	18830900	154.795 u
38b	2 : 15	Sample 75	1104.09 u	24.7425 u	8927950 u	259.290 u	289.441 u	30326.0 u	7338.72 u	779261 un	6438210 u	28353.0 u	20169600	157.833 u
39a	2 : 16	Sample 76	22.9164 u	41.5172 u	13353.6 u	23.3552 u	75.0251 u	1373.61 u	383.941 u	11992.8 u	8579.99 u	77.3074 u	9684.88 u	92.7644 u
39b	2 : 17	Sample 77	21.8339 u	35.7923 u	13200.5 u	44.0676 u	55.2302 u	1565.92 u	384.156 u	13066.7 u	8779.47 u	98.5443 u	7853.21 u	95.2333 u
40a	2 : 18	Sample 78	2916.15 u	62.9451 u	531785 ur	34.0168 u	1334.53 u	25004.7 u	6151.16 u	4613300 u	3516400 u	5129.24 u	388957 un	641.000 u
40b	2 : 19	Sample 79	2906.93 u	38.2452 u	647054 ur	26.0693 u	1494.16 u	26992.6 u	6403.57 u	4750080 u	3706370 u	5874.12 u	441469 un	660.290 u
41a	2 : 20	Sample 80	67.2746 u	26.4598 u	430.777 u	36.1417 u	59.8420 u	241.559 u	113.825 u	5396.66 u	2842.92 u	59.5439 u	3150.70 u	39.9573 u
41b	2 : 21	Sample 81	73.9754 u	24.2581 u	502.998 u	26.1846 u	37.4432 u	171.225 u	66.9412 u	7636.63 u	4700.56 u	42.3261 u	2695.62 u	28.2739 u
42a	2 : 22	Sample 82	183.787 u	17.3352 u	1929.92 u	22.4673 u	49.6586 u	488.229 u	141.029 u	24815.2 u	18813.3 u	88.6181 u	2249.15 u	16.2668 u
42b	2 : 23	Sample 83	2018.83 u	28.8057 u	41573.2 u	76.5564 u	220.811 u	9825.12 u	730.059 u	520565 un	293634 ur	500.740 u	47400.7 u	61.5198 u
43a	2 : 24	Sample 84	543.590 u	37.3945 u	3185.31 u	38.7109 u	138.115 u	2005.75 u	144.720 u	62080.3 u	32582.2 u	152.969 u	3907.84 u	35.0222 u
43b	2 : 25	Sample 85	2952.97 u	67.0625 u	237073 ur	26.7508 u	1332.73 u	97874.1 u	6052.76 u	4638450 u	2747040 u	3900.96 u	173208 un	654.172 u
44a	2 : 26	Sample 86	611.997 u	25.2376 u	1843200 u	50.3177 u	100.949 u	3704.32 u	5081.85 u	248457 un	9932970 u	5729.74 u	20640900	109.342 u

Appendix Table 3.3: Raw data on analysis of Cr (VI) with UV-Vis spectrophotometer

Sample ID	Descriptic	Concentra	Analyte (r	Ordinate (A)
Blank	Cr VI anal	0	0.0075	0.0002
Stand 0	Cr VI anal	0	0.0108	0
Stand 125 ul	Cr VI anal	0.5	0.5295	0.0302
Stand 250 ul	Cr VI anal	1	0.5884	0.0335
Stand 500 ul	Cr VI anal	2	2.286	0.1283
Stand 750 ul	Cr VI anal	3	2.4673	0.1384
Stand 1000 ul	Cr VI anal	4	4.9833	0.2789
Stand 1250 ul	Cr VI anal	5	4.4942	0.2516
Stand 1500 ul	Cr VI anal	6	6.7645	0.3784
Stand 1750 ul	Cr VI anal	7	6.7489	0.3776
Stand 2000ul	Cr VI anal	8	7.6487	0.4278
DB dry soil 1	Cr VI anal		0.0155	0.3869
DB dry soil 2	Cr VI anal		0.0145	0.3388
DB dry soil 3	Cr VI anal		0.0182	0.458
BO wet soil 1	Cr VI anal		0.2029	0.0399
BO wet soil 2	Cr VI anal		0.1919	0.0504
BO wet soil 3	Cr VI anal		0.179	0.0776
DB wet soil 1	Cr VI anal		0.1049	0.05
DB wet soil 2	Cr VI anal		0.216	0.0685
DB wet soil 3	Cr VI anal		0.2447	0.0143
BO dry soil 1	Cr VI anal		0.1058	0.2411
BO dry soil 2	Cr VI anal		0.1061	0.2613
BO dry soil 3	Cr VI anal		0.1145	0.3354
Control soil G. H 1	Cr VI anal		0.0011	0.1544
Control soil G.H 2	Cr VI anal		0.1011	0.1211
Control soil G.H 3	Cr VI anal		0.0102	0.1945
Spiked CRM 1	Cr VI anal		0.001	0.0514
Spiked CRM 2	Cr VI anal		0.013	0.0295
Spiked CRM 3	Cr VI anal		0.01	0.0743
DB control soil 1	Cr VI anal		0.0203	0.4988
DB control soil 2	Cr VI anal		0.0499	0.4223

RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:
Mr RO Ongon'g

Student No:
17007722

PROJECT TITLE: **Bioremediation of tannery-based chromium complexes in soils: The case of dump sites in Beit Ore Tannery in South Africa and Dogbone tannery in Kenya.**

PROJECT NO: SES/17/MEG/11/0510

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Prof JO Odiyo	University of Venda	Promoter
Dr JN Edokpayi	University of Venda	Co-Promoter
Mr RO Ongon'g	University of Venda	Investigator – Student

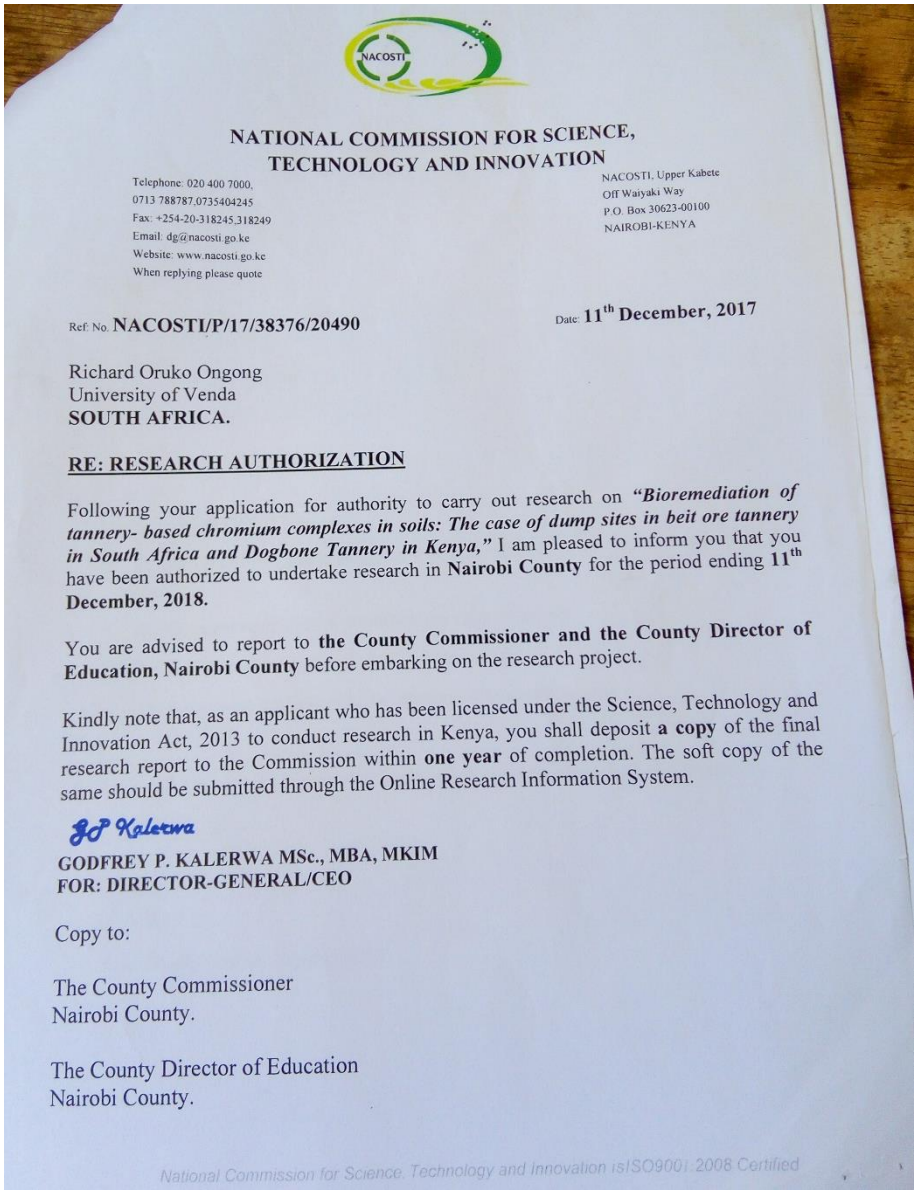
ISSUED BY:
UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE

Date Considered: October 2017
Decision by Ethical Clearance Committee Granted
Signature of Chairperson of the Committee: .....
Name of the Chairperson of the Committee: Senior Prof. G.E. Ekosse

UNIVERSITY OF VENDA
DIRECTOR
RESEARCH AND INNOVATION
2017 -10- 06
Private Bag X5050
Thohoyandou 0950

University of Venda
PRIVATE BAG X5050, THOHOYANDOU, 0950, LIMPOPO PROVINCE, SOUTH AFRICA
TELEPHONE (015) 962 8504/8313 FAX (015) 962 9060
"A quality driven financially sustainable, rural-based Comprehensive University"

Appendix Figure 3.2: Ethical clearance certificate from Univen Ethics committee



NACOSTI

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TECHNOLOGY AND INNOVATION

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When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. NACOSTI/P/17/38376/20490

Date: 11th December, 2017

Richard Oruko Ongong
University of Venda
SOUTH AFRICA.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on *"Bioremediation of tannery- based chromium complexes in soils: The case of dump sites in beit ore tannery in South Africa and Dogbone Tannery in Kenya,"* I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for the period ending **11th December, 2018.**

You are advised to report to **the County Commissioner and the County Director of Education, Nairobi County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

The County Director of Education
Nairobi County.

National Commission for Science, Technology and Innovation is ISO9001:2008 Certified

Appendix Figure 3.2: Ethical clearance certificate from National Commission for Science, Technology and Innovation (NACOSTI) Kenya novation (NACOSTI) Kenya

PERMISSION TO ACCESS PREMISE

I A. PUMJA on this day 16 of JANUARY ~~2017~~/2018 hereby consent to:

1. Request for permission to access and conduct academic research in my premise by RICHARD ORUKO,

on the topic entitled "BIOREMEDIATION OF TANNERY- BASED CHROMIUM COMPLEXES IN SOILS: THE CASE OF DUMP SITES IN BEIT ORE TANNERY IN SOUTH AFRICA AND DOGBONE TANNERY IN KENYA"

2. The permission allow principal researcher:-

1. Free movement into and out of the tannery during the period of the study.
2. Authority to collect soil, water, chromium effluent and plants samples from the dumpsite in the tannery.

I hereby acknowledge that the researcher has:

1. Discussed the purpose and objectives of this research project with me
2. Informed me about content of this agreement
3. Informed me about implications of signing this agreement.

As I sign this agreement the researcher undertake to:

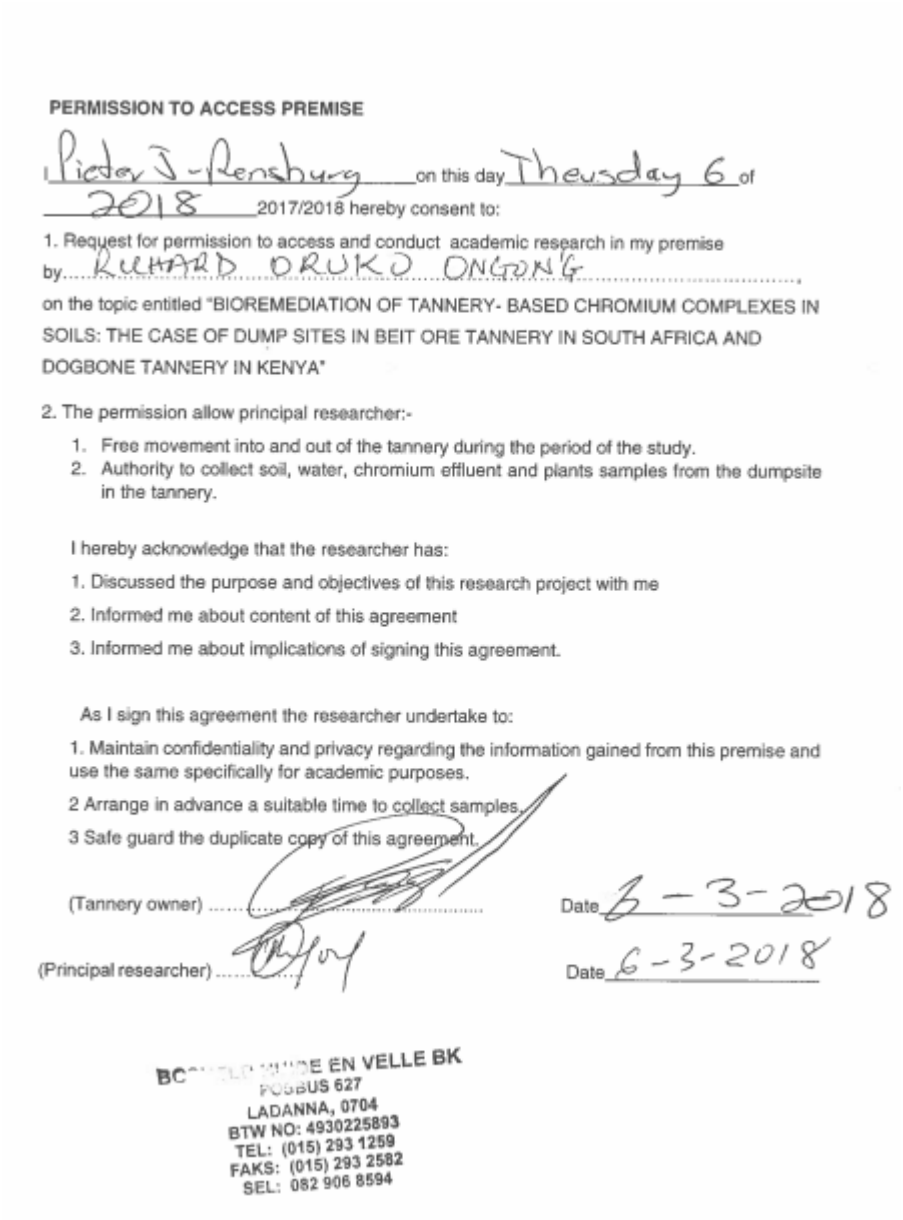
1. Maintain confidentiality and privacy regarding the information gained from this premise and use the same specifically for academic purposes.
2. Arrange in advance a suitable time to collect samples.
3. Safe guard the duplicate copy of this agreement.

(Tannery owner) ..... Date 16/1/18

(Principal researcher) ..... Date 16/1/2018

DOGBONES LIMITED
Date 16/1/2018
P.O. Box 78010, VIWANDANI

Appendix Figure 3.4: Permission and consent to access Dogbone tannery



PERMISSION TO ACCESS PREMISE

I Pieter J - Rensburg on this day Thursday 6 of 2018 2017/2018 hereby consent to:

1. Request for permission to access and conduct academic research in my premise by RICHARD ORUKO ONGONG,

on the topic entitled "BIOREMEDIATION OF TANNERY- BASED CHROMIUM COMPLEXES IN SOILS: THE CASE OF DUMP SITES IN BEIT ORE TANNERY IN SOUTH AFRICA AND DOGBONE TANNERY IN KENYA"

2. The permission allow principal researcher:-

1. Free movement into and out of the tannery during the period of the study.
2. Authority to collect soil, water, chromium effluent and plants samples from the dumpsite in the tannery.

I hereby acknowledge that the researcher has:

1. Discussed the purpose and objectives of this research project with me
2. Informed me about content of this agreement
3. Informed me about implications of signing this agreement.

As I sign this agreement the researcher undertake to:

1. Maintain confidentiality and privacy regarding the information gained from this premise and use the same specifically for academic purposes.
2. Arrange in advance a suitable time to collect samples.
3. Safe guard the duplicate copy of this agreement.

(Tannery owner) ..... Date 6-3-2018

(Principal researcher) ..... Date 6-3-2018

BC[illegible]E EN VELLE BK
POSBUS 627
LADANNA, 0704
BTW NO: 4930225893
TEL: (015) 293 1259
FAKS: (015) 293 2582
SEL: 082 906 8594

Appendix Figure 3.5: Permission and consent to access Beit Ore tannery

DOG BONE TANNERY TIME OF SAMPLE
14/02/2018 1530 Hrs E.A.Time

Field Manual for the Collection of Water, soil, plants and effluent samples AT DOGBONE TANNERY IN KENYA

Item	Comments
Dumpsite description: • Location of sample-collection sites: • Hydrologic and geologic information. • Name of landowner, tenant, or other responsible party. • Site access instructions (for example, call owner or site operator before arrival at site, obtain key to unlock security gate). • Photographs to document site conditions.	Dog bone tannery Dumpsite dry soil dumpsite environment. Land owned by the company. (A. Punja) Obtained Consent from the owner to access the site. photos taken of the site
Maps to site (State and local) County (Nairobi)	Latitude 1°15'49.91"S / -1.263891, Longitude 36°52'52.39"E / 36.881347
Profiles of cross section of dumpsite at sampling location(s). • Physical and chemical measurements.	Temp. 26°C Cloudy, intermittent clouds. Strong breeze.
Safety information: • Nearest emergency facilities. • Phone numbers (home) of study chief or supervisor. • Environmental hazards, such as exposure to toxic, weather and animals.	Mama Lucy Hospital +27748641941 Smell of hydrogen Sulphide (H_2S), fire, piercing metals,
Sampling schedule: • Laboratory analyses to be requested and associated codes. • When to collect samples (wet or dry season).	Chemical and physical analysis. Collected during dry period/season. ~~Dry~~ season
Bottle types needed for each analytical schedule.	Plastic and glass bottles used to collect samples.
Analytical Services Request form(s) and example of a completed form.	
Sampling instructions: • Equipment to use at various sample collection points. • Number of samples to collect for soil, plant, water and effluent.	Soil auger, measuring tape/ruler, gloves, dust coat, cutting knife, mouth mask, helmet Random sampling of soil from the dump site
Shipping instructions: • Amount of ice to use. • Mailing labels to and from laboratory. • Location of nearest post office or shipping agent/ airport	Ice packs will be used. Containers labelled and marked. Jomo Kenyatta Airport.
A tabulation sheet for each type of bacteria/fungus enumerated at the site (include example with date of sample, volumes filtered, dilutions, plate counts).	Not done in the field.
Plots of field-measured data Conductivity versus alkalinity. • Temperature versus time pH versus ORP	Will be done in the lab.
Special equipment needed to address site-specific conditions: • Sampling. • Safety	Heavy machinery to remove heavy dumped waste PPE, security marking tapes

BEIT ORE TANNERY TIME OF SAMPLE
6/3/2018 11.00 ~~am~~ SOUTH AFRICA TIME

Field Manual for the Collection of Water, soil, plants and effluent samples

Item	Comments
Dumpsite description: • Location of sample-collection sites: • Hydrologic and geologic information. • Name of landowner, tenant, or other responsible party. • Site access instructions (for example, call owner or site operator before arrival at site, obtain key to unlock security gate). • Photographs to document site conditions.	BEIT ORE TANNERY DUMPSITE Semi dry soil dumpsite Environment Pieter J. Rensburg. obtained official Consent from the owner to access site. photos taken of the site
Maps to site (State and local) Lowlanne Estate	Latitude Longitude 23°54'00"S and 29°27'00"E
Profiles of cross section of dumpsite at sampling location(s). • Physical and chemical measurements.	Temp t – Clear sunny hot day 30°C
Safety information: • Nearest emergency facilities. • Phone numbers (home) of study chief or supervisor. • Environmental hazards, such as exposure to toxic, weather and animals.	provincial hospital of Limpopo 0611311842 / +27748641941 Notifiable diseases (zoonotic diseases from raw hides, tanning chemical, fire, the smell from rotting protein
Sampling schedule: • Laboratory analyses to be requested and associated codes. • When to collect samples (wet or dry season).	Chemical and physical analysis, Collected during summer season in South Africa
Bottle types needed for each analytical schedule.	plastic and poly propylene vial used to collect samples. Hand trowel, plastic bucket, shovel/spade (for moist soil)
Analytical Services Request form(s) and example of a completed form.	
Sampling instructions: • Equipment to use at various sample collection points. • Number of samples to collect for soil, plant, water and effluent.	Soil auger, measuring tape, ruler, pH-meter, gloves, dust coat, cutting knife, spade, mouth mask Random sampling of soil from the dumpsite
Shipping instructions: • Amount of ice to use. • Mailing labels to and from laboratory. • Location of nearest post office or shipping agent/ airport	Icepack used during transportation from Polokwane to the univen lab. Containers and vials labelled.
A tabulation sheet for each type of bacteria/fungus enumerated at the site (include example with date of sample, volumes filtered, dilutions, plate counts).	Not done in the field
Plots of field-measured data Conductivity versus alkalinity. • Temperature versus time pH versus ORP	Will be done immediately in the lab.
Special equipment needed to address site-specific conditions: • Sampling. • Safety	Heavy machinery to lift heavy dumped waste. PPE, Security marking tapes

Appendix Figure 4.1: Sampled guide for Dogbone and Beit Ore tanneries dumpsites

Appendix Figure 5.1: The modified roots of *Vigna angularis* that germinated and grew in Cr (VI) levels of 456 mg/kg

Appendix Table 5.1. Raw data on plants root bioaccumulation

Sample ID	Description	Concentration	Analyte in mg ml-1	Absorbance
Blank	Cr VI analysis	0	0.0154	0
Std 0	Cr VI analysis	0	0.0158	0.0002
Std 0.5	Cr VI analysis	0.5	0.4657	0.1626
Std 1	Cr VI analysis	1	1.0102	0.3466
Std 2	Cr VI analysis	2	2.035	0.6931
Std 3	Cr VI analysis	3	3.01	1.0227
Std 4	Cr VI analysis	4	3.9611	1.3442
Std 5	Cr VI analysis	5	5.0459	1.7108
Std 6	Cr VI analysis	6	6.0827	2.0613
Std 7	Cr VI analysis	7	6.9053	2.3394
CpS root1	Cr VI analysis		0.4239	0.0332
CpS root2	Cr VI analysis		0.2106	0.0453
CpS root 3	Cr VI analysis		0.5292	0.0384
BeS root 1	Cr VI analysis		0.2031	0.0291
BeS root 2	Cr VI analysis		0.2134	0.0329
BeS root 3	Cr VI analysis		0.3106	0.099
SpS root 1	Cr VI analysis		0.001	0.0596
SpS root 2	Cr VI analysis		0.0211	0.0268
SpS root 3	Cr VI analysis		0.0012	0.0316
CkpS root 1	Cr VI analysis		0.3672	0.0204
CkpS root 2	Cr VI analysis		0.6141	0.0256
CkpS root 3	Cr VI analysis		0.4104	0.0514
AmS root 1	Cr VI analysis		0.0173	0.0445
AmS root 2	Cr VI analysis		0.1201	0.0842
AmS root 3	Cr VI analysis		0.117	0.1204
CpC1	Cr VI analysis		-0.0065	0.072
CpC2	Cr VI analysis		-0.01	0.0364
CpC3	Cr VI analysis		-0.0081	0.0744
SpC1	Cr VI analysis		-0.0178	0.0475
SpC2	Cr VI analysis		-0.0213	0.05
SpC3	Cr VI analysis		-0.0143	0.0339
BeC1	Cr VI analysis		-0.2246	0.0469
BeC2	Cr VI analysis		-0.3101	0.0621
BeC3	Cr VI analysis		-0.2109	0.0145
AmC1	Cr VI analysis		-0.0176	0.1579
AmC2	Cr VI analysis		-0.1461	0.047
AmC3	Cr VI analysis		-0.0253	0.0362
CkpC1	Cr VI analysis		-0.2781	0.0626
CkpC2	Cr VI analysis		-0.1352	0.0967
CkpC3	Cr VI analysis		-0.236	0.1294

Appendix Table 6.1: Gas composition from the GC analysis

Data File C:\CHEM32\1\DATA\TREVOR\BO 30G ORUKO T2 2019-05-13 12-00-25.D
Sample Name: BO 30g Oruko T2

Acq. Operator : SYSTEM Sample Operator : SYSTEM Acq. Instrument : BioGas Location : Vial 1 Injection Date : 5/13/2019 12:00:27 Inj Volume : 1000 µl Method : C:\CHEM32\1\METHODS\LUCIABIOGAS.M Last changed : 10/16/2018 08:07:37 by SYSTEM Method Info : Bio Gas Analysis

Sample Info : BO 30g Oruko T2

Signal 1: TCD1 A, Front Signal

RetTime Type Area Amt/Area Amount Grp Name [min] [25 µV*s] [mole%] -------|------|----------|----------|----------|--|---------- 1.071 VB S 2.84468e4 2.64047e-3 75.11305 N2 2.304 - - - Methane 3.840 BB 1145.24963 2.30787e-3 2.64309 CO2

Totals : 77.75613

Signal 2: TCD2 C, Aux Signal

Signal 3: FID3 B, Back Signal

RetTime Type Area Amt/Area Amount Grp Name [min] [pA*s] [mole%] -------|------|----------|----------|----------|--|---------- 1.511 - - - CH4

Totals : 0.00000

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

==========

==========
Summed Peaks Report
==========

Signal 1: TCD1 A, Front Signal Signal 2: TCD2 C, Aux Signal Signal 3: FID3 B, Back Signal
==========
Final Summed Peaks Report
==========

Signal 1: TCD1 A, Front Signal Name Total Area Amount [pA*s] [mole%] ----------|----------|----------| N2 2.84468e4 75.1130 Methane 0.00000 0.0000 CO2 1145.24963 2.6431

Totals : 77.7561

Signal 2: TCD2 C, Aux Signal

Data File C:\CHEM32\1\DATA\TREVOR\BO 30G ORUKO T2 2019-05-13 12-00-25.D Sample Name: BO 30g Oruko T2

BioGas 5/13/2019 12:05:00 SYSTEM Page 2 of 3

Signal 3: FID3 B, Back Signal Name Total Area Amount [pA*s] [mole%] ----------|----------|----------| CH4 0.00000 0.000

Totals : 0.0000

*** End of Report ***

Data File C:\CHEM32\1\DATA\TREVOR\DB CONTROL ORUKO T1 2019-05-13 13-10-24.D
Sample Name: DB CONTROL Oruko T1

Acq. Operator : SYSTEM Sample Operator : SYSTEM Acq. Instrument : BioGas Location : Vial 1 Injection Date : 5/13/2019 13:10:26 Inj Volume : 1000 µl Method : C:\CHEM32\1\METHODS\TREVORBIOGAS.M Last changed : 11/1/2018 11:41:44 by SYSTEM Method Info : Bio Gas Analysi

Sample Info : DB CONTROL Oruko T1

Signal 1: TCD1 A, Front Signal

RetTime Type Area Amt/Area Amount Grp Name [min] [25 µV*s] [mole%] -------|------|----------|----------|----------|--|---------- 1.073 VB S 2.76366e4 2.64047e-3 72.97371 N2 2.304 - - - methane 3.804 - - - CO2

Totals : 72.97371

Signal 2: TCD2 C, Aux Signal

Signal 3: FID3 B, Back Signal

RetTime Type Area Amt/Area Amount Grp Name [min] [pA*s] [mole%] -------|------|----------|----------|----------|--|---------- 1.511 - - - CH4

Totals : 0.00000

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

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Summed Peaks Report
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Signal 1: TCD1 A, Front Signal Signal 2: TCD2 C, Aux Signal Signal 3: FID3 B, Back Signal
==========
Final Summed Peaks Report
==========

Signal 1: TCD1 A, Front Signal Name Total Area Amount [pA*s] [mole%] ----------|----------|----------| N2 2.76366e4 72.9737 methane 0.00000 0.0000 CO2 0.00000 0.0000

Totals : 72.9737

Signal 2: TCD2 C, Aux Signal

Data File C:\CHEM32\1\DATA\TREVOR\DB CONTROL ORUKO T1 2019-05-13 13-10-24.D
Sample Name: DB CONTROL Oruko T1

Signal 3: FID3 B, Back Signal Name Total Area Amount [pA*s] [mole%] ------- --------|----------|----------| CH4 0.00000 0.0000

Totals : 0.0000

*** End of Report ***

Data File C:\CHEM32\1\DATA\TREVOR\DB 15G ORUKO T2 2019-05-13 12-58-55.D
Sample Name: DB 15g Oruko T2

Acq. Operator : SYSTEM Sample Operator : SYSTEM Acq. Instrument : BioGas Location : Vial 1 Injection Date : 5/13/2019 12:58:57 Inj Volume : 1000 µl Method : C:\CHEM32\1\METHODS\TREVORBIOGAS.M Last changed : 11/1/2018 11:41:44 by SYSTEM Method Info : Bio Gas Analysis

Sample Info : DB 15g Oruko T2

Signal 1: TCD1 A, Front Signal

RetTime Type Area Amt/Area Amount Grp Name [min] [25 µV*s] [mole%] -------|------|----------|----------|----------|--|---------- 1.074 VB S 2.79565e4 2.64047e-3 73.81837 N2 2.373 BB 166.45047 3.63983e-3 6.05851e-1 methane 3.842 BB 1380.92310 2.30787e-3 3.18699 CO2

Totals : 77.61121

Signal 2: TCD2 C, Aux Signal

Signal 3: FID3 B, Back Signal

RetTime Type Area Amt/Area Amount Grp Name [min] [pA*s] [mole%] -------|------|----------|----------|----------|--|---------- 1.514 BB 55.99414 1.02962e-2 5.76528e-1 CH4

Totals : 5.76528e-1

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Summed Peaks Report
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Signal 1: TCD1 A, Front Signal Signal 2: TCD2 C, Aux Signal Signal 3: FID3 B, Back Signal
==========
Final Summed Peaks Report
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Signal 1: TCD1 A, Front Signal Name Total Area Amount [pA*s] [mole%] ----------|----------|----------| N2 2.79565e4 73.8184 methane 166.45047 0.6059 CO2 1380.92310 3.1870

Totals : 77.6112

Signal 2: TCD2 C, Aux Signal Signal 3: FID3 B, Back Signal Name Total Area Amount [pA*s] [mole%] ----------|----------|----------| CH4 55.99414 0.5765

Data File C:\CHEM32\1\DATA\TREVOR\DB 15G ORUKO T2 2019-05-13 12-58-55.D
Sample Name: DB 15g Oruko T2

Totals : 5.7653e-1

*** End of Report ***

Data File C:\CHEM32\1\DATA\TREVOR\DB 30G ORUKO T1 2019-05-13 12-35-24.D
Sample Name: DB 30g Oruko T1

Acq. Operator : SYSTEM Sample Operator : SYSTEM Acq. Instrument : BioGas Location : Vial 1 Injection Date : 5/13/2019 12:35:26 Inj Volume : 1000 µl Method : C:\CHEM32\1\METHODS\TREVORBIOGAS.M Last changed : 11/1/2018 11:41:44 by SYSTEM Method Info : Bio Gas Analysis

Sample Info : DB 30g Oruko T1

Signal 1: TCD1 A, Front Signal

RetTime [min]	Type	Area [25 µV*s]	Amt/Area	Amount [mole%]	Grp	Name
1.079	VB S	2.55385e4	2.64047e-3	67.43364		N2
2.307	BB	4037.47266	3.63983e-3	14.69570		methane
3.799	BB	4185.11963	2.30787e-3	9.65871		CO_2

Totals : 91.78805

Signal 2: TCD2 C, Aux Signal

Signal 3: FID3 B, Back Signal

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mole%]	Grp	Name
1.513	BB	1372.21924	1.02962e-2	14.12866		CH4

Totals : 14.12866

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Summed Peaks Report
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Signal 1: TCD1 A, Front Signal Signal 2: TCD2 C, Aux Signal Signal 3: FID3 B, Back Signal
==========
Final Summed Peaks Report
==========

Signal 1: TCD1 A, Front Signal Name	Total Area	Amount [mole%]	Signal 2: TCD2 C, Aux Signal Signal 3: FID3 B, Back Signal Name	Total Area [pA*s]	Amount [mole%]
N2	2.55385e4	67.4336	methane	4037.47266	14.6957
CO2	4185.11963	9.6587			

Totals : 91.7880

Signal 2: TCD2 C, Aux Signal Signal 3: FID3 B, Back Signal Name	Total Area [pA*s]	Amount [mole%]
CH4	1372.21924	14.1287

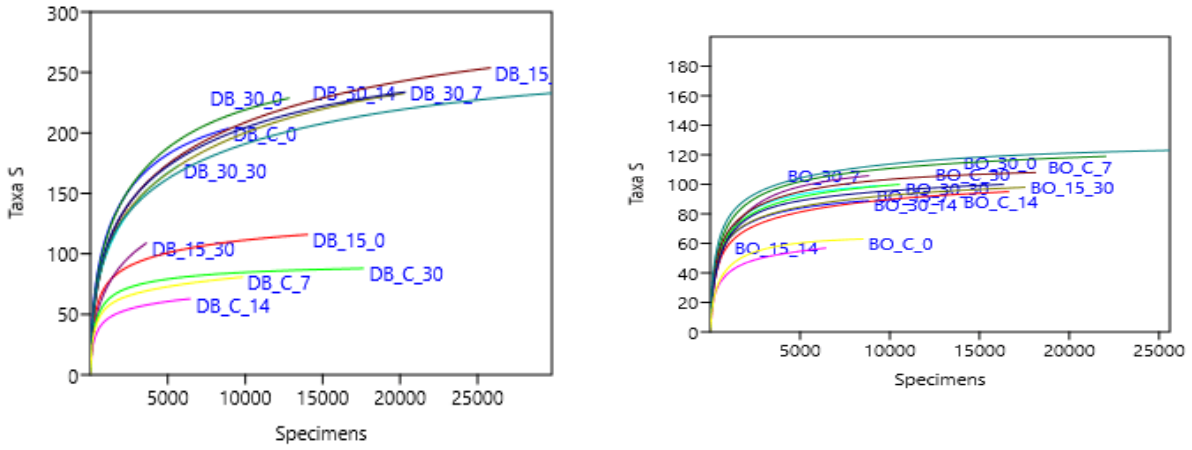
Data File C:\CHEM32\1\DATA\TREVOR\DB 30G ORUKO T1 2019-05-13 12-35-24.D
Sample Name: DB 30g Oruko T1

Totals : 14.1287

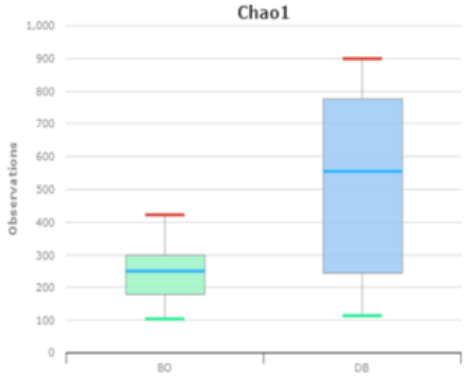
*** End of Report ***

Appendix Table 6.2: Bacterial community abundance and diversity indices during anaerobic digestion of chrome liquor samples supplemented with different levels of donkey dung based on 16S rDNA targeted amplicon sequencing.

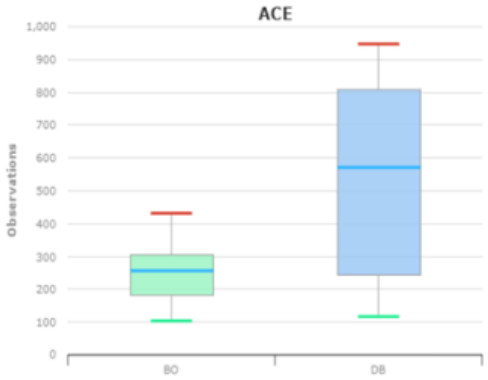
Chrome waste	Day	Donkey dung (gm)	Sample Name	ACE	CHAO	Jackknife	OTUs	Shannon	Simpson	Phylogenetic Diversity	Good's coverage (%)
BO	0	0	BO_C_0	117	119	123	108	1.60	0.526	261	99.8
		15	BO_15_0	326	313	335	301	3.98	0.058	548	99.9
		30	BO_30_0	361	347	375	331	3.89	0.054	499	99.8
	7	0	BO_C_7	288	283	300	271	3.40	0.095	487	99.9
		15	BO_15_7	257	254	268	247	4.09	0.042	407	99.8
		30	BO_30_7	433	421	457	411	3.80	0.092	448	99.5
	14	0	BO_C_14	194	189	202	176	2.29	0.358	377	99.9
		15	BO_15_14	105	101	108	91	1.91	0.344	232	99.7
		30	BO_30_14	173	168	180	162	2.97	0.205	351	99.8
	30	0	BO_C_30	255	247	264	239	3.38	0.106	420	99.9
		15	BO_15_30	273	264	282	260	3.71	0.059	434	99.9
		30	BO_30_30	252	244	261	225	3.42	0.099	421	99.7
DB	0	0	DB_C_0	655	642	687	553	4.73	0.024	817	98.5
		15	DB_15_0	290	597	307	248	3.09	0.202	471	99.7
		30	DB_30_0	840	528	898	663	4.44	0.054	1031	98.5
	7	0	DB_C_7	198	198	205	175	3.34	0.090	347	99.7
		15	DB_15_7	777	550	806	665	4.29	0.034	974	99.5
		30	DB_30_7	845	801	867	690	4.31	0.042	953	99.1
	14	0	DB_C_14	118	114	123	109	1.99	0.414	275	99.8
		15	DB_15_14	948	500	982	795	4.00	0.069	1072	99.3
		30	DB_30_14	771	733	787	619	3.80	0.089	962	99.2
	30	0	DB_C_30	200	196	207	190	3.69	0.055	371	99.9
		15	DB_15_30	301	391	307	219	2.68	0.288	405	97.9
		30	DB_30_30	491	469	499	376	4.17	0.041	626	97.9



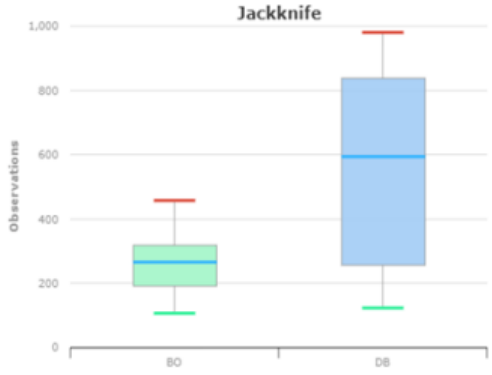
Appendix Figure 6.2: The rarefaction curve plot of the samples.



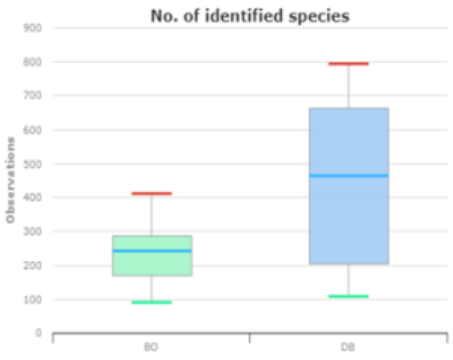
Wilcoxon rank-sum test: p=0.021*



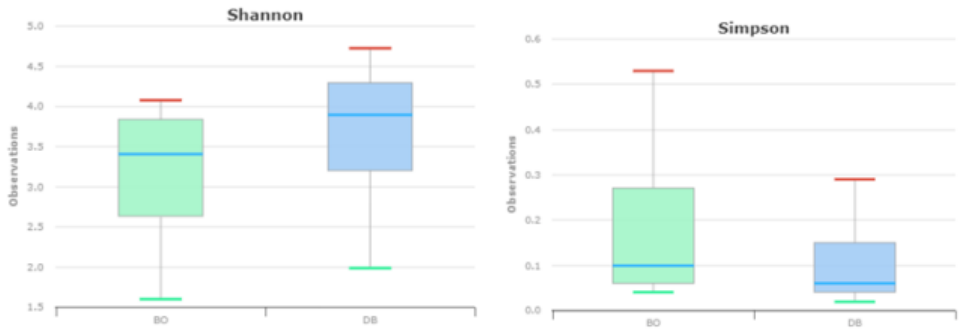
Wilcoxon rank-sum test: p=0.024*



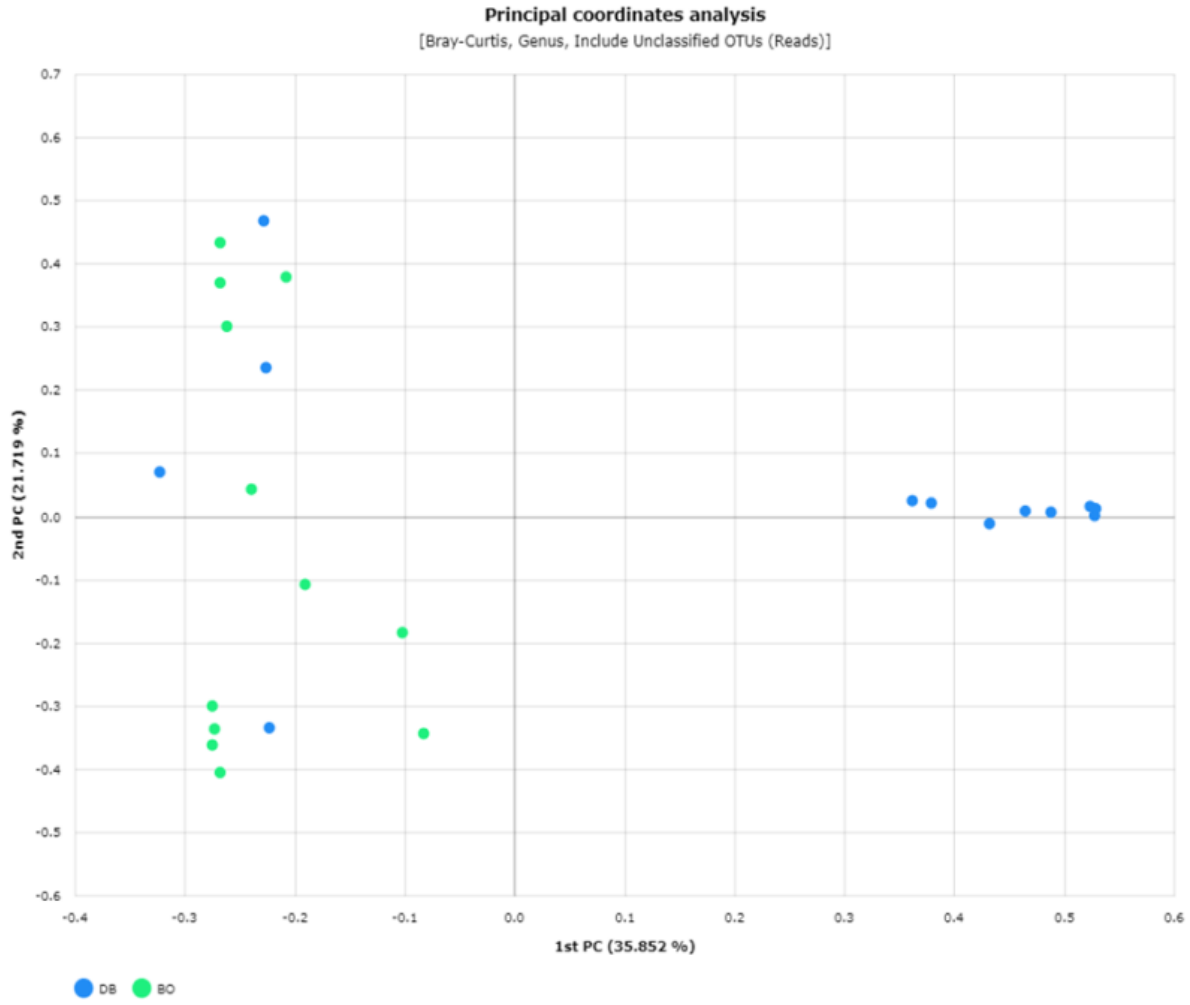
Wilcoxon rank-sum test: p=0.023*



Wilcoxon rank-sum test: p=0.073 (ns)



Appendix figure 6.3: Wilcoxon rank -sum test comparative analysis of bacterial community composition (richness) in BO and DB bioreactors.



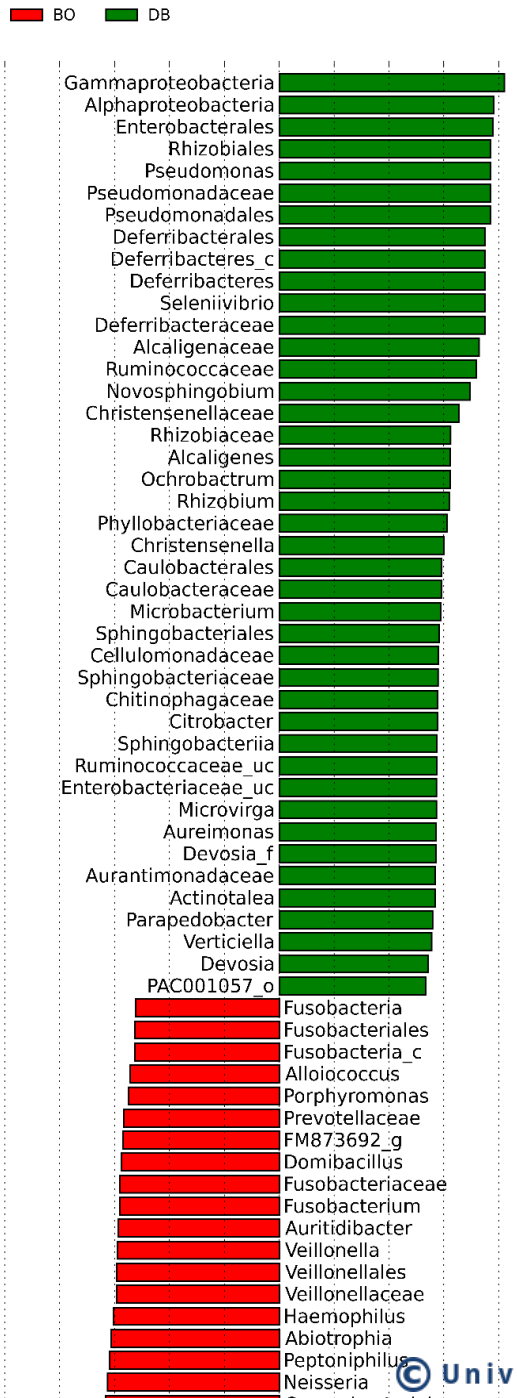
Appendix Figure 6.4: Principal coordinate analysis of bacterial community composition in DB and BO bioreactors

Appendix Table 6.3: Metagenomic data of bacteria at Phylum, Class and Genus

Phylum composition				
BO	**%**		**DB**	**%**
0,325703	32,5703	Proteobacteria	0,51698975	51,698975
0,16300967	16,300967	Actinobacteria	0,08695183	8,695183
0,01575817	1,575817	Bacteroidetes	0,05465	5,465
0,48283258	48,283258	Firmicutes	0,245655	24,5655
0,01269642	1,269642	ETC(under 1% in average)	0,00915308	0,915308
	0	Deferribacteres	0,08659992	8,659992
	Class composition			
BO	**%**		**DB**	**%**
0,03703833	3,703833333	Alphaproteobacteria	0,127241	12,7241
0,40292258	40,29225833	Bacilli	0,10220533	10,2205333
0,0331595	3,31595	Tissierellia	0,02057325	2,057325
0,16009642	16,00964167	Actinobacteria_c	0,08594008	8,59400833
0	0	Unclassified in higher taxonomic rank	0	0
0,03964708	3,964708333	Gammaproteobacteria	0,20442683	20,4426833
0,01042708	1,042708333	Negativicutes		#VALUE!
0,248068	24,8068	Betaproteobacteria	0,1839985	18,39985
0,03583975	3,583975	Clostridia	0,11355942	11,3559417
0,032801	3,2801	ETC(under 1% in average)	0,02722675	2,722675
	#VALUE!	Bacteroidia	0,0482275	4,82275
	#VALUE!	Deferribacteres_c	0,08659992	8,65999167
		Genus composition		

BO	%		DB	%
0,01632775	1,632775	Clostridium	0,02301958	2,301958
0,07852683	7,852683	Cutibacterium		0
0,01123808	1,123808	Haemophilus	0,00065075	0,065075
0,000901	0,0901	Unclassified in higher taxonomic rank	0,02220517	2,220517
0,0292535	2,92535	Streptomyces	0,02915125	2,915125
l0,08576425	8,576425	Staphylococcus	0,11211133	11,211133
0,21799033	21,799033	Delftia	0,03618017	3,618017
0,14646125	14,646125	Streptococcus		0
0,02073267	2,073267	Corynebacterium		0
0,01317892	1,317892	Abiotrophia		0
0,01179158	1,179158	Neisseria		0
0,027045	2,7045	Bacillus		0
0,04459333	4,459333	Rummeliibacillus		0
0,01765883	1,765883	Lysinibacillus		0
0,01984975	1,984975	Caryophanon		0
0,01929208	1,929208	Anaerococcus	0,31864	31,864
0,23939517	23,939517	ETC(under 1% in average)	0,02697758	2,697758
	0	Klebsiella	0,04005067	4,005067
	0	Brucella	0,08319925	8,319925
	0	Pseudomonas	0,01303583	1,303583
	0	Acetanaerobacterium	0,0103535	1,03535
	0	Ochrobactrum	0,03632933	3,632933
	0	Enterobacter	0,01499783	1,499783
	0	Bordetella	0,01449575	1,449575
	0	Achromobacter	0,01737833	1,737833
	0	Clostridium_g26	0,01611558	1,611558

	0	Escherichia	0,08659992	8,659992
	0	Seleniivibrio	0,01535925	1,535925
	0	Alcaligenes	0,01459508	1,459508
	0	Pantoea	0,01033492	1,033492
	0	Rhizobium	0,010625	1,0625
	0	Paracoccus	0,03538875	3,538875
	0	Parabacteroides	0,01220558	1,220558
	0	Christensenella		0



Appendix Figure 6.5. LDA score showing differentially abundant genera between BO and DB samples