DECONTAMINATION OF CHROME LEATHER WASTE AND ITS MODIFICATION FOR USE AS A COMMERCIAL FERTILIZER

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DECLARATION

I declare that this is my original work and has not been submitted for examination, award of a degree or publication. Where other people's work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirement

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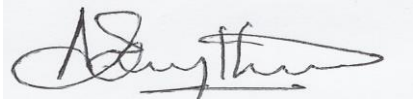

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DEDICATION

This thesis is dedicated to my dear loving wife, Mary Mukii Musyoka and my four children James Kavita, Mercy Ndululu, Emmanuel Muuo and Joshua Mumo.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrophotometry
ABNT	ABNT stands for the Brazilian “Associacao Brasileira de Normas Tecnicas.”
ANOVA	Analysis of variance
AP	Animal Production
ATSDR	Agency of Toxic Substances and Disease Registry
ATR	Attenuated Total Refluctance
BOD	Biochemical Oxygen Demand
BSE	Bovine Spongiform encephalopathy
CAN	Calcium Ammonium Nitrate
CEC	Cation Exchange Capacity
CI	Confidence Interval
COD	Chemical Oxygen Demand
CRD	Completely Randomized Design
CRFs	Controlled Release Fertilizers
Cr	Chromium
DAP	Di-Ammonium Phosphate
Dec	Dechroming
DEFRA	Department for Environment Food and Rural Affairs
DMY	Dry Matter Yield
ECe	Electrical Conductivity
EEF	Enhanced Efficiency Fertilizer
EPICH	Epichlorohydrin

ETP	Effluent Treatment Plant
FTIR	Fourier Transform Infrared
FVM	Faculty of Veterinary Medicine
GPS	Global Product Strategy
GPR	General Purpose Reagent
HERA	Human and Environmental Risk Assessment
HPs	Hydrolysed Proteins
HSD	Tukey Honest Significant Difference
HYP	Hydroxyproline
IR	Infrared
IULTICS	International Union of Leather Technologists and Chemists Society
KIRDI	Kenya Industrial Research and Development Institute
LARMAT	Land Resource Management and Agricultural Technology
MIP	Mercury Instruction Porosimetry
MN	Mineral Nitrogen
NBR	Navigation Base Reference
NEMA	National Environment Management Authority
NUE	Nutrient Use Efficiency
PAHs	Polycyclic Aromatic Hydrocarbons
PAR	Photosynthetic Active Radiation
PHPT	Public Health Pharmacology and Toxicology
Prolyl	Proline Residue (Residue C_4H_8NCO- of Proline)
PVA	Poly (Vinyl Alcohol)

RCBD	Randomized Completely Block Design
SPAD	Soil Plant Analysis Development
SPSS	Statistical Package for Social Sciences
SRFs	Slow-Release Fertilizers
SRM	Specified Risk Materials
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
UV-Vis	Ultra Violet-Visible
WRB	World Reference Base

DEFINITION OF TERMS

Anthropogenic	Environmental change caused/influenced by people directly or indirectly
Beamhouse	Tannery section dealing with preparatory stages for leather tanning
Bioaccumulation	Accumulation over time of a substance (contaminant) in a living organism
Dechroming	Extraction/removal of chromium from chrome tanned leather/leather waste
Detanning	Cleaving bonds between tanning agents and hide collagen to free the tans
Epoxidized PVA	Substituted PVA product that is generated by grafting epoxy groups to its OH^- group
Hydrolysis	Chemical process of decomposition involving bond splitting; $H^+ + OH^-$
N mineralization	Process by which inorganic nitrogen is obtained by decomposition of dead organisms and degradation of organic nitrogenous compounds, making it bioavailable as a nutrient for plant use
Pelt	Limed, delimed, bated or pickled hide or skin, which has not been tanned yet
Photosynthesis	Process by which plants use sunlight, $H_2O + CO_2$ to create O_2 + Glucose
Porosimetry	An analytical technique used to determine various quantifiable aspects of a material's porous structure e.g., pore diameter, surface area & bulk density
Recycling	Process of converting waste into reusable material
Tanning	The process of converting perishable raw hides and skins by the use of tanning agents into the permanent and imputrescible material; leather
Yield	Enhanced productivity (to supply or produce something positive; profit)

ABSTRACT

Chrome tanning is the most popular form of tannage worldwide due to the superior quality and versatility of the resultant leather compared to all other leather tanning materials. However, waste emanating from the production of chrome-tanned leather causes enormous disposal problems to human health and the environment due to the toxicity of chromium particularly in its hexavalent oxidation state. Besides, efforts to treat this type of leather waste either for chromium recovery, or for obtaining chrome-free collagen, have not been entirely successful for a number of reasons as investigated in this thesis. Thus, the current study assessed the amount of chrome leather waste generated by six selected tanneries operating in Kenya and mode of disposal of the waste. The assessment of leather waste generated in the six selected tanneries was done through a survey in which semi-structured questionnaires and key informant interviews were used as the instruments of data collection. Descriptive statistics were used to analyse the data collected in this study, and the analysis showed that 1,443,000kg of chromium-containing leather waste was generated by the 6 selected tanneries within a period of one month. This analysis further showed that out of the total amount of various types of leather solid wastes generated within one month in the selected tanneries, which was 2,112,560kg, 68.3% of this amount was actually chrome-tanned leather waste. The study also, established that the common methods of disposal of leather solid wastes at that time were; landfilling, open ground dumping and or incineration.

In addition, the study developed a new eco-friendly and cost-effective method for dechroming leather waste and modified the waste to enable its utilization as a slow-release organic fertilizer. The new method involved detanning, chromium extraction and complexation of the remaining traces of chromium with potassium oxalate. The method was able to extract up to 99.9% of chromium from the waste in 24 hours. The dechromed waste was subjected to mild phosphoric

acid hydrolysis to enhance controlled breakdown of peptide bonds in collagen to facilitate release of nitrogen and other nutrients, making them available for plant use. The collagen hydrolysate was modified further to make it more useful as a slow-release nitrogen fertilizer by treating it with 99% epichlorohydrin (EPICH). The modified collagen had 44% N, 21% P (as P_2O_5), 0.1% K (as K_2O), 0.2% Mg, and 1.8% Ca, 27.0% TOC and C:N ratio of 0.61. The slow-release organic fertilizer was formulated using modified collagen and ground maize cobs as filler material. The maize cob based filler material was found to have high carbon to nitrogen ratio (C:N) with the following nutrient composition: 2.8% N, 14.04% P (as P_2O_5), 0.3% K (as K_2O), 7.5% Mg, and 1.6% Ca, 38.12% TOC and C:N ratio of 13.61. The high C:N ratio contributed to the slow release of organic nitrogen into the soil in form of nutrients by slowing down the decomposition of the organic fertilizer to enable the release of nutrients at a rate that matches plant requirements. Besides, the filler contributed to the nutrient content of the new fertilizer.

This study also, assessed the N mineralization rate of the new fertilizer formulation in a laboratory soil incubation experiment to determine the rate at which inorganic nitrogen is obtained by decomposition of organic matter and degradation of organic nitrogenous compounds in the organic fertilizer, making it bioavailable for use by plants following fertilizer application to the soil. The highest mineralization rate of organic N into NO_3-N was observed during the 12^{th} week, and the one of organic N into NH_4^+-N was observed during the 16^{th} week of incubation.

Results of single application of the new fertilizer formulation during planting (under greenhouse fertilizer trials) were subjected to ANOVA using Genstat 14^{th} edition at 95% confidence interval (CI), and indicated that growth and productivity (yield) of kale and capsicum were comparable with the use of DAP and CAN conventional fertilizers during planting and top-dressing time, respectively. Application of the new fertilizer in growth and productivity of maize did not show

favourable results. However, use of different rates of this new fertilizer showed a significant difference ($P < 0.05$) on mean height of the maize stalk and chlorophyll content. The new fertilizer formulation (if applied at optimal rates), as found out in this study, was significantly better than DAP and CAN conventional fertilizers, particularly in terms of increasing chlorophyll content and number of leaves per capsicum plant. However, mean fruit yield and whole plant biomass for the capsicum crop under various fertilizer treatments did not show any significant difference ($P > 0.05$). The optimal rate of application for the new fertilizer formulation was 1238.50kg/ha fertilizer blended with 530.79kg/ha filler in enhancing growth and productivity of capsicum, while the optimal application rate of the new fertilizer toe enhance growth and productivity of kale was either 884.64kg/ha fertilizer blended with 884.64kg/ha filler or 1769.28kg/ha fertilizer without filler.

The study concluded that chrome leather waste can efficiently be dechromed using the new method described in this study and the resulting chrome free waste can be modified using 99% EPICH to make a useful organic fertilizer. Large-scale production of the new organic fertilizer will reduce disposal to the environment of chrome leather waste that usually causes environmental pollution. The study recommends up-scaling and optimization of the new leather wastes dechroming and collagen modification methods described in this study for large-scale decontamination of chrome leather waste and commercial production of the new slow-release organic fertilizer. In addition, the study recommends addition of potassium rich supplements to the organic fertilizer to boost its potassium levels, as well as test the suitability of the new fertilizer for use to grow various other types of crops.

CHAPTER ONE - INTRODUCTION

1.1.Background Information

The leather manufacturing process is widely known for generating enormous quantities of solid wastes, which if not properly disposed of, are highly problematic both to the environment and public health (Ramasami, (2001); Kanagaraj *et al.,* (2006); Fela *et al.,* (2011). Processing of one ton of hides from raw to finished stage generates approximately 800kg of tannery solid wastes as reported by Sundar *et al.,* (2011). Kanagaraj *et al.,* (2006) analysed such leather industrial wastes and found that they were constituted of fleshings (50-60%); chrome-shavings, chrome-splits and buffing dust (35-40%); skin trimmings (5-7%) and hair (2-5%). This is a clear indication that the highest proportion of tannery solid wastes emanates from the beamhouse (80%) followed by tanyard section of the tannery (19%) and lastly, the finishing section generates the remaining 1%. Recycling of such kind of solid wastes into useful products, which include; leather boards, carpets from recovered hair, gelatin (for food supplements), office glue, building bricks, fatliquors, cosmetics, photographic films, animal feeds and fertilizers among others, is possible, leading to reduced environmental contamination and an expanded revenue base for the leather industry (Ramasami, 1999). Literature values indicate that the United States of America (USA) alone, produces around 60,000 metric tons of chromium-containing leather waste (chrome-shavings), and the global production of this type of leather waste is 600,000 metric tons (Cabeza *et al.,* 1998).

Disposal of leather solid wastes (including chrome-tanned waste) has traditionally been done by landfilling. However, rapid increase of human population and urban development over the years has led to an acute shortage landfill sites vis a vis diminishing land availability (Gakungu *et al.,* 2011; Mwanzia *et al.,* 2013; Okalebo *et al.,* 2014). In addition, landfilling as a mode of solid

waste disposal is costly and environmentally unfriendly for a material that can be put into better utilization through recycling (Colak *et al.,* 2005). For example, chrome tanned solid wastes can be recycled into useful products for sale to generate revenue, or be used as raw material for other industries (Li *et al.,* 2019). Several researchers around the world have previously done a lot of work (but without much success due to efficiency issues and cost implications as well as sophistication of the methods involved), on the hydrolysis of leather waste to recover amino acids and peptides for use in various applications such as in the formulation of animal feeds and fertilizers (Brown *et al.,* 1996; Cabeza *et al.,* 1999; Silveira *et al.,* 2002; Mu *et al.,* 2003; Kamaludeen *et al.,* 2003; Rivela *et al.,* 2004; Saravanabhavan *et al.,* 2004). Besides, viable methods on chromium recovery from chrome tanned leather waste for re-use in tanning have also been developed (Sundar *et al.,* 2011; Jiang *et al.,* 2016).

Recycling of chromium-containing leather solid wastes can be done either directly or indirectly as described by Colak *et al.,* (2005) and Li *et al.,* (2019). utilizing the chrome tanned solid wastes as raw material for other industries after undertaking only a simple preparation (without having to extract the chromium first) is what is usually referred to as direct recycling. Exposing chrome tanned solid wastes to an environment with hot and humid conditions increases the chances of Cr^{3+} present in the wastes getting oxidised to its hexavalent state (Cr^{6+}), causing secondary pollution problems as a result (Imai and Okmura, 1991). A bulky of dechroming methods for tannery solid wastes that are currently in use have been found to cause secondary pollution, which is a type of pollution that results from the reaction between the main pollutants in the wastes (primary source) and other pollutants in the atmosphere such as greenhouse gases (e.g. SO_2, Cl_2, NO_2, etc.) (Ferreira *et al.,* 1999).

It has already been established in previous research that processing of one ton of wet-salted hides from raw to finish generates slightly above 600kg of solid wastes consisting of a considerable proportion of tanned waste (i.e., 200kg of shavings, trimmings, splits and buffing dust) with a minimum of 3.5kg of chromium if the tanning agent of choice was basic chromium (III) sulphate (Veeger *et al.,* 1993; Taylor *et al.,* 1998; Langmaier *et al.,* 1999). This state of affairs is common in nearly all tanneries worldwide due to the fact that chrome tanning is the most popular form of tannage globally with at least 90% usage in leather production as it forms relatively more stable cross-links with hide collagen carboxyl groups compared to other tannages (Sundar *et al.,* 2002; Aravindhan *et al.,* 2004). The versatility of chrome tanned leather (i.e., ability of wet-blue leather to be processed further into different types of finished leathers), high hydrothermal stability and strength characteristics of the final leather makes chrome tanning the most preferred choice of tannage in comparison with all other types of tannages (Scopel *et al.,* 2015).

It has been estimated that chrome tanned waste contains up to 4.5kg (w/w) of chromium (III) (Ozgunay *et al.,* 2007). Low concentrations of chromium (III) are needed in the human body as an essential nutrient. However, chromium (III) can easily be oxidised into its hexavalent oxidation state (Cr^{6+}) becoming carcinogenic as a result (Zayed and Terry, 2003; Welling *et al.,* 2015). The usefulness of trace levels of chromium (III) in the human body is its ability to form an organic complex known as Glucose Tolerance Factor (GTF) that regulates the uptake of glucose by human cells through its interaction with the pancreatic hormone, insulin for potentiation (i.e., enhancing insulin secretion) and normal glucose metabolism (Barrett *et al.,* 1985; ATSDR-HE-2001-2005). Toxicity of chromium (VI) compounds at both acute and chronic levels is well known, although the dose threshold effect of the chromium element is yet to be

determined accurately for ease of regulation in spite of some risk assessments that have already been undertaken in the past on the toxicity levels of chromium (VI) compounds (Belay, 2010).

Hexavalent chromium resulting from the oxidation of chromium (III) in dumped or landfilled chrome leather waste may be washed off from such waste and contaminate ground water, and become a health hazard as a result (Gauglhofer and Bianchi, 1991). It has already been found that improperly disposed of chromium-containing tannery solid wastes, can contaminate ground water with chromium present in the wastes and this chromium can persist in such water bodies for very many years without getting depleted (ATSDR-HE-2001-2005).

Generally, chromium-containing leather waste is a health hazard and a specified risk material (SRM) as far as human health and environmental pollution are concerned (Marchese *et al.*, 2008). These researchers (Marchese *et al.*, 2008), studied the accumulation rate of chromium in clams, crabs, fishes and four fresh water plant species, and found that there was a high concentration of chromium in all the specimens investigated in this experiment. This experiment demonstrated that the tendency for chromium bioaccumulation is very high. It was also, a clear indication that the problem of chromium accumulation in plant as well as animal species becomes prevalent and of grave concern to human beings, who are normally placed at the top of the food web, in terms of its hazardous and bioaccumulation effects.

Leather waste disposal in Kenyan tanneries is mainly carried out through a number of methods, the most common of which are; open dumping, incineration, landfilling, and use as a fuel source in most urban slum areas (Mwondu *et al.*, 2020). Open dumping and landfilling options are not sustainable because they pollute the environment causing toxicity of plants and animals (Jiang, 2016).

Studies done in recent decades by You, (2009); Yang, (2011); Dixit *et al.,* (2015) and Kanagaraj *et al.,* (2015), on the development of technologies to remove chromium from chrome-tanned leather solid wastes, and recovery of some useful products, such as collagen, have not been successful in completely removing chromium from the tanned leather waste to enhance its usefulness as an organic fertilizer. Even low levels of chromium in the waste can cause adverse effects on animal and human health and on the environment as described by Li *et al.,* (2003); Ding *et al.,* (2012) and Kanagaraj *et al.,* (2015). Cr (VI), which shows strong effects of toxicity (both acute and chronic) can easily be inhaled or ingested by humans and animals, and can also be taken in through skin ulcerations leading to high chances of causing cancer, due to its capacity to react with genetic material (Mordenti and Piva, 1997; Zayed and Terry, 2003).

It was established that toxicity in plants due to uptake of chromium from the soil is very real and that, its effects are primarily a function of the metal speciation (Shanker *et al.,* 2005). This speciation (gradual change of the metal's oxidation state) is the one that determines the uptake, translocation and ultimately; the accumulation of chromium in plants. In addition, chromium affects both growth and productivity of crops. Chromium accumulation in plants has been found to retard their growth and development (Cervantes *et al.,* 2001), particularly if it is in the form of Cr (VI). Previous research has established that the plant-available form of chromium in most soils occurs in very small concentrations, and its lack of solubility and hence low uptake by plants was demonstrated in the work done by Alloway, (1990) as low concentrations of the chromium element in plant samples that he investigated. It was found in the same study that the concentrations of chromium in the foliar parts of investigated plant samples had very little relationship with the total content of chromium present in soil where the plants had been grown.

Srivastava *et al.,* (1999) showed that the complexation of chromium with organic acids facilitates its availability to plants.

Chromium accumulation in plants has also, been shown to cause reduction in root systems in phaseolus and produced reddish brown colouration in the petioles and leaf veins of white beans (Rauser, 1987; Vazquez *et al.,* 1987; Corradi and Gorbi, 1993). Progressive stages of chromium toxicity in plants are usually manifested as chlorosis and necrosis symptoms. Work done by Ciavatta *et al,* (1985), and Oliveira, (2012) confirmed that the process of photosynthesis in plants can adversely be affected by chromium stress in the areas of enzyme activity, phosphorylation, electron transport and carbon dioxide fixation. Besides, studies undertaken by Tripathi and Smith, (2000), and also, Panda and Khan, (2003) reported reduced quantities of carotenoids, total chlorophyll content, chlorophyll a and chlorophyll b. It is against this background therefore, the present study aimed at developing a new method of extracting nearly all chromium from leather solid wastes using appropriate technology prior to modifying the chromium-free collagenic material for use in agricultural production as a value-added organic fertilizer.

1.2. Study Objectives

1.2.1. Overall objective

To decontaminate and modify chrome tanned leather waste for use as a commercial fertilizer.

1.2.2. Specific objectives

i. To assess the levels of chrome leather waste generated in selected Kenyan urban tanneries.

ii. To come up with a new method of dechroming leather solid wastes.

iii. To modify the collagenic material in dechromed leather waste to produce fertilizer.

iv. To assess the suitability of decontaminated and modified collagenic material as a fertilizer.

1.3.Hypothesis

H_o: Chrome tannery waste cannot be dechromed to enable collagen modification to make a useful organic fertilizer.

1.4.Problem Statement and Justification.

As a matter of necessity, the process of leather making leads to the generation of very large quantities of leather solid wastes. This scenario is caused by a number of reasons, the two major ones being; the fact that the leather making protein, collagen, must be freed from all non-collagenous skin components prior to its combination with tanning agents during leather processing. The other major reason for this scenario is the intrinsic nature of leather processing steps which also, involve a wide variety of chemicals leading to a considerable increase in the generated tannery solid wastes as a majority of the chemicals are offered in excess of the required quantities in order for them to function well (Sundar *et al.*, 2011). However, there was no data in the country documenting the amount of waste generated by tanneries and mode of disposal in Kenya. Huge quantities of waste are however a common site in the vicinity of tanneries in the country as a result of through open dumping.

Such huge quantities of tannery solid wastes, a great proportion of which is contaminated with chromium due to the usage of basic chromium (III) sulphate as a major tanning agent in the global leather industry, have posed major disposal problems both to the environment and public health for many years. This is because of the high possibility of the chromium (III) in such

wastes getting oxidised into chromium (VI), which has already been confirmed as toxic, mutagenic, carcinogenic and teratogenic especially if it is in high concentrations. In fact, chromium (VI) toxicity even at low concentrations, has been found to adversely affect a considerable number of plants and animals as well as bacteria inhabiting aquatic environments.

Previous research has shown that there has been very little or no effort at all over the years, to utilize tannery solid wastes in making useful products instead of leaving them unattended to in the environment of the tannery, dumping them in open grounds, landfilling and/or incineration as is currently practised in most tanneries around the world. Consequently, better options for disposal of tannery solid wastes (particularly chromium-containing wastes) are required so as to reduce environmental pollution and public health issues caused by such wastes if not properly managed. Work by a number of researchers to dechrome waste to enable utilization of the waste to produce value added products have not succeeded in removing all the chromium in the waste, with the various researchers reporting between 71-90% levels of chromium extraction. Therefore, the greatest challenge that needed to be addressed by this study was how to come up with new technologies for decontaminating chrome leather wastes that are less cumbersome, more efficient, eco-friendly and sustainable for their safe and economical disposal or utilization to make useful products such as fertilizer after a few modifications.

1.5. Study Limitations

The assessment of the levels and types of leather solid wastes generated and their mode of disposal was carried out only on a few pre-selected tanneries in Kenya due to limited resources in terms of finances and time as well as technical personnel and transport to cover the whole country. The level of engagement with respondents was below expectations due to their low awareness levels on leather waste management and poor data inventory.

CHAPTER TWO - LITERATURE REVIEW

2.1.Leather Tanning and its Environmental Impact

The process of converting hides and skins into leather is generally referred to as leather tanning. The process commonly uses chromium and other chemicals in production of strong and quality leather. Huge quantities of leather solid wastes are widely known to be generated in the course of leather processing. Such wastes are highly polluting to the environment and a threat to human health as reported by Cabeza *et al.,* (1998); Sundar *et al.,* (2011); Jiang *et al.,* (2016), and Li *et al.,* (2019). Leather manufacturing produces various types of waste, most of which, are contaminated with chromium which is toxic to animals, plants and to the environment in general. Chromium is well known as a major source of contamination to soil and ground water, especially in its Cr^{6+}oxidation state, because of its mutagenic and carcinogenic potential (Famielec, 2020). If the wastes from leather manufacturing are not handled properly, they could pose serious environmental and public health problems. According to Yilmaz *et al.,* (2007); Pati *et al.,* (2013); Kanagaraj *et al.,* (2020); Khatoon *et al.,* (2017), the increasing environmental and public health concerns of the leather industry due to such huge quantities of leather waste generation has triggered the need for renewed research and development for purposes of making the leather industry sustainable. The waste generated in the course of leather processing occurs in two categories namely; untanned waste - constituting of fleshings and scourings (50-60%), skin trimmings (5-7%) and hair or keratin (2-5%); and tanned waste – mainly composed of chrome tanned leather shavings, splits and buffing dust amounting to 35-40% as well as finishing waste (at least 1%) (Kanagaraj *et al.,* 2006).

2.2. Current Methods for Disposal of Tannery Waste

The common methods currently being used for leather waste disposal are, open dumping, incineration, landfilling, and use as a fuel source (Mwondu *et al.,* 2020). Open dumping and landfilling are the preferred methods of disposal of tannery waste by many tanners as they find these methods easier and more economical, in spite of the fact that it is not an environmental solution. The open dumped and landfilled material generates an environmental liability requiring monitoring for years (Rigueto *et al.,* 2020). Open dumping and landfilling leads to a secondary pollution problem due to the diffusion of chromium leachate from such wastes (Zhao *et al.,* 2022). The incineration method for tannery solid wastes disposal, as presented by Luo, (2014), is not safe as it leads to air pollution and a waste of collagen, which is a highly valuable resource in tannery solid wastes. The collagen accounts for approximately 90% by weight of tannery waste (Zhao *et al.,* 2022). Other methods of disposal include recycling and utilization to produce valuable products. However, recycling and utilization of this kind of waste without having to extract the chromium first, reduces the value of the collagen and limits its utilization. Therefore, it is important to extract the chromium first prior to its recycling and utilization in useful applications. However, extraction of chromium is costly and time consuming and hence, these methods have not been preferred by tanneries.

A number of ways of utilizing these solid wastes have been investigated in the past by Prentiss and Prasad IV, (1981); Lollar, (1981); Ramamoorthy *et al.,* (1989); Taylor *et al.,* (1990); Cot *et al.,* (2003), and Fella *et al.,* (2011), whereby chrome leather splits and trimmings have successfully been used as raw materials in the manufacture of protein flavour, reconstituted collagen, animal feeds and fertilizers as well as office glue and gelatin **(Figure 2.1).** Using these procedures; it has been possible to produce adhesives, gels and films of high molecular weight

gelable protein fraction from chrome leather shavings under mild alkaline conditions (Kanagaraj *et al.,* 2006).

These tannery solid wastes, which are usually rich in proteins, have been used (after hydrolysis with acids, alkalis, or enzymes) as raw materials in the manufacture of cosmetics and pharmaceutical products (Masilamani *et al.,* 2016). Tannery solid wastes have also been used as raw materials in the manufacture of biofuels and in the production of fats and oils (Sanek *et al.,* 2015). Efforts to valorize bio-collagen leather solid wastes to obtain activated carbon have been made by Cabrera-Codony *et al.,* (2020). According to Selvaraj *et al.,* (2020), cattle hides can be used to prepare high quality biopolymers, while leather collagen hydrolysates can be used as raw materials in the production of sound-absorbing materials.

It was demonstrated in some research work done by Bautista *et al.,* (2015); Majee *et al.,* (2019), and Sathish *et al.,* (2019) that, several useful products can be made from the protein fractions of tannery solid wastes including surfactants, organic NPK fertilizers and biodegradable re-tanning agents. However, direct utilization of chromium-containing solid wastes (without having to remove the chromium first) for manufacture of organic fertilizer is very risky due to the toxicity of chromium.

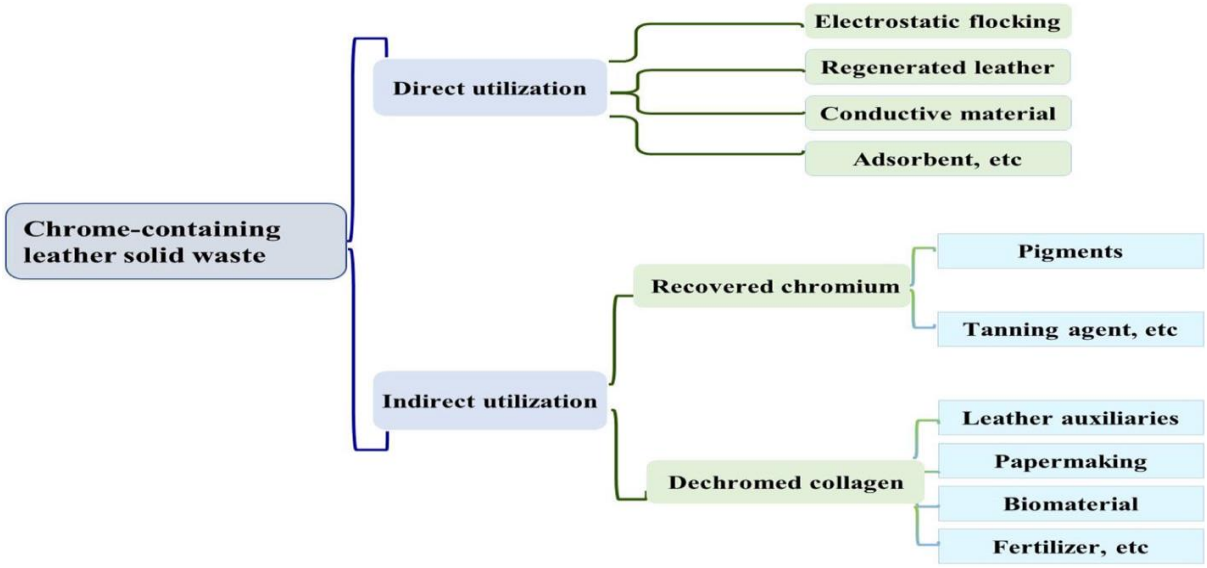


Figure 2.1: Direct and indirect utilization of chromium-containing leather solid wastes

2.3. Use of Chromium in the Leather Industry and its Toxicity

More than 90% of leather production worldwide (approximately 18 billion sq. ft. per annum) is done by chrome tannage as described by Belay *et al.*, (2010). The chromium tanning process is normally carried out with basic chromium (III) sulphate as the tanning agent of choice due to superior qualities and versatility of the resultant leather compared to all the other types of tanning materials (Thorstensen, 1993; Wachsmann, 1999). This tanning agent is also readily available and the tanning process takes much less time as compared to vegetable tanning, which takes 3 days for this part of the process (instead of the 24 hours or even less for the complete chrome tanning process leading to the production of high quality leather, which is stretchable and hence, excellent for manufacturing handbags and garments (Nigam *et al.*, 2015). However, one major disadvantage of using basic chromium (III) sulphate as the tanning agent of choice is that there is a possibility of the chromium (III) in this salt getting oxidised into chromium (VI) under conditions of high pH and temperature values, unsuitable storage and presence of UV lights as well as fatliquoring the leather with unsaturated fatliquors (Basaran *et al.*, 2008). It is in

this regard therefore, many countries around the world have imposed chromium (VI) limit levels of as low as 3-50ppm in leather emanating from tanneries within their respective jurisdictions (Chaudhuri and Sarkar, 2001). Although vegetable tanning agents are biodegradable, they are actually less environmentally friendly as compared to chrome tanning agents due to the fact that vegetable tannages produce relatively large volumes of sludge leading to high COD and BOD levels. Moreover, vegetable tanned leathers (unlike chrome tanned leathers, which are versatile), have specific applications, and have different physical properties (Belay *et al.*, 2010).

Unfortunately, out of the total chrome offer of around 8% (based on lime-fleshed pelt weight) in chrome tannage, only 60% of this chrome offer is fixed onto hide collagen, leaving the remaining 40% to go to the drain as chrome sludge (Belay *et al.*, 2010). Accumulation of chrome sludge in tannery wastewaters (effluent) has over the last few decades become a major environmental problem in the tanning industry in terms of the options available for its safe disposal as a by-product of tannery effluent treatment (Wionezyk *et al.,* 2006). According to Altaf *et al.,* (2008), tannery effluents contaminated with chrome sludge have been found to adversely affect the mitotic process of germinating seeds and also, retard the seed germination process in extensively cultivated pulse crops. Kolomaznik *et al.,* (2008) confirmed that chromium (VI) is toxic, mutagenic, carcinogenic, and teratogenic especially if it is in high concentrations. The reason for this scenario is that chromium (VI) possesses a very high membrane penetrative capacity and a strong oxidising ability (Paustenbach *et al.,* 2003). According to the priority list of hazardous substances, chromium is ranked at the 18th position (ATSDR, 2010).

It has been suggested in literature that there are two major routes through which heavy metals find their way into the human body namely; ingestion of food and contact of the human body

with any object or piece of clothing that are contaminated with such heavy metals (Basaran *et al.,* 2006). The three main oxidation states of chromium are +2, +3, and +6; with the trivalent oxidation state being the most stable. Cr^{3+}, an essential element to mammals is required in trace concentrations and due to its low solubility in water, it is less immobile in aquatic systems as compared to the other oxidation states. Cr^{6+} toxicity to a considerable number of plants and animals as well as bacteria inhabiting aquatic environments is more severe than Cr^{3+} toxicity even at low concentrations. However, it has been found that some of these bacteria are resistant to even high levels of Cr^{6+} toxicity (Altaf *et al.,* 2008). The same researchers also established that there was some relationship between the metal resistant/tolerant bacterial count in soil and total chromium content in the same soil. Cr^{3+} and Cr^{6+} have been found to co-exist in natural water bodies with contrasting toxicities, motilities, and bioavailability as described by Marchese *et al.,* (2008). However, Cr^{6+} has been shown by the same researchers to be relatively more toxic, soluble in water and with a strong oxidising ability that can quite easily damage cell membranes.

Global chromium contamination levels in soils has been rising steadily over the last few decades due to disposal of chromium-containing tannery wastes through landfilling, which is the predominant mode of disposal of such wastes under the assumption that the chances of Cr (III) present in the waste getting oxidised to its highly toxic Cr (VI) oxidation state are very slim (Belay, 2010). Contrary to this assumption, studies conducted by Mwondu *et al.,* (2020); Oruko *et al.,* (2021), and Mwondu *et al.,* (2021) have established that the chances of Cr (III) getting oxidised to Cr (VI) during leather production and/or landfilling of chromium-containing leather solid wastes are significantly high. Considerable quantities of Cr (VI) have been detected in soil samples, water bodies (both surface and underground water) and also, in the food chain; a

situation which raises critical questions on the way chrome tanned leather wastes are currently being disposed of (Mazumder *et al.,* 2013; Kinuthia *et al.,* 2020; Alam *et al.,* 2020).

Although Cr (III) is considered to have a high thermodynamic stability, if it comes into contact with certain naturally occurring mineral species (particularly the oxides of manganese) it gets oxidised to Cr (VI), which is highly biodegradable at high pH levels, mobile and consequently; causes the contamination of the ground water resource in soil (Avudainayagam *et al.,* (2003).

According to Fuck *et al.,* (2011), a significant number of leather and leather products have been found to contain traces of Cr (VI), which have caused major discomfort in the leather industry due to the fact that it is only Cr (III) that is used in leather tanning but not Cr (VI). Several researchers notably; Suresh *et al.,* (2001); Sundar *et al.,* (2002); Rius *et al.,* (2002), and Ganeshjeevan *et al.,* (2004), have done a lot of research work to establish why Cr (III) gets oxidised to Cr (VI) either during leather production or storage; with a view to reducing this menace, or possible complete elimination of the use of chromium in tanning. The outcome of these studies indicate that chrome tannage is most likely going to remain as the most popular form of tannage in the leather industry for a long time due to the superiority of the resultant leather that is produced through this type of tannage and also, the simplistic manner in which the chrome tanning process is carried out (Mazumder *et al.,* 2013).

Cleaner technologies in leather manufacturing aimed at reducing chromium in the effluent have been tried in some tanneries in the past (Moore and Ramamoorthy, 2001). Some of these technologies such as chrome recycling (whether directly or indirectly) and high exhaust chrome tanning are not able to completely get rid of chromium in tannery exhaust liquors especially the ones emanating from the post-tanning (wet-end) section of the tannery. For instance,

conventional chrome tanning produces exhaust chrome liquor amounting to 1500-3000ppm (parts per million) of Cr^{3+}, which can be reduced to as low as 500-1000ppm of chromium if high exhaust chrome tanning methods are followed (Aravindhan *et al.,* 2004). However, these discharge limits are still too high as the recommended discharge limits for Cr (III) into water bodies are supposed to be in range of 1-5 mg/l, while the discharge limits for Cr (III) into the municipal sewer systems should be in the range of 1-20 mg/l. Therefore, for tannery effluent treatment plants (ETPs) to cope with these discharge limits for Cr (III), they would need to be 200 fold more efficient in treating chromium-containing tannery effluent so as to be allowed to discharge the treated effluent directly into natural water bodies such as rivers or lakes, a situation that has been found to be impractical in most tanneries as described by Tadesse *et al.,* (2006).

Efforts to overcome this challenge by trying to use combination tannages to replace chrome tannage such as using combinations of organic tanning agents and some metallic cations particularly titanium, zirconium, magnesium and aluminium have not yielded satisfactory results in the resultant leathers as compared to chrome tannage in many aspects (Haroun *et al.,* 2008; Musa and Gasmelseed, 2013). Therefore, a suitable alternative to chrome tanning agents must possess the following characteristics; readily available in large quantities, cheap, environmentally friendly, non-toxic and offer superior or equivalent qualities on the resultant leather as compared to chrome tannage. When compared with some other elements such as Hg, Cd, Pb, Ni and Zn; the toxicity of Cr (III) to mammalian and aquatic organisms has been found to be relatively lower as described by Belay, (2010), who attributed this to the low solubility and mobility levels of Cr (III) compounds in soil. Consequently, such characteristics of Cr (III) compounds make them unavailable to plants. Contrary to this, Cr (VI) is very toxic. Kisku *et al.*, (1999) did some further investigations on Cr (VI) toxicity in fields contaminated with industrial

effluent and found that Cr (VI) compounds are potentially toxic to some selected plant species as they gave a mobilization ratio of less than 5. These researchers also found that weeds growing in the same fields had mobilization ratios of greater than 5 depicting healthy morphology in the beginning of flowering stage as opposed to the selected plant species that were investigated in the same experiment.

Four fresh water plant species together with some crabs, clams and fishes were used in a study carried out by Marchese *et al.,* (2008) to investigate chromium accumulation potential with the following outcome; all the crabs, clams and fishes as well as the four fresh water plant species showed high concentrations of chromium within their systems as a confirmation of the high accumulation potential of this metal. This is a clear indication that human beings, who are ranked at the top of the food web, are exposed to the highest risk of chromium toxicity and bioaccumulation effect especially chromium (VI).

2.4. Chromium Containing Tannery Waste

Chromium-containing tannery waste is mainly from tanned leather solid wastes, which include chrome shavings, splits and trimmings that are produced in huge quantities in the leather industry. According to Rao *et al.,* (2002), chrome tanned leather shavings amounting to approximately 800 million kg (0.8 million tons) are generated in the global leather industry every year. This is a clear indication that the challenge posed by chromium-containing leather solid wastes is real considering that the most common mode of disposal of these wastes has traditionally been either through landfilling or incineration as investigated by Mwondu *et al.,* (2020), and Zhao *et al.,* (2022). The main component in chrome tanned leather waste is collagen, which accounts for approximately 90% of the total weight of the waste (Zhao *et al.,* 2022). Due

to the toxicity of chromium, it is important to extract the chromium from this kind of waste prior to its utilization in various useful applications.

2.5. Methods for Dechroming Leather Waste

Many researchers, who most notably include; Taylor *et al.,* (1997); Sun *et al.,* (2003); Catalina *et al.,* (2007); Malek *et al.,* (2009); Paul *et al.,* (2013); Adeoye *et al.,* (2014); Ding *et al.,* (2015); Ariana *et al.,* (2018); Kokkinos *et al.,* (2019), and Famielec *et al.,* (2020), have tried several treatment options of chrome tanned waste to extract and recover the chromium in the past but without much success. These methods occur mainly in four categories namely; acidic hydrolysis method (Rai *et al.,* 2009), alkaline hydrolysis method (Liang *et al.,* 2010), enzymatic hydrolysis method (Chi *et al.,* 2012) and a hybrid of the various dechroming methods as described by Hu *et al.,* (2010); Ding *et al.,* (2015), and Mwondu *et al.,* (2021).

2.6. Use of Tannery Waste as Fertilizer

The substantial amounts of nitrogen and organic matter available in tannery wastes (mainly from collagen) have prompted researchers to make several investigations on the possibility of using this material as fertilizer, but the presence of chromium in such wastes has been a limiting factor (Barylska and Plucinska, 2015). These wastes are generally categorised as Class 1 dangerous/hazardous substances due to their direct toxicity emanating primarily from the chromium present in chrome tanned leather wastes (ABNT NBR 10005). It has been established that each piece of chrome tanned leather results in 2 to 3kg of chrome shavings (Alves and Barbosa, 2013). Experiments conducted by Zhao *et al.,* (2022) using zebrafish embryos to investigate the toxicity levels of collagen hydrolysates derived from chrome leather solid wastes showed that at concentrations of less than 0.45mg/L there was no effect on either the mortality or hatchability rate of the zebrafish embryos. However, at concentrations > 0.65mg/L high death

rates of the zebrafish embryos were recorded (Zhao *et al.,* 2022). This toxicity is mainly attributable to the residual chromium in such collagen hydrolysates when used in high concentrations. The recommended WHO safe limits for residual Cr^{6+} in wastewaters is 0.1mg/L.

Comparative assessment of fertilizer derived from tannery waste with other organic fertilizers found tannery waste derived fertilizer to be relatively better in terms of absorption/uptake by plants leading to their enhanced growth and productivity (yield) as well as resistance to diseases and increased rate of photosynthesis in such plants (Zhao *et al.,* 2022). However, the presence of high concentrations of heavy metals especially chromium, reduced the usefulness of this product as a fertilizer to promote plant growth and productivity (Rigueto *et al.,* 2020). Apart from chromium, collagen hydrolysate fertilizers have been found to contain very minimal amounts (if any) of other common heavy metals including; Ag, Cd, As, and Pb (Zhao *et al.,* 2022). Therefore, to enable successful use of collagen hydrolysates derived from tannery wastes as fertilizer, any chromium present in such wastes must be removed completely.

2.7.Nitrogen Mineralization and role of Collagen Based Fertilizer

Mineralization is the process through which nitrogen found in collagen is released and availed in the soil as a nutrient for use by plants (Kavita and Vinod, 2018). The process of mineralization occurs in two phases the first of which is ammonification (that is, the conversion of organic N into ammonium-N) and the second phase is known as nitrification, which involves the microbial conversion of ammonium- N into nitrite N and finally into nitrate-N. According to Zhu, (1997), and also, Kavita and Vinod, (2018), two processes namely; organic N mineralization and mineral N immobilization have been found to take place simultaneously in soil, and the relative rates at which they take place are directly proportional to the amount and the microbial susceptibility of carbonaceous compounds present in the collagenic waste-based fertilizers,

whose microbial decomposition provides energy for the mineralization and immobilization processes. Mineralization rates vary with soil temperature, moisture and the amount level of aeration of the soil (Zhu, 1997; Kavita and Vinod, 2018).

It has been found that for mineral N immobilization to take place more effectively, the readily decomposable carbon in the collagenous waste must be present in excess quantities as compared to the mineralization of organic N, and that the decomposition rate of these carbon compounds is inversely proportional to the gradual decrease of mineral N immobilization rate (Zhu, 1997). In addition to this, Zhu, (1997) also found that as the carbon compounds in the collagenic waste-based fertilizers got depleted, the rate of organic N mineralization overtook the rate of mineral N Immobilization leading to an overall mineralization process as a result.

The process of N mineralization has been articulated more elaborately in literature through several simulation methods as described by Zhang *et al.,* (2017), a phenomenon that had earlier been investigated by Stanford *et al.,* (1974) who simulated the N mineralization dynamics in a long-term aerobic incubation experiment using a one component first order kinetics model. At some later dates, Richter *et al.,* (1980); Gil *et al.,* (2011); Turrion *et al.,* (2012) used other kinetics models in the following order; double first-order kinetics model followed by mixed first-order kinetics model and then zero-order kinetics model. The simplistic nature of these models has made them very popular since they also, don't lose their theoretical foundation and in addition to this, they have traditionally been used specifically in studies involving the N mineralization process of paddy soil (Cabrera, 1993; Lu *et al.,* 2008; Gil *et al.,* 2011)

According to Stanford *et al.,* (1974), potentially mineralizable N (N_o) is the fractional portion N that is prone to mineralization. These researchers estimated N_o by use of a single first order

kinetics model, which was later made use of at different occasions by Karuku, (1989), and Karuku and Mochoge, (2018) adopting the following equation (*Eq. i*);

$$dN/dt = -kN \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (i)$$

A new equation Integrating this equation (*Eq. ii*) was derived from (*Eq. i*) through integration, thus;

$$Log\ (N_o - Nt) = Log\ N_o - kt/2.303 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (ii)$$

Where;

Nt = the amount of cumulative N mineralized within a period of t days,

N_o = N mineralization potential,

K = first order rate constant (day^{-1}).

From the above equation (*Eq. ii*), it was found that K was more or less equal for a large number of soil samples in a two weeks' laboratory soil incubation experiment carried out immediately after short-term preincubation, making it reasonably possible to estimate the mineralization potential (N_o) (Stanford *et al.*, 1974; Karuku and Mochoge, 2018). This then made it possible to make use of a simplified equation (*Eq. iii*); thus,

$$N_o = 9.77\ Nt \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (iii)$$

Where;

N_o = N mineralization potential,

Nt = amount of N mineralized in a two weeks' incubation period.

Nitrogen (N), phosphorus (P) and potassium (K) are the three major macromolecules that are required as nutrients by plants for their growth and development. Nitrogen (N) is known for its role in increasing the rate of photosynthesis for enhanced chlorophyll synthesis, which facilitates the plant growing process, determines the effects of photosynthesis and regulates the amount of organic matter in the young growing plant (seedling) (Zhao *et al.,* 2022). In the same study, these researchers also found that the major form of carbohydrate that is formed as a result of photosynthesis is soluble sugar (glucose), which is a source of energy for optimal growth performance of young plants and provision of resistance of the seedlings to adverse environmental conditions. The synthesis process of vitamins and enzymes as well as the regulation of the physiological activities of plants are triggered by the presence of N, which is known to occupy a primary position in the life activities of young growing plants (Zhao *et al.,* 2022). The major role of both phosphorus (P) and potassium (K) is their involvement in regulating plant growth and also, the metabolism of carbohydrates in young growing plants (Zhao *et al.,* 2022). Nogueira *et al.,* (2010) incorporated mineral P and K into chrome tanned leather wastes for application as fertilizer after extracting the chromium using acid hydrolysis at 50^0C, after which the collagen hydrolysate was added into a mixed solution of P and K, then dried and milled into small fragments of various particle sizes. This fertilizer formulation was compared with commercial fertilizer in the growth of rice plants; and it was found that the leather waste-based fertilizer caused an increase in plant productivity with reduced dry matter yield (DMY); a clear indication that it was a good nutrient source for the rice crop.

CHAPTER THREE:
STATUS OF LEATHER WASTE GENERATION IN KENYA

3.1. Abstract

The leather industry is known to generate large quantities of various types of solid leather wastes that pose great risks to both the environment and public health due to the presence of hazardous chemical substances they contain. A study on the status and disposal mode of leather waste generation in some 6 pre-selected urban tanneries in Kenya was carried out for purposes of assessing the current levels of leather waste management in the country. The study aimed at identifying and quantifying the various types of leather waste generated in Kenyan tanneries as compared to the corresponding quantities of hides and skins processed all the way from raw stage to finished leather in these tanneries. The study also aimed to assess the tannery solid wastes management methods used by various tanneries in Kenya. Six tanneries were selected for this study including three located in Nairobi, one in Nakuru, one in Athi River, and one in Limuru.

The study recorded generation of a total amount of 2,112,560kg of tanned solid wastes by the 6 tanneries during the one-month study period. Out of this, a total of 1,443,000kg (68.3%) was chrome tanned leather waste, which comprised of 32.09% chrome shavings and 36.21% chrome splits & trimmings. The remaining proportion was constituted as follows; 9.14% vegetable shavings, 14.91% vegetable splits and trimmings, 3.53% crust trimmings, 2.35% buffing dust and 1.77% finished trimmings. This waste was disposed of through; landfilling, open ground dumping and or incineration causing environmental pollution.

These findings showed the need for development of appropriate methods for utilization of these wastes to produce value added products, a move that can go a long way to curb environmental

pollution due to inappropriate disposal of these wastes. Such value-added products may include leather boards, cosmetics, carpets, office glue, gelatin (for food supplements), photographic films, animal feeds and fertilizers, which can also be an extra source of income for tanners.

3.2.Introduction

The leather industry worldwide is known for its generation of very large quantities of leather solid wastes (Ramasami, 2001; Fela, *et al.,* 2011). Such wastes pose great risks both to the environment and public health as they contain hazardous chemical substances some of which are difficult to remove even after preliminary treatment of the wastes prior to their disposal or utilization (Kanagaraj *et al.,* 2006). According to these researchers, the leather industry solid wastes generation, which has been estimated to be 800kg in weight for every ton of wet-salted hides processed up to the finished stage of leather manufacturing, are constituted as follows; fleshings (50-60%), chrome shavings, splits and trimmings (35-40%), skin trimmings (5-7%) and hair (2-5%). Majority of tannery solid wastes emanate mainly from the tannery beamhouse process steps; especially the unhairing/liming stage (80%), with the rest being generated during tanning (19%) and finishing (1%) (Kanagaraj *et al.,* 2006). A wide variety of useful products can be processed from tannery solid wastes, some of which are; leather boards from chrome shavings, fatliquors and tallow from recovered fats, building blocks from both vegetable and chrome shavings, cosmetics and office glue from skin trimmings, carpets and brushes from recovered hair, gelatin (for food supplements), photographic films, animal feeds and fertilizers from hydrolysed collagenic material as a way of reducing environmental pollution as well as revenue generation for the respective tanneries (Ramasami, 1999). Literature values indicate that the worldwide generation of chrome shavings alone is to the tune of 600,000 metric tons, 10% (i.e., 60,000 metric tons) of which is produced in the USA (Cabeza *et al.,* 1998). Traditionally,

solid wastes generated in the leather industry have been disposed of through landfilling, but in recent years this has become very problematic due to a number of factors which include; acute shortage of landfill sites resulting from rapid increase of human population growth vis a vis limited land availability, escalating costs of land disposal of hazardous solid wastes and increasing risks of environmental pollution posed by such wastes (Li *et al.*, 2019).

Several attempts have been made by a considerable number of researchers in the past, to come up with leather waste hydrolysis methods aimed at recovering amino acids and peptides for use in soil conditioning or animal feed additives as well as trying to recover the chromium in chrome tanned leather wastes for re-use in tanning (Sundar *et al.*, 2011; Jiang *et al.*, 2016; Li *et al.*, 2019). The current study therefore, assessed the various types and quantities of tanned solid wastes generated in the Kenyan tanning industry to establish the relationship between such wastes and the corresponding quantities of hides and skins processed from raw to the finished stage of leather making. The current mode of disposal of tanned solid wastes generated in the local tanneries as well as the level of their utilization was also, carried out as part of this study.

3.3.Materials and Methods

3.3.1 Study areas

The study was conducted in two large and four medium size pre-selected tanneries distributed in different parts of Kenya as follows; Nairobi - 3, Limuru -1, Nakuru -1 and Athi River -1. Three of the four medium size tanneries are situated in the capital city, Nairobi. They are; Reddamac (located along the Eastern By-Pass), Aziz and East Africa tanneries (located in Kayole-Njiru areas). They process hides and skins into leather at semi-processed wet-blue and crust stages as well as finished stages. The large-scale tanneries are; Bata Shoe Company, Limuru, (about 23km

from the capital – along the Nairobi-Nakuru highway) and Alpharama (located in Athi River Township of Machakos County, around 19km from the capital city, off Mombasa Road at the Namanga junction). The installed daily production capacity of the large-scale tanneries is 40 tons of hides and 20,000 pieces of skins (goat and sheep) processing up to finished leather, while the medium size tanneries have a full installed daily production capacity of approximately 20 tons of hides and 10,000 pieces of skins (goat and sheep). Nakuru tanners, which is a medium size tannery, specializes mainly with wet-blue leather production for export. At the moment, the Bata Shoe Company tannery is only processing leather starting from the wet-blue stage. This tannery stopped beamhouse operations due to the high pollution loads of effluents emanating from such operations and the challenges associated with their treatment costs as well as their disposal around the suburbs of Limuru Township, which are densely populated with human settlements.

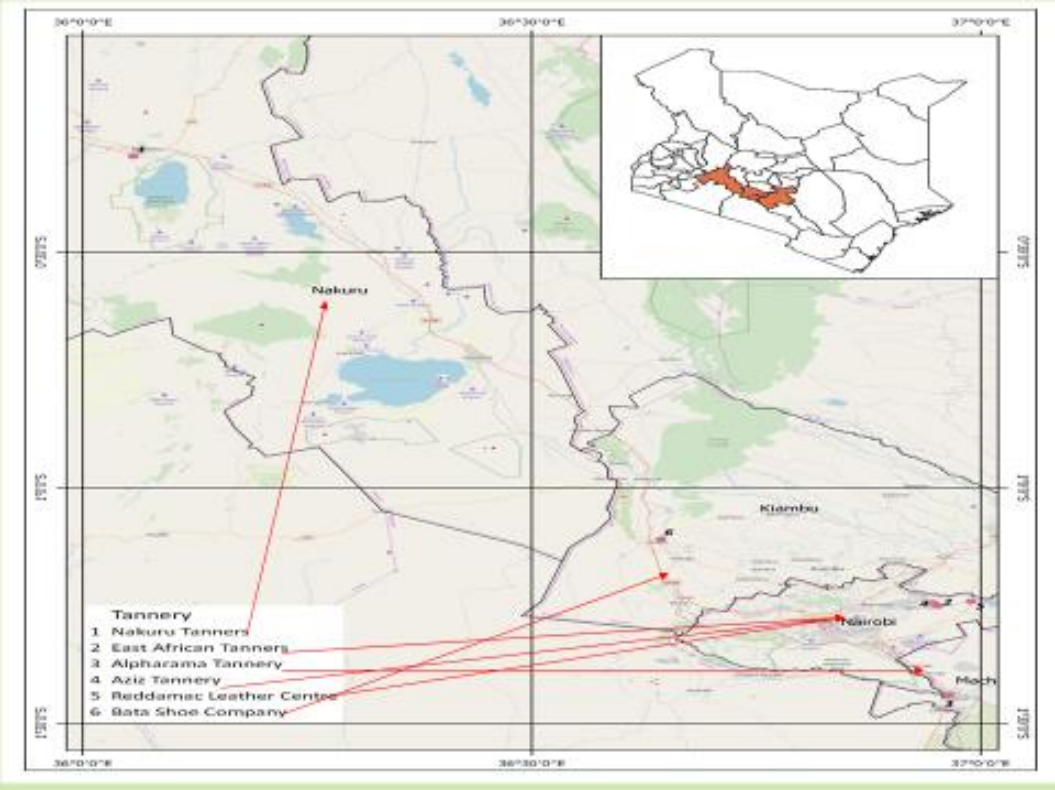


Figure 3.1: Kenyan Map showing the locations of the 6 pre-selected tanneries for this study

Source: *www.spectregeospatial.co.ke*

3.3.2 Study design

The study used cross sectional design utilizing mixed methods.

3.3.3 Sampling method and data collection

Purposive sampling method of the homogeneous type was the preferred method of sampling in selecting the 6 tanneries that were used for this study as described by Kombo and Tromp, (2006). The reason for preferring this sampling method was that the pre-selected tanneries had similar operational characteristics with the rest of the tanneries in Kenya. Data capturing quantities and types of leather waste generation as well as the common methods used in disposing of leather waste and its utilization thereof, if any, covering the 6 pre-selected tanneries for this study, was collected using a structured questionnaire, key informant interviews and in some cases, personal observations **(the questionnaire sample is shown in Appendix 4).** Data on the prevailing challenges in the Kenyan leather industry in terms of leather production, supply of raw materials, tanning chemicals and machinery as well as marketing of finished leather products and current leather waste management practices in the Kenyan tanning industry was also, captured using the same structured questionnaires.

3.3.4 Sample size determination

Sample size determination for this study, which was 200 respondents, was done using the formula described by Rao and Richard, (2016). It was about testing the difference between two proportions of the respective respondents; that is, the management and technical personnel working in the tanneries that were covered by this study. In this formula, a decision was made

based on the power of the test whereby an 80% (1 – β) of the test was considered, leading to the sample size determination as follows;

$$n = \frac{2(Z\alpha + Z\beta)^2 pq}{d^2}$$

where;

Zα is 1.96, which is the critical value at 5% level of the normal distribution rounded off as 2

p = rate at which false information may have been given by the respondents expressed as a percentage

q = rate at which true information may have been given by the respondents expressed as a percentage (p + q = 100%)

The standard error of p is given by the formula $\sqrt{pq/n}$

α = Type I error β = Type II error (often type I error is considered)

d = difference tolerated = 10 (i.e., $p_1 - p_2$)

Then, Zα = 1.96, Zβ = 0.842 (from statistical tables), p = 15 and q = 85

Sample size $n = \frac{2(1.96 + 0.842)^2 \times 15 \times 85}{10^2} = 200$

The sample size of 200 respondents was taken from all the technical departments of the respective tanneries; viz. beamhouse, tanyard, wet-end, machinery and finishing. The respondents were distributed as follows; 30 each from Bata, Alpharama, Nakuru, Aziz and East

Africa tanneries and only 26 from Reddamac tannery as this tannery had relatively a smaller number of employees at the time of the current study. Structured questionnaire survey was conducted on all the 176 technical personnel drawn from these tanneries, while key informant interviews were done on the remaining 24 respondents (i.e., 4 members of the management staff in each of the 6 pre-selected tanneries for this study).

3.3.5 Data analysis

SPSS (statistical package for social sciences) was the statistical tool that was used for entry and analysis of data obtained from leather waste generation status in the 6 pre-selected tanneries covered by this study according to Rao and Richard, (2016). Descriptive statistics were used in the calculation of mean levels of the different types of leather waste generated in the respective tanneries and results compared with set standards using a t-test as described by Annadurai, (2007) and alluded to by Oruko *et al.,* (2014).

3.4.Results

3.4.1 Proportions and types of leather waste generated in selected tanneries

The levels of various types of leather waste generated on a monthly basis in selected Kenyan tanneries are indicated in **Table 4.** Chromium-containing leather solid wastes (including chrome shavings, splits and trimmings) were found to constitute approximately 68.3% of the total tanned solid wastes generated in the 6 pre-selected tanneries every month, while the average constitution of vegetable tanned solid wastes (i.e. vegetable shavings, splits and trimmings) generated in the same period was 24% **(Table 3.1.).** Other types and proportions of leather solid wastes generated every month in the same tanneries that were covered by this study are; crust trimmings (3.5%), buffing dust (2.4%) and finished trimmings (1.8%) as shown in **Table 3.1.**

Table 3.1: Proportions and types of monthly leather waste generation in selected Kenyan tanneries

Type of waste (kg)	Nakuru Tanners	East Africa	Alpharama	Aziz	Reddamac	Bata	Total (Kg)	Proportion (%)
Chrome shavings	150,000	100,000	148,000	60,000	40,000	180,000	678,000	32.1
Chrome splits& trimmings	175,000	120,000	205,000	15,000	20,000	230,000	765,000	36.2
Vegetable shavings	0	10,000	92,000	6,000	10,000	75,000	193,000	9.1
Vegetable splits & trimmings	0	12,000	165,000	3,000	5,000	130,000	315,000	14.9
Crust trimmings	0	4,500	38,000	2,000	5,000	25,000	74,500	3.5
Buffing dust	0	6,500	18,560	10,000	4,000	10,500	49,560	2.4
Finished trimmings	0	6,500	8,500	5,000	5,000	12,500	37,500	1.8
Total	325,000	259,5000	675,060	101,000	89,000	663,000	2,112,560	100
Total average amount of leather waste generated monthly in the 6 pre-selected tanneries is 2,112,560kg								

Table 3.2: Quantities of hides processed (kg) in the month of study versus leather waste generated in selected Kenyan tanneries

Tannery	Quantities of hides processed in the month of study (Kg)	Quantities of leather solid wastes generated in month of study (kg)
Nakuru Tanners	1,027,230	325,000
East Africa Tannery	811,414	259,500
Alpharama Tannery	2,128,420	675,060
Aziz Tannery	314,289	101,000
Reddamac	283,180	89,000
Bata	2,077,992	663,000
Total	**6,642,525**	**2,112,560**
Proportion (%) – leather solid wastes generated in the 6 pre-selected tanneries in Kenya = 31.8% of the total hides processed (kg) in the month of study		

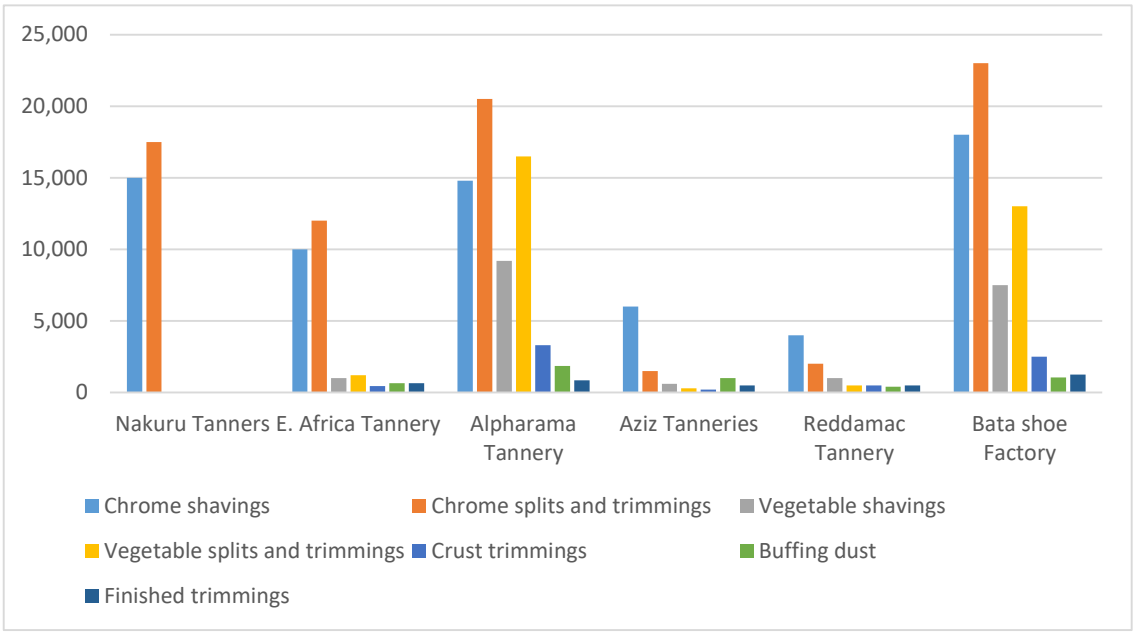


Figure 3.2: A graphical representation of the types and quantities (kg) of leather waste generated by selected Kenyan tanneries in the month of study

3.4.2 Current disposal and utilization methods of tanned solid wastes in Kenya

As shown in **Table 3.3,** the most common methods used in the disposal of leather waste in Kenya are; open dumping, incineration, landfilling and in some cases, use of this kind of waste as a source of fuel. According to the findings of this study; no recycling, reuse or any other form

of leather waste utilization was being practised in all the tanneries that were investigated **(Table 3.3.).** This scenario therefore, poses a major challenge to the Kenyan tanning industry in terms of searching for safe and economical disposal methods of leather waste as well as its utilization; particularly, chrome tanned waste.

Table. 3.3: Current modes of leather waste disposal and/or utilization in selected Kenyan tanneries

Tannery	Leather waste disposal/utilization mode
Nakuru Tanners (Nakuru town)	Landfilling mode of disposal No documented method of recycling or re-using of leather waste
East Africa Tannery (Nairobi)	Dried fleshings and trimmings are disposed of by: Open dumping Incineration Being used as a source of fuel No recycling of leather waste is done.
Alpharama Ltd. (Athi River)	Landfilling is the only mode of disposal of leather waste in this tannery. Neither recycling nor re-using of leather waste is done.
Aziz Tannery Ltd. (Nairobi)	Leather waste disposal is done by the following methods: Open dumping Incineration. There is no documented mode of either recycling or re-using of leather waste in this tannery.
Reddamac Leather Centre (Zingo Investments, Nairobi)	Leather waste disposal is done by the following two modes: Open dumping Landfilling. Neither recycling nor re-using of leather waste is done.
Bata Shoe Company (Limuru)	Landfilling is the only mode of disposal Neither recycling nor re-using of leather waste is done.

Figure 3.3: Landfilling of chrome tanned waste

3.4.3 Management of tannery solid wastes in Kenya and its challenges

The current study documented a number of challenges various tanneries face in tannery waste disposal in Kenya. **Table 3.4** outlines the challenges in the management of tannery wastes that hinder growth of the leather industry in the 6 selected tanneries that were covered by this study.

Table. 3.4: Challenges faced by selected Kenyan tanneries in the management of tannery solid wastes

Tannery	Challenges in tannery solid wastes management
Nakuru Tanners Ltd.	High transportation costs of the leather waste to landfills Bad smell
East Africa Tannery	High transportation costs of the tannery solid wastes to the dumping site High handling and treatment costs for chromium containing leather solid wastes
Alpharama Tannery	Difficulty in assessing the buffer zone area/dumping site because of poor road infrastructure (especially during the rain seasons) Pollution due to solid waste decomposition/degradation Soil and ground water contamination due to leaching
Aziz Tannery	Environmental pollution due to lack of proper disposal and treatment mechanisms for both tannery solid wastes and effluent

Figure 3.4: Chrome leather shavings and trimmings dumped in the environment of a tannery

3.5.Discussion

As established in this study, tanned leather waste amounting to 2,112,560kg was generated in the 6 pre-selected Kenyan tanneries from the processing of 6,642,525kg of raw hides into finished leather within a period of 30 days (one month). This was about 31.8% of the total amount of hides processed as shown in **Table 3.2**. These findings correlated very well with literature values as indicated in the work done by Simeonova and Dalev, (1996), who found that a middle capacity tannery processing approximately 100 tons of hides on a daily basis was able to generate about 30 tons of tanned solid wastes. This figure correlates very well with the findings of other researchers who pointed out that tanned waste (including; shavings, trimmings, splits and buffing dust) to the tune of 200kg for every 1000kg of wet-salted hides processed into finished leather in addition to a considerable amount (50-100kg) of dyed and finished leather trimmings, were generated (Veeger, 1993; Taylor *et al.,* 1998; Langmaier *et al.,* 1999).

The total number of tanneries currently operating in Kenya are 14, with an average installed capacity of 70% producing 40 million tons of wet blue leather daily (Oruko *et al.,* 2014). This is the basis for the relatively high quantities of chrome leather waste (chrome splits and trimmings) generated monthly in each of the 6 selected tanneries that were covered by this study as compared to the other types of leather waste, which are given in **Table 3.1** and represented graphically in **Figure 3.2.).** The quantities of chrome shavings generated in the same tanneries were also, relatively high in comparison with the other types of leather waste; a clear indication that wet blue leather production in Kenyan tanneries is the main form of tannage.

The levels and constituent components of the leather waste generated is a function of many factors including but not limited to the procedures used in tanning such as the number, type and sequence of unit operations – as some of the operations are not mandatory, chemical offer of the

different types of reagents used in the tanning process and the control systems used in specific process variations. The results given in **Table 3.1** indicate that the levels of both chrome splits and trimmings (36.2%) as well as chrome shavings (32.1%) ranked amongst the highest of the different types of leather solid wastes that were generated in the six tanneries, which were involved in this study. According to the findings of this study, chromium-containing leather solid wastes were the most difficult to dispose of due to the adverse effects they pose on the environment. This then calls for concerted efforts to try and come up with suitable disposal and/or treatment facilities for tannery wastes, particularly those that are contaminated with chromium as alluded to by Abebaw, (2015).

The reason for the relatively high levels of chromium-containing leather waste generation is that slightly more than 80% of all the operational tanneries in Kenya manufacture leather up to the wet-blue stage for export in that condition due to shortage of the prerequisite technology and facilities for manufacturing finished leather. The international market for finished leather is also very restrictive as far as fashion, customer and product specifications are concerned. Other factors that have been found to contribute significantly to the generation of such huge quantities of chrome tanned solid wastes are of regional and social economic nature as described by Padilla-Rizo *et al.,* (2018).

According to the key findings of this study, landfilling, open dumping and incineration; following one another in that order, were found to be the most common modes of tannery solid wastes disposal in the Kenyan tanning industry as indicated in **Table 3.3** and also, **Figure 3.3** as well. However, disposal of solid wastes by landfilling is the least recommended option of solid waste management according to DEFRA, (2011).

In addition to the shortage of land availability in urban areas for landfilling of solid wastes, escalating costs associated with the transportation of such wastes to the landfills is also, a challenge that needs to be dwelt with. All the challenges associated with the management of tannery solid wastes and factors hampering the growth of the leather industry in Kenya are given in **Table 3.4.** Most of the landfilling sites are also, poorly designed, a scenario that facilitates the leaching of contaminants in the landfilled wastes (e.g., chromium) leading to the contamination of ground water as a result (Kirk *et al.,* 2002; Ahmed and Kashif, 2014). Landfills have also been found to be a source of methane gas emissions, and this gas is known as a significant contributor to global warming (Sekaran *et al.,* 2007). Disposal of such wastes by incineration is similarly, detrimental to the environment in spite of the fact that this mode of disposal is more efficient than landfilling in reducing tannery solid wastes in the environment. This is due the imminent production of air pollutants e.g., SO_2 and NOx, which must be removed by use of air pollution control devices as described by Kanagaraj *et al.,* (2006). Work done by Sekaran *et al.,* (2007) established that thermal incineration of leather solid wastes produces ash, which is a major source of pollutants notably; chromium (Cr), halogenated organic compounds and polycyclic aromatic hydrocarbons (PAHs).

Another key finding established in this study is that chrome tanned waste formed the highest percentage (68.3%) amongst all the different types of leather solid wastes generated in the 6 pre-selected tanneries during the month of study and it was also, the most problematic type of waste to get rid of from the environment of the respective tanneries **(Table 3.1** and **Figure 3.4.).** Chromium-containing leather waste is not biodegradable and due to this scenario, previous studies have highlighted its disposal problems both to the environment and public health (Ahmed and Kashif, 2014). In this regard therefore, thorough research needs to be carried out so as to

obtain accurate findings on the consequences of leaving chrome tanned solid wastes unattended to in the environment of the tannery or dumping them in landfills/open grounds without prior treatment to recover the chromium first. This will go a long way in helping researchers and policy makers to make appropriate decisions that are aimed at reducing the environmental impact of such hazardous wastes.

Work done by Parvin *et al.,* (2017) showed that handling of chrome tanned waste in the environment of the tannery-by-tannery workers, exposes them to the highest risk of contracting cancer related ailments as compared to all the other heavy metals, which are found in leather waste. These researchers reported several cases of workplace exposure to chromium during a typical working day in a tannery; one prominent case being a situation where chromium was found in both the blood and serum of a sample of tannery workers in Ontario, Canada (Parvin *et al.,* 2017). Investigations carried out by Randall and Gibson, (1987) on the same topic, revealed that there was no correlation between chromium levels in the body of the victims of chromium exposure and the length of employment of such victims in the tannery. However, these researchers found that there was some correlation between the lengths of employment of the respective victims in the tannery and the amount of exposure to chromium during a typical working day (Randall and Gibson, 1987).

Extra care should be taken in the management of chromium-containing leather waste so as to reduce as much as possible, the adverse effects of chromium on the environment and human health. A considerable number of leather waste management strategies have been put into practice as one of the objectives of achieving the United Nations sustainable development goals, with the best four being given priority in the following order; source reduction, recycling and composting, combustion (waste-to-energy) and, landfilling (DEFRA, 2011). However, these

strategies have not been fully implemented in most countries of the world including Kenya; where the implementation of solid waste recovery and disposal laws (legal instruments) is uncoordinated and poorly done to date. As stipulated by NEMA, (2014), the following approach should be adopted in the management of solid wastes; re-use and recycling, enacting/enforcing the laid down regulatory and supervisory statutes as well as formulating relevant policies, legislations and economic instruments geared at reducing the amount of generated solid wastes. According to Onyuka, (2010), a viable option of leather solid wastes management would be to recycle such wastes with the aim of producing useful products as this would go a long way in reducing environmental challenges posed by the untreated leather wastes and at the same time, create employment in the leather sector.

3.6. Conclusions and Recommendations

According to the findings of this study, leather waste amounting to approximately 31.8% by weight, of the total quantities of hides processed into finished leather was generated. This proportion of generated leather waste was constituted as follows; chrome splits and trimmings-36.2%, chrome shavings-32.1%, vegetable splits and trimmings-14.9%, vegetable shavings-9.1%, crust trimmings-3.5%, buffing dust-2.4% and finished trimmings-1.8%. Amongst all the different types of leather solid wastes that were generated in the selected Kenyan tanneries covered by this study, chrome tanned waste was ranked the highest at 68.3% and it was also, the one that posed the greatest challenge in terms of its disposal from the environment of the respective tanneries.

Common disposal methods of tannery wastes in most tanneries are, open dumping, incineration, landfilling and in some cases, use of the wastes as a fuel source. These disposal methods have been found to create environmental problems and public health issues due to the obnoxious smell

and also, the presence of hazardous substances (e.g., chromium) in a majority of such wastes. It is recommended that chromium-containing leather wastes be treated using appropriate methods to remove all the chromium prior to their disposal or use in other applications. There is need to explore a sustainable and an environmentally sound method of decontaminating these huge amounts of chrome leather waste so that the waste can be utilized in other useful applications. A wide variety of useful products can be processed from tannery solid wastes, some of which are; leather boards from chrome shavings, fatliquors and tallow from recovered fats, building blocks from both vegetable and chrome shavings, cosmetics and office glue from skin trimmings, carpets and brushes from recovered hair, gelatin (for food supplements), photographic films, animal feeds and fertilizers from hydrolysed collagenic material as a way of reducing environmental pollution as well as revenue generation for the respective tanneries.

CHAPTER 4: DEVELOPMENT OF AN ECO-FRIENDLY METHOD FOR DECHROMING TANNERY WASTES

4.1. Abstract

Chrome tanning process is the most widely practised form of tannage in leather production worldwide. Consequently, there is an inevitably very high generation of chromium-containing leather solid wastes in the global leather industry. Chrome leather waste has over the years created major disposal problems due to the presence of chromium, which is highly toxic (especially in its hexavalent oxidation state) and environmentally unfriendly. A few reseachers have previously attempted to dechrome solid leather waste to enable utilization of the waste, but they have not been able to succeessfully remove all the chromium from the chrome contaminated leather waste. This study developed a new method that is user-friendly, cost-effective and environmentally sound for dechroming leather waste to enable its utilization in the manufacture of an organic fertilizer. The new method involved detanning, chromium extraction and complexation of the remaining traces of chromium with potassium oxalate

Chrome leather waste was detanned by mixing 175g of lime with 5kg of chrome leather waste and 7.5 litres of water and the mixture allowed to act for a period of 12 hrs. After 12 hours, the contents were filtered using 2mm seive and strainer and filtrate discarded. The solid waste was delimed using 0.2% ammonium sulphate in 200% float for 30 minutes after which the contents were filtered to remove excess fluid. Chromium was extracted from chrome waste by adding 85% formic acid to the delimed waste and mixture allowed to act for 12 hours. After 12 hrs the mixture was filtered to remove excess formic acid and extracted chromium. Further extraction of chromium from the waste was done by mixing 25g of potassium oxalate for every 1 kg dried formic acid extracted leather waste in 100% fresh float and mixture allowed to act for 12 hours.

After 12 hours, the mixture was filtered and filtrate containing extracted chromium discarded. The dechromed leather waste was dried in an oven and chromium levels determined using Atomic Absorption Spectrophotometer.

The level of chromium remaining in the waste using the new method was compared with three other dechroming methods previously described by other researchers. The levels of chromium extracted from the chrome-tanned leather wastes using the new method reached up to 99.90% with % TKN in chrome-free collagen hydrolysate being 52.89%. Total ash and total organic carbon levels in the dechromed and hydrolysed leather wastes were also, relatively high at 12.42% and 23.27%, respectively. The performace of the new dechroming method developed in this study was found to be significantly better than existing methods ($P < 0.05$) in terms of efficiency, convenience, environmental pollution potential and cost-effectiveness. The statistical significance on the efficiency of the investigated dechroming methods in terms of amount of chromium extracted per unit time was tested by the t-distribution test, which confirmed that the means were significantly different at 95% confidence interval. The study concluded that the new method of dechroming chrome-tanned leather wastes achieved complete removal of toxic chromium from chrome contaminated leather waste and the resulting waste was safe for its utilization to make economically valuable products.

4.2. Introduction

The leather industry is well known for the generation of huge quantities of tannery solid wastes (approximately 800kg for every ton of wet-salted hides processed from raw to finished stage) as investigated by Veeger, (1993) and Langmaier *et al.,* (1999). Bulk of these wastes (in the range of 50-60%) are constituted of fleshings while 80% of the remaining proportion is largely constituted of chrome shavings, splits and buffing dust as pointed out by Taylor *et al.*, (1998).

The generation of such enormous quantities of solid wastes during leather production are mainly associated with some facts about the leather making process namely; opening up of the hide fibre structure during the initial stages of leather making (beamhouse processing) to free the leather making protein – collagen, from non-collagenous skin components, and use of a wide variety of chemicals majority of which are not fully utilized due to the intrinsic nature of the many process-steps involved in leather making as well as the inevitable use of machinery operations such as fleshing, splitting, shaving and buffing. These tannery solid wastes, majority of which are contaminated with chromium, are mainly disposed of through landfilling and/or incineration (Sundar *et al.,* 2011). However, due to the adverse effects caused by particularly; chromium-containing tannery wastes on both the environment and public health, there is a dire need for the exploration of better options for their disposal and/or utilization (Avudainayagam *et al.,* 2003). some of the treatment options for such wastes that have been suggested by previous researchers (e.g. recovery of useful components in the waste, recycling of the waste into other useful products and re-use of either the entire waste or some of its components as a raw material for other industries) still pose a major challenge to circular economy and green chemistry to date (Pfaltzgraff *et al.,* 2013; Sole *et al.,* 2019).

Previous studies have shown that for every 1 ton of wet-salted hides processed into finished leather slightly more than 600kg of tannery solid waste is generated, one-third of which is actually tanned waste composed of shavings, trimmings, splits and buffing dust with at least 3.5kg of chromium in the waste if the tannage followed was chrome tannage (Taylor *et al.,* 1998; Langmaier *et al.,* 1999; Ozgunay *et al.,* 2007). Chrome tannage is the most widely practised form of tannage globally (occupying slightly more than 90% share as the most preferred leather manufacturing process) due to the relatively high hydrothermal stability of the resultant leather

compared to other tannages (Sundar *et al.,* 2002; Aravindhan *et al.,* 2004). According to these researchers, the tanning agent of choice in this type of tannage, basic chromium (III) sulphate, has a very high affinity for hide collagen carboxyl groups – the interaction of which leads to the formation of stable cross-links. Other qualities of chrome tannage that make it attractive to the tanner include; versatility (i.e., ability to make a wide variety of finished leathers from this type of tannage) and superiority of the resultant leather in terms of stretchability, dyeability and resistance to tear and flexural failure as compared to other types of tannages (Scopel *et al.,* 2015). Further, chrome tanning salts (especially basic chromium (III) sulphate) have proved to be very efficient in leather tanning and they are readily available at a reasonable cost to the tanner (Sole *et al.,* 2019).

Many scientific groups have pooled their research resources together to find a method to recycle and treat chrome tanned waste (Brown *et al.,* 1996; Marmer *et al.,* 1999; Silveira *et al.,* 2002; Mu *et al.,* 2003; Kamaludeen *et al.,* 2003; Rivela *et al.,* 2004; Saravanabhavan *et al.,* 2004). Further efforts to recycle such chromium-contaminated leather waste by utilizing it either directly or indirectly into other useful applications were reviewed by Colak *et al.,* (2005), and Li *et al.,* (2019). Direct recycling is a term used to refer to the treatment of chromium-containing leather waste for use in other applications without having to dechrome it first. However, a study carried out by Velusamy *et al.,* (2020) confirmed the possibility of chromium (III) oxidation into its hexavalent oxidation state (Cr^{6+}) if direct recycling of chrome-tanned waste is performed, which then leads to secondary pollution problems. This is a type of pollution that is witnessed in the atmosphere due to reactions taking place between one major pollutant and other minor pollutants emanating from a primary source. Narasimhan *et al.,* (1980); Cabeza *et al.,* (1998); Sundar *et al.,* (2011); Zehra *et al.,* (2014); Jiang *et al.,* (2016); Dang *et al.,* (2019), and Pahlawan

et al., (2019), made several attempts at different occasions to formulate organic fertilizers using chromium-containing leather scraps and shavings as the raw material through thermal or enzymatic techniques, but the presence of chromium in such wastes was found to be a limiting factor in the success of those techniques. The techniques themselves were also found to cause secondary pollution problems in addition to being cumbersome, costly and less efficient to carry out (Katsifas *et al.,* 2004).

The protein fractions obtained from the hydrolysis of chrome-tanned leather wastes can be used in the manufacture of low-cost surfactants, high chrome fixation auxiliaries and fillers for paper as reported by Munoz *et al.,* (2002); Yilmaz *et al.,* (2007); Santos and Gutterres, (2007); Sundar *et al.,* (2011), and Zehra *et al.,* (2014). In this regard therefore, a major part of the current research work was devoted to the development of a sustainable and environmentally sound technology that can effectively be used to dechrome, hydrolyse and modify chromium-containing tannery wastes for possible use as a value added organic fertilizer.

4.3.Materials and Methods

4.3.1 Study design.

This was an experimental study design.

4.3.2: Reagents for dechroming process

The chemical reagents required for the various experiments that were carried out in this study were sourced from OSHO CHEMICALS (K) Ltd. They were of the analytical reagent grade. Common laboratory reagents were used in the various experiments, tests and analyses that were conducted in this study.

4.3.3 Sample collection and preparation

Fresh chrome-tanned leather waste samples composed of shavings (10kg), trimmings and unusable splits for leather making (10kg) were collected from Alpharama tannery situated in Athi River Township, Kenya. Due to their relatively large sizes, chrome splits and trimmings were chopped into smaller pieces prior to their further preparation, which was done by mixing them thoroughly for homogeneity according to the procedure described by Ozgunay *et al.* (2007)**.** The two (2) categories of leather waste samples, chrome shavings on one hand, and chopped up chrome splits and trimmings on the other, were dried for two (2) days on polythene sheets of paper. Thereafter, the two categories of leather waste samples were each sub-divided further into two (2) 5kg duplicate samples so as to enable a more representative analysis of the various variables that were being assessed by this study.

4.3.4 Determination of the characteristics of tannery wastes

The International Union of Leather Technologists and Chemists Society (IULTCS) official methods of analysis (2001) was used to determine chromium concentration in the dried leather waste samples (IUC 8). This method is also referred to as the the wet oxidation method and it was summarized as follows:

- Accurately weighed (2.5g), prepared and characterized sample was put into a conical flask and 15ml of mixed sulphuric and perchloric acids, together with a few drops of anti-bumping granules added.
- The neck of the conical flask was then covered with a funnel after which the sample contents were heated to boiling on a wire gauze over a moderate flame.

- The flame was lowered immediately after the reaction mixture started to turn orange.
- The sample contents in the conical flask were then heated for a further 2 minutes after complete change to the orange colour.
- Thereafter, the sample contents were allowed to cool in air for 5 minutes and then diluted to approximately 200ml with distilled water.
- Further heating (at boiling point) was done for 10 minutes to eliminate chlorine after which the flask was allowed to cool and then 15ml of orthophosphoric acid added to mask any iron.
- A solution of potassium iodide (20ml) was added into the flask after which the flask was immediately stoppered and left to stand in the dark for 10 minutes.
- The contents of the flask were then titrated with 0.1M Sodium Thiosulphate solution and towards the end of the titration, starch indicator solution (5ml) was added and titration continued up to the end point, which was signaled by the solution in the flask turning light green.

Moisture content in the same dried samples (prior to conditioning or wetting back) was determined using the International Union of Leather Technologists and Chemists Society (IULTCS) official methods of analysis (IUC 5, 2001) and also, according to Aneja, (2007). Total nitrogen and hide substance**,** total lipid-fat content, humidity, total ash and pH levels in the dried leather waste samples were determined using International Union of Leather Technologists and Chemists Society (IULTCS) official methods of analysis - IUC 10, (2001); IUC 4, (2021); IUC

5, (2021); IUC 7, (2021) and ISO 4045, (1977), respectively. Total organic carbon (TOC) in the respective samples was determined by the Walkley-Black method, (1934).

Four different dechroming methods were investigated, and the rate at which the chromium was being extracted from chrome tanned leather waste was monitored by setting up the extraction protocols for each of the four extraction methods, and sample aliquots were drawn at different intervals during the extraction process. Sample aliquots were analysed for Cr content using Atomic Absorption Spectrophotometry (AAS), Model No. 210 VGP, 115-230 Volts (Bulk Scientific).

4.3.5 Wetting back of tannery waste samples

After determining the characteristics of interest in the dried leather waste samples, the samples were ground using a laboratory grinding mill (to increase their surface area for enhanced chromium extraction) and then subjected to wetting back to around 50% moisture content prior to the subsequent detanning process that was to follow immediately after this final preparation. The wetting back exercise was necessary so as to increase the chances of extracting as much chromium as possible after the detanning process step was completed.

4.3.6 Detanning of chrome tannery waste samples

After wetting back, the ground leather waste samples were then detanned as follows;

- Water amounting to 7.5 liters was added to 5kg of sample in a plastic container and the experiment replicated 4 times.

- Approximately 175g of pure lime was added to each of the 5kg sample replicates and the contents allowed to mix in the plastic containers for 1 hour after which the pH of the liquor was checked and adjusted to 12.5 with some alkali (lime).
- The contents of each sample were then put in an experimental tannery drum and the drum allowed to run intermittently 10 minutes every hour for a total period of 12 hours.
- After 12 hours, the contents of each sample in the experimental tannery drum were carefully offloaded into a plastic container ensuring that no liquor was lost.
- The respective sample contents were then filtered using a 2mm sieve and a strainer, and thereafter delime-washed for 30 minutes with 0.2% ammonium sulphate in 200% fresh float (based on the weight of original leather waste sample) to remove excess lime. This filtration process contributed considerably to the reduced quantities of formic acid that would be required later in the subsequent step of chromium extraction from the detanned samples.
- Extracted chromium was collected in the filtrate.
- The filtration process was repeated to ensure complete extraction of chromium from each sample replicate.
- Atomic absorption spectrophotometry (AAS) was used to determine the amount of chromium extracted from each sample replicate, after which the mean value was then calculated.

4.3.7 New method for extraction of chromium from detanned leather waste samples

The new method for dechroming chrome leather is a modification of a procedure used previously by Malek *et al.,* (2009). In the modified method, formic acid was used as the major reagent for extracting chromium from detanned chrome leather waste as opposed to the use of potassium tartrate as a chelating agent in the Malek *et al.,* (2009) method. The following steps were followed in the new dechroming method;

- Formic acid (85% pure) amounting to 200ml was added to 1kg of detanned chrome leather waste samples in a plastic container containing 100% fresh float (based on the weight of detanned chrome leather waste sample) and the experiment replicated 4 times.
- The sample contents were allowed to mix for 1 hour in the horizontal shaker after which the pH of the liquor was checked and adjusted to 3 with a few drops of formic acid.
- The sample contents were transferred to the experimental tannery drum and the drum allowed to run intermittently 10 minutes every hour for a total period of 12 hours.
- After 12 hours, the contents of each sample in the experimental tannery drum were offloaded into a plastic container ensuring that no liquor was lost.
- The respective samples were subsequently filtered using a 2mm sieve and a strainer while collecting all the filtrate in a plastic container, and then the volume of all the collected filtrate was measured.
- The respective samples were then mechanically squeezed out using a tannery vacuum drier for 2 minutes at a temperature of 80^0C to remove any remaining excess water,

while ensuring that the water was being collected in another plastic container and its volume noted.

- The formic acid dechromed samples were subsequently kept in the laboratory oven at 40^0C overnight to dry uniformly in preparation for a repeat Cr extraction procedure using potassium oxalate instead of formic acid.
- The percentage of chromium extracted with formic acid was then calculated on the basis of the volume of water collected both after filtration and after mechanical dewatering of the samples following the method described by Sun *et al.*, (2003).
- Samples were kept in the laboratory oven at 40^0C overnight for uniform drying.

The above chromium extraction procedure was repeated using potassium oxalate (25g for every 1kg of the detanned chrome leather waste samples in 100% fresh float), which replaced formic acid as a means of extracting the remaining chromium from the respective samples. Atomic absorption spectrophotometry (AAS) was used to determine the amount of chromium extracted from each sample replicate after this further Cr extraction with potassium oxalate. Determination of the few traces of chromium (VI) remaining in the respective samples after this further Cr extraction procedure with potassium oxalate was determined using UV-Vis spectrophotometer (U2900 model SP-291 - 200V). Preparation of samples for this determination was done according to the procedure described in the official methods of analysis of the society of leather technologists and chemists (SLC 22) (IUC 18, 2001), and the ensuing calculation for any traces of residual chromium (expressed as % Cr^{6+}) in the dechromed collagen hydrolysate was done using the following formula:

$$\% Cr^{6+} = \frac{[\text{Absorbance Graph reading x Dil.Factor (12.5)x Initial Vol.Of sample (100ml)}]}{\text{Weight of sample (300g) x 1,000,000}} \text{x100}$$

The rate at which the chromium was being extracted was investigated by measuring chromic oxide content levels after 0.5, 1, 2, 4, 8, 16, 24, 36 and 72 hours of the dechroming process. The calculation for the dechroming ratio or percentage was done according to the formula described by Sun *et al.,* (2003) as follows:

Dechroming ratio (%) = 100 [(Crwaste – Crdec)/Crwaste];

where,

Crwaste = concentration of chromic oxide (Cr_2O_3) in leather waste sample before dechroming.

Crdec = concentration of chromic oxide (Cr_2O_3) in leather waste sample after dechroming.

4.3.8 Extraction of chromium from leather waste using previously described methods

The new method of extracting chromium from chrome-tanned leather waste, whose procedure is described in section 4.3.7, was compared with three other methods previously used by other researchers to dechrome tanned leather wastes namely; normal alkaline hydrolysis method by Sun *et al.,* (2003), the alkaline hydrolysis method by Paul *et al.,* (2013), and modified alkaline hydrolysis method by Adeoye *et al.,* (2014).

4.3.8.1 Sample collection and preparation

The sample collection and preparation techniques for the various dechroming methods were carried out just as described in section 4.3.3.

4.3.8.2 Detemination of characteristics of waste samples

Determination of the various characteristics of interest and wetting back (conditioning to 50% moisture content) before carrying out the different dechroming methods for comparison purposes was done according to the International Union of Leather Technologists and Chemists Society (IULTCS) official methods of analysis (2001), details of which are given in section 4.3.4.

4.3.8.3 Dechroming of tanned leather waste samples using previously described methods

i). The standard dechroming method: Normal alkaline hydrolysis according to Sun et al., (2003)

The method aimed at extracting chromium from the prepared and characterized leather waste samples without interferring with the original structure of the hide collagen fibres, and it was carried out as follows:

- 100g/l of the prepared and characterized chrome leather waste sample was measured and replicated 4 times.
- Each of the measured sample replicate (100g/l) was stirred in a mixed solution containing 10% sodium sulphate and 10% sodium carbonate for15 minutes.
- Hydrogen peroxide (8%) was then added to the sample + mixed solution and stirred for 2 hours.
- Thereafter, filtration was done to remove the liquid from the solid waste sample using a 2mm sieve.
- The sample was subsequently washed with 10% sodium sulphate solution for 15 minutes and then filtered using a 2mm sieve.

- ❖ This washing step with 10% sodium sulphate solution for 15 minutes was repeated three times after which the sample was put in a solution containing 6% sodium chloride, 0.29% hydrogen peroxide and 1.0% sulphuric acid and then stirred in this mixture for 30 minutes.
- ❖ The sample was thereafter filtered and subsequently washed in a 6% sodium chloride solution for 15 minutes after which it was filtered again.
- ❖ This washing step in a 6% sodium chloride solution for 15 minutes was repeated twice.
- ❖ The dechromed sample was then allowed to air-dry at room temperature for 2 days.

The rate at which the chromium was being extracted was investigated by measuring chromic oxide content levels after 0.5, 1, 2, 4, 8, 16, 24, 36 and 72 hours of the dechroming process. The calculation for the dechroming ratio or percentage was done according to the formula described by Sun *et al.,* (2003) as follows:

Dechroming ratio (%) = 100 [(Crwaste – Crdec)/Crwaste];

where,

Crwaste = concentration of chromic oxide (Cr_2O_3) in leather waste sample before dechroming.

Crdec = concentration of chromic oxide (Cr_2O_3) in leather waste sample after dechroming.

ii). Dechroming method according to Paul et al., (2013): Alkaline hydrolysis method

This dechroming method was carried out on the prepared and characterized chrome leather waste samples, which had been dried at room temperature to a constant weight and thereafter

pulverized using a 2mm mesh size electric mill. The procedure for this method was executed just as described by Paul *et al.*, (2013) in the following manner:

- 20g of the pulverized leather waste samples were measured in 3 replicates.
- Each of the measured sample replicates (20g) was placed in 100ml solution containing a mixture of 5% w/v sodium sulphate and 4% w/v sodium carbonate and left for 30 minutes.
- 3% w/v Calcium hydroxide was then added and allowed 1 hour for the reaction to take place.
- 0.1% w/v Sodium hydroxide solution was subsequently added followed by hydrogen peroxide (10% v/v) and the mixture stirred for two days.
- Filtration was then carried out to remove water and the filtered sample subjected to washig (x 3) with 10% w/v sodium sulphate solution after which it was filtered again.
- The washed and filtered sample was immersed in a mixed solution of sodium chloride (6% w/v) and sulphuric acid (1% v/v) for a period of 1 hour to allow for acid steeping to take place after which the sample was then filtered again.
- Another round of washing (x 2) was carried out on the sample in a mixed solution of 10% w/v sodium sulphate and 6% w/v sodium chloride and then filtered.
- The dechromed sample (product) was thereafter allowed to air-dry at room temperature for 2 days and then assessed for the residual chromium using atomic absorption spectrophotometer.

The rate at which the chromium was being extracted was investigated by measuring chromic oxide content levels after 0.5, 1, 2, 4, 8, 16, 24, 36 and 72 hours of the dechroming process. The calculation for the dechroming ratio or percentage was done according to the formula described by Sun *et al.,* (2003) as follows:

Dechroming ratio (%) = 100 [(Crwaste – Crdec)/Crwaste];

where,

Crwaste = concentration of chromic oxide (Cr_2O_3) in leather waste sample before dechroming.

Crdec = concentration of chromic oxide (Cr_2O_3) in leather waste sample after dechroming.

iii) Modified alkaline hydrolysis method of dechroming leather waste (Adeoye et al., 2014)

Some of the pulverized leather waste samples were dechromed using the modified alkaline hydrolysis method according to the procedure developed by Adeoye *et al.,* (2014), which was summarized as follows:

- 20g of the pulverized leather waste samples were measured in 3 repilcates.
- Each of the measured sample replicates (weighing 20g) was dissolved in 100ml water and sodium carbonate (5% w/v) solution added after which a duration of 30 minutes was allowed for the reaction to take place.
- 2% w/v **s**odium hydroxide solution was then added followed immediately by 15% v/v hydrogen peroxide, which was added under air-tight conditions after which a duration of 30 minutes was allowed for the reaction to take place.
- Thereafter, filtration was carried out on the sample to remove water.

- ❖ The sample was then washed 3 times using 10% w/v sodium sulfate solution and subsequently filtered.
- ❖ The washed and filtered sample was thereafter immersed in a mixed solution of sodium chloride (6% w/v) and sulphuric acid (1% v/v) for a period of 1 hour to allow for acid steeping to take place after which the sample was then filtered again.
- ❖ Another round of washing (x 2) was carried out on the sample in a mixed solution of 10% w/v sodium sulphate and 6% w/v sodium chloride and then filtered.
- ❖ The dechromed sample (product) was thereafter allowed to air-dry at room temperature and then assessed for the residual chromium using atomic absorption spectrophotometer.

The rate at which the chromium was being extracted was investigated by measuring chromic oxide content levels after 0.5, 1, 2, 4, 8, 16, 24, 36 and 72 hours of the dechroming process. The calculation for the dechroming ratio or percentage was done according to the formula described by Sun *et al.,* (2003) as follows:

Dechroming ratio (%) = 100 [(Crwaste – Crdec)/Crwaste];

where,

Crwaste = concentration of chromic oxide (Cr_2O_3) in leather waste sample before dechroming.

Crdec = concentration of chromic oxide (Cr_2O_3) in leather waste sample after dechroming.

4.4.Results

4.4.1 Estimation of total chromium content in raw chrome leather waste

Determination of total chromium content in raw chrome leather waste samples was done following the method described in the International Union of Leather Technologists and Chemists Society (IULTCS) official methods of analysis (IUC 8, 2001), which is commonly referred to as the wet-oxidation method due to the oxidation of Cr (III) in the waste using $KMnO_4$ in acidified solution. The results of this experiment are given in **Table 4.1,** where the average extractable total chromium content (usually expressed as chromic oxide (Cr_2O_3)) in raw chrome leather waste samples was found to be 3.67%.

Table 4.1: Estimation of total chromium content (expressed as Cr_2O_3) in raw chrome leather waste samples by the wet oxidation method

Raw chrome leather waste	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Mean
Cr_2O_3 content (%)	3.17	3.77	3.97	3.91	3.34	3.89	3.67

4.4.2 Extraction of chromium from tanned leather waste

The calculated mean concentration value for chromium in raw chrome leather waste samples prior to dechroming using various dechroming methods (as determined by the atomic absorption spectrophotometry (AAS) on 3 sample replicates in each case) was 218.65ppm as indicated in **Table 4.2.),** which also shows results of residual chromium concentration (ppm) in the same leather waste samples after dechroming using the respective dechroming methods. The methods were four in number and gave the following mean values of residual chromium concentration: dechroming method according to Paul *et al.*, (2013) – 62.52ppm, Sun *et al.*, (2003) – 37.83ppm, Adeoye *et al.*, (2014) – 21.67ppm, and the new method developed in this study – 0.22ppm.

Results indicate that after extraction, the chromium reduced in all the methods used but at different levels **(Table 4.4.).**

Table 4.2: Concentration of chromium in raw chrome leather waste before and after extraction using various dechroming methods as determined by AAS

Sample type	Concentration of chromium (ppm)			
	1st trial	2nd trial	3rd trial	Mean
Raw tanned leather waste before extraction	218.65	218.63	218.66	218.65
Tanned leather waste after extraction by Paul et al., (2013) method	69.35	55.29	62.91	62.52
Tanned leather waste after extraction by Sun et al., (2003) method	35.20	39.14	35.64	37.83
Tanned leather waste after extraction by Adeoye et al., (2014) method	19.94	23.53	21.53	21.67
Tanned leather waste after extraction by the new method	0.24	0.19	0.23	0.22

4.4.3 Efficiency of chromium extration by various methods.

The efficiency of chromium extraction differed from one method to another. The new method led in efficiency of chromium extraction at (99.9%) while the other methods followed in this order; Adeoye *et al*., (2014) at (90.1%), Sun e*t al.*, (2003) at (82.7%), and Paul *et al*., (2013) at (71.4%) as indicated in **Table 4.3.**

Table 4.3: Percentage of chromium extracted from chrome leather waste using the various extraction methods

Chromium extraction Method	Efficiency of chromium extraction (%)			
	Trial 1	Trial 2	Trial 3	Mean
Method by Sun *et al.,* (2003), Normal alkaline hydrolysis	83.50	81.20	83.40	82.70
Method by Paul *et.al.,* (2013), Alkaline hydrolysis	71.30	71.00	71.90	71.40
Method by Adeoye *et al.,* (2014), Modified alkaline hydrolysis	89.60	89.20	91.50	90.10
New method (Modified Malek *et al.,* 2009)	99.89	99.92	99.89	99.90

4.4.4 Rate of chromium extracted by different types of methods

Table 4.4. shows the rate (%) of chromium extraction from leather waste and the residual chromium remaining in the waste sample using different methods. Extraction was completed at various periods for each of the methods. The results shown in **Table 4.4.,** were subjected to a t-distribution test, where the calculated $t._{025}$ –value was found to be 12.62 as opposed to the value given in statistical tables of 2.306 at 95% confidence interval with 8 degrees of freedom. This is a confirmation that there was a significant difference at 95% confidence interval, on the rate of chromium extracted by different dechroming methods that were investigated in this study.

Table 4.4: Rate (%) of chromium extraction from leather waste using different dechroming methods

Duration (hrs)	Method 1 (Sun *et al.,* 2003)	Method 2 (Paul *et al.,* 2013)	Method 3 (Adeoye *et al.,* 2014)	Modified Malek *et al.,* (2009) New method (Method 4)
0	0	0	0	0
0.5	5.0	2.9	11.3	20.0
1.0	7.2	6.8	20.2	28.6
2.0	15.2	13.0	28.9	31.8
4.0	36.8	22.3	42.7	62.8
8.0	68.8	48.2	66.6	77.1
16	82.7	53.1	72.5	89.4
24	82.7	62.6	83.4	99.4
36	82.7	68.2	90.1	99.9
72	82.7	71.4	90.1	99.9
Residual Cr in sample	**17.3%**	**28.6%**	**9.9%**	**0.1%**

Key

Method 1: Dechroming method as described by Sun *et al.*, (2003); 82.7% Cr extracted in 16 hrs.

Method 2: Dechroming method as described by Paul *et al.*, (2013); 71.4% Cr extracted in 36 hrs.

Method 3: Dechroming method as described by Adeoye *et al.*, (2014); 90.1% Cr extracted in 36 hrs.

Method 4: New dechroming method developed in this study; 99.9% Cr extracted in 24 hrrs.

The remaining traces of chromium in the dechromed and hydrolysed leather waste samples (using various dechroming methods) was determined as chromium (VI) by the 1, 5 Diphenyl carbazide method (IULTICS; IUC 18 or SLC 22, 2001). The results of this determination were tabulated as shown in **Table 4.5.** after measuring the absorbance at 540nm with UV/Vis spectrophotometer (U2900 200V model SP-191 made in Japan).

Table 4.5: Absorbance graph readings at 540nm for residual Cr^{6+} in collagen hydrolysate using UV/Vis spectrophotometer (U2900 200V model SP-191)

Dechromed collagen hydrolysate sample	Absorbance at 540nm) (mg/l)	Residual Cr^{6+} present in sample (%)
A1	1.97	0.0008
A2	2.01	0.0008
B1	8.51	0.0035
B2	8.72	0.0036
C1	1.99	0.0008
C2	10.45	0.0043
D1	10.55	0.0044
D2	10.51	0.0044
Mean residual Cr^{6+} present in sample (%)		**0.0028**

Key

Samples A_1 & A_2 – Dechromed collagen hydrolysate sample concentration (50mg/l)

Samples B_1 & B_2 – Dechromed collagen hydrolysate sample concentration (100mg/l)

Samples C_1 & C_2 – Dechromed collagen hydrolysate sample concentration (150mg/l)

Samples D_1 & D_2 – Dechromed collagen hydrolysate sample concentration (200mg/l)

Table 4.6: Chemical characteristics of dechromed leather waste after dechroming using the new method

Chemical characteristic	Measured value (%)
Level of moisture (moisture content)	12.61
Total organic carbon (TOC)	23.27
Total ash	12.42

Chemical characteristic	Measured value (%)
TKN (Total Kjeldahl Nitrogen)	52.89
pH	10.01
Chromium extracted	99.90
Residual chromium	0.10

Collagen hydrolysate obtained using the new dechroming and hydrolysis method gave the highest mean value for total protein content of 52.89% as indicated in **Table 4.6.,** which was roudend to 53% as shown in **Table 4.7.** This value was higher than values obtained using other methods that were covered by this study as well as those obtained from literature review, regardless of the time taken for the respective dechroming and hydrolysis reactions to go to completion. (Taylor *et al.*, 1997; Catalina *et al.*, 2007; Malek *et al.*, 2009; Ding *et al.*, 2015). Similarly, the new method of extracting chromium from chrome-tanned leather waste gave the highest value for the ratio of TKN to residual chromium, which was 2,409,091 (sample E) as compared to the other chromium extraction methods that were investigated in this study (**Table 4.7.).** In the new dechroming method, P-Value of < 0.05 was considered significant in all statistical comparisons for TKN concentrations in the dechromed and hydrolysed leather waste samples as shown in **Table 4.8.**

Table 4.7: Ratio of TKN to residual chromium concentration after various dechroming methods

Sample	TKN (%)	TKN (ppm)	Residual chromium (ppm)	TKN/Residual chromium ratio
A	35.00	350,000	218.65	1,601
B	45.00	450,000	62.52	7,198
C	44.00	440,000	37.83	11,630
D	47.00	470,000	21.67	21,689
E	53.00	530,000	0.22	2,409,091

Key

Sample A: Raw chrome tanned leather waste.

Sample B: Dechromed leather waste according to Paul *et al.,* (2013); alkaline hydrolysis method.

Sample C: Dechromed leather waste according to Sun *et al.,* (2003); normal alkaline hydrolysis method.

Sample D: Dechromed leather waste according to Adeoye *et al.,* (2014); modified alkaline hydrolysis method.

Sample E: Dechromed leather waste according to the new method (chromium extraction using formic acid and potassium oxalate).

Table 4.8: ANOVA for TKN concentrations in dechromed and hydrolysed leather waste samples (P<0.05) using the new dechroming method

Sample	Sum of squares	Degrees of freedom ($x\ 10^{-5}$)	Means of squares ($x\ 10^{-5}$)	Sample Variance	F_{ratio}
1	0.0222	1	0.0037	6.7512	0.0230
2	0.0273	1	0.0046	0.0769	0.0002
3	0.0272	1	0.0045	3.7152	0.0070
4	0.0271	1	0.0045	0.1479	0.0003
5	0.0312	1	0.0052	1.7880	0.0038
6	0.0280	1	0.0047	0.0000	0.0000

4.5. Discussion

Among the three previous methods of dechroming tannery waste that were investigated in this study, the one developed by Adeoye *et al.,* (2014) was on the lead in terms of chromium extraction efficiency as it reduced the mean concentration of chromium in leather waste from 218.65ppm to 21.67ppm, which translated into 90.1% reduction leaving an average of 9.9% residual chromium in the leather waste samples as a result **(Table 4.4.).** The dechroming method according to Sun *et al.,* (2003) followed closely in chromium extraction efficiency as it managed

to reduce chromium in the leather waste samples by 82.7% on average (i.e. from 218.65ppm in raw tanned leather waste, to 37.83ppm in the dechromed leather waste) leaving only 17.3% residual chromium in the sample as shown in **Table 4.2, Table 4.3 and Table 4.4,** respectively. The third previously used dechroming method that was investigated in this study was the one according to Paul *et al.,* (2013), which achieved an average of only 71.4% chromium extraction efficiency (i.e. from 218.65ppm in raw tanned leather waste samples to 62.52ppm in the dechromed samples) leaving approximately 28.6% as residual chromium in the dechromed samples **(Table 4.2., Table 4.3., Table 4.4.).** Results obtained from AAS analysis of the amount of chromium extracted from leather solid wastes as a function of time using the new dechroming method established that this new method was the best so far, in terms of efficiency and effectiveness when compared with values obtained from previous methods that were covered by this study **(Table 4.4.)**, and also from literature review. The new dechroming method managed to reduce chromium concentration in raw tanned leather waste samples from 218.65ppm to a neglegible concentration of only 0.22ppm in the dechromed samples on average as shown in **Table 4.2.** This means that the new method was able to extract 99.9% chromium from the leather waste leaving only 0.1% as residual chromium in the dechromed samples **(Table 4.2., Table 4.3., Table 4.4.).** After subjecting the leather waste samples dechromed using this new method to further analysis with UV/Vis spectrophotometer (U2900 200V model SP-191) for purposes of determining any traces of chromium (VI) that may have remained after potassium oxalate complexation, it was found that only a negligible quantity remained (i.e. 0.0028% Cr^{6+}) which was far below the recommended safe limits in wastewater and in the soil **(Table 4.5.).** The WHO recommended safe limits for Cr(VI) in wastewater, soil and drinking water ranges between 0.05-0.10ppm.

The newly developed dechroming procedure was found to be a suitable method of extracting chromium from chrome tanned solid wastes without destroying the collagen tissues as the hydrolysis part was conducted using formic acid, a weak acid which works best at room temperature. The method was able to extract 99.9% chromium and increased %TKN in the waste to 52.89%. Besides, the relatively very high ratio of TKN to residual chromium - 2,409,091 (sample E) as reflected in **Table 4.7.**, is a clear indication that this new dechroming method was very effective. This is attributable to the use of potassium oxalate, which was able to complex any remaining traces of chromium into chromium oxalate whose high solubility in water enabled it to be washed away very quickly leaving an almost chrome-free collagenic material with relatively high protein content. The levels of total ash and total organic carbon were also relatively high in the resultant collagen hydrolysate (i.e. 12.42% and 23.27%, respectively as indicated in **Table 4.6.).** This was attributed to the normally high presence of organic matter in leather and by extension leather products, and even solid wastes emanating from the leather industry as a majority of the chemicals used in leather production are organic in nature. The hide collagen itself, which constitutes the largest proportion of leather, is also an organic material. The new method was more efficient than the dechroming methods previously described by Sun *et al.,* (2003), Paul *et al.,* (2013) and Adeoye *et al.,* (2014). The results obtained from the rate of chromium extraction using the new dechroming method were analysed statistically by the F-test method (ANOVA) and the observed variance ratio F_{ratio} (observed) for six different samples calculated as shown in **Table 4.8.** Since the probability of F as found from the Table of F-distribution (F_{theory}) was small, i.e. $P < 0.05$, the results obtained from this study suggest that there was a significant difference between the population means from which the respective samples were drawn. This is a clear indication that the results obtained from the new dechroming

method were statistically significant (i.e. did not happen by chance) as confirmed by Rao and Richard, (2016).

Each of the four different dechroming methods that are being discussed here had their own strengths and weaknesses. The Cr extraction method according to Sun *et al.*, (2003), had the following strengths; Cr extraction was done without affecting the properties of the original collagen fibres, the extraction process was completed within the first 16 hours of dechroming (achieving 82.7% Cr extraction), excess water was removed by filtration (there was no need for mechanical dewatering after dechroming), the dechroming rate was relatively fast from the 2nd to the 8th hour of dechroming unlike in the other three methods, the resultant collagen hydrolysate was air-dried at room temperature as opposed to the other methods. However, there were some drawbacks (weaknesses) that caused this method to be less efficient which were; involvement of a lot of washing leading to high water consumption, use of hydrogen peroxide leading to secondary pollution problems, involvement of a lot of calculations leading to less accurate results and the high costs incurred in setting up the experiment and huge chunks of time spent in preparation.

Although the dechroming method described by Paul *et al.*, (2013) was the least efficient among those studied in this work, it had two major advantages namely; the results obtained from the method were accurate (reliable) and drying of the dechromed waste was conducted at room temperature. However, it was time-consuming (involving a lot of steps) and costly (consuming a lot of water and chemical reagents). Other weaknesses of this method were; secondary environmental problems emanating from it and its inefficiency (it managed to extract a maximum limit of only 71.4% Cr from the leather waste samples in 36 hrs).

The dechroming method according to Adeoye *et al.,* (2014) had a number of strengths that included; its capacity to extract a maximum limit of 90.1% Cr in 36 hrs, excess water was removed by filtration only, there was no need for mechanical dewatering of the dechromed samples and the drying process was done at room temperature. However, the method had several weaknesses which can be summarized as follows:

- The resultant collagen hydrolysate is of low quality due to digestion of the collagen fibres by strong alkaline hydrolysis involved in this method.
- Use of hydrogen peroxide in this method leads to secondary pollution problems.
- The leather waste needs to be pulverized first before dechroming making the method costly.

The new dechroming method had more strengths than the other three discussed methods as can be seen in the summary below.

- It is the most efficient method of dechroming leather wastes so far (can extract 99.9% Cr in just 24 hours).
- The remaining traces of chromium in the resultant collagen hydrolysate can be complexed with potassium oxalate to form a complex of potassium chromate, which can easily be washed away during the subsequent washing steps as it is highly soluble in water.
- There is minimum input of energy (very little heating is required) and chemicals making the technology very simple as a result.

- The resultant collagen hydrolysate remains intact as no strong alkalis, acids, or enzymes are used in the hydrolysis part of the Cr extraction process. Further, the process is carried out at a controlled temperature of 50^0C to avoid the dissolution of collagen.
- The method is environmentally-friendly and cost-effective making it sustainable as a result.
- It leads to very high levels of total protein content (expressed as %TKN) and very low levels of residual chromium in the resultant collagen hydrolysate.

The few weaknesses of this new dechroming method are overwhelmingly overshadowed by its numerous strengths as they are only two in number namely;

1) The resultant collagenic material needs oven-drying at 40^0C for uniform drying.
2) Excess water needs to be removed mechanically.

According to (Kanagaraj *et al.,* 2006), the reaction between the tanning agent and collagen during leather making normally takes place in spaces where the collagen fibres are surrouded by a sheath of connective tissue termed as sarcolemma, whose structural characteristics have been illustrated in the work done by Bella *et al.,* (1994). The findings of the current study have shown that previously used dechroming methods have not been able to extract all the chromium in chrome-tanned leather waste as the residual chromium remains trapped within the collagen fibres in regions enclosed by the sarcolemma sheath. However, this problem was overcome in the new dechroming method as the use of potassium oxalate in the second part of the dechroming procedure was able to complex the remaining chromium into potassium chromate, which is highly soluble in water and hence, easily washed away in subsequent washing steps. This

complexation reaction between residual chromium in the dechromed leather waste also led to the formation of a few traces of chromium oxalate, which have been found to play a key role as an essential plant nutrient for food crops production (Cary *et al.,* 1997). This contributed positively to the resultant collagen hydrolysate fertilizer, which was eventually formulated in this study. Further work done by Hammond, (1995) found that organic nitrogen in dechromed leather wastes containing less than 0.5% residual chromium (as shown in **Table 4.6.),** undergoes degradation very quickly due to the large quantities of organic matter present in such wastes. This makes properly dechromed leather wastes very good candidates for fertilizer production.

The environmental impact of all the reagents used in the new method of dechroming leather wastes was found to be acceptable. For instance, the ammonium sulphate used in this method is non-toxic due to the presence of the ammonium ion (NH_4^+). An equilibrium exists between the toxic ammonia (NH_3), which is unionised, and the non-toxic ammonium ion (NH_4^+) as a function of existing or introduced environmental changes; mainly temperature and pH.

According to *GPS Safety Summary Ammonium sulphate – Arkema.com,* the ammonia to ammonium ratio is 1 to 3000 at the pH value of around 6 whereas this ratio decreases drastically to 1 to 30 when the pH is raised to 8 by environmental factors. The representative equation for this equilibrium reaction is given below.

$$NH_{3(aq)} + H_2O \text{ .....} \rightarrow\leftarrow \text{.....} NH_3 \cdot H_2O_{(aq)} \text{ .....} \rightarrow\leftarrow \text{.....} NH^+_{4(aq)} + OH^-_{(aq)}$$

(NH_3 + water) (NH_4^+ in water)

Another reagent that was used in this new dechroming method is ammonium sulphate, which has no known adverse effects to aquatic life even after prolonged exposure to aquatic environmets. However, as reported in the ARKEMA GPS Safety Summary, this compound has been

documented as toxic to fish but not to algae and invertebrates living in aquatic environments. According to *GPS Safety Summary Ammonium sulphate – Arkema.com,* ammonium sulphate dissociates immediately it is disposed of to the environment into its ionic constituents, which are environmentally friendly. Formic acid was used in the first part of the Cr extraction process in this new dechroming method, and due to its high rate of evaporation leaving no residues behind, it is a very efficient weak organic acid making it environmentally sound as a result (Xiang *et al.,* 2015). Potassium oxalate, which was used in the second part of the new dechroming method, doesn't pose any threat to the environment even in the areas of chrome toxicity, severe/acute toxicity, irritation due to corrosion, or possible hazardous effects emanating from normal industrial activities *(Safety Data Sheet for Potassium Oxalate, 2015).* However, although this compound tends to be inert (i.e. non-reactive) under normal conditions, releasing it into the environment is not recommeded as it has been reported to have some bioaccumulation tendancy (Geoff, 2015).

4.6. Conclusions and Recommendations

The study reached the conclusion that chrome-tanned leather waste can efficiently be dechromed using the new method described herein to produce chrome-free collagen hydrolysate that has great potetial in the production of a useful organic fertilizer. The study recommends up-scaling and optimization of the waste dechroming method described herein for large scale decontamination of chrome leather waste.

CHAPTER 5: HYDROLYSIS AND MODIFICATION OF DECHROMED LEATHER WASTE

5.1. Abstract

Organic fertilizer mainly consists of organic materials that must be broken down by microbial activity before the nutrients are available to the plants. These fertilizers usually take a long time to release plant nutrients and, in some cases, the nutrients may not be available when the plant needs them. This study focused on hydrolysis of collagen from dechromed leather waste using mild orthophosphoric acid, followed by modification with Epichlorohydrin (EPICH) to enhance availability of nutrients for use by plants. The nutrient content of the resulting modified collagen material including total organic carbon, ammonium-N, nitrate-N, calcium, magnesium, phosphorus and transferable potassium were determined using standard methods. The study also determined the nutritional content of ground maize cobs and tested its suitability for use as a filler material in the formulation of slow-release high nitrogen organic fertilizer. The modified collgen material had the following nutrient composition: Total Nitrogen, 44%, Phosphorous, 21.02 %, Exchangeable potassium 0.1%, Magnesium 0.2%, Calcium, 1.8%, Total Organic Carbon 27% and C:N ration of 0.61. On the other hand, the ground maize cob filler material had the following nutrient composition: Total Nitrogen, 2.8%, Phosphorous, 14.04 %, Exchangeable potassium 0.3%, Magnesium 7.5%, Calcium, 1.6%, Total Organic Carbon 38.12% and C:N ration of 13.61. Determination of the rate of N mineralization was carried out by mixing soil samples with different rates of fertilizer and filler combinations and aerobically incubating the mixture for 16 weeks. The highest mineralization rate of organic N into NO_3-N was observed during the 12^{th} week, while, the highest mineralization rate of N into NH_4^+-N was observed during the 16^{th} week of incubation. It was concluded that EPICH modification of dechromed tannery waste released carbon, nitrogen and phosphorus from collagen making them available

for plant use, and that the use of maize cobs powder as filler material during formulation enhanced the quality of the organic fertilizer. In addition, the highest mineralization rate observed during the 16th week of incubation was an indication of the slow-release nature of the formulated organic fertilizer.

5.2. Introduction

Organic fertilizer mainly consists of organic materials that must be broken down by microbial activity before the nutrients are available to the plants (Lazcano *et al.*, 2021). In general, fertilizers derived from organic sources usually take a long time to release plant nutrients and these nutrients may not be available when the plant needs them (Guertal, 2009; Ding *et al.*, 2016). According to Langmaier *et al.,* (2005), there is a possibility of EPICH reacting with collagen protein matrix by activating the protein nucleophile groups (particularly $–NH_2$ or –OH groups) to form epoxide compounds which then react with the rest of the nucleophile groups in the polypeptide to open up epoxide rings. Once the epoxide rings are opened (at elevated temperatures), the nitrogen becomes more available for release. It has been shown that in aqueous environments, EPICH can react with the primary amino groups of the hydrolysed collagen as a mono-functional agent via its chlorine atom; reaching an equilibrium within 1 hour if the temperature is kept at 60^0C. Reaction of the oxirane ring of EPICH then proceeds if the temperature is considerably increased to around 200^0C (Langmaier *et al.*, 2005). EPICH tends to target those amino acids with high concentration of nitrogen atoms in their structures and strategically positioned for ease of crosslinks formation with them (e.g., histidine and arginine). EPICH reacts more specifically with guanidine or imidazole groups in arginine and histidine amino acids, respectively (Baumert and Fassold, 1989; Feichtinger *et al.*, 1998). The use of EPICH as a crosslinking agent can be an advantage in the development of slow-release nitrogen

fertilizers from collagen hydrolysates derived from dechromed leather solid wastes. Using the method developed by Ritter and Drake, (1945) to obtain pore volume distributions through high-pressure mercury porosimetry, with some few modifications according to Ritter and Erich, (2002); it was also observed that the volume fractions of solids, liquids and gases may be influenced by heat changes. Alterations in protein-water interactions and hydration phenomenon have also been linked to such influence on the volume fractions of solids, liquids and air. In this regard therefore, there is a possibility that crosslinking collagen hydrolysate with EPICH at an increased temperature of 120-200^{0}C triggers a crosslinking reaction between the EPICH oxirane rings and specific amino acids (e.g. histidine and arginine) leading to the formation of multiple epoxy epoxides, which then at elevated temperatures, can trigger cross-linking reactions with the functional groups present in peptides such as amines, hydroxyl groups, carboxylic groups, and sulfhydryl groups (Greg, 2013). These reactions are illustrated in **Figure 5.1.**

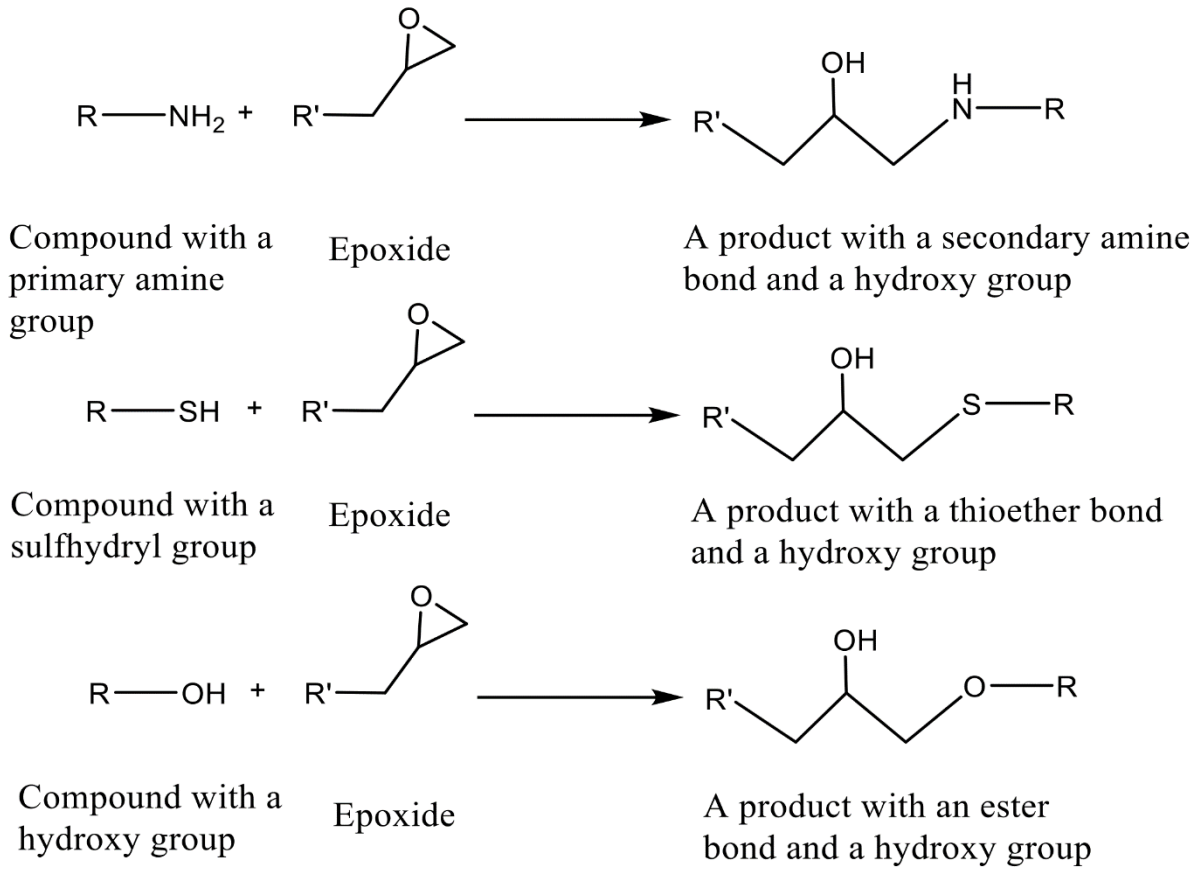


Figure 5.1: Reactions between epoxy groups and; (a) primary amine groups, (b) sulfhydryl groups and, (c) hydroxyl groups

At lower temperatures (surrounding temperature), the reaction between epoxy groups and amines was found to take place faster than reaction between the same epoxy groups and other functional groups including; carboxylic or hydroxyl groups as described by Shechter *et al.,* (1956). However, at elevated temperatures (120-200^{0}C), crosslinking with EPICH is usually very successful and tends to target carboxylic or hydroxyl groups more than amines as established by Li *et al.,* (2003); Motawie, (2010); Kristufek *et al.,* (2016); Shui *et al.,* (2020). The current study found that the crosslinking reaction of EPICH with peptides at temperatures between 120 and 200^{0}C leads to the formation of epoxides with a very high tendency to react with carboxylic functional groups making the heteroatom carbon more available for release as a result. **Figure**

5.2 shows the pathway of poly (vinyl alcohol) (PVA) epoxidation using epichlorohydrin (EPICH) as demonstrated by Shechter *et al.,* 1956).

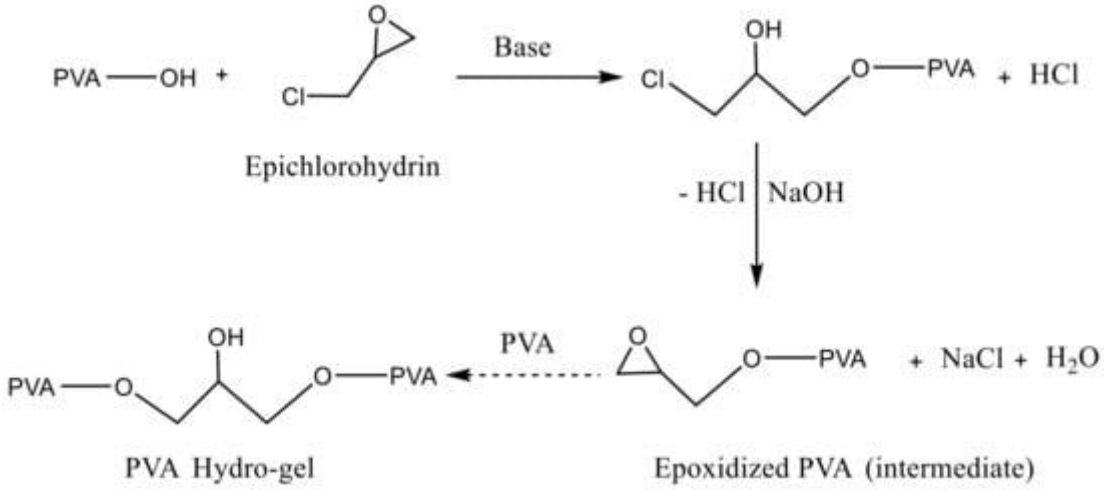


Figure 5.2: PVA epoxidation reaction pathway using epichlorohydrin (EPICH).

Shui *et al.,* (2020) tried to improve the crosslinking capacity of EPICH, by introducing an epoxide group into PVA to epoxidize it; enhancing its reactivity as a result. The same researchers (Shui *et al.,* 2020), also found that due to the very high reactivity of epoxidized PVA with specific risk materials (SRM) derived peptides (through their amino and carboxylic acid functional groups), the epoxidized PVA is normally very effective in forming crosslinks with SRM – derived peptides such as chrome-tanned leather wastes. This study investigated the suitability of epichlorohydrin as a crosslinking agent for chrome-tanned leather waste hydrolysate (after chromium removal and mild alkaline hydrolysis with orthophosphoric acid) in the development of a slow-release nitrogen fertilizer.

Typically, the percentage of free amino acid content in hydrolysed protein fertilizers ranges from 5-40% (Cavani *at al.,* 2003). Glycine tops the list of the most abundant amino acids in

hydrolysed proteins (HPs), which amounts to approximately 26-50% of the total. The nitrogen content in glycine is also the highest in collagen hydrolysates, standing at 28% of the total. Other amino acids which are also particularly abundant are proline and hydroxyproline, ornithine and glutamine as well as histidine and arginine (Corte *et al.,* 2013).

The arginine amino acid was found to play a crucial role in the formation of crosslinks with EPICH due to the presence of a guanidine moiety in its side-chain, which is known to interact very intimately with enzymes or receptors through formation of hydrogen bonds and/or electrostatic interactions as described by Feichtinger *et al.,* (1998) who also reported the extensive use of this nitrogen organic base in the production of heterocyclic compounds having at least two nitrogen atoms. Amino acids containing imidazole moieties such as histidine tend to form epoxides (five-membered rings with two heteroatoms) more readily than others with two heteroatoms (e.g., pyridine and pyrrole) due to the strategic location of the available lone pair of electrons. Since in this case one of the heteroatoms must be nitrogen, and the ring system is numbered starting with those heteroatoms ranked higher in terms of their respective atomic numbers, nitrogen is usually the heteroatom that comes first in numbering. Epichlorohydrin is favoured by this strategic positioning of nitrogen in the protein structure and easily forms oxirane rings as a result. Crosslinking proteins with EPICH is also advantageous due to its character to undergo a two-stage process (Langmaier *et al.,* 2005). The environmental impact: health hazards and safety precautions associated with epichlorohydrin (chemical formula: C_3H_5ClO) are described in **Appendix 3.**

For tannery waste to be useful as a fertilizer, there should be high level of mineralization of the waste when applied into the soil. Mineralization being the process by which nitrogenous compounds present in organic matter are decomposed, or oxidized into readily available forms

for use by plants as nutrients, it increases the rate at which such nutrients particularly nitrogen, phosphorus and sulphur are made bioavailable in the decomposing organic matter. According to Kavita and Vinod, (2018), the microbiota in soil (e.g., fungi, bacteria and earthworms) is the one that triggers the process of N mineralization in organic molecules. Calculating the difference between the final and the initial total inorganic N (nitrate + ammonium) concentration after the process of mineralization, gives the N mineralization potential (Kavita and Vinod, 2018). This study assessed N mineralization rate of the modified material to determine the rate at which nutrients will become available to plants following its application to the soil.

5.3.Material and methods

5.3.1 Reagents

The common laboratory reagents required for the hydrolysis and modification of collagen derived from the dechromed leather solid wastes were sourced from OSHO CHEMICALS (K) Ltd., and were all of analytical reagent grade. Usual laboratory chemicals were used in the experiments and during all the analyses. Epichlorohydrin (99%) was sourced from Sigma-Aldrich in Germany. The 0.01M caustic soda (NaOH) used to adjust pH of the collagen hydrolysate to 11 prior to the modification process with EPICH was sourced from OSHO Chemicals (K) Ltd. Orthophosphoric acid used for mild hydrolysis of the dechromed leather waste prior to its modification with EPICH was also sourced from OSHO Chemicals (K) Ltd.

Soil was sampled and the samples dried and ground using mortar and pestle, after which the samples were then sieved using 2 sieves of different sizes; 2mm and 0.5mm (the 2mm sieve was placed on top of the 0.5mm sieve so that whatever did not pass through the 2mm sieve was discarded). Samples collected in the 0.5mm sieve were used to determine total N and organic

carbon. The reason for using the 0.5mm samples was to ensure that the soil was completely homogenized and the organic part of the samples could easily be determined in fine soil samples. Weight of 1g for each of these 0.5mm soil samples was used in the determination of N and organic carbon, pH, N, available P, exchangeable K, total organic carbon and CEC determinations.

5.3.2 Mild hydrolysis of the dechromed leather waste samples

After mechanical dewatering and oven drying of the dechromed leather waste samples, they were given a mild hydrolysis with orthophosphoric acid added at a rate of 100ml perkg of leather waste in 300% float for a period of 1 hour. The experiment was conducted in a stainless-steel tannery experimental drum at KIRDI, and the temperature of the drum was maintained at 50^0C as higher temperatures would destroy the collagen fibres making them useless as a raw material for the intended fertilizer formulation. After running the drum for 1 hour, an extra 100ml of orthophosphoric acid perkg of sample was added through the drum axle and the drum continued running for another 1 hour under the same conditions of temperature (50^0C) after which it was stopped and the sample allowed to cool overnight. The drum was drained the following morning after being allowed to run for 30 minutes and its contents offloaded, mechanically dewatered by pressing and squeezing the sample through a strainer, and at the same time, collecting some of the squeezed liquor for total chromium content analysis using atomic absorption spectrophotometry (AAS). The hydrolysed collagenic material was then put in the laboratory oven at 40^0C overnight for uniform drying in preparation for EPICH modification process that was to follow next.

5.3.3 Modification of Collagenic material in dechromed leather waste hydrolysates

Approximately 10kg of the oven dried collagen hydrolysate was put back into the stainless-steel tannery experimental drum in KIRDI containing 50% float. The pH of the float was adjusted to 11.0 with 0.01M sodium hydroxide according to the procedure described by Langmaier *et al.*, (2005). About 10ml of epichlorohydrin (EPICH) (99%) was then added and the drum temperature controls set at 60^0C after which it was allowed to run for I hour and then stopped. After resting for I hour and checking pH (the pH was maintained at 11.0 throughout the reaction process) an extra 10ml of epichlorohydrin (EPICH) (99%) was added and the temperature controls set at 120^0C (to trigger the reaction of the epichlorohydrin oxirane ring). The drum was then allowed to run for I hour under these temperature conditions and thereafter, the drum contents were offloaded and spread on polythene papers to cool down (overnight). After cooling, the EPICH modified collagenic material was vacuum dried at 80^0C for 2minutes using the tannery vacuum drier at KIRDI to mechanically squeeze out excess water present. The purpose of this mechanical removal of excess water was to allow for concentration of the protein fractions and further modification of the relevant amino acids.

The modified collagen hydrolysate fertilizer was thereafter dried at 40^0C for 3 days using the laboratory oven to ensure complete drying. The dried samples were then prepared for the analysis of different parameters of interest as described herein. Infrared (IR) spectroscopy was used to identify the functional groups of interest in the collagen hydrolysate fertilizer (both before and after modification with epichlorohydrin). This analysis was carried out at the Department of Chemistry (University of Nairobi). The analysis was performed following the new technique of Fourier Transform Infrared (FTIR) spectroscopy usually known as Attenuated Total Refluctance (ATR) spectra, using the Japan made Iraffinity – IS model). The fertilizer

sample to be analysed was ground into a powder before loading it into the ATR for ease of reading.

5.3.4 Formulation of the new organic fertilizer

Maize cobs powder was used as filler material. The milled maize cobs powder was selected as filler material after conducting some preliminary laboratory tests and relevant literature review. Dry maize cobs were shredded and milled to make powder and samples of the EPICH modified collagenic material were blended at different rates of filler material. The use of maize cobs powder keeps the resultant slow-release fertilizer from drying out, hardening and clumping during storage. The filler was also intended to condition the fertilizer to have some special traits such as can be seen in the research findings of this study. The nutrient content of the filler was determined to see if the filler could contribute to the nutrient value of the fertilizer.

5.3.5 Total organic carbon determination in trial fertilizer and filler samples

Total organic carbon (TOC) determination in soil, trial collagen hydrolysate fertilizer and filler samples was carried out according to Walkley-Black Method, (1934) described in Soil Analysis Routine Methods with some few modifications (Nelson and Sommers, 1996). A small amount of the sample was weighed in duplicate (between 0.9 and 1.6g) and transferred to 250ml Erlenmeyer conical flask. Excess 1N potassium dichromate (10ml) was then added to the Erlenmeyer flask and a blank also included (i.e., an empty conical flask containing only potassium dichromate but no sample). The contents were then mixed with 20ml conc. Sulfuric acid. Addition of this acid was done quickly by means of an automatic pipette, which directed the stream into the suspension for enhanced oxidation process. This was followed immediately by gentle swirling of the flask to allow for thorough mixing of the sample and reagents after

which the contents were allowed to stand for 20 minutes to cool down. Distilled water (approximately 100ml) was then added followed by a few drops of (1, 10 phananthroline hydrochloride ($C_{12}H_9ClN_2H_2O$) 99.5% AR) indicator. The contents were titrated using approximately 0.5N ferrous sulphate to an end-point of reddish-purple colour.

The percentage of total organic carbon (TOC) was done using the following formula:

$$\text{Percentage TOC} = \frac{[(V_{blank} - V_{sample}) \times 0.3 \times N]}{\text{Weight of the sample}} \times \frac{100}{77}$$

Where:

V_{blank} - volume of ferrous sulphate used to titrate the blank

V_{sample} - volume of ferrous sulphate used to titrate the sample

0.3 is a multiplication factor

N is the actual Normality of ferrous sulphate

$N = 10/V_{blank}$ (10 ml is the volume of $K_2Cr_2O_7$ used in the experiment. Apart from oxidation of the organic carbon present in the sample, the potassium dichromate was also used to standardize the sample solution).

Percentage organic matter in the sample = % TOC x 1.724

5.3.6 Determination of percentage total nitrogen in trial fertilizer, filler and soil samples

The determination of total nitrogen (i.e. TKN + nitrate + nitrite) in trial soil, fertilizer and filler samples was carried out using the Kjeldahl steam distillation method (Bremner, 1996), but with

some few modifications. Approximately 0.3g of fertilizer or filler (or 1g of soil) samples were digested using HCL. This was followed by distillation of 10ml of sample mixed with 2% boric acid (20ml) and phenolphthalein indicator (2 drops). Thereafter, 40% sodium hydroxide was added gently into the distilling flask until the mixture turned pink (indicating full neutralization of the acid). The trapped NH_3-boric acid in the distilling flask was then titrated with 0.1N sulfuric acid (for fertilizer and filler samples, and 0.01N sulfuric acid for soil samples) until the greenish colour changed to pale pink. The titration volume was subsequently recorded for all samples and then calculations for total nitrogen in the trial fertilizer, filler and soil samples done as follows% N =

$$\frac{\text{Titre volume x Normality of acid x Relative Atomic Mass of N x Volume extracted}}{\text{Weight of sample (g) x 1000 (dilution factor) x Volume distilled (10)}} \times 100$$

For a fertilizer sample titre volume of 2.0ml, the calculation for percentage total nitrogen (%N) gave 46.7%

For a filler sample titre volume of 12.1ml, the calculation for percentage total nitrogen (%N) gave 2.8%

Amount of soil sample weighed for %N determination was 1.0g whereas the normality for sulfuric acid used in titration was 0.01N. Therefore, using the equation for %N determination, and with a titre volume of 0.9ml, %N for the soil sample was calculated as 0.063%.

5.3.7 Determination of ammonium and nitrate nitrogen in the new fertilizer and filler

The determinations for ammonium and nitrate nitrogen in the newly developed fertilizer, filler and trial soil samples were carried out according to Black, (1965). Fertilizer and filler samples were digested in a solution, which consisted of Lithium sulfate (14g), selenium powder (0.42g), hydrogen peroxide (350ml) and concentrated sulfuric acid (420ml). An aliquot (0.3g) of the fertilizer as well as filler samples were weighed (in duplicate) and then mixed with 10ml of the prepared digestion mixture after which the contents were digested for 4 hours at 360^0C in the digestion apparatus. The digestion was continued until the samples turned whitish in colour indicating complete digestion.

For N determination in the respective soil samples, a mixed catalyst was added prior to the digestion process. The catalyst consisted of 1g of a mixture of potassium sulfate (160g), copper sulfate, (10g) and selenium powder (3g). Eight (8ml) of concentrated sulphuric acid was added into the soil flask before digestion and then the digestion was carried out for 1½hours at 360^0C until the contents of the soil flask turned gray (ash).

After digesting overnight, the following morning the samples were made up to volume by dissolving them in distilled water up to the 50ml mark in 50ml plastic containers. The samples were then distilled for both NH_4-N and NO_3-N using 2% boric acid (20ml) and mixed indicator (Bromocresol green (0.11g) and Methyl red (0.009g). The digested fertilizer and filler samples were titrated using 0.005N sulphuric acid as the titrant.

The experiments for these determinations were conducted at the soil chemistry laboratory in the department of LARMAT (University of Nairobi) using analytical grade laboratory reagents

obtained from OSHO CHEMICALS LTD. The respective standards used were prepared as described in **Appendix 1.**

5.3.8 Determination of calcium and magnesium in trial fertilizer and filler samples

The calcium and magnesium contents in the respective trial fertilizer, filler and soil samples were determined by the potassium chloride (IM KCl 1:10) method as described by Bailer *et al.*, (1981). The elements were extracted from digested samples (0.3g in case of fertilizer and filler samples, and 1g in case of soil samples) using KCl solution (1M); a soil to solution ratio of 1:2.5 and stirred for 1 hour as described by (Vona *et al.*, 2020). Distilled water was used to make the volume of these solutions up to 50ml and then 5ml of this solution pipetted into a 50ml volumetric flask and the volume made up to the mark with distilled water prior to taking readings for calcium using AAS (Buck Scientific Model 210 VGP made in USA).

Prior to taking the readings for calcium, the instrument was calibrated with the following range of standards; 20ppm, 15ppm, 10ppm, 5ppm. The standards were taken from a stock solution of 100ppm Ca^{2+}, which was prepared with $CaCl_2$. Distilled water was used to auto zero the instrument.

The formula used in calculating magnesium and calcium contents in fertilizer, filler and soil samples was as follows:

$$\text{Ca/Mg (ppm)} = \frac{\text{Absorbance x volume extracted}}{\text{Weight of sample}}$$

Volume of sample extracted was 50ml

Calculations (in percentage) for calcium (from the ppm AAS results) in trial fertilizer, filler and soil samples were done using the following formula:

$$\text{Mol/kg Ca} = \frac{\text{ppm x volume extracted x 100 x dilution factor}}{\text{weight of sample x 1000 x atomic weight}}$$

Volume of sample extracted in this case was 5ml, which, was diluted tenfold with distilled water prior to taking absorbance readings for calcium using AAS. Calculations (in percentage) for magnesium (from the ppm AAS results) in trial fertilizer, and filler were done using the following formula:

$$\text{Mol/kg Mg} = \frac{\text{ppm x volume extracted x 100}}{\text{weight of sample x 1000 x atomic weight}}$$

Volume extracted in this case was 50ml.

5.3.9 Determination of available phosphorous (P) in trial fertilizer and filler samples

This determination was done using the colorimetric method as described by Mylavarap, (2002). The method targets Al- and Fe- phosphates as well as P adsorbed in colloidal surfaces in soil samples. The test was done at a pH range of 6.0 to 7.2. An aliquot (0.2ml) for each of the respective samples (i.e. fertilizer, filler and soil samples) was mixed with 25ml of distilled water in a 50ml volumetric flask and then 8ml of a solution containing (12g Ammonium molybdate, 0.27g Potassium tartrate, 1.05g Ascorbic acid in one liter of 5N H_2SO_4) added. This solution is usually referred to as Reagent B. The solution was actually constituted with reagents A and B whose formulations are described in **Appendix 2.** Topping up was done with distilled water to develop colour. When the colour was not developing, some more aliquot (0.2ml) of the sample was added in each case. For the filler sample, the colour could not develop even after adding the

extra 0.2 aliquot of the sample. Therefore, some more 2.8ml of the filler sample was added making a total of 3ml.

The intensity of the blue colour developed was measured using a UV-Visible spectrophotometer at 882nm wavelength within 10 minutes after development of the blue colour. The UV-Visible spectrophotometer was zeroed with distilled water and mixed with 8ml of reagent B before reading the absorbance. The actual values for elemental P in the fertilizer, filler and soil samples were calculated using the following formula:

$$P\ (ppm) = \frac{\text{graph reading (absorbance) x volume extracted (ml)}}{10{,}000} \times 100\%$$

The value of P in form of P_2O_5 was then calculated by multiplying the value obtained for the elemental P x 2.29.

5.3.10 Determination of exchangeable potassium (K) in trial fertilizer and filler

Exchangeable K^+ is the amount of K^+ which can be extracted from the soil by a routine chemical procedure (Affinnih *et al.,* (2004). Potassium that is available for plant uptake was extracted from the fertilizer, filler and soil samples using the Colwell K method as described by Bota, (2015). This was an identical extraction method as used for phosphorus, and it was done by shaking the respective samples with ammonium acetate/acetic acid solution (0.5M) for 30 minutes. This was meant to displace the potentially available K^+ ions. The potassium content of the filtered extract was subsequently determined using a Flame Photometer. According to Bota, (2015), the Colwell K method is meant to measure extractable K in the soil solution by estimating the readily available and potentially available K in the soil. However, the exchangeable K test, measures only the readily available K in the sample. The formula used in

calculating the readily available potassium content in fertilizer, filler and soil samples was as follows:

$$K\ (ppm\ or\ mg/kg) = \frac{Absorbance \times volume\ extracted}{Weight\ of\ sample}$$

Volume extracted was 50ml.

Multiplying the value for K (ppm) obtained in the above formula by 100 gave the percentage of exchangeable K in the sample, which was then multiplied by 1.2051 to convert it to % K_2O.

Calculations for available potassium in mol/kg K were conducted following the formula given below.

$$Mol/kg\ K = \frac{Graph\ reading\ (Abs) \times volume\ extracted}{Weight\ of\ sample \times 100} \times 100$$

5.3.11 Determination of N mineralization rate of modified collagenic material

Laboratory incubation study on the soil samples that were randomly collected and bulked from the University of Nairobi's Faculty of Agriculture field station was conducted in the soil physics laboratory (department of LARMAT). The clay-loam soil samples used for this study were first dried at room temperature and then passed through a 2mm sieve. They were thereafter allowed to pre-incubate for one week at 60% water holding capacity before being treated with different rates of the newly formulated slow-release N fertilizer. This treatment was performed in 10 pots with 3 replicates each weighing 1kg. The first three replicates were not given any treatment while the rest were treated with different rates of fertilizer and filler combinations and then covered with perforated asbestos to give room for exchange of air. The respective treatments were as follows:

1. Negative control - no Fe or Fi in 1kg of incubated soil sample ≡ 0.00kg/ha Fe + 0.00kg/ha Fi
2. 0.325g Fe without Fi in 1kg of incubated soil sample ≡ 46.00kg/ha Fe + 0.00kg/ha Fi
3. 0.65g Fe without Fi in 1kg of incubated soil sample ≡ 92.00kg/ha Fe + 0.00kg/ha Fi
4. 0.625g Fe and 0.625g Fi in 1kg of incubated soil sample ≡ 88.46kg/ha Fe + 88.46kg/ha Fi
5. 1.25g Fe without Fi in 1kg of incubated soil sample ≡ 176.93kg/ha Fe + 0.00kg/ha Fi
6. 1.75g Fe and 0.75g Fi in 1kg of incubated soil sample ≡ 247.70kg/ha Fe + 106.16kg/ha Fi
7. 2.5g Fe without Fi in 1kg of incubated soil sample ≡ 353.86kg/ha Fe + 0.00kg/ha Fi
8. 1.563 g Fe and 1.563g Fi in1kg of incubated soil sample ≡ 221.23kg/ha Fe + 221.23kg/ha Fi
9. 3.125g Fe without Fi in 1kg of incubated soil sample ≡ 442.32kg/ha Fe + 0.00kg/ha Fi
10. 2.188g Fe and 0.938g Fi in 1kg of incubated soil sample ≡ 309.70kg/ha Fe + 132.77kg/ha Fi

Fi = Filler material (milled maize cobs – 2 mm particle size)

Fe = Fresh trial fertilizer sample (dechromed, milled and EPICH modified leather waste – 2.0 mm particle size)

One week after pre-incubation, the first sampling (from each of the 10 pots) was done in duplicate where one set of the samples was dried, ground with mortar and pestle, and sieved with a 2mm sieve placed on top of 0.50mm sieve in preparation for pH, available P, cation exchange capacity and exchangeable basis K determination. Whatever did not pass through the 2mm sieve was discarded. The texture class of the soil was also done from the 2mm soil samples. Samples collected in the 0.50mm sieve were used to determine total N and total organic carbon. The

reason for using the 0.50mm sieve to collect the fine dried soil samples is because the samples needed to be completely homogenized and also, the organic part of the sample is more easily determined in very fine soil samples. The other set of soil samples, which was kept wet, under refrigeration, were later digested and analysed for N and K using UV-Vis spectrophotometer and flame photometer, respectively. Thereafter, subsequent samplings were done during the 2^{nd}, 4^{th}, 8^{th}, 16^{th} weeks of incubation period and after the 16^{th} week. A total of 6 sets of samples were collected (i.e. at the onset of incubation, and then during the 2^{nd}, 4^{th}, 8^{th}, 16^{th} and finally, after 16 weeks incubation period). The mineralization rate of organic N into ammonium-N and nitrate-N in the respective sets of sampled soil, was determined by the Kjeldahl steam distillation method according to Black, (1965).

5.4.Results

5.4.1 Nutrient content of the fertilizer

Table 5.1 shows the nutrient content of unmodified and EPICH modified collagenic hydrolysate as well as that of the filler material. This can be seen from the table that EPICH modification improved the nitrogen, phosphorus, magnesium and calcium content of the collagenic fertilizer. In addition, it is clear from the table that the filler material could contribute to the nutrient content of formulate fertilizer as it had high levels of important nutrients.

Table 5.1: Calculated mean values for total N, available P, exchangeable K, Mg, Ca, & TOC

Type of nutrient	Unmodified collagen hydrolysate	Modified collagen hydrolysate	Filler
Total N (%)	35	44	2.8
Available P (% P_2O_5)	8.27	21.02	14.04
Exchangeable K (% K_2O)	0.25	0.1	0.3
Mg	0.002	0.2	7.5
Ca	0.03	1.8	1.6
TOC (%)	22.15	27.0	38.12
C:N ratio	0.47	0.61	13.61

Type of nutrient	Unmodified collagen hydrolysate	Modified collagen hydrolysate	Filler
pH	6.79	6.79	6.76

Table. 5.2: Physico-chemical characteristics of soil used in the mineralization experiments

Physico-chemical characteristics	Amount
Total N (%)	0.06
Available P (%)	12.93
Exchangeable K (%)	11.00
Mg (%)	Trace
Ca (%)	Trace
TOC (%)	27.0
pH	6.39
Electrical conduct. (ds/m)	0.23
CEC (Cmol(+) kg-1	14.26

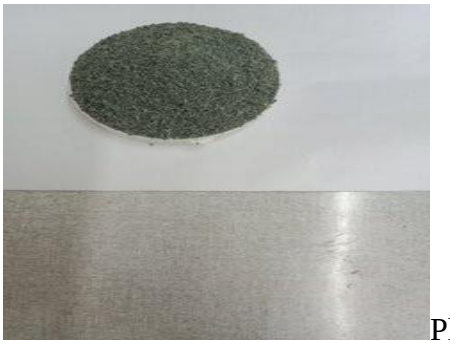


Plate 5a

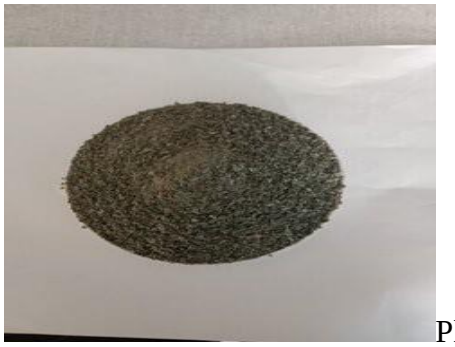


Plate 5b


Plate 5c

Key:

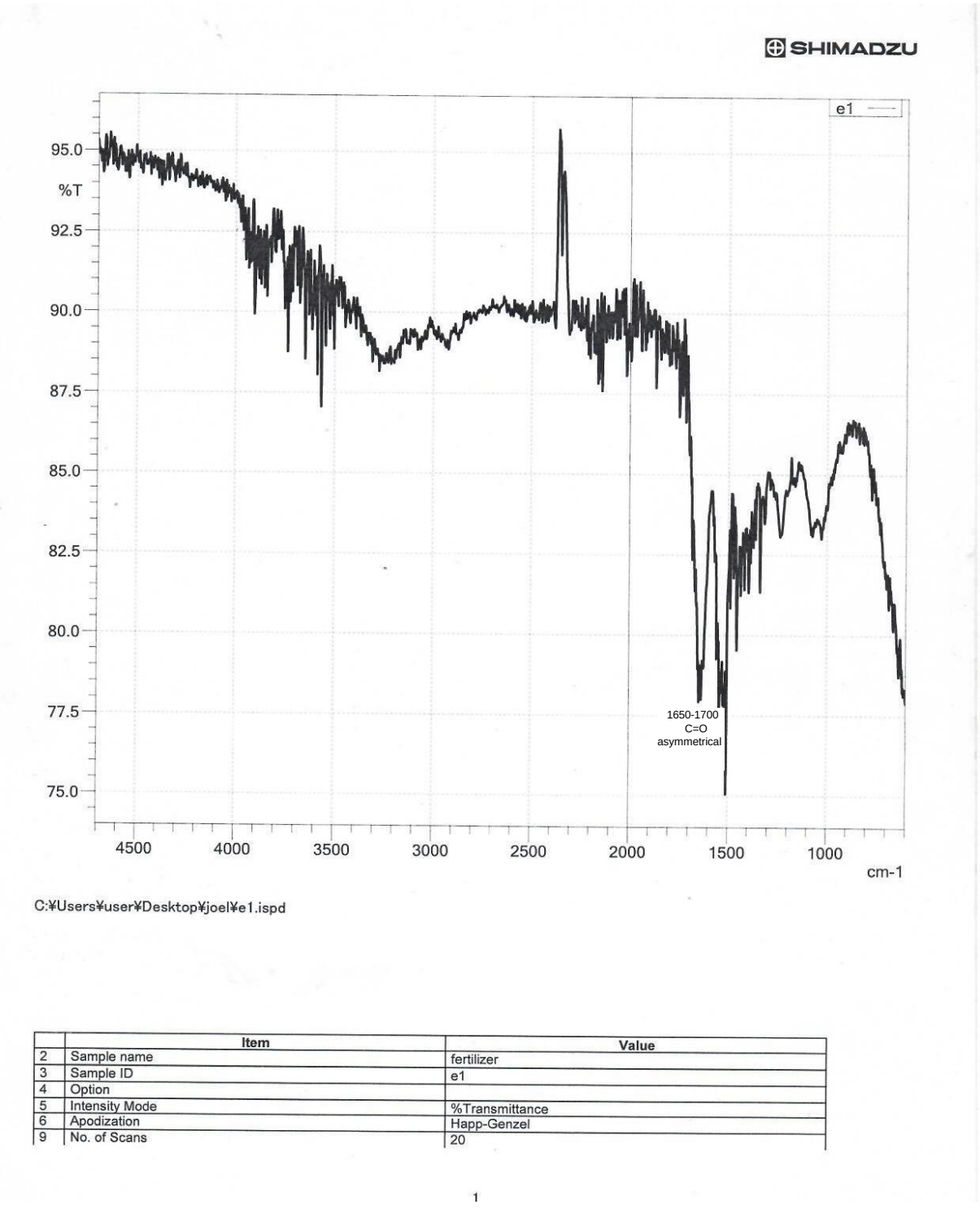
Plate 5a = Dechromed collagen hydrolysate fertilizer (unmodified)

Plate 5b = Dechromed collagen hydrolysate fertilizer (EPICH modified)

Plate 5c = Dechromed collagen hydrolysate fertilizer (EPICH modified and blended with filler)

5.4.2 Functional groups detected in modified collagen

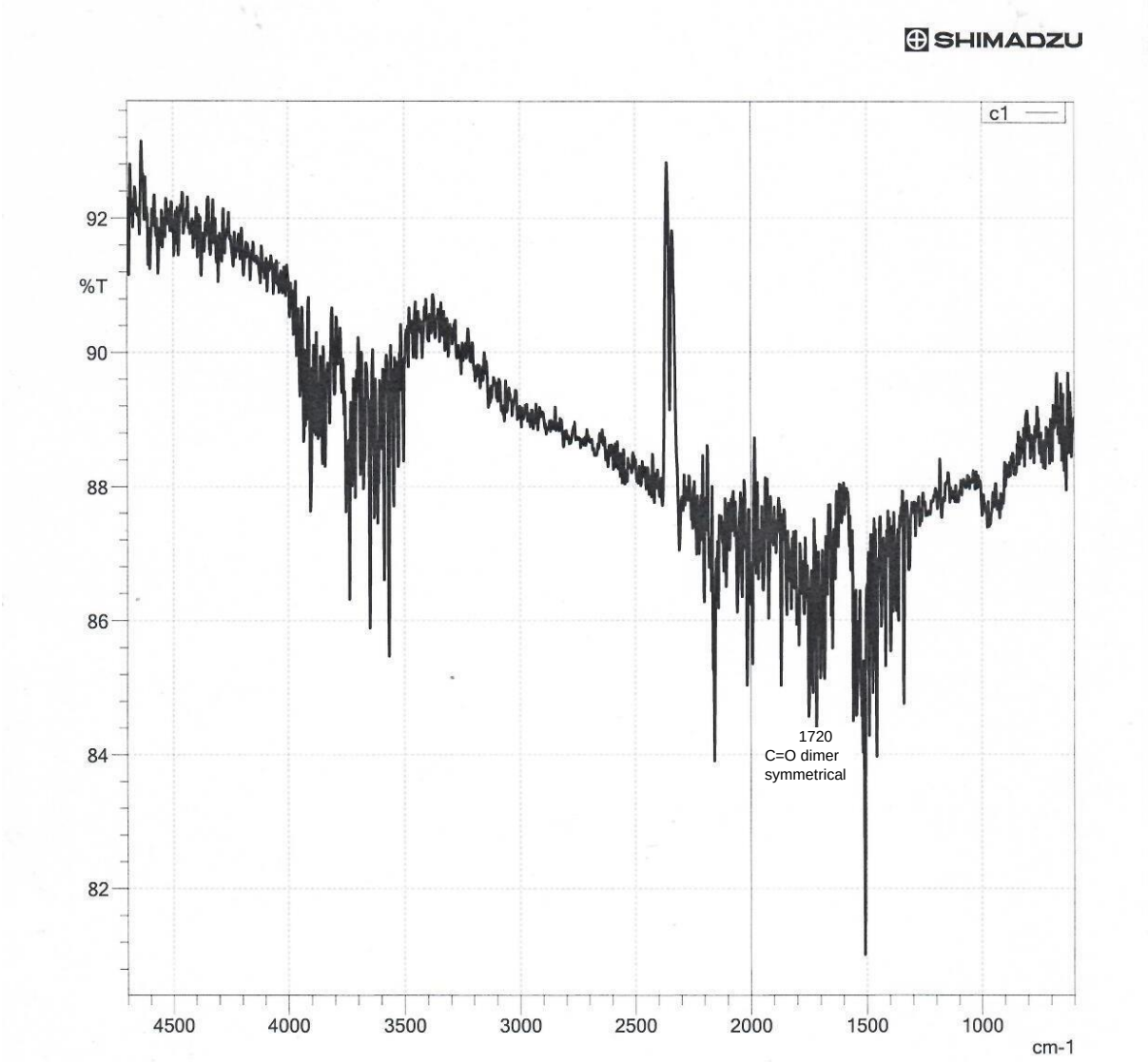
Figure 5.3 and **Figure 5.4** show the IR spectra for unmodified and EPICH modified collagen hydrolysate fertilizer, respectively. The compounds identified that contained the carbonyl functional group include; ketones at 1710cm^{-1} (lowest peak), aldehydes at 1720cm^{-1} (medium peak), esters at 1810cm^{-1} (medium peak), anhydrides at 1760cm^{-1} (medium peak) and acid halides at 1800cm^{-1} (strong peak). There was also a possibility that EPICH modification of the collagen hydrolysate fertilizer availed more nitrogen, a heteroatom of relatively higher atomic number, to come into contact with the increased presence of carboxylic acid groups broadening the carbonyl peak further as a result. Two asymmetrical carbonyl functional groups will usually show two strong peaks at around 1650-1700cm^{-1} in the IR spectra **(Figure 5.3.).** However, if symmetrical they will overlap into one peak at around 1720cm^{-1} as can be seen in the IR spectra for the EPICH modified collagen hydrolysate fertilizer **(Figure 5.4.).**



	Item	Value
2	Sample name	fertilizer
3	Sample ID	e1
4	Option	
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	20

1

Figure 5.3: IR spectra for unmodified collagen hydrolysate fertilizer.



	Item	Value
2	Sample name	fertilizer
3	Sample ID	c1
4	Option	
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	20

Figure 5.4: IR spectra for EPICH modified collagen hydrolysate fertilizer.

5.4.3 Nitrogen mineralization in incubated soil

The results on mineralization of N into (NH_4^+-N + NO_3-N) in incubated soil as observed during 16 weeks incubation period, are given in **Table 5.3** (mean concentration of NH_4^+-N (ppm)), and **Table 5.4** (mean concentration of NO_3-N (ppm)). The results showed no significant increase in the mineralization of N into both NH_4^+-N and NO_3-N during the initial periods of incubation under all the 10 different treatments that were involved in this study. However, there was some notable decrease (though not statistically significant) in the average concentration of NH_4^+-N (ppm) during the 12th week of incubation **(Table 5.3.).** This is an indication that there was some decline in the mineralization of N to form NH_4^+-N during this period. During the same period (i.e. 12th week of incubation), a significant decline in the mineralization of N ($p < 0.05$) into NO_3-N was witnessed, and the results for this observation are given in **Table 5.4.**

Table. 5.3: Mean concentration of NH_4^+-N (ppm) during 16 weeks incubation period

Mean concentration of NH_4^+-N (ppm) during 16 weeks incubation period						
Treatment	**2 weeks**	**4 weeks**	**8 weeks**	**12 weeks**	**16 weeks**	**After 16 weeks**
1.	32262a	24389a	9811a	5811a	47626ab	24389a
2.	19474a	19474a	12128a	7481a	6219a	200900a
3.	25597a	5470a	5470a	7127a	4401a	5470a
4.	65926a	1338a	35595a	6711a	7269ab	1338a
5.	117699a	19352a	25154a	8878a	93032b	19352a
6.	43804a	14845a	18117a	8009a	10162ab	14845a
7.	33437a	15884a	20330a	7781a	9118ab	15884a
8.	24005a	65088a	18413a	7100a	8876ab	65088a
9.	19413a	200900a	5244a	7479a	5193a	23368a
10.	24229a	13190a	22363a	7328a	11568ab	13190a
P-Value	P < 0.196	P < 0.559	P < 0.789	P < 0.976	P < 0.016	P < 0.559

One letter (a or b) in the same column indicate that treatments are not significantly different Different letters in the same column indicate that treatments are significantly different

Key:

1 = Negative control - no Fe or Fi in 1kg of incubated soil sample ≡ 0.00kg/ha Fe + 0.00kg/ha Fi

2 = 0.325g Fe without Fi in 1kg of incubated soil sample ≡ 46.00kg/ha Fe + 0.00kg/ha Fi

3 = 0.65g Fe without Fi in 1kg of incubated soil sample ≡ 92.00kg/ha Fe + 0.00kg/ha Fi

4 = 0.625g Fe and 0.625g Fi in 1kg of incubated soil sample ≡ 88.46kg/ha Fe + 88.46kg/ha Fi

5 = 1.25g Fe without Fi in 1kg of incubated soil sample ≡ 176.93kg/ha Fe + 0.00kg/ha Fi

6 = 1.75g Fe and 0.75g Fi in 1kg of incubated soil sample ≡ 247.70kg/ha Fe + 106.16kg/ha Fi

7 = 2.5g Fe without Fi in 1kg of incubated soil sample ≡ 353.86kg/ha Fe + 0.00kg/ha Fi

8 = 1.563 g Fe and 1.563g Fi in1kg of incubated soil sample ≡ 221.23kg/ha Fe + 221.23kg/ha Fi

9 = 3.125g Fe without Fi in 1kg of incubated soil sample ≡ 442.32kg/ha Fe + 0.00kg/ha Fi

10 = 2.188g Fe and 0.938g Fi in 1kg of incubated soil sample ≡ 309.70kg/ha Fe + 132.77kg/ha Fi

Fi = Filler material (milled maize cobs – 2 mm particle size)

Fe = Fresh trial fertilizer sample (dechromed, milled and modified leather waste – 2.0 mm particle size)

Table 5.4: Mean concentration of NO_3-N (ppm) during 16 weeks incubation period

Mean concentration of NO_3-N (ppm) for specific periods of incubation.					
Treatment	**2 weeks**	**4 weeks**	**8 weeks**	**12 weeks**	**16 weeks**
1.	0.1427a	0.1843a	0.2613a	0.1173a	1.0187a
2.	0.0827a	0.6790a	0.1933a	0.1120a	0.3940a
3.	0.1227a	0.1980a	0.2490a	0.2960abc	0.2653a
4.	0.2307a	0.2160a	0.3933a	0.1740ab	0.6427a
5.	0.5093a	0.1357a	0.3853a	0.3707abc	0.3090a
6.	0.1097a	0.3797a	0.4712a	0.5780abc	0.3800a
7.	0.1367a	0.3537a	0.6400a	0.7110c	0.6907a
8.	0.1093a	0.3120a	0.3583a	0.3087abc	0.7493a
9.	0.1653a	0.3253a	0.6317a	0.5760abc	0.5397a
10.	0.1813a	0.5637a	0.4947a	0.6587bc	0.3020a
Significance	P-Value < 0.530	P-Value < 0.573	P-Value < 0.038	P-Value < 0.001	P-Value < 0.691

Notes: One letter (a or b) in the same column indicate that treatments are not significantly different Different letters in the same column indicate that treatments are significantly different

Key:

1 = Negative control - no Fe or Fi in 1kg of incubated soil sample ≡ 0.00kg/ha Fe + 0.00kg/ha Fi

2 = 0.325g Fe without Fi in 1kg of incubated soil sample ≡ 46.00kg/ha Fe + 0.00kg/ha Fi

3 = 0.65g Fe without Fi in 1kg of incubated soil sample ≡ 92.00kg/ha Fe + 0.00kg/ha Fi

4 = 0.625g Fe and 0.625g Fi in 1kg of incubated soil sample ≡ 88.46kg/ha Fe + 88.46kg/ha Fi

5 = 1.25g Fe without Fi in 1kg of incubated soil sample ≡ 176.93kg/ha Fe + 0.00kg/ha Fi

6 = 1.75g Fe and 0.75g Fi in 1kg of incubated soil sample ≡ 247.70kg/ha Fe + 106.16kg/ha Fi
7 = 2.5g Fe without Fi in 1kg of incubated soil sample ≡ 353.86kg/ha Fe + 0.00kg/ha Fi
8 = 1.563 g Fe and 1.563g Fi in1kg of incubated soil sample ≡ 221.23kg/ha Fe + 221.23kg/ha Fi
9 = 3.125g Fe without Fi in 1kg of incubated soil sample ≡ 442.32kg/ha Fe + 0.00kg/ha Fi
10 = 2.188g Fe and 0.938g Fi in 1kg of incubated soil sample ≡ 309.70kg/ha Fe + 132.77kg/ha Fi
Fi = Filler material (milled maize cobs – 2 mm particle size)
Fe = Fresh trial fertilizer sample (dechromed, milled and modified leather waste – 2.0 mm particle size)

The highest mineralization rate of organic N into NH_4^+-N was observed during the 16th week of incubation. The mineralization results were at their peak during this period showing a mean concentration of 11568 NH_4^+-N (ppm) with treatment 10, where 309.70kg/ha trial fertilizer combined with 132.77kg/ha filler, had been applied **(Table 5.3.).** However, the highest mineralization rate of organic N into NO_3-N in this incubation study was witnessed during the 12th week giving peak results of 0.6587 NO_3-N (ppm) with treatment 10 (309.77kg/ha trial fertilizer + 132.77kg/ha filler) as indicated in **Table 5.4.** At P value of 0.05, a significant difference was observed in the mean concentration of NH_4^+- N (ppm) during the 16th week of incubation period, whereas no significant difference was witessed in the mean concentration of NO_3-N (ppm) even towards the end of the incubation period (16 weeks) ($P > 0.05$). Due to its high total organic carbon content (TOC) with mean value of 39.12%), the rate of mineralization of the organic nitrogen in the new fertilizer into plant available forms such as nitrates could be slowed down.

5.5.Discussion

5.5.1 Modification of dechromed and hydrolysed tannery waste

The dechromed and hydrolysed collagen hydrolysate was modified by reacting it with epichlorohydrin (EPICH) so as to increase its potential in the formulation of a slow-release nitrogen fertilizer to match plant nutrient requirements. Previous research has shown that if reaction conditions for EPICH are favourable, it can successfully be used to activate the nucleophile groups normally present in a polypeptide matrix (notably $–NH_2$ or –OH groups) to form epoxide compounds which then react with the rest of the nucleophile groups in the entire protein structure (Langmaier *et al.,* 2005). Once the epoxide rings are opened (at elevated temperatures), the nitrogen becomes more available for release. The same researchers (Langmaier *et al.,* 2005) also found that if EPICH is put in an aqueous environment it behaves as a mono-functional agent through its chlorine atom triggering its reaction with the primary amino groups of the hydrolysed collagen as a result. If the temperature is raised to around 60^0C, this reaction reaches equilibrium in about one hour. Reaction of the oxirane ring of EPICH then proceeds if the temperature is considerably increased to around 200^0C (Langmaier *et al.,* 2005).

EPICH tends to target those amino acids with high concentration of nitrogen atoms in their structures and strategically positioned for ease of crosslinks formation with them (e.g. histidine and arginine). EPICH reacts more specifically with guanidine or imidazole groups in arginine and histidine amino acids, respectively (Baumert and Fassold, 1989; Feichtinger *et al.,* 1998). This is an added advantage when EPICH is used as a crosslinking agent in the development of slow-release nitrogen fertilizers from collagen hydrolysates derived from dechromed leather solid wastes.

EPICH was preferred as the crosslinking agent for collagen hydrolysate used in this study due to its high affinity for guanidine and imidazole groups allowing the protein structure to make more nitrogen available for release. Total N in modified collagen hydrolysate increased to a mean value of 44% from 35% in the unmodified form after modification with EPICH **(Table 5.1.).** The two-stage process of EPICH modification of collagen hydrolysate favoured the development process for a slow-release nitrogen fertilizer that was being investigated in this study.

In addition, the amount of available phosphorous (% P_2O_5) in the developed slow-release fertilizer increased from 8.27% (in the unmodified collagen hydrolysate) to 21.02% after modification with EPICH as indicated in **Table 5.1.** This can be attributed to the fact that mild collagen hydrolysis with phosphoric acid (which was the case in this study) is less efficient as compared to other agents of hydrolysis such as enzymatic, alkaline or even strong acid hydrolysis. According to Valcarcel *et al.,* (2021), collagen hydrolysis is usually more efficient with papain than alcase, but the sum of pyrolidine amino acids is lower in phosphoric acid treatments. These amino acids are some of the targets for EPICH modification of collagen hydrolysates, and there is a possibility that the phosphoric acid held up electrostatically within the collagen hydrolysate protein structure was involved in crosslinking reactions with EPICH availing more phosphorous in the resultant slow-release nitrogen fertilizer. According to Cohen-Solal *et al.,* (1979), only 20% of the phosphorous present in collagen hydrolysates is bound covalently by the collagen fibres. Therefore, the remaining 80% P is only bound electrostatically within the protein structure and thus, readily available for crosslinking with EPICH during the modification process carried out in this study.

EPICH modified collagen hydrolysate was slightly lighter in appearance as compared to the unmodified form due to the presence of relatively more available phosphorus after this

modification process **(Plates 5a and 5b).** The choice of orthophosphoric acid for mild hydrolysis of the dechromed collagenic material prior to its modification with EPICH was also informed by the fact that this acid does not have harmful effects on the environment particularly if it is released into the environment in small doses. This is because once in the environment, the acid is either neutralised into phosphate salts or gets diluted so much that it becomes harmless. However, as reported in the *Australian Government, Department of Agriculture, Water and Environment; Natural Pollutants Inventory;* if large quantities of this acid are released into the environment, there will be a problem of soil and water acidification.

On the other hand, the calculated mean values for exchangeable potassium (K) in form of % K_2O decreased significantly from 0.25% to 0.1% after modification of the collagen hydrolysate with EPICH **(Table 5.1.).** This can be attributed to the fact that prior to EPICH modification and phosphoric acid hydrolysis, the chrome tanned leather waste had been complexed with potassium oxalate leading to the formation of potassium chromate which is highly soluble in water. Thus, it was consequently washed away in subsequent washing steps, which were carried out on the collagen hydrolysate in preparation for the final modification with epichlorohydrin. The mean values of all the other parameters (i.e. Mg, Ca and pH) were not affected significantly by this final modification of collagen hydrolysate with EPICH. However, there was some significant increase in the amount of carbon content in the EPICH modified collagen hydrolysate from 22.15 to 27% (**Table 5.1.).** This could possibly be due to the fact that collagen hydrolysate porous space (pore distribution size) inside the protein matrix gets reduced due to shrinkage when treated with a specific crosslinking agent at a certain characteristic temperature as can be attested by the mercury instruction porosimetry (MIP) method carried out by Fathima *et al.*, (2002). These researchers (Fathima *et al.,* 2002) investigated how positive changes in

temperature (for instance, increasing from 20-120^{0}C) affects the porous nature (i.e. pore connectivity) of the collagen matrix using two different methods namely; MIP and scanning electron microscopy (SEM), the findings of which were that the percentage porosity decreases with thermal shrinkage and also, the type of crosslinking agent used.

Identification of functional groups in the EPICH modified collagen hydrolysate fertilizer was done using Fourier transform infrared (FTIR) spectroscopical analysis. Infrared (IR) spectra for the unmodified collagen hydrolysate fertilizer gave a very narrow carbonyl peak (C═O) at around 1650-1700cm^{-1} (wavenumbers) as shown in **Figure 5.3.,** signaling a heavy presence of this functional group (strong peak). This is a clear indication that the unmodified collagen hydrolysate fertilizer contained very little amount of carboxylic acid groups as compared to the EPICH modified collagen hydrolysate, whose IR spectra is shown in **Figure 5.4.,** as a broad peak at around 1700cm^{-1}.

Presence of carboxylic acid compounds as opposed to other compounds that are also known to contain the carbonyl functional group (C═O) was identified by a very strong and broad peak in the IR spectra occurring at 1720cm^{-1} (strong peak) **(Figure 5.4.).** This is an indication that the carboxylic acid group identified in the EPICH modified collagen hydrolysate fertilizer developed in this study, was converted into a dimer during the modification process. If the carboxylic acid group is a monomer, the IR spectra usually occurs at 1730cm^{-1} and if it is a dimer, the IR spectra occurs at 1720cm^{-1} (Emmeluth *et al.,* 2003; Max and Chapados, 2004; Giubertoni *et al.,* 2019; Socha and Dracinsky, 2020).

5.5.2 Assessment of the suitability of maize cobs powder as a filler material

This study established that the fertilizer filler derived from ground maize cobs is highly cost effective, and 100% biodegradable and ecofriendly. The filler had high total organic carbon (TOC) content (mean value of 38.12%) and a high carbon to nitrogen ratio (C:N) which was 38.12/2.80 = 13.6. C:N ratio values should not be very high to avoid compromising the N in the soil as it requires a lot of N consumption for the fertilizer to breakdown.

Other contributions of filler material to the quality of the new fertilizer include, better physical condition and ease of application of the blended mixtures of fertilizer and filler, addition of more plant nutrients such as P (14.04% P_2O_5), TOC (38.12%), Mg (7.5%), Ca (1.6%), K (0.3% K_2O) and N (2.8%) **(Table 5.1.),** and enhanced uniformity in particle size of the final fertilizer as the filler was a fine maize cobs powder which was screened for uniformity in size. High values for Carbon-Nitrogen ratio (C:N) in organic matter such as the filler material used in this study, ensures there is a considerable slowing down of the rate at which organic nitrogen is being released into the soil in form of nitrates. However, C:N ratio values should not be very high to avoid compromising the N in the soil as it will require a lot of N consumption for the fertilizer to breakdown. If the C:N ratio is too little, it will decompose too fast for the crop to benefit from the organic fertilizer. The C:N ratio for manure ranges from 12 to 20 (Flavel and Murphy, 2006).

5.5.3 Mineralization

There was low rate of mineralization of nitrogen in this fertilizer formulation during the first few weeks of incubation, a scenario attributable to the lag phase of microbial activity. During this lag phase the microorganisms do not multiply, but are normally preoccupied with immobilization of nutrients to nourish and increase their size visibly (Deenik and Yost, 2008; Karuku and

Mochoge, 2016; Tambone and Adani, 2017). For maintenance and growth to take place optimally, the microorganisms will need access to sufficient quantities of inorganic nutrients, carbon sources, trace elements and most importantly, water (Rop *et al.,* 2018).

However, average concentration of NH_4^+-N (ppm) during the 12th week of incubation decreased noticeably, though this decrease was not statistically significant ($P > 0.05$) as shown in **Table 5.3.** This is an indication that there was some decline in the mineralization of N to form NH_4^+-N during this period. During the same period (i.e. 12th week of incubation), a significant decline in the mineralization of N ($P < 0.05$) to form NO_3-N was witnessed as indicated in **Table 5.4.** This decline in mineral nitrogen content (MN) during the 12th week of incubation was possibly due to depletion of mineralizable nitrogen. There is also a possibility that the microbes responsible had gone past the stationary phase (in which the bacterial population remains constant) and already had started to decline in the death or decline phase, which is also sometimes referred to as the endogenous growth phase, slowing down the immobilization process as a result.

The ratio of filler to fertilizer in this newly formulated slow-release N fertilizer seemed to have a significant influence in the N mineralization rate from the beginning of the 12th week up to the end of the 16th week of incubation. It was observed that, filler to fertilizer ratio of 0.43 gave the highest mineralization rate of N into NO_3-N during the 12th week of incubation while the highest mineralization rate of N into NH_4^+-N was observed during the 16th week of incubation with the same fertilizer to filler ratio of 0.43. As can be seen in **Table 5.3,** treatment 6 (106.16 filler : 247.70 fertilizer ratio = 0.43) had a mean concentration of 10162 NH_4^+-N (ppm) while treatment 10 with the same ratio of filler to fertilizer (i.e. 132.77 : 309.70 = 0.43) had a mean concentration of 11568 NH_4^+-N (ppm) in the 16th week of the incubation period. Similarly, the mean concentration of NO_3-N in treatment 6 was 0.5780ppm during the 12th week of incubation while

in treatment 10 (with the same ratio of filler to fertilizer), the NO_3-N mean concentration was 0.6587ppm in the same period of incubation.

In this regard therefore, the filler in this new fertilizer, and the ratio in which it was combined, seemed to have some considerable significance in N mineralization rate. The C:N ratio in the filler was 13.61 which was neither too low (leading to excessive N mineralization rate) nor too high (slowing down the N mineralization rate). The ratio of filler to fertilizer combinations in treatments 6 and 10 seemed to significantly favour the N mineralization rate from the beginning of the 12^{th} week up to the end of the 16^{th} week of incubation. N mineralization as a function of the fertilizer chemical constituents notably the N content and C:N ratio among other factors as well, was confirmed by Masunga *et al.,* (2016). N mineralization being a biological process, then the C:N ratio inevitably influences mineralization rate through the action of microorganisms, which cause the breakdown of carbon bonds/chains in the organic material for their energy requirements by immobilizing N according to Dong *et al.,* (2012); Karuku and Mochoge, (2016); Tambone and Adani, (2017).

5.6. Conclusions

- EPICH modification of dechromed tannery waste released carbon, nitrogen and phosphorus from collagen making them available for plant use
- The use of maize cobs powder as filler material during formulation enhanced the quality of the organic fertilizer

5.7.Recommendations

- EPICH modification of collagen hydrolysate from dechromed tannery waste and formulation of the slow-release organic fertilizer should be upscaled and optimized for commercial manufacture.

CHAPTER 6: ASSESSMENT OF THE PERFORMANCE OF THE NEW FERTILIZER IN A GREENHOUSE SETTING

6.1. Abstract

Leather manufacture produces various types of waste that is contaminated with chromium, which is toxic to animals, plants and to the environment in general. The substantial amounts of nitrogen and organic matter available in tannery wastes (mainly from collagen) have prompted researchers to make several investigations on the possibility of using this material as fertilizer, but the presence of chromium in such wastes has been a limiting factor. Collagen hydrolysates derived from dechromed leather solid wastes have been found to contain high amounts of nitrogen that can be a source of plant nutrients.

This chapter describes an assessment of the new organic fertilizer developed from dechromed and modified solid leather waste, as previously described in this study to determine its plant nutrient content and efficiency of the fertilizer on growth and productivity of selected crops in a greenhouse setting. The new organic fertilizer was tested as a nutrient source on growth and productivity of two commonly grown vegetable crops namely; kale (*Brassica oleracea var; sabellica*) and capsicum (*Capsicum anuum*) and one cereal crop, maize (*Zea mays*), under a greenhouse experimental setting. Analysis of the fertilizer to determine its plant nutrient content gave the following mean results; 44% N, 23% P (as P_2O_5), 0.1% K (as K_2O), 0.2% Mg, 1.8% Ca and 22.15% TOC. The performance of the new fertilizer on growth and productivity of the two vegetable crops (kale and capsicum) was comparable with the use of conventional DAP and CAN fertilizers. However, the new fertilizer had an advantage of good performance after a single application at transplanting time without the need for top-dressing with any other form of

fertilizer. Application of this new organic fertilizer on growth and productivity of maize did not show favourable results. However, application of different rates of this fertilizer on mean height of the maize stalk and chlorophyll content gave a significantly improved difference in performance ($P < 0.05$) compared to the use of conventional DAP and CAN fertilizers. The study established that the new fertilizer had adequate nutrients to support plant growth and was highly effective on vegetable crops. It was concluded that the organic fertilizer as formulated can be used successfully in growing vegetable crops such as kale and capsicum. It was recommended that the new fertilizer be improved by addition of magnesium, calcium and potassium to enable its utilization for growth of maize and other crops that require substantial amounts of these nutrients.

6.2. Introduction

Organic fertilizer formulations mainly consist of organic materials that must be broken down by microbial activity before the nutrients are available to the plants (Lazcano *et al.,* 2021). In general, fertilizers derived from organic sources usually take a long time to release plant nutrients and these nutrients may not be available when the plant needs them (Guertal, 2009; Ding *et al.,* 2016). Studies on the development of slow-release fertilizers have provided a conducive environment for the improvement of fertilizer use efficiency and reduction of environmental pollution due to fertilizer losses (Azeem *et al.,* 2014).

Organic fertilizer formulations are becoming very popular due to the controlled release tendency of plant nutrients and reduced associated labour costs in such fertilizers (Itelima *et al.,* 2018). Besides, application of these fertilizer formulations plays a key role in timely provision of proper nutrition to the plant and in restricting the growth of other plant species in the crop field (Majee *et al.,* 2020). Several technologies to effectively address plant nutrient requirements using

fertilizers that release plant nutrients slowly have been developed with the aim of slowing down or even controlling the rate at which such nutrients are released into the soil (Trenkel, 2010; Timilsena *et al.*, 2015).

Collagen hydrolysates derived from dechromed leather solid wastes have been found to contain high amounts of nitrogen that can be a source of plant nutrients. According to Nogueira *et al.*, (2011), wet-blue leather contains an average of 140g/kg N (dry weight) which can effectively be utilized as nitrogen-enriched organic fertilizer after extracting the chromium. This study aimed at assessing the performance of the new organic fertilizer derived from dechromed and EPICH modified leather waste in terms of growth and yield of crops grown under greenhouse conditions.

Adequate amounts in the soil of the following nutrients N, Ca Mg, K and P are important for crop growth and good yields. High TOC levels cause the soil to retain more water leading to better crop yield, increased plant nutrient retention, reduced soil erosion and increased biological diversity (Pimentel and Burgess, 2014). Soil organic carbon is chemically known to improve the cation exchange capacity (CEC) of the soil, and it has already been established that 20 to 80% of the cation exchange capacity is attributable to soil organic matter (Ciotta *et al.,* 2003). The cation exchange sites are crucial for retention of plant nutrients particularly nitrogen, phosphorus and sulphur which, upon decomposition, provide slow release of nutrients for plant growth. In addition, soil organic matter provides binding sites for a considerable number of anthropogenic chemicals leading to the minimization of hazardous chemicals through the soil profile (or making them less available); this phenomenon reduces soil toxicity as a result according to the work done by Delgado *et al.*, (2013).

6.3.Materials and Methods

6.3.1 Determination of optimal fertilizer application rates

Determination of the optimal amount of a specific fertilizer that needs to be applied per crop in order to meet the exact nutritional requirements of that crop was done using the yield response quadratic function (*Eq. 1*). According to Hartinee *et al.*, (2010), the productivity (yield) of the crop was found to be directly proportional to the amount of fertilizer applied, but on reaching the maximum limit, the yield started to decline in a mirror image of the increments in fertilizer application. The agronomic optimal fertilizer application rate (xagrkgha^{-2}) was determined following the procedure described by Wang *et al.*, 2014; Luce *et al.*, 2015; Puntel, 2016) using (*Eq. 2*).

$$y_{fer} = y - y_o = a + bx + cx^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. \quad (1)$$

$$xagr = -\frac{b}{2 * c} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \quad (2)$$

Where,

y_{fer} stands for the increase in crop productivity response with increase in the amount of fertilizer applied (fertilizer-derived yield, mgha^{-1});

y and y_o are the crop productivity rates with and without fertilizer application, respectively;

x stands for the fertilizer application rate (kgha^{-1}) and,

a is the intercept while b and c are linear and quadratic coefficients, respectively.

6.3.2 Greenhouse planting trials and assessment of crop yields

The completely randomized design (CRD) design, which is also sometimes known as randomized completely block design (RCBD), was adopted for the greenhouse planting trials and assessment of crop yields in this study. The experimental design used to determine crop health and yields under different fertilizer: filler ratios had 7 treatments and 3 blocks (i.e., replicates) as shown below.

1. 7.00 grams Trial Fertilizer + 3.00 grams Filler ═ 990.80kg/ha Fertilizer + 424.63kg/ha Filler
2. 10.00 grams Trial Fertilizer + 0.00 grams of Filler ═ 1415.43kg/ha Fertilizer + 0.00kg/ha Filler
3. 6.25 grams of Trial Fertilizer + 6.25 grams Filler ═ 884.64kg/ha Fertilizer + 884.64kg/ha Filler
4. 12.50 grams Trial Fertilizer + 0.00 grams Filler ═ 1769.28kg/ha Fertilizer + 0.00kg/ha Filler
5. 8.75 grams Trial Fertilizer + 3.75 grams Filler ═ 1238.50kg/ha Fertilizer + 530.79kg/ha Filler
6. 123.55kg/ha DAP at transplanting time + 123.55kg/ha CAN (2 weeks after transplanting) + 123.55kg/ha (4 weeks after transplanting) (positive control)
7. 0.00 grams Trial Fertilizer + 0.00 grams Filler (negative control) ═ 0.00kg/ha fertilizer + 0.00kg/ha Filler

The test crops used in this greenhouse experiment were maize (H513 variety), kale and capsicum whose planting arrangement was as done in a set of 3 pots (replicates) for each of the 7 different treatments follows; 2 maize seeds were sown at a depth of 4cm in the first set of pots while 2 four weeks old kale seedlings per pot, were transplanted in the second set. In the third set of pots, only 1 kale four weeks old seedling per pot was transplanted. Capsicum seedlings (2 four weeks

old seedlings per pot) were transplanted in the 4th set of pots. The 5th and 6th sets of pots were dedicated to both positive and negative control tests for kale and capsicum while in the 7th set of pots a negative control test for maize was conducted. The crops (i.e., maize, kale and capsicum) were watered by sprinkling every 3 days and uprooting of weeds done appropriately as the need arose. Pest control was done by applying the pesticide Imidacloprid at a rate of 1ml per 2 liters of water once every 5 days.

Growth parameters of interest in this study were monitored from the 4th week after planting. This involved counting the number of leaves and measuring plant; height of stalk, thickness of stalk, as well as length and maximum width of the leaves. The plant girth was estimated from the stem diameter, which was measured at half the plant height using a Vernier caliper. A tape measure was used to measure the plant height, leaf length and maximum width. Average chlorophyll content in the respective experimental crops was measured in the leaves using a soil plant analysis development (SPAD) chlorophyll meter (MC-100,). A few leaves per plant (5-6) were sampled and the chlorophyll concentration measured at different spots on each leaf and then the mean chlorophyll concentration per plant calculated from the different readings recorded by this device (Parry *et al.*, 2014). This was a non-destructive procedure of measuring chlorophyll concentration where measurements are taken by the chlorophyll meter on the ratio of radiation transmittance from two wavelengths (red, strongly absorbed by chlorophyll, and near infrared, not absorbed by chlorophyll), making the measurements non-destructive and nearly instantaneous as a result. This measurement is very swift as it takes less than 3 seconds enabling the device to take rapid measurements of multiple leaves within a very short span of time.

The SPAD chlorophyll meter used in this study was able to measure the chlorophyll concentration relatively over a leaf area of approximately 64mm^2 (circle with 9mm diameter).

For leaves narrower than 9mm diameter, there was a view reducer system fitted in the device to reduce the sampling area to approximately $20mm^2$ (circle with 5mm diameter).

Harvesting of kale leaves was done every two weeks starting from the 7^{th} week after transplanting on 3 occasions and mature capsicum fruits harvested on a continuous basis from the 12^{th} week for a period of 6 weeks. The fresh weights measured in the day of harvest were each used in the determination of cumulative yields. The above ground biomass (dry matter) for both maize and capsicum, was cut at final harvest and oven dried at 60^0C. For kale, cumulative dry weight of harvested leaves was summed up with that of the stalk at final harvest.

Salient chemical characteristics of final soil samples after both maize and kale harvesting were determined, and the results obtained from these determinations used as a basis for calculating the quantities of the different nutrients of interest absorbed by a specific crop under different rates of trial fertilizer and filler applications. The wet-oxidation method was used to extract NPK from the dried soil samples after pulverization as explained by Anderson and Ingram, (1993). The micro-Kjeldahl method was used to determine N according to Bremner, (1996) while the determination of phosphorus was done using the molybdenum blue method as described by Murphy and Riley, (1962). Potassium was determined by flame photometry.

6.3.3 Data analysis

Data collected from the greenhouse experiment were subjected to ANOVA using Genstat 14^{th} edition at 95% confidence interval (CI) whereas, comparison and assessment of the significance of the mean values at $P < 0.05$ was done using the Tukey honest significant difference (HSD) post hoc test.

6.4.Results

6.4.1 Physicochemical properties of soil used for planting experiments

Table 6.1., gives the physico-chemical characteristics of soil used in the greenhouse experiment for this study. The nature of the soil was clay-loam and it had low levels of nutrients including Ca, Mg, N, P and K and was slightly acidic. In addition, the soil had low conductivity (0.23ds/m)

Table 6.1: Physico-chemical characteristics of soil used in the greenhouse experiment

Physico-chemical characteristics	Measured value	Suitability for crops
Total N (%)	0.06	Very low
Available P (%)	12.93	Low
Exchangeable K (%)	11.0	Low
Mg (%)	Trace	Very low
Ca (%)	Trace	Very low
TOC (%)	27.00	Satisfactory
pH	6.39	Slightly acidic
Electrical conduct. (ds/m)	0.23	Low
CEC (Cmol(+) kg-1	14.26	Satisfactory

6.4.2 Growth and productivity of maize

Growth parameters of interest and productivity of maize are indicated in **Table 6.2.** No significant difference (P-Value > 0.05) was witnessed in the use of different rates of the newly developed slow-release N fertilizer on the mean number of leaves and thickness of stalk per plant as well as the mean whole plant biomass. However, a significant difference (P-Value < 0.05) was observed in terms of mean height of the stalk and leaf chlorophyll content on using different rates of this new fertilizer formulation.

Table. 6.2: Maize growth parameters and yield (two plants per pot)

Treatment	Mean whole plant biomass Dry weight (g)	Mean No. of leaves per plant	Mean height of the stalk per plant (cm)	Mean thickness of stalk per plant (cm)	Mean chlorophyll content (SPAD)
1.	0.3013a	9.667a	22.58ab	3.883a	35.40ab
2.	0.3007a	9.500a	27.72ab	3.883a	45.27b
3.	0.3020a	9.000a	31.40b	4.450a	33.53ab
4.	0.3027a	8.667a	27.35ab	4.217a	42.30b
5.	0.3013a	8.000a	21.60ab	3.783a	22.77ab
6.	0.3010a	7.500a	16.22a	2.633a	13.50a
P-Value	P < 0.117	P < 0.835	P < 0.006	P < 0.046	P < 0.011

One letter (a or b) in the same column indicate that treatments are not significantly different Different letters in the same column indicate that treatments are significantly different

Key:

1 = 7.00 grams Fertilizer + 3.00 grams Filler = 990.80kg/ha Fertilizer + 424.63kg/ha Filler

2 = 10.00 grams Fertilizer + 0.00 grams of Filler = 1415.43kg/ha Fertilizer + 0.00kg/ha Filler

3 = 6.25 grams of Fertilizer + 6.25 grams Filler = 884.64kg/ha Fertilizer + 884.64kg/ha Filler

4 = 12.50 grams Fertilizer + 0.00 grams Filler = 1769.28kg/ha Fertilizer + 0.00kg/ha Filler

5 = 8.75 grams Fertilizer + 3.75 grams Filler = 1238.50kg/ha Fertilizer + 530.79kg/ha Filler

6 = 0.00 grams Fertilizer + 0.00 grams Filler (negative control) = 0.00kg/ha fertilizer + 0.00kg/ha Filler

Note:

Filler material = milled maize cobs – 2 mm particle size)

Trial fertilizer sample = dechromed, milled and modified leather waste – 2.0 mm particle size

The maize stalk height and chlorophyll content per plant increased at different rates (as a function of different rates of fertilizer application) in the begining of the 4th week and continued up to the 8th week after planting **(Table 6.2.).** Treatment 3 (884.64kg/ha Fertilizer + 884.64kg/ha Filler) gave the highest mean stalk height of the maize crop (31.40cm per plant) at the 8th week after planting (as compared to treatment 6 (negative control – No fertilizer or filler), which gave the lowest mean stalk height (16.22cm per plant) at the same period **(Figure 6.1a and 6.1b).** There was some notable increase in mean chlorophyll content per plant (of the maize crop) under

treatment 2 (1415.43kg/ha Fertilizer + 0.00kg/ha Filler), though not statistically significant ($P > 0.05$), by the 8th week after planting (45.27SPA units). Treatment 3 also, gave the highest statistically significant mean leaf chlorophyll content per plant (33.53SPA units), by the 8th week after planting ($P < 0.05$). This was compared with treatment 6 (negative control), where neither fertilizer nor filler was applied. It gave the lowest mean value for the leaf chlorophyll content (13.50SPA units).

Figure 6.1a: Maize crop – 4 weeks old.

Figure 6.1b: Maize crop – 8 weeks old.

Treatment 6 – Transparent pot (Negative control – Nil Fertilizer + Nil Filler)

Treatment 2 – Purple pot (1415.43kg/ha Fertilizer + 0.00kg/ha Filler)

Treatment 3 – 3 Blue pots in the near background (884.64kg/ha Fertilizer + 884.64kg/ha Filler)

There was a general steady increase in the average thickness of stalk and leaf area as well as the number of leaves per plant from the 4th week to the 12th week after planting in all treatments (1 to 6), especially for treatments 1, 2 and 3; although these growth parameters were not significantly

different amongst the various treatments. However, some decrease in such growth parameters was observed in the 12th week after planting **(Figure 6.2.).**

Figure 6.2: Maize crop – 12 weeks old.

Treatment 2 – Purple pots in the middle background (1415.43kg/ha Fertilizer + 0.00kg/ha Filler)

Treatment 3 - 3 Blue pots in the near background (884.64kg/ha Fertilizer + 884.64kg/ha Filler)

Treatment 4 – Light-green pot in the fore background (1769.28kg/ha Fertilizer + 0.00kg/ha Filler)

Treatment 6 – Transparent pot in the far background (Negative control – Nil Fertilizer + Nil Filler)

6.4.3 Growth and productivity response for kale

The growth parameters and productivity response for kale as well as the whole plant biomass for this crop (one plant per pot) are shown in **Table 6.3.** Cumulatively, treatment 5 gave the highest crop yield per plant (139.5.2g dry weight after 3 harvestings), though not statistically significant.

Treatment 4, which consisted of 1769.28kg/ha fertilizer + 0.00kg/ha filler, compared very well with treatment 6 (positive control), where 123.55kg/ha DAP fertilizer was applied 2 weeks after transplanting and top dressing done with 123.55kg/ha CAN (4 weeks after transplanting), though not significantly different (P > 0.05). Treatment 2 (with 1415.43kg/ha fertilizer + 0.00kg/ha filler) gave slightly higher cumulative crop yield compared to the positive control, although the difference was not statistically significant **(Table 6.3).** Treatment 4 seemed to favour the vegetative growth of kale more than the other treatments as it gave the highest whole plant biomass (22.08g dry weight) and the highest mean chlorophyll content (55.74 SPAD units) as indicated in **Table 6.3** and also, demonstrated in **Figure 6.3.** Nevertheless, these differences did not show any statistical significance (P-Value > 0.05) despite being very conspiquous.

Table. 6.3: Kale growth parameters, crop yield and whole plant biomass (one plant per pot)

Treatment	Mean whole plant biomass Dry weight (g)	Mean crop yield (1st Harvesting) Dry weig.ht (g)	Mean crop yield (2nd Harvesting) Dry weight (g)	Mean crop yield (3rd Harvesting) Dry weight (g)	Mean chlorophyll content (SPAD)
1.	12.76a	6.640a	11.45a	5.260a	42.33a
2.	19.38a	6.253a	23.17a	7.310a	55.38a
3.	15.20a	6.937a	17.22a	6.040a	51.70a
4.	22.08a	5.580a	20.61a	7.307a	55.74a
5.	20.63a	5.703a	18.48a	9.333a	48.58a
6.	19.77a	3.573a	20.18a	9.870a	47.91a
7.	2.56a	2.273a	3.27a	0.940a	42.71a
P-Value	P < 0.062	P < 0.086	P < 0.081	P < 0.069	P < 0.072
One letter (a or b) in the same column indicate that treatments are not significantly different					

Key:

1 = 7.00 grams Fertilizer + 3.00 grams Filler = 990.80kg/ha Fertilizer + 424.63kg/ha Filler

2 = 10.00 grams Fertilizer + 0.00 grams of Filler = 1415.43kg/ha Fertilizer + 0.00kg/ha Filler

3 = 6.25 grams of Fertilizer + 6.25 grams Filler = 884.64kg/ha Fertilizer + 884.64kg/ha Filler

4 = 12.50 grams Fertilizer + 0.00 grams Filler = 1769.28kg/ha Fertilizer + 0.00kg/ha Filler

5 = 8.75 grams Fertilizer + 3.75 grams Filler = 1238.50kg/ha Fertilizer + 530.79kg/ha Filler

6 = 123.55kg/ha DAP at transplanting time + 123.55kg/ha CAN (2 weeks after transplanting) + 123.55kg/ha (4 weeks after transplanting) (positive control)

7 = 0.00 grams Fertilizer + 0.00 grams Filler (negative control) = 0.00kg/ha fertilizer + 0.00kg/ha Filler

Note:

Filler material = milled maize cobs – 2 mm particle size)

Trial fertilizer sample = dechromed, milled and modified leather waste – 2.0 mm particle size

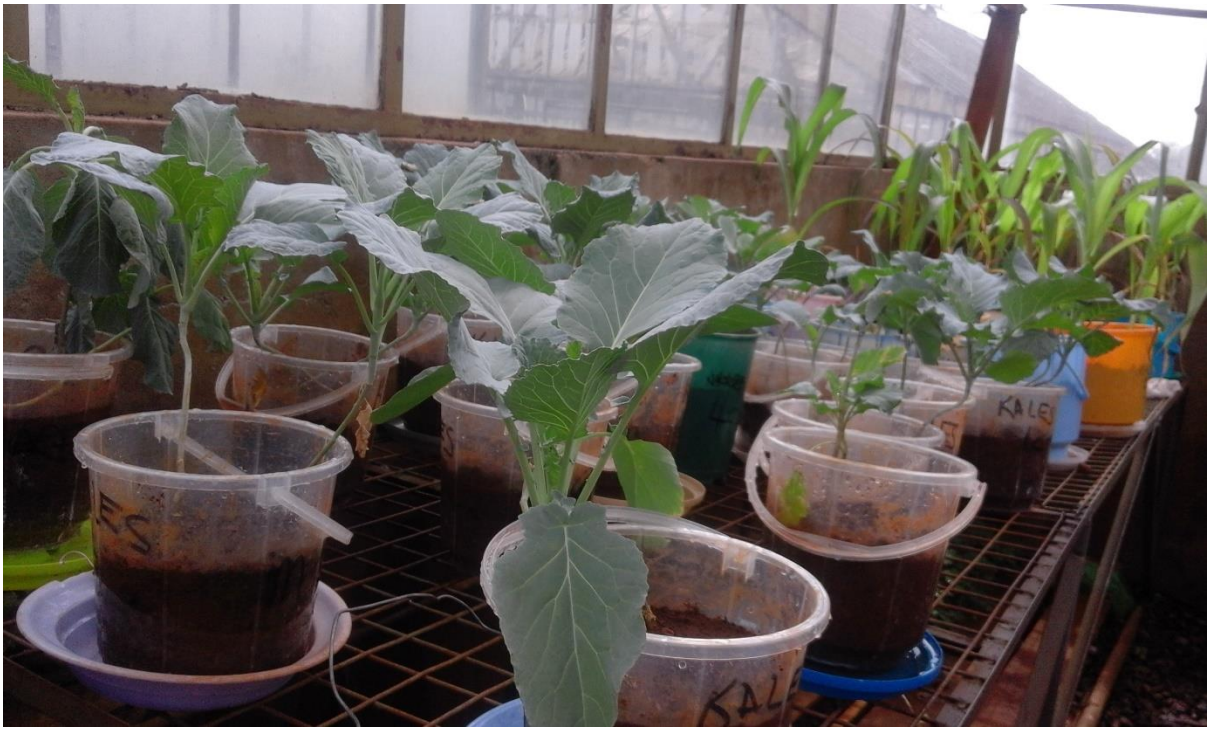


Figure 6.3: Kale crop growth parameters under different fertilizer application rates (8 wks.)

Treatment 1 (Extreme row to the right)

Treatment 2 (Extreme row to the left)

Treatment 3 (Extreme row in the background)

Treatment 4 (Foreground)

Treatment 5 (Middle background)

Treatment 6 (second row to the left)

Table. 6.4: Kale growth parameters, crop yield and whole plant biomass (two plants per pot)

Treatment	Mean whole plant biomass Dry weight (g)	Mean crop yield (1st Harvest) Dry weight (g)	Mean crop yield (2nd Harvest) Dry weight (g)	Mean crop yield (3rd Harvest) Dry weight (g)	Cumulative mean yield of leaves; Dry weight (g)
1.	15.74a	8.887b	8.247ab	6.580a	23.714
2.	12.74a	9.093b	9.267ab	9.267ab	27.627
3.	13.94a	6.657ab	7.293ab	7.293a	21.243
4.	20.10a	5.667ab	14.460b	17.833b	37.96
5.	8.55a	3.803ab	6.180ab	6.180a	16.163
6.	6.82a	6.187ab	6.207ab	4.500a	16.894
7.	7.00a	0.967a	1.033a	0.927a	2.927
P-Value	P < 0.040	P < 0.018	P < 0.024	P < 0.002	

One letter (a or b) in the same column indicate that treatments are not significantly different
Different letters (ab) in the same column indicate that treatments are significantly different

Key:

1 = 7.00 grams Fertilizer + 3.00 grams Filler = 990.80kg/ha Fertilizer + 424.63kg/ha Filler

2 = 10.00 grams Fertilizer + 0.00 grams of Filler = 1415.43kg/ha Fertilizer + 0.00kg/ha Filler

3 = 6.25 grams of Fertilizer + 6.25 grams Filler = 884.64kg/ha Fertilizer + 884.64kg/ha Filler

4 = 12.50 grams Fertilizer + 0.00 grams Filler = 1769.28kg/ha Fertilizer + 0.00kg/ha Filler

5 = 8.75 grams Fertilizer + 3.75 grams Filler = 1238.50kg/ha Fertilizer + 530.79kg/ha Filler

6 = 123.55kg/ha DAP at transplanting time + 123.55kg/ha CAN (2 weeks after transplanting) + 123.55kg/ha (4 weeks after transplanting) (positive control)

7 = 0.00 grams Fertilizer + 0.00 grams Filler (negative control) = 0.00kg/ha fertilizer + 0.00kg/ha Filler

Note:

Filler material = milled maize cobs – 2 mm particle size)

Trial fertilizer sample = dechromed, milled and modified leather waste – 2.0 mm particle size

Figure 6.4: Kale crop under different treatments of greenhouse fertilizer trials (8 weeks old)

Treatment 1 (second row to the left)

Treatment 2 (Foreground row)

Treatment 4 (Extreme row to the left)

Treatment 6 (Extreme row to the right)

Figure 6.4 shows kale growth in soil amended with the newly formulated slow release nitrogen fertilizer as compared with conventional DAP and CAN fertilizers; 8 weeks after transplanting. Treatment 4 (1769.28kg/ha Fertilizer + 0.00kg/ha Filler) gave the highest kale vegetative growth as confirmed by the cumulative mean yield of harvested leaves, 37.96g (dry weight), indicated in **Table 6.4.** The mean value for the kale crop yield in treatment 4, after second harvesting (week 8 after transplanting) was significantly higher (approximately twice) than the one observed in treatment 6 (positive control), where conventional fertilizers were used **(Table 6.4.).** This was a confirmation that the new fertilizer formulation developed in this study, was more favourable for

vegetative growth of kale crop than the use of DAP and CAN conventional fertilizers **(Figure 6.4).**

The new fertilizer formulation was highly deficient in exchangeable K, comprising only 0.1% in the form of K_2O **(Table 5.2).** K deficiency was particularly observed in those treatments with low application rates of this new fertilizer, manifesting as bright yellowish colour in the leaf margins (edges) on otherwise green leaves **(Figure 6.5; Treatments 1 & 7).** Potassium deficiency is often characterized by curling of leaf tips and chlorosis (yellowing) between leaf veins as well as brown scorching (https://en.m.wkipedia.org.). Treatment 7 was used as a negative control, where no fertilizer was added to the experimental soil. Symptoms of P deficiency were also observed especially in Treatment 7 (negative control), which were characterized with purplish colouration originating from the leaf tip as demonstrated in **Figure 6.5.**

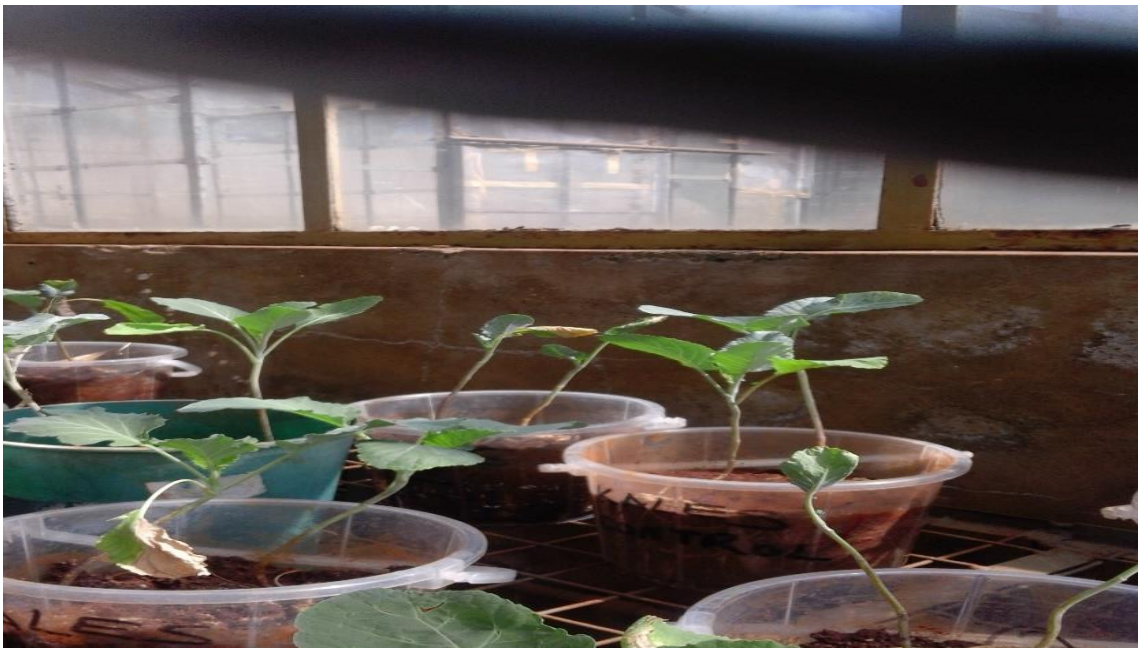


Figure 6.5: Kale crop under (a); very low fertilizer application rate (Treatment 1 - Foreground row), and (b); nil fertilizer (Treatment 7 - background row)

6.4.4 Capsicum growth parameters, fruit yield and whole plant biomass

Capsicum growth parameters, fruit yield and whole plant biomass are given in **Table 6.5.** A record high mean number of leaves and chlorophyll content per plant were observed in treatment 5 by week 8 after transplanting, where the new fertilizer formulation had been applied at the rate of 1238.50kg/ha fertilizer + 530.80kg/ha filler **(Figure 6.6.).**

Table. 6.5: Capsicum growth parameters, fruit yield and whole plant biomass

Treatment	Mean whole plant biomass Dry weight (g)	Mean No. of leaves per plant	Mean fruit yield Fresh weight (g)	Mean chlorophyll content (SPAD)
1.	8.057a	29.67ab	31.84a	55.28b
2.	9.343a	22.67ab	61.44a	45.38b
3.	7.907a	28.67ab	19.29a	45.72b
4.	8.857a	26.33ab	37.49a	48.12b
5.	7.150a	30.67b	33.21a	56.09b
6.	9.327a	29.67ab	20.94a	55.90b
7.	2.423a	14.67a	3.98a	25.73a
P-Value	P < 0.227	P < 0.021	P < 0.117	P < 0.001

One letter (a or b) in the same column indicate that treatments are not significantly different
Different letters (ab) in the same column indicate that treatments are significantly different

Key:

1 = 7.00 grams Fertilizer + 3.00 grams Filler = 990.80kg/ha Fertilizer + 424.63kg/ha Filler

2 = 10.00 grams Fertilizer + 0.00 grams of Filler = 1415.43kg/ha Fertilizer + 0.00kg/ha Filler

3 = 6.25 grams of Fertilizer + 6.25 grams Filler = 884.64kg/ha Fertilizer + 884.64kg/ha Filler

4 = 12.50 grams Fertilizer + 0.00 grams Filler = 1769.28kg/ha Fertilizer + 0.00kg/ha Filler

5 = 8.75 grams Fertilizer + 3.75 grams Filler = 1238.50kg/ha Fertilizer + 530.79kg/ha Filler

6 = 123.55kg/ha DAP at transplanting time + 123.55kg/ha CAN (2 weeks after transplanting) + 123.55kg/ha (4 weeks after transplanting) (positive control)

7 = 0.00 grams Fertilizer + 0.00 grams Filler (negative control) = 0.00kg/ha fertilizer + 0.00kg/ha Filler

Note:

Filler material = milled maize cobs – 2 mm particle size)

Trial fertilizer sample = dechromed, milled and modified leather waste – 2.0 mm particle size

Figure 6.6: Capsicum crop under optimal fertilizer application rates (8 weeks old)

Treatment 5 (the first 3 pots in the middle row)

Treatment 6 (the first 3 pots in the left row)

6.5.Discussion

6.5.1 Growth and yield of maize

Generally, treatments 3 and 4 showed some improvement (as compared to the other treatments in this study) on mean whole plant biomass after final harvesting i.e. Dry matter yield (DMY), though not significant **(Table 6.2.).** Hatfield and Parkin, (2014) encountered a similar situation in their evaluation of the effects of enhanced efficiency fertilizers (EEFs) relative to their non-EEF forms on grain yield and biomass of corn. According to this research work, no significant effect of EEFs on the biomass was recorded as compared to non-EEF treatments. The same researchers (Hatfield and Parkin, 2014), also observed that the non-significant effect of EEFs on the whole plant biomass was somehow related to increased leaf chlorophyll index that increased the ability

of corn canopy to capture photosynthetic active radiation (PAR) consequently converting it into higher yields. This same observation was witnessed in the current study whereby the newly developed SRF derived from dechromed and EPICH modified leather solid wastes had non-significant effect on the whole plant biomass, but significantly increased the mean leaf chlorophyll content ($P < 0.05$) as indicated in **Table 6.2.** However, the decline in some growth parameters such as average number of leaves, leaf area and thickness of stalk per plant in the 12th week as indicated in **Table 6.2** and also, demonstrated in **Figure 6.2;** could be attributed to senescence. Rop *et al.,* (2019) described senescence as a phenomenon that is usually caused by heat stress, age related development and N limitation at the plant's maturity stage. According to Woo *et al.,* (2013), senescence is an oxidative process involving degradation of cellular and sub-cellular structures and macromolecules (e.g., chlorophyll), followed by; mobilization of degradation products especially thylakoid proteo-lipids, to other parts of the plant. This phenomenon then results to a decline in photosynthetic rate and ultimately, death of the plant.

Overall, this new fertilizer formulation was not favourable for growth and productivity of maize as the percentages of both P and K in such fertilizer were below the recommended rates for healthy growth and yield of the crop **(Table 6.2.).** P deficiency was observed as early as the 4th week after planting particularly for treatment 2, manifesting itself as purplish colour in the stalk **(Figure 6.1a.).** The mean value of available P in the soil was also too low for successful growth and productivity of maize **(Table 6.1.).** This deficiency was most likely due to inadequate supply of P, and, possibly P fixation by clay as a result of prolonged interaction of soluble P with soil particles (Maize crop requires relatively higher amounts of P compared to kale and capsicum). It is common knowledge that the nitisols in the Kenya highlands are predominantly made up of

kaolinitic clay as established by Karuku, 2011; Karuku *et al.,* 2012). It has also been found that kaolinitic clay possesses high P sorption capacity (WRB, 2015).

6.5.2 Growth and yield of kale

The relatively higher values for whole plant biomass and chlorophyll content in treatments 2 and 4 as compared to treatments 1, 3 and 5, are attributable to availability of P in the increased application rates of the EPICH modified new fertilizer **(Table 6.3** and **Figure 6.3.).** This new fertilizer also gave better results than DAP and CAN in terms of whole plant biomass and chlorophyll content as indicated in Treatment 6 due to the same reason (i.e. higher P content). This invariably increased the availability and assimilation of P as confirmed in similar studies, where insignificantly higher biomass yield in different types of slow-release fertilizer formulations was observed when compared with conventional fertilizers having the same nutrient content (Li *et al.,* 2013; Rop *et al.,* 2019). According to these researchers, the increased biomass yield was attributed to enhanced NUE and reduced nutrient loss in the new slow-release fertilizer formulations.

6.5.3 Growth and yield of capsicum

The new fertilizer formulation (if applied at optimal rates as seen in Treatment 5) was significantly better than DAP and CAN conventional fertilizers **(Treatment 6)** particularly in terms of increasing chlorophyll content and number of leaves per capsicum plant. As indicated in **Table 6.5** and demonstrated in **Figure 6.6.,** the mean number of leaves per capsicum plant under treatment 6 (during week 8 after transplanting) was 29.67 compared to 30.67 under treatment 5 during the same period. It was also observed that the mean chlorophyll content per capsicum

plant under treatment 6 (during week 8 after transplanting) was 55.90 SPAD units compared to 56.09 SPAD units under treatment 5 (during the same period).

There was no significant difference ($P > 0.05$) on mean fruit yield and whole plant biomass for the capsicum crop under the various treatments as can be seen in **Table 6.5.** Even increasing fertilizer application rates did not seem to favour higher fruit yield as well as whole plant biomass of the capsicum crop. In a similar investigation conducted by Reyes *et al.*, (2008), who compared a slow-release fertilizer (SRF) with conventional calcium nitrate fertilizer; found out that capsicum crop was doing better, though not statistically significant ($P > 0.05$), in SRF compared to $Ca(NO_3)_2$ treatment in terms of fruit yield and whole plant biomass due to improved availability of N. Contrary to the findings of this study, Stagnary and Pisante, (2012) observed that urea and $Ca(NO_3)_2$ amendments gave statistically higher capsicum DMY and fruit yields compared to SRF amendment due to delayed N release.

6.5.4 Conclusions

The performance of the newly formulated leather-based fertilizer in growth and productivity (yield) of kale and capsicum was comparable with the use of DAP and CAN conventional fertilizers. However, the new fertilizer was applied only once at transplating time and there was no need for top-dressing with any other form of fertilizer. As described by Rop *et al.*, (2018), although microbes at the exponential growth phase are well satiated, more are normally left for maintenance and the rate of mineralization is much higher than immobilization. The newly formulated slow-release N fertilizer is therefore more suited for growing vegetables such as kale and capsicum, which require low uptake of N at establishment and development stages, but higher uptake of N at both reproductive and maturity stages. This is in contradiction to growing

other annual crops such as maize, which require small doses of N uptake at establishment, much higher doses at both development and reproductive phases, but very little N uptake at maturity (Rop *et al.,* 2018).

CHAPTER 7: GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1. General Conclusions

- Tanned solid waste that is generated during leather manufacture was found in this study to be 31.8% by weight, of the total amount of hides processed.

- The highest percentage of tanned solid waste (68.3%) was found in this study to be contaminated with chromium and was also, the most difficult to dispose of from the environment of the tannery.

- Tanneries studied disposed all their solid wastes including chrome contaminated solid wastes mainly by open dumping, incineration and landfilling; leading to environmental contamination.

- A new dechroming method developed in this study managed to extract 99.90% of the chromium in chrome tanned leather wastes in a period of 24 hours, and the remaining traces of chromium complexed with potassium oxalate to form potassium chromate, which is highly soluble in water and hence, easily washed away.

- Hydrolysis and modification of collagen in the dechromed leather wastes released N from the collagenic material making it accessibe for use by plants.

- The dechromed and hydrolysed leather solid wastes had the following plant nutrient levels: 44% N, 23% P (as P_2O_5), 0.1% K (as K_2O), 0.2% Mg, 1.8% Ca and 22.15% TOC.

- Maize cobs powder was successfully used as filler to formulate a slow release nitrogen fertilizer with added advantages of contributing to plant nutrient levels and being biodegradable.

- The new fertilizer formulation had the following levels of plant nutrients; 46.8% N, 35.06% P (as P_2O_5), 0.4% K (as K_2O), Mg 7.7% Mg, 3.4% Ca and 65.12% TOC.

- The new fertilizer had showed a high rate of N mineralization; a process by which organic nitrogenous compounds present in organic matter are decomposed, or oxidized into readily available forms for use by plants as nutrients.

- A single application of the new fertilizer formulation during planting enhanced growth and productivity of kale and capsicum with results comparable with those produced by conventional DAP and CAN fertilizers that were applied during planting and top-dressing respectively. However, the new fertilizer did not show favourable results in growth and productivity of maize.

- The new fertilizer formulation was deficient in exchangeable K, which was only 0.4% in the plant available form of K_2O. This deficiency was manifested as bright yellowish colour in the leaf margins, particularly in kale crop where there was conspicuous brown scorching and curling of leaf tips.

7.2. General Recommendations

- Up-scale and optimize the new leather solid wastes dechroming and collagen modification methods described in this study for large scale decontamination of chrome leather waste and commercial production of the new slow-release organic fertilizer.

- Improve the new fertilizer formulation by addition of potassium rich supplements in order to enhance the level of potassium in the fertilizer.

- More studies to be done to understand why maize performed poorly with this new fertilizer formulation.
- More studies to be done to test the suitability of the new fertilizer for use in other types of crops.

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APPENDICES

Appendix 1: Preparation of the standard for ammonium nitrogen (NH_4-N) using ammonium chloride

To prepare 1000 ppm from the ammonium salt (ammonium chloride), its molecular weight was taken and then the amount of ammonium ions (NH_4^+) in the salt calculated:

Molecular weight of NH_4Cl = 14 + (1 x 4) + 35.5 = 53.5g

The amount of NH_4^+ in 53.5g of NH_4Cl = 14 + (1 x 4) = 18g

In that salt (i.e. NH_4Cl), there are 18g of NH_4^+ ions

Therefore, 1g of NH_4^+ is equivalent to 53.5/18 = 2.9722g NH_4^+

Preparation of the standard for nitrate nitrogen (NO_3-N)

To prepare 1000 ppm of nitrate nitrogen (NO_3-N) from the sodium salt, the amount of NO_3^- in 85g of $NaNO_3$ (i.e. molecular weight of $NaNO_3$) was calculated as follows:

85g of $NaNO_3$ = 14 + 48 = 62g

In that salt ($NaNO_3$) there are 62g of NO_3^-

1g of NO3- 85/62 x 1 = 1.37097g

This is equivalent to 1.37g of $NaNO_3$; 1g = 1000mg = 1,000,000µg; ppm = mg/l (solid/liquid) = mg/kg (solid/solid) = µg/ml = µg/g

Weighed 1.3709g of $NaNO_3$ and put it in the 1000ml capacity conical flask; the volume was then made up to the mark with distilled water. The contents of the flask were then

1,000,000µg/1000ml of water = 1000µg/ml = 1000ppm NO_3^-

From the 1000 ppm NO_3^- any quantity of ppm NO_3^- can be made for the standard using the formula:

$C_1V_1 = C_2V_2$ to make the various stock solutions.

The sodium nitrate used in this case was of analytical reagent grade (anala), hygroscopic, and was dried overnight in the oven at 105^oC +/- 0.2.

Appendix 2: Formulations of Reagents A and B

Formulation of Reagent A

1) Take 12 g of ammonium molybdate
2) Take 0.27 g of potassium tartrate
3) Take 1L of 5 N H_2SO_4

Mix 1), 2) and 3) in a 2L volumetric flask and top up to the mark with distilled water.

That is then known as Reagent A.

Formulation Reagent B

1) Take 200 ml of Reagent A and put it in a beaker
2) Add 1.05 g ascorbic acid and shake well to mix

The Reagent is then ready for use.

Appendix 3: Environmental Impact of Epichlorohydrin (C_3H_5ClO): Associated Health Hazards and Safety Precautions

Composition

The technical product is more than 99% pure.

Depending on its source, epichlorohydrin contains different impurities, among which may be chlorinated ethers, 1, 4-dichloromethane, and several chlorinated propenes.

Uptake. Metabolism, and Excretion

Epichlorohydrin is absorbed rapidly into the body through the skin, and after ingestion or inhalation. It is distributed widely throughout the body. Most absorbed epichlorohydrin is metabolised rapidly, part being excreted as carbon dioxide via the lungs and part as water-soluble compounds via the urine.

Health Hazards Associated with Epichlorohydrin (EPICH)

- Epichlorohydrin vapour irritates the skin, eyes, nose, throat, and lungs, and may cause excessive accumulation of fluid in the lungs (oedema).
- The liquid is severely irritating to the skin and eyes after local contact, and to the mouth, throat and stomach, after ingestion.
- EPICH can sensitize the skin. The compound affects the central nervous system and liver, and is suspected carcinogen.

Safety Precautions on Exposure to EPICH

- Do not smoke, drink, or eat in the work place.
- In case of overexposure the victim should leave, or be removed from, the contaminated area to fresh air as rapidly as possible.
- Remove contaminated clothing and shoes, and wash with plenty of water and soap.
- Flush affected eye(s) with water for at least 15 minutes.

Appendix 4: Questionnaire on leather waste management in tanneries

1.0 **BACKGROUND**

1.1 Name of interviewee and date of interview

……

……

1.2 Name of tannery and address

……..

……..

1.3 How long has he/she worked in this tannery and in which department(s)?

……..

…….

2.0 **PRODUCTION CAPACITY OF THE TANNERY**

NO.	**PRODUCT**	**% EXPORT**	**% LOCAL SALES**
2.1	Wet-blue		
2.2	Crust		
2.3	Finished leather		
2.4	Footwear		
2.5	Leather goods		

3.0 **LEATHER WASTE GENERATION**

3.1 How much of the following types of leather waste does the tannery approximately generate per month?

……

……..

3.1.1 Chrome shavings.....

3.1.2 Chrome splits and trimmings .....

3.1.3 Vegetable shavings.....

3.1.4 Vegetable splits and trimmings.....

3.1.5 Crust trimmings.....

3.1.6 Buffing dust.....

3.1.7 Finished trimmings.....

4.0 UTILIZATION AND DISPOSAL OF LEATHER WASTE

4.1 How do you dispose of the various types of leather waste mentioned in 3.1 above?

(i) Landfilling

(ii) Open dumping

(iii) Incineration

(iv) Digestion

(v) Composting

(vi) Burying

(vii) Extraction

4.2 Which of these wastes are you recycling or re-using?

……..

…….

4.3 If the answer to 4.2 above is recycling; what is the secondary product that is being produced?

……

……

4.4 Do you think you comply with NEMA/CITY COUNCIL standards in relation to treatment and disposal requirements?

……

……..

4.5 Are you aware of any regulations governing the release of chemicals and toxic solid waste from industries to the environment?

……

……..

5.0 CONSTRAINTS TO GROWTH

5.1 What challenges do you face in managing and disposing of waste from the tannery in light of clean technology requirements and restrictive environmental legislation?

…….

…….

5.2 What are some of public health concerns of leaving solid waste unattended to on the environment of the tannery?

……

……

5.3 How much money per month do you spend on waste disposal?

……

……

5.4 What in your opinion do you consider to be some of the major constrains to the development of the local leather sector?

(i)..... …….

(ii) ……..

(iii) …….

(iv) ……..