

**ASSESSMENT OF THE EFFECTS OF SELECTED
AGROCHEMICALS ON SOIL MACRO-INVERTEBRATES IN SUGAR
CANE (*Saccharum officinarum L.*) PLANTATIONS IN MASINDI
DISTRICT, UGANDA**

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DECLARATION

This Dissertation is my original work and has not been presented for a degree
in any other University

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APPROVAL

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DEDICATION

In a special way, I dedicate this dissertation to my dad Mr. Biingi Abel Amooti who has been the main sponsor for this study.

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LIST OF ABBREVIATIONS AND ACRONYMS

2,4-D:	2,4-Dichlorophenoxyacetic acid
ANOVA:	Analysis Of Variance
EU:	Experimental Units
FAO:	Food Agriculture Organisation
GPS:	Geographic Positioning System
LSD:	Least Significant Difference
NPK:	Nitrogen, Phosphorus and Potassium
SIR:	Substrate Induced Respiration
SOM:	Soil Organic Matter
TSBF:	The Soil Biology and Fertility
USMA:	Uganda Sugar Manufacturers Association

ABSTRACT

The increasing demand for sugar has intensified sugarcane cultivation, leading to extensive use of agrochemicals such as herbicides and fertilizers. This study aimed to evaluate the effects of 2,4-D herbicide and NPK fertilizer on soil macro-invertebrates in sugarcane plantations in Masindi District, Uganda. Using a completely randomized block design over eight weeks, sugarcane was planted in 12 plots subjected to four different treatments, including a control. Soil samples were collected using the core sampler method to assess soil properties and the presence of soil-dwelling macro-invertebrates. Data were analysed using IBM SPSS version 26, applying one-way ANOVA at a 0.05 significance level. Results showed that 2,4-D and NPK significantly altered soil properties. Specifically, 2,4-D increased soil alkalinity, while NPK reduced pH due to nitrification. Both treatments also influenced soil texture—NPK increased clay content, while chemical applications raised silt levels. Biologically, short-term application of these agrochemicals did not significantly affect the abundance or morphology (length, segment number, coloration) of soil macro-invertebrates such as earthworms and centipedes. However, biodiversity and ecological stability were highest in untreated control plots. Notably, 2,4-D alone suppressed macro-invertebrate diversity, whereas the combined application with NPK moderated this effect. The study concludes that short-term agrochemical use alters soil chemistry but not macro-invertebrate morphology, while still negatively impacting biodiversity. It recommends reduced herbicide use, promotion of agro-ecological practices, long-term environmental monitoring, and farmer education on the ecological risks of agrochemicals to support sustainable soil health and biodiversity conservation.

CHAPTER ONE: INTRODUCTION

1.1 Background

Macro invertebrates are small, spineless animals visible to the naked eye, commonly found in freshwater habitats like rivers, lakes, and wetlands. They play essential roles in ecosystems by aiding in nutrient cycling, decomposing organic matter, and serving as a food source for fish and wildlife (González *et al.*, 2021). Due to their sensitivity to environmental changes, they are widely used as bioindicators of water quality (Beasley *et al.*, 2020; Zhao *et al.*, 2023). Taxonomic groups include insects (e.g., mayflies, dragonflies), crustaceans, mollusks, worms, and water mites (Fernandes *et al.*, 2022). Functionally, they are classified into feeding groups namely; shredders, collectors, scrapers, and predators and this highlights their diverse ecological roles in maintaining the health of freshwater systems (Jacobsen *et al.*, 2020; Li *et al.*, 2023).

The global population has been rising steadily since the early 20th century, with a current growth rate of about 1.05%. It is projected to exceed 9.7 billion by 2050 (Miller *et al.*, 2019), increasing the demand for food and energy and putting significant pressure on agriculture to boost productivity sustainably. Sugarcane is a key global crop used for sugar, ethanol, and bioenergy, with Brazil, India, and China among the top producers (FAO, 2022; USDA, 2023). Countries like Brazil and Australia have adopted large-scale, mechanized sugarcane farming, utilizing irrigation, improved crop varieties, and agrochemicals to enhance yields (Silva *et al.*, 2021; Santos *et al.*, 2020). However, this intensive approach raises environmental and health concerns. Fertilizer and pesticide runoff contaminates water bodies, causing

eutrophication and harming aquatic ecosystems (Raghavan *et al.*, 2021). Additionally, excessive agrochemical use degrades soil health by reducing microbial diversity and altering pH levels (Fernandes *et al.*, 2022). Biodiversity loss, especially of pollinators and soil invertebrates, has also been linked to pesticide exposure (Chand *et al.*, 2021), underscoring the need for more environmentally sustainable sugarcane cultivation practices.

Sugarcane cultivation in Uganda was introduced during the early 20th Century, specifically between 1926 and 1934. This period marked the introduction of various sugarcane varieties into the country, which laid the foundation for the sugar industry that would develop in subsequent decades (Mwavu *et al.*, 2018).

The establishment of sugar factories further facilitated the growth of sugarcane farming. Notably, the Vithaldas Haridas & Company initiated sugar production in 1920 by acquiring land for a sugar factory in Kakira, which began operations in 1930 (Guloba *et al.*, 2023). Similarly, the Sugar Corporation of Uganda Limited was developed in 1924, marking significant milestones in Uganda's sugar industry history (Nabalegwa *et al.*, 2022).

More agricultural land has been converted to sugarcane cultivation in Uganda and East Africa due to rising global demand for sugar and other sugarcane products (Martiniello, 2021). Sugarcane has thus grown to be one of the region's most important cash crops (FAO, 2021).

With an annual output of over 600,000 metric tons as of October 2021, Uganda is the East African leading producer of granular brown sugar (Guloba *et al.*, 2023). The majority of sugar produced in Uganda comes from private

estates, namely the Madhvani Group at Kakira near Jinja in Busoga district, the Mehta Group estate at Lugazi in East Mingo region, and Kinyara sugar in Masindi district in the country's southwest (Guloba *et al.*, 2023).

According to a study, by Cherry *et al.*, (2017) sugarcane crops require a large amount of pesticides and fertilizers to maintain the crop growth and health given that they are affected by a variety of pests at different developmental stages. These include the sugar cane Stalk Borer (*Chilo infuscatellum*), Nematodes Meloidogyne and *platylenchus sp.*, termites, Isoptera, and vermin (Cherry *et al.*, 2017). Given the increasing demand, sugarcane has been intensively produced in the monoculture production system (Semie *et al.*, 2019). This evidently has detrimental effects on biodiversity conservation, including a decline in the populations of soil macro invertebrates which results in decline in soil fertility, as a result of the heavy use of agrochemicals in sugarcane cultivation and weeding (Tayyab *et al.*, 2021).

As a result, the use of agricultural chemicals such as fungicides, insecticides, and inorganic fertilizers (2,4-D herbicide and NPK fertilizer) has increased to maintain high crop productivity and minimize losses (Breil *et al.*, 2023). Sugarcane production has not been exceptional as farmers use various inorganic chemicals, including pesticides, herbicides, and fertilizers, in order to meet increasing demand for sugar.

Intensive use of fertilizers and herbicides in sugarcane cultivation has been shown to significantly reduce the abundance and diversity of soil macro invertebrates (Huang *et al.*, 2021). This decline is attributed to the toxic effects of agrochemicals, which can lead to soil degradation and a decrease in

biodiversity (Mello *et al.*, 2019). For instance, studies indicate that soil macro invertebrate populations, such as earthworms and myriapods, are particularly sensitive to these chemicals, resulting in lower taxonomic richness and biomass in heavily treated soils compared to those managed organically (Colegio *et al.*, 2020).

An assessment of an ecological dynamic balance and biodiversity can be made by evaluating the relationship between land use and soil macro invertebrates (Siqueira *et al.*, 2016). Due to their high sensitivity to both chemical and physical soil conditions, soil macro invertebrates can serve as ecological indicators of agricultural practices when searching for sustainable methods of producing sugarcane. As an indication of soil quality, soil macro invertebrates must be included. In this sense, their ability to break down organic materials and create intricate food webs that are necessary for an ecosystem to function makes them significant in soil ecosystems (Barnes *et al.*, 2016), as well as supporting most agriculture production systems (production services) through beneficial services that they mediate: soil formation, nutrient cycling and primary production (Lavelle *et al.*, 2022).

Due to the belief that agrochemicals like pesticides, fertilizers, and herbicides increase harvests, their use has grown quickly, and pesticides are bought by both major producers and smallholder farmers. In most developing nations, pesticide subsidies have decreased since the 1990s as a result of structural adjustment measures and the emergence of sustainability theory (Sharma *et al.*, 2019). Presently, over four million tonnes of pesticides are used annually worldwide, of these include herbicides (47.5%) and then insecticides (29.5%),

fungicides (17.5%), and other categories like nematicides and rodenticides (5.5%) (Sharma *et al.*, 2019).

Agrochemicals often have side effects that impact various living forms both above and below the soil surface, despite the fact that they are crucial for preventing losses in agriculture production. Soil faunal populations play a crucial role in the functionality of ecosystems through their direct and indirect interactions with plants, nutrient cycling, and organic matter (Wardle *et al.*, 2012).

Soil macro invertebrates are closely linked to the availability of nutrients in the soil and are susceptible to changes in the physicochemical conditions of the soil and roots, which can be brought about by both organic and inorganic fertilizers (Yan *et al.*, 2018). Concerns about soil degradation and the need for sustainable soil management have prompted increasing scientific interest in soil quality bio indicators in recent years (Hou *et al.*, 2020).

When cane is established, it grows for 14 to 16 months and can reach a height of up to four meters before being harvested when matured. Agrochemicals are primarily used in the preparation of the soil for planting and the early stages of the cane's growth (Meena *et al.*, 2020).

Agrochemical use decreases taxonomic richness, density, and biomass, reflecting severe soil ecosystem disturbance due to intensive farming practices like cultivation and fertilization (Twagirayezu *et al.*, 2024). Studies in Cuba found higher macro-invertebrate density and biomass in secondary forests than in sugarcane monocultures (Caperra *et al.*, 2011). Earthworms and myriapods are especially vulnerable, and soil type also affects invertebrate diversity

(Siqueira *et al.*, 2016). These findings highlight the need for sustainable agriculture to protect soil biodiversity and ecosystem health.

1.2 Problem statement

The rise in sugar demand has led to increased sugarcane cultivation, driving extensive use of agrochemicals like pesticides, herbicides, and fertilizers (FAO, 2022). While these chemicals boost crop yields, they harm soil macro-organisms such as earthworms, beetles, and millipedes, which are vital for soil fertility and ecosystem functions (Pelosi *et al.*, 2021; Lavelle *et al.*, 2016). Agrochemical exposure disrupts these organisms, compromising soil health and ecosystem resilience (Goulson *et al.*, 2020).

An estimated 385 million herbicides poisonings occur globally each year, resulting in around 11,000 deaths (Boedeker *et al.*, 2020). Soil degradation costs U.S. corn farmers over half a billion dollars annually due to reduced fertility (Doulder, 2021). In Uganda yield potential is often 3–4 tons per hectare, but actual yields average around 1.5 tons/ha, largely due to poor soil fertility (Okoboi *et al.*, 2012). These figures highlight the urgent need to reassess current farming practices. The widespread use of agrochemicals in sugarcane production poses significant risks to both ecological health and public safety, requiring immediate research and policy changes.

The widespread use of agrochemicals in sugarcane farming contradicts Sustainable Development Goal (SDG) 2, which focuses on sustainable agriculture and environmental restoration (United Nations, 2015). In Uganda's western districts, land is increasingly being converted to sugarcane plantations (UBOS, 2023), yet there is limited eco-toxicological data on the effects of

agrochemicals like NPK fertilizer and 2,4-D herbicide on soil macro-invertebrates in tropical ecosystems (Munyuli *et al.*, 2022). For this reason, this research aimed to study the effects of using NPK fertilizer and 2,4-D herbicide on the soil macro invertebrates in sugar cane plantations.

1.3 Objectives of the study

1.3.1 General objective

To assess the effects of application of 2,4-D Amine herbicide and NPK fertilizer on soil macro invertebrates and soil physical and chemical properties in sugar cane (*Saccharum officinarum*) plantations in Bulima town council, Masindi District western Uganda.

1.3.2 Specific objectives

- i. To determine the effects of 2,4-D herbicide and NPK fertilizer application on soil physicochemical properties in sugarcane plantations.
- ii. To determine the effect of 2,4-D herbicide and NPK fertilizer application on the abundance and diversity of soil macro invertebrates in sugarcane plantations.
- iii. To determine the effects of 2,4-D herbicide and NPK fertilizer application on the morphological features of soil macro invertebrates in sugarcane plantations.

1.4 Research hypothesis

H₀₁: 2,4-D herbicide and NPK fertilizer application have no significant effect on soil physical and chemical properties in sugarcane plantations.

H02: 2,4-D and NPK fertilizer application have no significant effect on the abundance and diversity of soil macro invertebrates in sugar cane plantations.

H03: 2,4-D herbicide and NPK fertilizer have no significant effect on the morphological features of soil macro invertebrates in sugar cane plantations.

1.5 Scope of the study

The study first established sugarcane plantation for field testing, soil samples of the virgin land were taken to establish the baseline data. Soil preparation involved removing vegetative material and preparing a tith adequate for sugarcane growth. The study was in five operations; sub-soiling, ploughing (18, 34" discs), harrowing (24 discs), furrowing and bed formation, after which the sugarcane was planted manually. This was followed by collection of soil samples, to identify soil types, assess soil health, numerate the visible soil macro invertebrates, classify them and assess their health based on their morphological features. The soil health of the sample collected were assessed based on physical parameters: texture, colour, porosity density and temperature and chemical parameters; pH, salinity, soil organic matter content. The soil macro invertebrates were hand sorted, counted and recorded for each species in each sample to determine abundance and diversity and compare their size, length and colour with the standard parameters of normal functioning of the macro invertebrates. The researcher developed macro invertebrate chart that was used to classify and compare the macro invertebrate morphological attributes with their standard morphological features.

1.6 Significance of the study

The study provides valuable information for conservationists to understand the short-term impacts of agricultural practices on ecosystems and to advocate for more sustainable management practices that protect biodiversity and water resources.

The study provides significant for advancing ecological knowledge, promoting sustainable agricultural practices, fostering interdisciplinary research, and enhancing educational frameworks in academia.

The study gives vital information for policymakers by providing empirical evidence on the ecological consequences of agrochemical use in sugarcane plantations, thereby guiding regulatory measures, promoting sustainable practices, and ensuring the protection of vital ecosystem services.

Findings of the study provide guidance to sugar cane farmers on how to set the stage for agronomic practices and possibly policy makers to follow in terms of sustainable restoration of degraded soils and enhanced soil ecosystem services, which will lead to higher agricultural productivity. Our understanding of the relationships between the intensity of agrochemical application, soil biodiversity, and soil health will be substantially improved by being able to investigate these changes in both space and time.

1.7 Justification

The primary producers of soil organic matter (SOM), macro invertebrates are the subject of critical study. Agrochemical use upsets vital soil processes (such as primary production, organic matter breakdown, and nutrient cycling) by having direct and indirect effects on soil organisms. Understanding the effects

of agrochemicals on these organisms is crucial, the student first provided a general description of macro invertebrate communities, morphological features, diversity and abundance in the plots before treatment. The data analysis, conceptual models, and simulations were used to provide dependable projections for a general typology and patterns of the macro invertebrate communities. This provided unambiguous information on the abundance and diversity of this fauna at the study plots.

1.8 Conceptual Framework

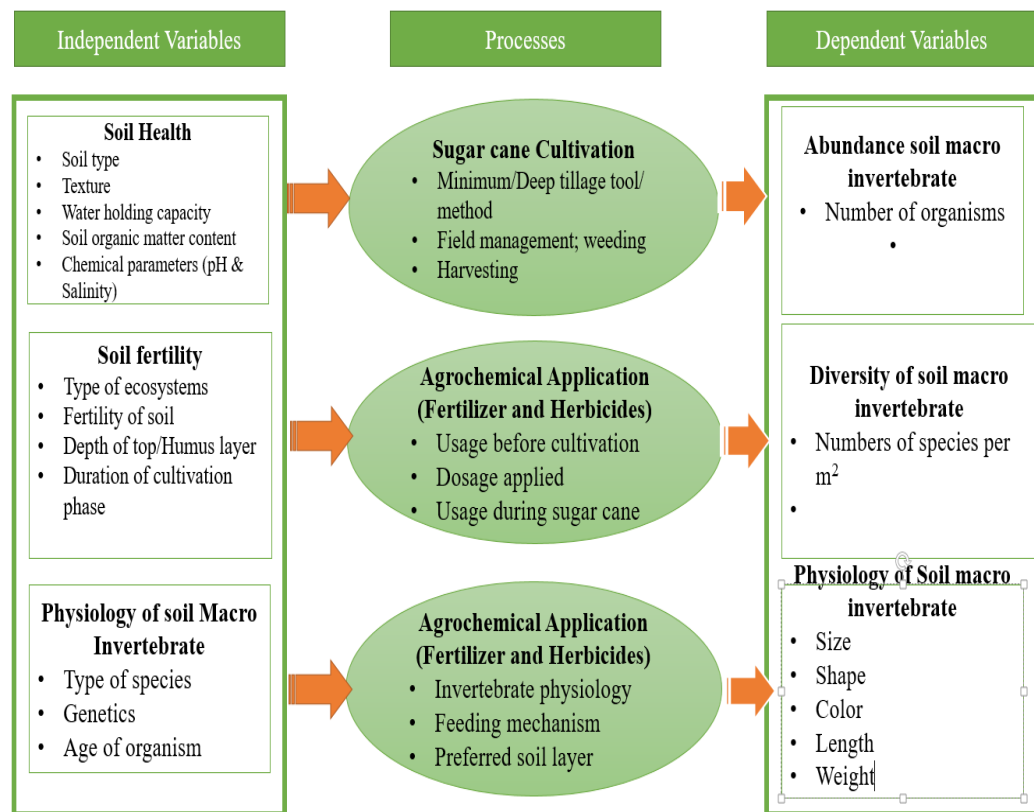


Figure 1.1: Conceptual framework

Independent Variables for the study are Soil Health, Soil fertility and morphological features of soil Macro Invertebrates. These factors largely

influence the prevalence, abundance, diversity and morphological features of the soil macro vertebrates in the soil (Bufebo *et al.*, 2021).

Soil health is commonly influenced by Texture, pH, Water holding capacity, Soil organic matter content, Salinity, soil type, type of vegetation grown and land use changes that have happened on the land. The soil health was used as an indicator prevalence of the target macro invertebrates in the study. Healthy soils create favourable conditions for the existence and growth of macro invertebrates (Lavelle *et al.*, 2022).

Soil fertility which is influenced by many factors including available if nutrients, the type of ecosystems, depth of top/Humus layer, types of crops grown and their nutrient demand, the duration of cultivation phase among others. The study established the soil fertility and thus determined its impact on the abundance of soil macro vertebrates (Wurst *et al.*, 2018).

Morphological features of soil macro invertebrate: the morphology of organisms was determined by the type of species, its genetics, and the age of organism. The study assessed the health of the target macro invertebrates based on the standard attributes described in literature (Lavelle *et al.*, 2022).

Dependent variables of the study were abundance soil macro invertebrates, diversity of soil macro invertebrates and the morphology of soil macro invertebrates.

The Abundance soil macro invertebrate was determined by manual counts of the number of soil macro vertebrates per m² regardless of the species.

Diversity of soil macro invertebrate was determined by identification of the numerated macro vertebrates and the numbers of species per m²

CHAPTER TWO: LITERATURE REVIEW

2.1 Soil macro invertebrates

Soil macro invertebrates are organisms that live within the soil and are visible to the naked eye, typically exceeding 2 mm in body size. They play a crucial role in maintaining soil structure, nutrient cycling, organic matter decomposition, and enhancing soil aeration and water infiltration (Lavelle *et al.*, 2020). These organisms are critical indicators of soil health and ecological functioning, particularly in agro-ecosystems and forest soils.

Macro invertebrates are broadly categorized based on their ecological roles into three functional groups: ecosystem engineers, litter transformers, and microbial grazers. Ecosystem engineers, such as earthworms and some ant species, modify the physical structure of the soil, influencing water dynamics and root growth (Jouquet *et al.*, 2021). Litter transformers, such as millipedes and isopods, fragment organic residues, thereby facilitating microbial decomposition. Microbial grazers, including various beetle larvae and mites, feed on soil microorganisms, regulating microbial populations and enhancing nutrient mineralization (Orgiazzi *et al.*, 2022).

2.1.1 Categories of Soil macro invertebrates

The major taxonomic categories of soil macroinvertebrates include:

Annelids (e.g., Earthworms – *Lumbricidae*): Often regarded as key ecosystem engineers, earthworms influence soil aggregation and nutrient availability through burrowing and casting activities (Tondoh *et al.*, 2021).

Arthropods (e.g., Ants – Formicidae, Termites – Termitidae, Beetles – Coleoptera): Arthropods are diverse and contribute significantly to soil

processes such as litter decomposition, predation, and soil bioturbation. Termites, in particular, play essential roles in tropical soils, breaking down tough plant materials and contributing to humus formation (Mando & Bationo, 2020).

Mollusks (e.g., Land snails and slugs): These organisms contribute to organic matter breakdown and nutrient cycling, although their roles are less studied compared to annelids and arthropods (Gholami *et al.*, 2023).

Recent studies emphasize the sensitivity of macroinvertebrate communities to land use changes, agricultural intensification, and chemical inputs, making them valuable bio-indicators for soil quality assessment (Cardoso *et al.*, 2022; Silva *et al.*, 2023). In agroecosystems, the application of fertilizers and pesticides has been shown to alter the diversity and abundance of soil macroinvertebrates, potentially disrupting ecological balance and reducing soil productivity (Nkongolo *et al.*, 2021).

In conclusion, soil macroinvertebrates are integral to sustainable soil management and ecosystem health. Understanding their taxonomy and ecological functions is vital for designing strategies aimed at promoting soil biodiversity and resilience.

2.1.2 Soil health

Soil health, also referred to as soil quality, is broadly defined as the continued capacity of soil to function as a vital living system that sustains plants, animals, and humans. It encompasses physical, chemical, and biological properties that interact to support ecosystem productivity, environmental quality, and plant and animal health (Lehmann *et al.*, 2020).

Over the past few years, there has been an increasing focus on biological indicators as key components of soil health, including microbial diversity, macroinvertebrate populations, enzyme activities, and organic matter content (Bünemann *et al.*, 2021). These biological indicators are dynamic and respond sensitively to changes in land use, agricultural practices, and climate.

A healthy soil exhibits good structure and porosity, retains and filters water efficiently, contains balanced nutrients, and hosts diverse and active biological communities. It is also resilient to erosion, compaction, and degradation, making it a cornerstone of sustainable land management and food security (Rojas *et al.*, 2022).

In agricultural landscapes, soil degradation—including erosion, nutrient depletion, compaction, salinization, and pollution—has been a major threat to soil health. Globally, about one-third of soils are moderately to highly degraded due to unsustainable land use practices (FAO & ITPS, 2020). The loss of soil organic carbon (SOC), for instance, impairs fertility, water retention, and soil structure.

Recent research has emphasized regenerative agriculture and conservation practices such as cover cropping, reduced tillage, organic amendments, and crop rotation as key strategies for improving and restoring soil health (Lal, 2020; Schulte *et al.*, 2023). These practices enhance organic matter, support soil biodiversity, and improve overall resilience to climate variability.

The development of soil health assessment frameworks and decision support tools has advanced, particularly with the integration of remote sensing, digital soil mapping, and big data analytics. These tools aim to support farmers and

land managers in making informed decisions that enhance soil function while minimizing environmental impacts (Singh *et al.*, 2021).

Despite these advancements, a major challenge remains the context-specific nature of soil health indicators. Soil properties vary significantly by region, land use, and climate, and thus, soil health assessments must be localized and integrative (Panagos *et al.*, 2022).

2.1.3 Role of macro invertebrates in soil health and fertility

Soil macroinvertebrates, such as earthworms, millipedes, ants, termites, beetles, and other larger invertebrate fauna, play a pivotal role in enhancing soil health and fertility. Their activities influence various soil processes including organic matter decomposition, nutrient cycling, soil structure formation, and microbial interactions making them key bioindicators and functional agents in sustainable soil management (Bignell & Moreira, 2020).

These organisms contribute directly to soil fertility through the fragmentation and incorporation of plant residues into the soil, which accelerates microbial decomposition and nutrient mineralization (Brown *et al.*, 2021). Earthworms, for example, are often referred to as “ecosystem engineers” due to their ability to bioturbate the soil, enhance aggregation, and increase the availability of essential nutrients such as nitrogen, phosphorus, and potassium (Lavelle *et al.*, 2020).

In agro-ecosystems, macroinvertebrates help improve soil structure by creating networks of burrows that enhance porosity, aeration, and water infiltration, thereby promoting root growth and reducing the risk of erosion (Rossi *et al.*, 2022). These physical changes not only improve soil resilience to

compaction and drought but also enhance its capacity to store water and nutrients.

Moreover, the interactions between macroinvertebrates and soil microorganisms are crucial for soil biological functioning. Many macroinvertebrates create microhabitats that support microbial activity and diversity. For instance, dung beetles and termites facilitate nutrient recycling by redistributing organic matter across soil layers, while ants and millipedes influence microbial colonization through their nest-building and feeding activities (Sapkota *et al.*, 2021).

The loss or decline of soil macroinvertebrate diversity due to intensive land use, chemical inputs, and habitat degradation poses a significant threat to soil health. Recent studies have emphasized the need to conserve and manage these organisms to ensure ecosystem sustainability and productivity, particularly in tropical and subtropical agricultural systems (Nkongolo *et al.*, 2023; Fiera & Popescu, 2024).

Efforts to restore soil macroinvertebrate populations through conservation agriculture such as no-till practices, organic amendments, and agroforestry have been shown to enhance soil biological activity, leading to improved crop yields and long-term soil fertility (Carrijo *et al.*, 2020).

2.2 Sugarcane production

Sugarcane (*Saccharum officinarum L.*) is a major commercial crop cultivated in tropical and subtropical regions for sugar, bioethanol, and other industrial products. Globally, it contributes to more than 70% of sugar production and

plays a significant role in food security, employment, and renewable energy generation (Balsalobre *et al.*, 2020; Khan *et al.*, 2023).

In 2022, Uganda's sugar production was approximately 822,000 metric tonnes, comprising 720,000 metric tonnes of brown sugar and 102,000 metric tonnes of white industrial sugar. This output is modest compared to global figures; for instance, Brazil and India are leading producers (Mwanake *et al.*, 2023). Regarding soil degradation, Uganda faces significant challenges, with soil erosion and nutrient depletion impacting agricultural productivity. Studies have identified soil erosion as a critical issue affecting the country's croplands (Bamutaze *et al.*, 2021). Chemical-intensive farming contributes to soil degradation, reduced yields, and biodiversity loss. Herbicides, in particular, have decreased the abundance and diversity of soil invertebrates, including essential pollinators and decomposers (Pelosi *et al.*, 2021).

Recent advancements in agronomic practices, varietal improvement, and biotechnology have contributed to enhanced productivity and sustainability of sugarcane cultivation. Genetic improvements through conventional breeding and molecular techniques have led to the development of high-yielding, disease-resistant, and drought-tolerant varieties (Biswas *et al.*, 2021). Studies highlight the use of genomic selection and CRISPR-Cas technologies for improving traits such as sucrose content, ratoon performance, and pest resistance (Gouy *et al.*, 2022).

Soil health and nutrient management remain crucial in sugarcane production. Integrated nutrient management combining organic amendments and synthetic fertilizers has been shown to improve yield and soil fertility while reducing

environmental degradation (Tiwari *et al.*, 2020). Additionally, precision agriculture tools such as remote sensing and GIS mapping are being adopted to monitor crop health and optimize resource use (Sharma *et al.*, 2021).

Climate change is emerging as a major challenge in sugarcane production, especially due to its sensitivity to changes in temperature, rainfall variability, and water availability. Adaptive strategies such as the use of drought-resistant cultivars, efficient irrigation systems (like drip and furrow), and altered planting dates are increasingly being explored to mitigate climate impacts (Kandpal *et al.*, 2022).

Sustainable sugarcane production also includes a focus on ratooning abilitygrowing multiple harvests from a single planting which reduces input costs and land disturbance. However, ratoon productivity often declines due to pests, diseases, and nutrient depletion, calling for improved ratoon management practices (Patil *et al.*, 2023).

Moreover, sugarcane is a promising crop for bioenergy production, especially in the form of bioethanol. The use of sugarcane bagasse and molasses as by-products for cogeneration and distillation contributes significantly to renewable energy portfolios in producing countries like Brazil, India, and Thailand (Zhou *et al.*, 2020).

2.3 Agrochemical usage in crop production

Agrochemicals, including fertilizers, pesticides, herbicides, and fungicides, play a significant role in enhancing crop productivity and protecting plants from pests, diseases, and nutrient deficiencies. Their use has become integral to modern agriculture, particularly in intensive cropping systems where

maximizing yield is critical for food security (Jatav *et al.*, 2020; Adeola & Olorunfemi, 2022).

2.3.1 Role of Agrochemicals in Enhancing Crop Productivity

Agrochemicals have contributed significantly to the Green Revolution and remain essential in achieving high crop yields, especially in densely populated countries. Fertilizers provide essential macro- and micronutrients to crops, supporting plant growth and development. Pesticides and herbicides protect crops against a wide range of biotic stresses, including weeds, insects, and microbial pathogens (Yadav *et al.*, 2021). According to Singh *et al.* (2023), the timely and appropriate use of agrochemicals increases crop output by up to 40%, particularly in cereal and vegetable production.

2.3.2 Concerns over Excessive Use and Environmental Impact

Despite their benefits, the excessive or improper use of agrochemicals raises serious concerns about environmental degradation, human health, and biodiversity loss. Residues of pesticides have been found in soil, water, and food products, posing risks to both ecosystem and human health (Wang *et al.*, 2021). Agrochemical leaching into groundwater and runoff into aquatic ecosystems can lead to eutrophication and toxicity to aquatic organisms (Kumar *et al.*, 2022). Moreover, long-term use can lead to the development of pesticide resistance in pest populations, rendering some chemical controls ineffective (Baral *et al.*, 2023).

2.3.3 Adoption of Integrated Pest and Nutrient Management

In response to the negative impacts of overreliance on agrochemicals, the adoption of Integrated Pest Management (IPM) and Integrated Nutrient

Management (INM) has gained traction. These strategies combine chemical, biological, and cultural control measures to sustainably manage crop health. IPM, for example, minimizes pesticide use by incorporating resistant crop varieties, crop rotation, and biological control agents (Moussa *et al.*, 2020). INM enhances soil fertility while reducing dependence on synthetic fertilizers through the use of organic manure, compost, and biofertilizers (Sharma *et al.*, 2022).

2.3.4 Trends in Precision Agriculture and Smart Agrochemical Use

Technological innovations in precision agriculture are revolutionizing agrochemical application. Tools such as drones, sensors, and satellite imagery enable site-specific application of agrochemicals, reducing waste and minimizing environmental impact (Chandio *et al.*, 2020). Smart agrochemical delivery systems, such as nanoformulations, are also being explored for more efficient and targeted application (Bhardwaj *et al.*, 2021).

2.3.5 Policy and Regulatory Perspectives

Governments and international organizations have introduced stricter regulations to promote safer agrochemical use and reduce environmental harm. For instance, the European Union has pushed for a 50% reduction in chemical pesticide use by 2030 under its Farm to Fork strategy (European Commission, 2021). Many developing countries are also implementing farmer training programs and promoting organic farming to reduce agrochemical dependency (FAO, 2022).

Uganda has several policies regulating agrochemical use, but none are specific to sugar production. The Sugar Act (2020) governs production and marketing

but does not address agrochemical use directly. The Agricultural Chemicals (Control) Act and the National Environment Act (2019) regulate the importation and environmental impact of agrochemicals broadly, including those used in sugarcane farming. Uganda also promotes safer practices through the Organic Agriculture Policy (2019) and the Agricultural Extension Policy (2016), which encourage reduced chemical use and farmer training. International frameworks like the Rotterdam Convention and the FAO/WHO Code of Conduct on Pesticide Management guide safer agrochemical use, while the Sustainable Development Goals influence environmental protection efforts. However, herbicides like 2,4-D, commonly used in sugarcane, are regulated only under general laws. There is a clear gap in sugar-specific agrochemical policy, highlighting the need for integrated pest management and stricter sector-focused regulation to ensure sustainable sugar production.

2.4 Sugarcane production and soil macro invertebrates

Sugarcane (*Saccharum spp.*) cultivation significantly influences soil macroinvertebrate communities, which are vital for maintaining soil health and fertility. These organisms, including earthworms, ants, beetles, and millipedes, play crucial roles in organic matter decomposition, nutrient cycling, and soil structure enhancement.

2.4.1 Impact of Sugarcane Cultivation on Soil Macroinvertebrates

Research indicates that converting natural vegetation to sugarcane plantations can reduce the abundance and diversity of soil macroinvertebrates. A study in Brazil found that land use changes for sugarcane cultivation significantly decreased the richness and abundance of edaphic mesofauna, particularly in

clay soils (Chi *et al.*, 2020). Similarly, in Belize, sugarcane fields exhibited lower macroinvertebrate diversity compared to secondary forests, with notable reductions in earthworm species and other decomposers (Vanolli *et al.*, 2024).

2.4.2 Fertilization Practices and Macroinvertebrate Communities

The type of fertilization used in sugarcane farming plays a crucial role in shaping soil macroinvertebrate populations, which are important indicators of soil health and ecosystem function. In a study conducted in Belize, researchers compared the effects of organic amendments such as vinasse and filter mud with those of chemical fertilizers on soil macroinvertebrate abundance. The findings revealed that the use of organic inputs did not significantly increase macroinvertebrate populations compared to chemical fertilization (Vanolli *et al.*, 2024). This suggests that while organic matter inputs are often promoted for their ecological benefits, their impact may vary depending on other environmental factors. Notably, the study found that soil type and seasonal variations such as changes in moisture and temperature had a more substantial influence on macroinvertebrate communities than the type of fertilizer applied. These results highlight the complexity of soil ecosystems and the need to consider multiple environmental variables when assessing the ecological impacts of agricultural practices.

2.4.3 Ecological Implications

Soil macroinvertebrates are indicators of soil quality and ecosystem health. Their decline in sugarcane plantations suggests potential degradation of soil functions, which can impact crop productivity and environmental sustainability. Maintaining diverse and abundant macroinvertebrate communities is essential for the resilience of agricultural systems.

2.5 Effects of 2,4-D herbicide and NPK fertilizer application on soil physicochemical properties in sugarcane plantations

The application of agrochemicals such as 2,4-D herbicide and NPK fertilizer in sugarcane (*Saccharum officinarum L.*) plantations plays a vital role in crop productivity, but it also significantly influences the physicochemical properties of soil. Understanding these effects is crucial for maintaining sustainable soil health and ensuring long-term agricultural productivity.

2,4-Dichlorophenoxyacetic acid (2,4-D) is a systemic herbicide commonly used in sugarcane production to manage broadleaf weeds. Although it is effective in weed control, 2,4-D can persist in the soil and influence various physicochemical characteristics. Research has shown that the presence of 2,4-D can alter soil pH and reduce soil enzyme activities, which are essential for organic matter decomposition and nutrient cycling (de Souza *et al.*, 2022). Moreover, the herbicide may negatively impact microbial diversity and biomass, potentially disrupting soil ecological functions (Silva *et al.*, 2023). These effects highlight the need for careful monitoring of herbicide use in sugarcane plantations.

NPK fertilizer, which supplies essential macronutrients nitrogen (N), phosphorus (P), and potassium (K) is widely applied to boost sugarcane growth and yield. However, its repeated and excessive use can result in significant changes in soil chemistry. For instance, nitrogen-containing fertilizers, particularly those based on ammonium, tend to lower soil pH, causing acidification over time and thereby affecting nutrient availability and microbial processes (Kumar *et al.*, 2021). Additionally, continuous reliance on

synthetic fertilizers may reduce soil organic matter, which is crucial for soil structure, moisture retention, and fertility (Sharma & Verma, 2020). In some cases, imbalanced fertilizer application can lead to nutrient antagonism, where an excess of one nutrient inhibits the uptake of others, resulting in deficiencies (Singh *et al.*, 2022).

The combined use of 2,4-D herbicide and NPK fertilizer in sugarcane fields may have synergistic or antagonistic effects on soil physicochemical properties. For example, the acidifying effect of NPK fertilizers may slow down or speed up the degradation of 2,4-D in the soil, influencing its persistence and toxicity (Patel *et al.*, 2021). Furthermore, the disruption of microbial communities caused by 2,4-D can hinder nutrient cycling, potentially reducing the effectiveness of applied fertilizers (Gupta & Rao, 2023). These interactions underscore the importance of integrated management strategies that consider the cumulative effects of agrochemicals.

To minimize the adverse effects of agrochemical use on soil health, sustainable practices are essential. Integrated nutrient management (INM), which combines the use of organic and inorganic fertilizers, has been shown to enhance soil fertility while maintaining soil structure and microbial health (Meena *et al.*, 2020). Similarly, rotating herbicides with different modes of action and incorporating non-chemical weed control methods can reduce the ecological burden of 2,4-D (Thakur *et al.*, 2022). Regular soil testing and monitoring are also critical, as they allow for the adjustment of fertilizer and herbicide applications based on soil conditions and crop requirements (Joshi & Sharma, 2021).

2.6 Effects of 2,4-D herbicide and NPK fertilizer application on the abundance and diversity of soil macro invertebrates in sugarcane plantations

The application of 2,4-D herbicide and NPK fertilizers in sugarcane plantations is a common agricultural practice aimed at controlling weeds and enhancing crop productivity. However, these agrochemicals can significantly affect soil biota, particularly the abundance and diversity of soil macroinvertebrates. Macroinvertebrates, including earthworms, beetles, ants, and other organisms, play crucial roles in soil health by influencing soil structure, nutrient cycling, and organic matter decomposition. The effects of 2,4-D herbicide and NPK fertilizers on these soil organisms are multifaceted and depend on factors such as the chemical composition, application rates, and duration of exposure.

2,4-D is a selective herbicide widely used in sugarcane cultivation to control broadleaf weeds. Despite its efficacy in weed management, 2,4-D can have detrimental effects on soil macroinvertebrate populations. Several studies have reported a decline in the abundance and diversity of soil invertebrates in soils treated with 2,4-D. For example, Ferreira *et al.* (2021) found that the application of 2,4-D at recommended doses reduced the abundance of earthworms and other soil macroinvertebrates due to its toxicity, which disrupted their respiration and feeding behaviors. Similarly, Souza *et al.* (2022) highlighted that the herbicide's persistence in soil could result in long-term suppression of macroinvertebrate populations, particularly affecting species sensitive to chemical pollutants.

The mechanism behind this reduction in abundance and diversity can be attributed to the toxic nature of 2,4-D, which interferes with the physiological functions of soil organisms. According to Santos *et al.* (2023), 2,4-D can alter microbial communities in the soil, indirectly affecting macroinvertebrates by disrupting the decomposition of organic matter, which is a primary food source for many soil-dwelling organisms. The herbicide can also decrease the richness of macroinvertebrate communities by favoring pesticide-tolerant species over others, thereby reducing overall biodiversity.

NPK fertilizers, which provide essential nutrients for plant growth, can also significantly impact soil macroinvertebrate communities. The application of these fertilizers can alter soil pH, moisture, and nutrient availability, influencing the habitat suitability for macroinvertebrates. Research by Silva *et al.* (2020) showed that the excessive use of NPK fertilizers, especially nitrogen-rich fertilizers, can lead to soil acidification, which in turn affects the abundance and diversity of earthworms and other macroinvertebrates. The increased nitrogen levels can disrupt the microbial balance in the soil, affecting nutrient cycling processes and reducing the food availability for macroinvertebrates (Kumar & Kumar, 2021).

Moreover, studies have indicated that imbalanced fertilizer applications—where nitrogen exceeds phosphorus or potassium—can negatively affect soil macroinvertebrates. For instance, Hossain *et al.* (2022) observed that excessive nitrogen levels in fertilized soils led to a decrease in the diversity of soil macroinvertebrates due to changes in soil organic matter decomposition rates. These findings suggest that the imbalanced use of NPK fertilizers can

harm not only the diversity but also the ecological functions of soil invertebrate communities.

The combined application of 2,4-D herbicide and NPK fertilizers may have synergistic or antagonistic effects on soil macroinvertebrates. The interaction between herbicide and fertilizer could exacerbate the negative impacts on soil organisms. A study by Patel *et al.* (2023) found that the simultaneous use of 2,4-D and NPK fertilizers in sugarcane fields resulted in a significant reduction in the population of macroinvertebrates, particularly those that rely on organic matter for nutrition, such as earthworms and beetles. The herbicide's toxicity, compounded by the altered soil conditions from fertilizer application, created an unfavorable environment for soil invertebrates, leading to reduced biodiversity and ecosystem function.

However, other studies have suggested that moderate levels of fertilization may mitigate some of the negative effects of herbicides. For example, Sharma and Joshi (2021) reported that when NPK fertilizers were applied at appropriate rates, they could help maintain the abundance of certain macroinvertebrate species that are less sensitive to herbicide toxicity. This interaction suggests that integrated pest and nutrient management strategies, including balanced fertilization and selective herbicide use, may help minimize adverse effects on soil macroinvertebrates.

2.7 Effects of 2,4-D herbicide and NPK fertilizer application on the morphological features of soil macro invertebrates in sugarcane plantations

The use of 2,4-D herbicide and NPK fertilizers in sugarcane plantations is widespread due to their effectiveness in controlling weeds and enhancing crop productivity. However, these agrochemicals can have significant effects on the morphological features of soil macroinvertebrates, which are vital components of soil ecosystems. Macroinvertebrates, including earthworms, beetles, ants, and other organisms, contribute to soil structure, nutrient cycling, and organic matter decomposition. Changes in the morphological features of these organisms as a result of agrochemical exposure can indicate shifts in their ability to perform these ecological functions, ultimately influencing soil health and fertility.

2,4-D is a widely used systemic herbicide in sugarcane fields for controlling broadleaf weeds. While 2,4-D targets plant growth, it can have unintended side effects on soil macroinvertebrates, particularly in terms of their morphological features. These effects are often a result of the herbicide's toxicity, which interferes with the normal physiological processes of soil organisms.

Studies have shown that exposure to 2,4-D herbicide can lead to alterations in the body size, shape, and behavior of soil macroinvertebrates. For instance, research by Ferreira *et al.* (2021) demonstrated that earthworms exposed to 2,4-D exhibited a reduction in body length and weight compared to non-exposed individuals. The herbicide's toxic effects may lead to morphological

changes in earthworm segments, such as shortening or deformations, which can impair their ability to burrow and perform soil aeration (Souza *et al.*, 2022). In addition, certain beetles and ants showed a decrease in body size and appendage length, potentially limiting their mobility and feeding efficiency (Santos *et al.*, 2023). These morphological alterations are often associated with the disruption of vital processes like nutrient cycling, which can negatively impact soil health.

The morphological changes observed in these organisms are indicative of a compromised physiological state, with reduced survival and reproductive success. According to Silva *et al.* (2020), herbicide-induced morphological changes in soil macroinvertebrates can lead to diminished community resilience, thus affecting overall soil biodiversity and function.

NPK fertilizers are essential for promoting plant growth, but their application in sugarcane plantations can also affect the morphology of soil macroinvertebrates. The high nutrient concentrations, particularly nitrogen, can alter the soil environment, affecting the organisms living within it. Excessive nitrogen or phosphorus in fertilized soils can lead to changes in the body size, structure, and overall health of soil macroinvertebrates.

Studies have indicated that NPK fertilizers can cause changes in the morphological features of earthworms, beetles, and other invertebrates by altering soil pH, organic matter content, and moisture levels. Kumar and Kumar (2021) found that earthworms exposed to soils treated with high levels of nitrogen-rich NPK fertilizers showed reduced body length and increased mortality rates. The elevated nutrient levels can induce stress in soil

organisms, leading to changes in body mass, reproductive organs, and appendage development. For example, studies by Hossain *et al.* (2022) noted that excessive fertilization could cause earthworms to exhibit stunted growth and reduced fertility, likely due to imbalances in soil pH and microbial activity that affect their food sources.

In addition to direct morphological changes, high fertilizer concentrations can affect the overall physiology of macroinvertebrates, reducing their ability to adapt to environmental stresses and survive. Silva *et al.*, (2020) found that the combined effect of high phosphorus and potassium levels in soil reduced the mobility and feeding behaviour of soil macroinvertebrates, leading to a decrease in their size and number. This effect could be linked to changes in the quality and quantity of organic matter, which serves as food for these organisms.

The combined use of 2,4-D herbicide and NPK fertilizers in sugarcane plantations can have compounded effects on the morphological features of soil macroinvertebrates. When both agrochemicals are applied together, they may exacerbate the negative impacts on the organisms' physiology and morphology. Research by Patel *et al.*, (2023) demonstrated that earthworms and beetles exposed to both 2,4-D and NPK fertilizers exhibited severe reductions in body length, weight, and overall body condition. The synergistic effects of herbicides and fertilizers can create an environment where the organisms' metabolic and physiological functions are stressed, leading to more pronounced morphological changes.

Furthermore, the interaction between these chemicals can alter the soil's microhabitat, affecting food availability and habitat suitability for soil invertebrates. According to Sharma and Joshi (2021), the presence of both 2,4-D and fertilizers can alter the chemical composition of the soil, affecting the organic matter that these organisms rely on for nutrition. As a result, macroinvertebrates may experience altered development, with noticeable reductions in body size and reproductive potential. This is particularly concerning because reduced organismal size can lead to diminished soil ecosystem functions, such as nutrient cycling and organic matter decomposition, which are critical for maintaining soil health.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

3.1.1 Location

The study was conducted in the western Uganda in the district of Masindi, at Bulima Town council (Fig 3.1), in the Marongo cell with GPS coordinates 31°44'E and 01°41'N where the experiment was set up. It is situated 216 kilometers from Kampala in the western region of Uganda. The following are its borders: Kyakwanzi in the south, Hoima in the south-west, Buliisa in the west, Nakasongola in the south-east, Nakaseke in the south-east, Nwoya in the north, and Kiryandongo in the north-east. Situated between longitudes 310 22' and 320 23' East of Greenwich, and between latitudes 10 22' and 20 20' North of the Equator, the district has an average elevation of 1,295 meters above sea level.

3.1.2 History

Historically Masindi gained a district status from Bunyoro District Administration in 1976 and it's one from which both Kiryandongo and Buliisa districts were curved off. 9,326 square kilometers (3,601 sq mi) make up the District's total area; 8,087 square kilometers (3,122 sq mi) or 86.7% of it island, 2,843 sq mi, or 1,098 sq km (30.5%) There is a national forest reserve covering 1,031 square kilometers (398 sq mi) (11.1%), a national wild reserve area, and open water covering 799.6 square kilometers (308.7 sq mi).

3.1.3 Climate

The district's annual temperature is 0.62% higher than Uganda's average at 24.09°C (75.36°F). Three main climate zones high rainfall (>1000mm),

medium rainfall (800–1000mm), and low rainfall (<800mm) define the district. The district receives 1,304 mm of rain on average per year. However, during the dry season, Kimengo and Masindi port sub-counties have significant water shortages. The soils and environment, which average 250 degrees Celsius annually, are ideal for agriculture. Masindi experiences 282.63 wet days (77.43% of the time) and 264.04 millimeters (10.4 inches) of precipitation on average each year.

3.1.4 Geology and Soils

The district of Masindi has a diverse range of soil types. Among them are vertisols, which are made up of kaiso beds that are periodically laterized, black clays, and sandy alluvial. the mostly freely-drained ferruginous tropical soils and lissols, which are horizonless soils typically composed of young, naked rocks or stones. Ferrallitic soils are also widespread and indicate a nearly complete stage of weathering where little to no mineral reserve remains. Alluvial and lacustrine sands as well as alluvial clays comprise the other type. There are 17 soil mapping units that can be used to categorize the district's soils. Over 70% of the soil units in the Masindi district are mostly composed of pre-Cambrian rocks, the parent rocks of which are restricted to the Cenozoic and alluvium rock types.

3.1.5 Economic activity

Masindi is mostly an agricultural district that depends heavily on the production of food and cattle. Farming is the main source of income for the entire population. Cassava, bananas, sweet potatoes, beans, and maize are among the food crops produced in Masindi. The main cash crops farmed there are coffee, cocoa, and important note. The majority of the District's land area

has been occupied by sugar cane production, which has become a significant economic activity and impacted food production.

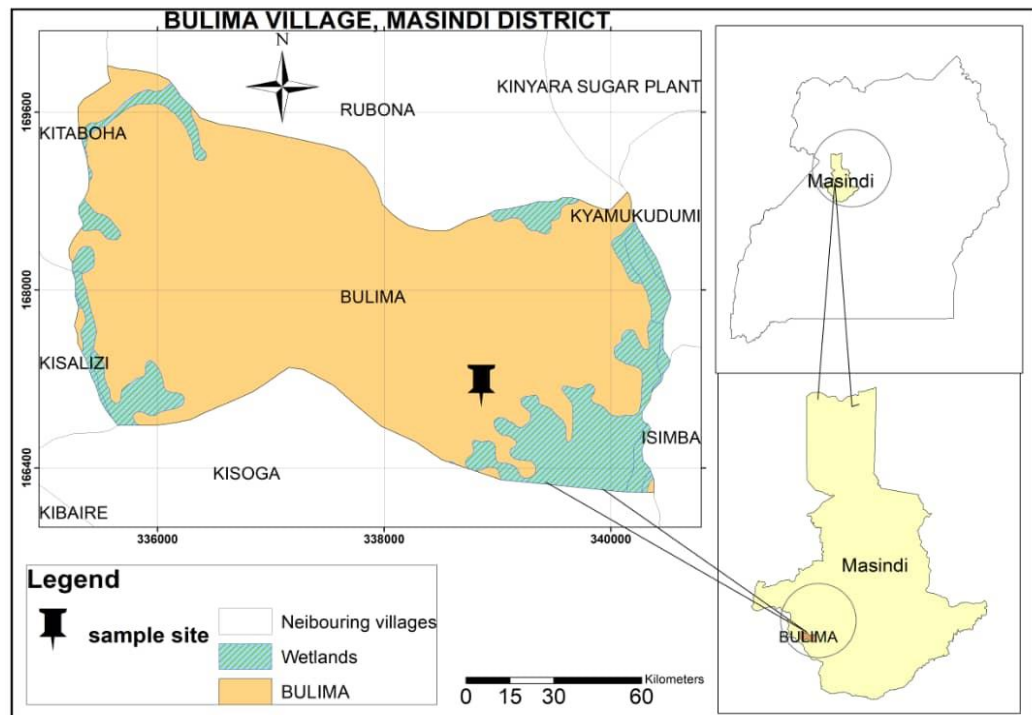


Figure 3.1: Map of Bulima showing the study site

3.2 Research design

The study took eight (8) weeks and this was followed by an experimental design on fresh Land which has not been in use for the last five (5) years. Soil preparation was done by removing vegetative material and ploughing using (18, 34" discs) and then harrowing and furrowing was done manually using hand hoe to make the garden ready for bed formation.

3.2.1 Experimental Design

Sugar cane was planted in 4 plots per block with 4 treatments that were replicated 3 times giving a total of 12 experimental units (EU). Each Block were divided into 4 replication plots which were subjected for treatments with 2,4-D (2,4 Dichlorophenoxyacetic acid) because it's mostly used herbicide for

control of broadleaf weed in sugar cane fields and NPK fertilizer since its applied in sugar after planting to increase growth of sugarcanes. One plot was left untreated to act as control for the experiments. Sugarcane was planted manually at 40cm depth with 4 rows in each plot at a spacing of 1.5m and the plot size was 5m×10m (50m²) drawn from the total land area of (600m²) which was used for the entire study.

Plots were subjected to different treatments, one plot in each block was left untreated to serve as control. NPK fertilizer with composition of nitrogen (N) 3%, Phosphorus (P) 5% and Potassium (K) 5% of quantity 5kg was applied by broadcasting method in each replicate plot (Figure 3.2).

Table 3.1: Randomized experimental design

Block I		Block II		Block III	
Plot	T	Plot	t	Plot	t
1	FTZ	5	Control	9	FTZ
2	HCD	6	HCDFTZ	10	Control
3	HCDFTZ	7	FTZ	11	HCD
4	Control	8	HCD	12	HCDFTZ

FTZ: fertilizer, **HCD:** herbicides, **HCDFTZ:** herbicides plus fertilizer

3.2.2 Experimental layout

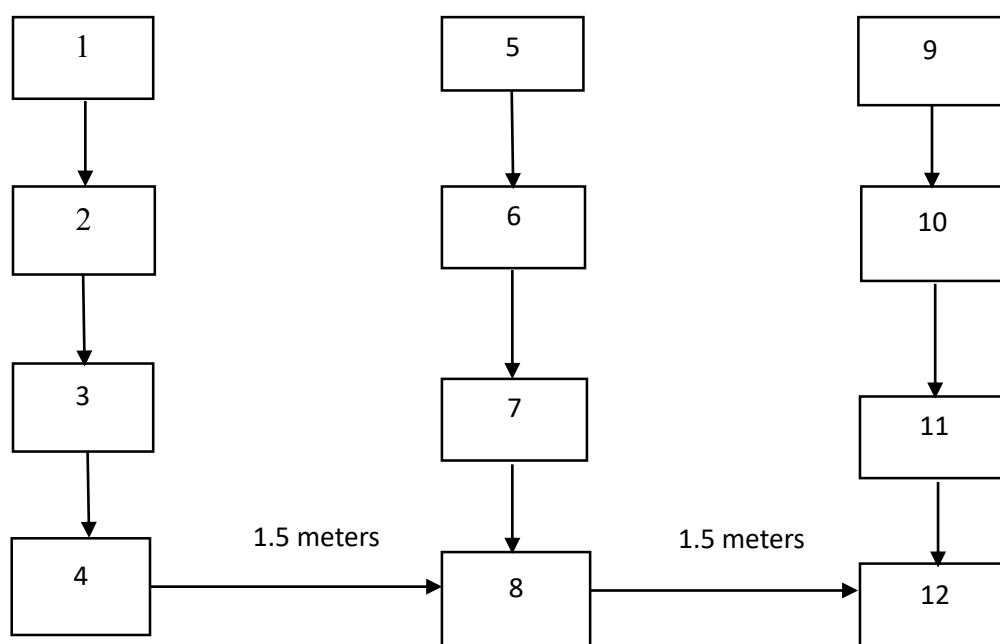


Figure 3.2: Experimental layout

3.3 Sampling and sample size

Soil Sampling was done according to the Soil Biology and Fertility (TSBF) method (Nezomba *et al.*, (2015) were a monolith of 25 x 25 x 30 cm was dug into the soil at each sampling point along the line transect and soil samples collected at depth of 30cm. A total of 192 samples for the abundance and diversity and 48 samples for soil physical and chemical properties. Three samples were drawn from each line transect, mixed up together in one bucket and a sub sample of quantity 500g of soil was collected per plot packed in plastic zipper bags and transported to laboratory for analysis (James and Wells 2010).

3.3.1 Sampling interval

Soil samples were collected from the plots by a core sampler method to analyze soil properties and evaluate presence soil dwelling macro invertebrates. Soil physical and chemical properties, soil dwelling macro

invertebrates of the research plots at soil depth 30 cm was assessed. Each plot size was 12.5m² and was separated from each other by 1m space within the field. NPK fertilizer and 2,4-D Amine was applied 30 days after planting of sugarcanes. NPK was broadcasted along the lines of the sugarcanes as top dress and this was allowed to dissolve into the soils. Recommended dose of 2,4-D Amine herbicide was mixed in 20litre spray pump and sprayed to plots as guided in the experimental design. Weather conditions such as rain fall and wind was monitored (Table 3.2).

Due to the nature of the herbicides which was used in this study, they tend to persist or be active after application and lose concentration with time up a period of 6 month.

Table 3.2: Soil sampling intervals for the study

Sampling phase	Sampling interval
Phase 1	Sampling was done in all the three blocks before any treatment to obtain baseline data.
Phase 2	Sampling was done two weeks after treatments of the plots
Phase 3	Sampling was done four weeks after initial treatments of the plots
Phase 4	Sampling was done six weeks after initial treatments of the plots
Phase 5	Sampling was done eight weeks after initial treatments of the plots

The samples were drawn in four phases in two months between the dry and wet season months of February, March, and April since some macro-invertebrates such as earthworms tend to be dormant in dry seasons and high in rainy seasons (Huert *et al.*, 2008). The researcher also carried out laboratory

assessments for the samples collected from the field. The concentrations were measured based on the recommended dose from the manufacturer's instructions for application in sugarcane plantations.

Tools: Invertebrate Chart (compare invertebrates discovered with the standard), Push probes, hammer probes, and bucket augers, electronic weighing scale, sampling bags, and Plastic buckets

3.4 Soil physical and chemical properties in sugarcane plantations

3.4.1 Determination of soil pH

Using a pH meter, the pH of the soil was ascertained. A sample of soil was taken and allowed to air dry. A 2 mm sieve was used to filter the material. A part of the soil was combined in a ratio of 1:1 or 1:2.5 with distilled water. After giving the mixture a good shake, it was allowed to settle for half an hour. Using pH indicator strips or a calibrated pH meter, the pH of the soil was determined.

3.4.2 Determination of soil moisture content

The Gravimetric Method was used to calculate the moisture content of the soil. A sample of soil was taken and wet weighted. The soil sample was dried for twenty-four hours at 105°C in an oven. Weighing the dried soil sample yielded the dry weight. Moisture content was calculated using the formula:

$$\text{moisture content (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100$$

3.4.3 Determination of soil organic matter

The amount of organic matter in the soil was determined using the Loss on Ignition (LOI) method as described by Gerenfes et al., (2022) as follows. After

the soil sample was dried at 105°C, the moisture was removed. The dry sample was weighed. The sample spent two to four hours at 360 to 400 degrees Celsius in a muffle furnace. After cooling in a desiccator, the sample was weighed once again. The weight decrease was indicative of the amount of organic materials.

3.4.4 Determination of soil total porosity

The bulk density and particle density were used to calculate the overall porosity of the soil (Lavelle *et al.*, 2022). The bulk density (BD) of the soil was first determined using the formula:

$$\text{Bulk density } \left(\frac{\text{g}}{\text{cm}^3} \right) = \frac{\text{dry soil mass}}{\text{soil volume}}$$

The particle density (PD) was measured. The total porosity was calculated using the formula:

$$\text{total porosity (\%)} = \left(1 - \frac{BD}{PD} \right) \times 100$$

3.4.5 Determination of soil texture

According to Colby and Crouse (2017), the hydrometer method was used to determine the soil texture as follows:

Sifted soil (50g) of known weight was added to a 250 ml Erlenmeyer flask. Using a wash bottle and distilled water, the flask's sides were cleaned. The graduated cylinder holding the sample was filled with 100 ml of distilled water and 10 ml of sodium hexametaphosphate solution (500 g/l) from a dispersion bottle. Stirring was done to the mixture. Once more, distilled water was used to wash the flask's sides. After that, a para-film was applied to the flask and it was labelled.

The sample was chemically dispersed for 24 hours, and then it was put into a metal dispersion cup. Distilled water was then added to half of the cup, and the mixture was stirred for 5 minutes. The sample was then put into a sedimentation cylinder that held one litre. The entire capacity of the cylinder was filled. Using the plunger method, all of the soil samples were suspended in the sedimentation cylinder. Time was noted each time the stirring was stopped and the plunger was taken out. The hydrometer was cautiously and slowly introduced. After 40 seconds, the first hydrometer reading (g/l) was obtained. After seven hours, another hydrometer value (g/l) was obtained.

Calculations of the different percentages of clay, silt and sand were obtained as follows;

The 7 hour reading indicated the amount of clay in the soil;

$$\text{Percent Clay} = \frac{\text{Corrected 7hr reading}}{\text{Mass of dry sample}} \times 100\%$$

The 40-second reading was used to calculate the amount of silt and clay;

$$\text{Percent silt + clay} = \frac{\text{Corrected 40S reading}}{\text{Mass of dry sample}} \times 100\%$$

The percentages of silt and sand were determined as follows;

$$\text{Percent silt} = (\text{Percent silt + Percent clay}) - \text{Percent clay}$$

$$\text{Percent sand} = 100 - (\text{Percent silt} + \text{Percent clay})$$

Once soil fractionation was complete, a soil textural triangle was used to determine the soil texture (Hunt and Gilkes, 1992).

3.4.6 Determination of bulk density

Soil bulk density was determined using the core method (FAO, 2020) as follows:

A known-volume metal core was employed. Without changing the core's contents, it was shoved into the ground to the appropriate depth and then carefully withdrawn. Extra soil was scraped off the ring with a knife with a flat blade. After carefully removing the soil sample from a metal ring and placing it on sealable paper, the samples were labelled appropriately. Weighing was done on the field moist sample and its bag. The sample was not included in the weight of the sampling bag. The wet soil sample's real weight was determined by deducting the sampling bag's weight from the total weight of the wet soil sample and the bag. The soil's wet weight (Wt) was the cause of this variation.

The paper bags containing the soil samples were put in the oven dryer. At 105°C, the samples were dried for roughly 24 hours. The dry sample (soil with sampling bag) was weighed after it had dried. The weight of the sampling bag was deducted from the combined weight of the dried soil sample and sampling bag in order to get the dry sample's true weight. The soil's dry weight (Wd) made a difference in weight. To compute bulk density, the following formula was used.

$$\text{Bulk density (g/cm}^3\text{)} = \frac{W_d}{V}$$

Where; W_d = Weight of oven dry soil (g) and V = Volume of soil (cm³)

3.4.7 Determination of soil colour

Munsell soil colour chart was used to determine the colour of soil mixed with 2.5g and 5g of wood ash and soil sample without wood ash (control) respectively. The following procedures as described by Nodi et al., (2023) were used;

Twelve soil samples were collected from different experimental sites of the plots using soil auger, the samples were put in a plastic bag and mixed to form a composite. This was taken for laboratory analysis at Kyambogo University Agriculture department. Using the HM-519 Munsell soil colour book, the composite samples were compared.

The tabbed chart is a standard tool, it includes a two –page grey chart for submerged soil covers weak Chroma’s and neutrals of blue and green hues with different colour ranges as; 10R, 7.5R, 5R, 2.5R, 5YR, 7.5YR, 10YR, 2.5Y, 5Y, and 10Y-5GY. The white page was used to describe carbonates, silica, soluble salt participates and others. The Munsell colour chart- Indicating the Value (Figure 3.3).

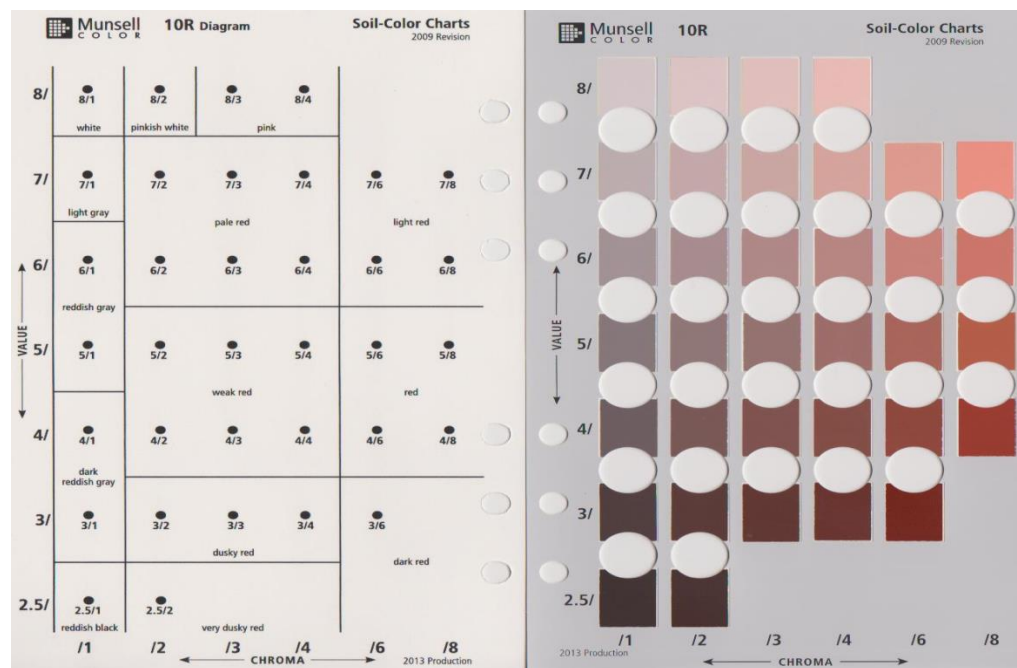


Figure 3.3: Munsell colour chart indicating the value Hue and Chroma
Source: Munsell colour (2013)

In this chart, the value is indicated with a number such as 2,4,6 and others, these value scale runs vertically and moves from lightest (at the top) to darkest (at the bottom) in the descending order. Thus a 2 is darker than a 6.

Hue is the colour such as red, green, blue and others, this can be coded as Red (R), Blue (B), and green (G) and so on. Chroma therefore, is how weak or strong a colour. Thus, the major colour attributes are; chroma, value and hue. Each colour is designated by the colour notation such as; 5R 7/2, where 5R is the Hue (colour), 7 is the value (lightness/darkness) and 2 is the Chroma (weak/strong) and all was compared on the HM-519 Munsell soil colour book (Odeh and Mcbratney, 2005).

3.4.8 Abundance and diversity of Soil-dwelling macroinvertebrate communities in agrochemical-treated sugarcane plots and untreated sugar cane plot

The study assessed the abundance and diversity of three (3) soil macro invertebrates' size >2mm<15mm of earthworms (*Lumbricidae*), Millipedes (*Diplopoda*), and Termites (Isoptera). Hand sorting of soil dwelling macro invertebrates from each soil samples was done, counted and classified or grouped according to their phylum, order and species (Anderson & Ingram, 2013).

Soil dwelling macro-invertebrates retained on sieves after previously hand-sorted soils was counted and the soils were washed carefully through sieves to recover any the residual soil macro invertebrates (Lavelle & Spain, 2011). The separation technique described in the ISO/TSBF method was applied to increase accuracy and consistence for soil macro invertebrates extraction

efficiencies because the efficiency of the hand-sorting technique varied greatly with organism size, color, habitat, mobility and among operators of the study (Edwards & Bohlen, 2016).

3.4.9 Morphological features of soil dwelling macro invertebrates upon exposure to selected agrochemicals

After hand sorting of soil dwelling macro invertebrates recovered from 25cm x 25cm and down to depth of 30cm (ISO, 2011; Velasquez, 2020), their morphological characteristics such as colour, body segmentation, legs, antennae, exoskeleton, eyes, and movement were assessed as described by Zhang et al., (2023) as follows: and recorded. Sampling ensured three species of taxonomic groups were examined, reflecting their ecological characteristics and function within the habitat.

All soil macro invertebrates collected were assessed visually using a hand lense and then arrayed on a clear white paper for proper visibility and then assessed. Scores using a score sheet that is based on the standard attributes of the soil macro invertebrate. This was carried out to form a baseline data set of what is present in the site, which could be used to provide a picture of soil macro invertebrate populations before and after a period of development or treatment of the blocks, giving a measure of effects of 2,4-D herbicide and NPK fertilizer on soil macro invertebrates in the area (ISO, 2011; Velasquez, 2020).

3.5 Data Analysis

3.5.1 Effects of 2,4-D herbicide and NPK fertilizer application on soil physicochemical properties

IBM SPSS version 26 was used for analysis of data. To analyze the effects of 2,4-D herbicide and NPK fertilizer application on soil physicochemical properties, a combination of descriptive and inferential statistical tests was done. Descriptive statistics such as mean, standard deviation, and range were summarized for each soil property. Before proceeding with inferential tests, normality of the data was assessed using the Shapiro-Wilk test to determine the appropriate statistical approach. Since the data met parametric assumptions, a one-way ANOVA was employed to compare soil properties across treatment groups Control, NPK, 2,4-D, and the combination of NPK and 2,4-D.

3.5.2 Effect of 2,4-D herbicide and NPK fertilizer on the abundance and diversity of soil macro invertebrates

IBM SPSS version 26 was used for analysis of data. To analyse the effect of 2,4-D herbicide and NPK fertilizer on the abundance of soil macroinvertebrates, descriptive statistics such as the mean and standard deviation were calculated for each treatment group to summarize the distribution of macroinvertebrate counts. Inferential statistical analysis was then conducted using Analysis of Variance (ANOVA) to determine if there were significant differences in abundance among the different treatment groups. Post hoc test such as Tukey's Honest Significant Difference (HSD) test for ANOVA were used to identify specific differences between treatment pairs.

For diversity analysis, biodiversity indices were calculated to assess species richness and distribution. Using the Simpson's Diversity Index (D) for dominance.

3.5.3 Effects of 2,4-D herbicide and NPK fertilizer application on the morphological features of soil macro invertebrates

IBM SPSS version 26 was used for analysis of data. Descriptive statistics were used to determine the proportion of individuals exhibiting morphological deformities within each treatment group, providing an overview of the distribution of abnormalities. To compare mean differences in body size and deformity scores across treatments, Analysis of Variance (ANOVA) was applied where data meet parametric assumptions. Chi-square tests were conducted to assess whether there are significant differences in the frequency of deformities among the various treatment groups. Additionally, correlation analysis using either Pearson's correlation coefficients was performed to explore potential relationships between levels of chemical exposure and observed morphological changes in the macro invertebrates.

3.6 Research approval

The researcher obtained approval letters from the Directorate of Research and Graduate Training of Kyambogo University. Consent was sought from Masindi District local government. Furthermore, the data collected was validated using the ISO/TSBF method Macro-fauna database because all the data collected from sites sampled using the ISO/TSBF method was stored in the Macro-fauna database. The ISO/TSBF method has been applied independently in a large number of different projects with different purposes

since the earliest publication of the method, in 1989, and their data was accessed to validate the findings in this study.

CHAPTER FOUR: RESULTS

4.0 Introduction

This chapter presents the findings of the study with respect to the objectives

4.1 Effect of 2,4-D and NPK application on soil physical and chemical properties in sugar cane plantation

4.1.1 Diagnostic tests

4.1.1.1 Normality Testing

To determine whether the data for each soil parameter (pH, organic matter, moisture content, bulk density, and total porosity) were normally distributed across treatment groups, the Shapiro-Wilk test was employed due to its robustness for small sample sizes (Table 4.1).

Table 4.1: Normality Testing

Parameter	Shapiro-Wilk Test	p-value	Interpretation
pH	0.973	0.785	Normally distributed
Organic Matter (%)	0.961	0.691	Normally distributed
Moisture Content (%)	0.947	0.545	Normally distributed
Bulk Density	0.978	0.853	Normally distributed
Total Porosity (%)	0.951	0.581	Normally distributed

$P > 0.05$: Data was normally distributed

4.1.1.2 Homogeneity of Variance

The Levene's Test was conducted to assess the assumption of equal variances across treatments for each parameter. The assumption of homogeneity of variance (HoV) was confirmed throughout. This shows strong evidence that

the risk of Type I and Type II errors were minimized in the analyses (Table 4.2).

Table 4.2: Homogeneity of Variance test

Parameter	Levene's Statistic	p-value	Interpretation
pH	1.284	0.320	HoV confirmed
Organic Matter (%)	2.133	0.126	HoV confirmed
Moisture Content (%)	0.894	0.471	HoV confirmed
Bulk Density	0.432	0.733	HoV confirmed
Total Porosity (%)	0.761	0.528	HoV confirmed

$P > 0.05$: Homogeneity of variance confirmed

4.1.2 Soil physical and chemical properties after application of treatments

Generally, the soil physical and chemical properties of the soil varied with the treatments. The highest soil pH was recorded from plots treated with 2,4-D (8.35 ± 0.18) while the lowest soil pH was in control plots (7.45 ± 0.20). The study findings also showed that there was a significant difference between soil pH with the treatment types (LSD = 0.475; $P < 0.05$). The highest amount of organic matter was observed in plots with control treatments (22.15 ± 1.96) while the lowest amount of organic matter was in plots treated with NPK fertilizer alone (15.27 ± 1.24). There was no statistically significant difference between organic matter and the treatment types (LSD = 5.160; $P > 0.05$) (Table 4.3 – 4.4).

The highest moisture content was recorded from plots treated with NPK application (30.80 ± 2.31) while the lowest moisture content was obtained from plots treated with control (29.00 ± 2.28). There was no statistically significant difference between moisture content with the treatment types (LSD = 7.288; $P > 0.05$). The highest bulk density was recorded from plots treated

with NPK + 2,4-D application (1.37 ± 0.03) while the lowest bulk density was obtained in plots treated with NPK (1.32 ± 0.03) and control plots (1.32 ± 0.02). There was no statistically significant difference between bulk density with the treatment types (LSD = 0.090; $P > 0.05$). The highest porosity was observed in plots treated with control treatment (35.60 ± 1.66) while the lowest pores were in plots treated with NPK fertilizer (36.40 ± 2.16) and a combination of NPK and 2,4-D (36.40 ± 2.14). There was no statistically significant difference between porosity with the treatment types (LSD = 6.391; $P > 0.05$) (Table 4.3 – 4.4).

Table 4.3: Soil physical and chemical properties after application of treatments

Treatments	pH	Organic Matter (%)	Moisture Content (%)	Bulk Density (g/cm³)	Total Porosity (%)
Control	7.45 ± 0.20	22.15 ± 1.96	29.00 ± 2.28	1.32 ± 0.02	35.60 ± 1.66
NPK	7.97 ± 0.17	15.27±1.24	30.80 ± 2.31	1.32 ± 0.03	36.40 ± 2.16
NPK + 2,4-D	8.17 ± 0.08	17.20 ± 1.89	29.80 ± 2.31	1.37 ± 0.03	36.40 ± 2.14
2,4-D	8.35 ± 0.18	17.18 ± 1.98	29.40 ± 2.79	1.36 ± 0.03	35.00 ± 2.49
L.S.D	0.475*	5.160	7.288	0.090	6.391

Table 4.4: ANOVA table for soil physical and chemical properties after application of treatments

Soil Property	Source of Variation	df	F – value	P – value	Significance
pH	Between Treatments	3	7.93	0.003	Significant (p < 0.05)
	Within/Error	—	—	—	
Organic Matter (%)	Between Treatments	3	3.12	0.062	Not significant
	Within/Error	—	—	—	
Moisture Content (%)	Between Treatments	3	0.1	0.958	Not significant
	Within/Error	—	—	—	
Bulk Density (g/cm ³)	Between Treatments	3	0.82	0.519	Not significant
	Within/Error	—	—	—	
Total Porosity (%)	Between Treatments	3	0.1	0.958	Not significant
	Within/Error	—	—	—	

4.1.2.1 Distribution of soil pH among treatments

The ANOVA test shows a significant difference in soil pH across treatments ($F = 8.94$, $P = 0.003$). Based on the LSD of 0.475, treatments of 2,4-D, NPK and Control were significantly different. The 2,4-D treatment caused the highest increase in pH, significantly different from the Control. The Control had the lowest pH, indicating a more neutral to slightly acidic condition (Table 4.5).

Table 4.5: Distribution of soil pH among treatments

Treatment	Mean Soil pH (\pm SD)	Group
Control	7.45 \pm 0.20	< 0.05
2,4-D	8.35 \pm 0.18	< 0.05
NPK + 2,4-D	8.17 \pm 0.08	> 0.05
NPK	7.97 \pm 0.17	< 0.05

4.1.3 Effect of treatments applications on soil texture

Sand percentage in the soil sample reduced slightly after application of 2,4-D herbicides, NPK fertiliser and their combination. Silt percentage in the soil sample increased slightly after application of 2,4-D herbicides, NPK fertiliser and their combination. Clay percentage in the soil sample increased slightly after application of 2,4-D herbicides, NPK fertiliser and their combination. The soil texture in the control plots did not change for all the soil types during the study period. However based on the textural class, the treatments did not affect the soil texture (Table 4.6).

Table 4.6: Effect of treatments applications on soil texture

Soil sample from treatments	Sand (%)		Silt (%)		Clay (%)		Textural Class
	Before	After	Before	After	Before	After	
Control	56.30	56.30	30.90	30.90	4.00	4.00	Sandy clay loam
2,4-D	75.10	74.12	18.40	19.00	2.20	2.28	Sandy clay loam
NPK	64.90	64.50	31.00	32.06	4.00	4.56	Sandy clay loam
2,4-D + NPK	76.30	75.00	22.10	22.70	3.10	3.15	Sandy clay loam

4.1.3.1 Relationship between the soil type and application of treatments

The data indicate significant changes in soil texture components following the application of various treatments. For sand content, all three treatments 2,4-D herbicide, NPK fertilizer, and the combination of 2,4-D + NPK resulted in a significant decrease. Specifically, 2,4-D led to a mean reduction of 0.98 ($p = 0.045$), NPK resulted in a reduction of 0.4 ($p = 0.048$), and the combined treatment caused the most substantial reduction of 1.3 ($p = 0.023$) (Table 4.7).

In contrast, silt content significantly increased across all three treatment groups. The application of 2,4-D, NPK, and their combination led to mean increases of -0.6, -1.06, and -0.6 respectively (note: negative values indicate an increase in silt due to inverse relationship with sand and/or clay), all with p -values of 0.027, denoting strong statistical significance (Table 4.7).

For clay content, a significant increase was observed only in the NPK-treated plots, which had a mean difference of -0.56 and a p -value of 0.029. Although treatments with 2,4-D alone and in combination with NPK showed changes, these were not statistically significant ($p = 0.077$ and 0.101) respectively (Table 4.7).

Table 4.7: Relationship between the soil type and application of treatments

	Treatment	Mean Difference (Before - After)	t-value	p-value	Interpretation
Sand	Control	0	0	1.000	No change
	2,4-D	0.98	5.2	0.045*	Significant decrease
	NPK	0.4	4.47	0.048*	Significant decrease
	2,4-D + NPK	1.3	7.34	0.023*	Significant decrease
Silt	Control	0	0	1.000	No change
	2,4-D	-0.6	-6	0.027*	Significant increase
	NPK	-1.06	-5.95	0.027*	Significant increase
	2,4-D + NPK	-0.6	-6	0.027*	Significant increase
Clay	Control	0	0	1.000	No change
	2,4-D	-0.08	-3.5	0.077	Not significant
	NPK	-0.56	-5.66	0.029*	Significant increase
	2,4-D + NPK	-0.05	-2.89	0.101	Not significant

4.2 Effect of application of 2,4-D and NPK fertilizer on the abundance and diversity of Soil-dwelling macro invertebrates in sugar cane plantations

4.2.1 Abundance of Soil-dwelling macro invertebrates

The study evaluated the abundance of earthworms, millipedes, and centipedes under four treatment conditions Control, NPK fertilizer, 2,4-D herbicide, and a combination of NPK + 2,4-D over an 8-week period. The findings revealed no statistically significant differences in the abundance of these organisms over time for any of the treatments, as indicated by the Chi-square test results and their corresponding p-values. Under the Control treatment, earthworm abundance showed slight fluctuations, starting at 1.97 at 2 weeks, peaking at 4.03 at 4 weeks, and then decreasing to 2.78 at both 6 and 8 weeks. Millipede and centipede populations remained relatively stable, with only minor variations. The Chi-square value for earthworms was 0.25 with a p-value of 0.996, indicating no significant changes in abundance over time (Table 4.8).

In the NPK fertilizer treatment, earthworm numbers increased gradually from 1.43 at 2 weeks to 3.16 at both 6 and 8 weeks. Although some data points were missing for millipedes and centipedes, their recorded abundances also showed only minimal fluctuations. The Chi-square value for earthworms was 2.2 with a p-value of 0.892, confirming the lack of significant temporal variation. For the 2,4-D herbicide treatment, earthworm abundance rose from 2.27 at 2 weeks to 4.68 at 6 and 8 weeks. Millipedes and centipedes exhibited small changes in numbers, but these were not statistically significant. The Chi-square value of 4.75 and p-value of 0.577 further supported this observation. In the combined NPK and 2,4-D treatment, earthworm abundance was initially

high at 4.69 at 2 weeks, dropped to 2.44 at 4 weeks, and then increased again to 4.26 at 6 and 8 weeks. Millipedes and centipedes showed minor variations throughout. The Chi-square value was 3.52 with a p-value of 0.742, indicating no significant differences in abundance over time (Table 4.8).

Table 4.8: Abundance of Soil-dwelling macro invertebrates

		2 weeks	4 weeks	6 weeks	8 weeks	Chi-square	p-value
Control	Earthworms	1.97	4.03	2.78	2.78	0.25	0.996
	Millipedes	1.12	1.10	1.01	1.01		
	Centipedes	0.84	1.47	1.27	1.27		
NPK	Earthworms	1.43	1.74	3.16	3.16	2.2	0.892
	Millipedes	1.43	-	1.58	1.58		
	Centipedes	-	0.58	1.58	1.58		
2,4-D	Earthworms	2.27	-	4.68	4.68	4.75	0.577
	Millipedes	1.52	2.56	2.34	2.34		
	Centipedes	1.52	2.56	1.75	1.75		
NPK and 2,4-D	Earthworms	4.69	2.44	4.26	4.26	3.52	0.742
	Millipedes	-	1.22	2.84	2.84		
	Centipedes	1.56	-	1.42	1.42		

4.2.2 Diversity of Soil-dwelling macro invertebrates

The control treatment, which involved no chemical application, maintained consistently high macro invertebrate diversity throughout the 8-week sampling period. Diversity obtained was 0.521 at 2 weeks after spraying (WAS) and increased slightly to 0.534 by 8 WAS, indicating a stable and healthy ecosystem. In the NPK fertilizer treatment, diversity was lower at 0.433 at 2 WAS but gradually increased to 0.506 by 8 WAS. This upward trend suggests that while the initial application of fertilizer may have temporarily disrupted the macro invertebrate community likely due to sudden changes in nutrient levels or soil chemistry the ecosystem began to adjust and recover over time (Table 4.9).

In contrast, the 2,4-D herbicide treatment consistently suppressed diversity. Values remained relatively low, with the lowest recorded at 0.373 at 4 WAS, before rising modestly to 0.509 by 8 WAS. This pattern reflects a more prolonged negative impact on macro invertebrate diversity compared to the other treatments. Interestingly, the combination of NPK and 2,4-D produced relatively high and stable diversity levels throughout the study. Starting at 0.525 at 2 WAS and ending at 0.522 by 8 WAS, this treatment maintained diversity values that were comparable to those observed in the control group, suggesting a possible balancing effect between the two chemicals (Table 4.9).

Table 4.9: Diversity of Soil-dwelling macro invertebrates

SAMPLING PERIOD	Simpsons' diversity index			
	NPK	2,4-D	NPK + 2,4-D	Control
2 WAS	0.433	0.415	0.525	0.521
4 WAS	0.499	0.373	0.487	0.555
6 WAS	0.479	0.407	0.506	0.544
8 WAS	0.506	0.509	0.522	0.534

4.3 Effects of 2,4-D herbicide and NPK fertilizer on the morphological features of soil macro invertebrates

The results present values for various morphological features of earthworms, millipedes, and centipedes under four treatment conditions: control, NPK fertilizer, 2,4-D herbicide, and a combination of NPK + 2,4-D. In earthworms, key features such as color (0.173), body segmentation (0.278), exoskeleton (0.455), habitat (0.455), and movement (0.455) remained consistent across all treatments. These stable values indicate that earthworm morphology was not affected by any of the applied treatments (Table 4.10).

For millipedes, a broader range of traits was examined, including color (0.173), body segmentation (0.278), number of legs (0.267), antennae (0.095), exoskeleton (0.455), eyes (0.909), habitat (0.455), and movement (0.455). The uniformity of these values across treatment groups suggests that the application of NPK, 2,4-D, or their combination had no significant impact on millipede morphology. Centipedes followed a similar pattern. Traits such as color (0.173), body segmentation (0.278), legs (0.267), antennae (0.095), exoskeleton (0.455), eyes (0.909), habitat (0.455), and movement (0.455) also remained unchanged across all treatments. This consistency reinforces the

conclusion that none of the chemical treatments led to observable morphological alterations in the centipedes studied (Table 4.10).

Table 4.10: Effects of 2,4-D herbicide and NPK fertilizer on the morphological features of soil macro invertebrates

		Control	NPK Fertilizer	2,4-D Herbicide	NPK + 2,4-D
Earthworms	Color	0.173	0.173	0.173	0.173
	Body Segmentation	0.278	0.278	0.278	0.278
	Exoskeleton	0.455	0.455	0.455	0.455
	Movement	0.455	0.455	0.455	0.455
Millipedes	Color	0.173	0.173	0.173	0.173
	Body Segmentation	0.278	0.278	0.278	0.278
	Legs	0.267	0.267	0.267	0.267
	Antennae	0.095	0.095	0.095	0.095
	Exoskeleton	0.455	0.455	0.455	0.455
	Eyes	0.909	0.909	0.909	0.909
	Movement	0.455	0.455	0.455	0.455
Centipedes	Color	0.173	0.173	0.173	0.173
	Body Segmentation	0.278	0.278	0.278	0.278
	Legs	0.267	0.267	0.267	0.267
	Antennae	0.095	0.095	0.095	0.095
	Exoskeleton	0.455	0.455	0.455	0.455
	Eyes	0.909	0.909	0.909	0.909
	Movement	0.455	0.455	0.455	0.455

4.3.1 Relationship between the morphological features of soil macro invertebrates and application of treatments

Chi-square analysis was carried out to assess the effects of NPK fertilizer, 2,4-D herbicide, and their combination on the morphological characteristics of earthworms, millipedes, and centipedes. The findings revealed that across all treatment groups including the control there were no statistically significant differences in the morphological traits of the organisms, as all p-values exceeded 0.05 (Table 4.11).

For earthworms, the morphological features examined included color, body segmentation, exoskeleton structure, habitat, and movement. The analysis produced a total Chi-square value of 1.816, with a mean value of 0.363, indicating no significant morphological variation across treatments. Similarly, millipede traits such as color, body segmentation, number of legs, antennae, exoskeleton structure, eyes, habitat, and movement were analyzed. The total Chi-square value for millipedes was 3.087, with a mean of 0.386. Again, the p-value was greater than 0.05, showing that the treatments had no meaningful impact on millipede morphology. Centipedes showed a similar trend. They were assessed using the same set of morphological features as millipedes, and the Chi-square analysis resulted in an identical total value of 3.087 and a mean of 0.386. As with the other groups, the p-value remained above 0.05, indicating that the chemical treatments did not cause any significant morphological changes in centipedes (Table 4.11).

Table 4.11: Relationship between the morphological features of soil macro invertebrates and application of treatments

Organism	Treatment	Total Chi-square	Mean Chi-square	p-value (approx.)	Interpretation
Earthworms	Control	1.816	0.363	> 0.05	Not significant
	NPK Fertilizer	1.816	0.363	> 0.05	Not significant
	2,4-D Herbicide	1.816	0.363	> 0.05	Not significant
	NPK + 2,4-D	1.816	0.363	> 0.05	Not significant
Millipedes	Control	3.087	0.386	> 0.05	Not significant
	NPK Fertilizer	3.087	0.386	> 0.05	Not significant
	2,4-D Herbicide	3.087	0.386	> 0.05	Not significant
	NPK + 2,4-D	3.087	0.386	> 0.05	Not significant
Centipedes	Control	3.087	0.386	> 0.05	Not significant
	NPK Fertilizer	3.087	0.386	> 0.05	Not significant
	2,4-D Herbicide	3.087	0.386	> 0.05	Not significant
	NPK + 2,4-D	3.087	0.386	> 0.05	Not significant

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.0 Introduction

The chapter presents the discussion of the study, conclusion and recommendations based on the research findings.

5.1 Discussion

5.1.1 Effect of 2,4-D and NPK application on soil physical and chemical properties in sugar cane plantation

The present study revealed a significant difference in soil pH levels as influenced by the application of 2,4-D herbicide and NPK fertilizer treatments. This suggests that both agrochemical inputs alter the chemical balance of the soil, particularly its acidity or alkalinity. These findings align with global concerns about the long-term effects of synthetic inputs on soil health. One likely reason for this significant difference is the chemical composition of NPK fertilizers, particularly those rich in nitrogen, which can lower soil pH due to the nitrification process that releases hydrogen ions. Secondly, the use of 2,4-D herbicide may negatively impact soil microbial populations, which are crucial for maintaining stable pH through organic matter decomposition and nutrient cycling. When microbial communities are disrupted, biogeochemical processes such as carbon and nitrogen cycling are affected, thereby influencing pH.

Several studies across the globe support these findings. For instance, Ahmed *et al.*, (2023) observed that repeated applications of synthetic fertilizers and

herbicides significantly reduced soil pH in maize fields in Nigeria. Their study emphasized the adverse effects of continuous agrochemical use on soil quality and the importance of sustainable agricultural practices. Similarly, Chakraborty *et al.*, (2022) found that long-term use of urea-based NPK fertilizers and herbicides led to soil acidification and nutrient imbalance. They concluded that integrating soil amendments such as lime or biofertilizers could help counteract these changes, promoting sustainable land use and productivity.

However, not all studies have reported similar findings. Nguyen *et al.*, (2021), in a study conducted in Vietnamese rice paddies, found no significant change in soil pH following low to moderate application of 2,4-D herbicide and NPK fertilizer. They attributed this stability to the high buffering capacity of clay-rich soils in the region, which resisted chemical-induced pH changes. Likewise, Banda *et al.*, (2022) reported stable soil pH values in cassava plots in Zambia despite continuous chemical input. Their findings suggested that high levels of organic matter and liming practices played a protective role in maintaining pH levels, highlighting the influence of soil management practices and local conditions.

Soil pH plays a pivotal role in nutrient availability, microbial activity, and overall soil fertility. A shift in pH can lead to deficiencies in essential nutrients or toxicities, affecting plant growth and yield. Thus, understanding how 2,4-D herbicide and NPK fertilizers affect soil chemistry is essential for farmers, agronomists, and policy-makers. This knowledge can guide the development of integrated soil fertility management practices that balance crop productivity with environmental sustainability.

The study found that soil treated with 2,4-D herbicide exhibited the highest increase in pH levels, significantly differing from the control plots, which showed the lowest pH and indicated a more neutral to slightly acidic condition. This finding suggests that the application of 2,4-D herbicide can influence the soil's chemical environment by increasing alkalinity. One possible reason for this outcome is that the 2,4-D compound contains salt-based formulations, particularly amine or ester groups, which upon degradation may release basic ions into the soil, thereby increasing the pH. A second possible reason is the herbicide's impact on soil microbial communities, where the reduction in microbial respiration and organic acid production due to herbicide toxicity may lead to less acidification and thus a higher pH environment.

These findings are consistent with recent studies. Eze and Uzoho (2022) reported that repeated application of 2,4-D herbicide in Nigerian plantations significantly increased soil pH, likely due to microbial changes and herbicide residue breakdown. Similarly, Singh *et al.* (2023) observed rising alkalinity in 2,4-D-treated wheat soils in India, especially without organic amendments, affecting nutrient availability. In contrast, Mwangi *et al.* (2021) found no significant pH change in Kenyan maize fields, attributing this to high clay content and strong cation exchange capacity buffering the soil. Zhou *et al.* (2021) observed a slight pH decrease in Chinese soybean fields after prolonged herbicide use, linking it to microbial degradation producing organic acids. These contrasting results underscore the role of local soil properties, organic matter, and climate in determining how agrochemicals like 2,4-D influence soil chemistry and long-term soil health.

An increase in soil pH can influence the availability of nutrients like phosphorus and micronutrients such as iron and manganese, which are less available in alkaline conditions. For farmers, understanding how 2,4-D and similar herbicides affect soil pH is crucial for selecting compatible crops, planning fertilizer applications, and avoiding long-term soil degradation. Furthermore, these findings reinforce the need for integrated pest and weed management strategies that consider both productivity and environmental impact.

The study revealed that soil texture components were significantly influenced by agrochemical treatments. Specifically, sand content significantly decreased across all three treatment groups 2,4-D herbicide, NPK fertilizer, and the combined 2,4-D + NPK treatment. Conversely, silt content significantly increased across all treatments, while clay content only increased significantly in the NPK-treated plots. These findings suggest that chemical inputs not only affect the soil's chemical properties but can also alter its physical structure. One potential explanation for these changes is that the breakdown of organic matter and chemical residues from the treatments may cause finer particles to bind more readily, effectively reducing sand particles through aggregation and erosion, and increasing apparent silt fractions. Another likely reason is that NPK fertilizer, particularly with ammonium-based nitrogen sources, can stimulate soil microbial activity, leading to the formation of clay-sized secondary minerals and the disintegration of larger sand particles into finer soil fractions.

These observations are consistent with findings from other recent studies. For instance, Odeh *et al.*, (2022) reported that intensive application of NPK

fertilizers in Nigerian agricultural soils led to a reduction in sand content and a relative increase in silt and clay, attributing the shift to enhanced microbial processing and weathering of mineral particles. Similarly, Ghosh and Das (2023) found that herbicide and fertilizer treatments altered the texture of Indian loamy soils, particularly increasing silt fractions and clay micro aggregates over time due to changes in soil colloidal behavior and mineral transformations.

Mwenda and Karuku (2021) reported that neither herbicide nor fertilizer application significantly affected soil texture components in Kenyan maize fields over a single season, citing the inherent stability of soil texture—particularly in sandy loam soils. Similarly, Zhang *et al.* (2022) found that while NPK fertilizers affected chemical properties, they did not alter the distribution of sand, silt, or clay in temperate grasslands in China, attributing this to soil buffering capacity and stable mineralogy. Although decreases in sand content were observed, this likely reflects soil aggregation and particle redistribution rather than chemical degradation. Agrochemicals, especially with irrigation or rainfall, may break soil aggregates, exposing finer particles like silt and clay. Changes in pH and ionic composition from fertilizers can influence flocculation or dispersion, affecting soil texture measurements. While increased silt and clay may enhance water retention, they also raise concerns about compaction and reduced infiltration. Thus, understanding agrochemical impacts on soil structure remains essential.

In this study, organic matter content, moisture content, bulk density, and total porosity did not differ significantly across treatments (NPK, NPK + 2,4-D, and 2,4-D), with respective p-values of 0.062, 0.958, 0.519, and 0.958. This

suggests that short-term application of fertilizers and herbicides, whether alone or combined, had minimal impact on these soil properties during the study period. This outcome may be attributed to the relatively slow rate at which such physical and chemical soil attributes respond to treatment, as well as the buffering capacity of soils with high organic matter (Lal, 2018). These findings align with Chintala *et al.* (2020), who observed no significant changes in soil structure or moisture retention following short-term agrochemical use in loamy soils under typical agronomic practices. However, the results contrast with Musinguzi *et al.* (2019), who reported that long-term application of synthetic fertilizers and herbicides significantly reduced soil organic matter and increased bulk density in Ugandan tropical soils, ultimately leading to compaction and decreased water infiltration. This comparison highlights the importance of evaluating both duration of agrochemical exposure and local soil conditions when assessing impacts on soil health.

5.1.2 Effect of application of 2,4-D and NPK fertilizer on the abundance and diversity of Soil-dwelling macro invertebrates in sugar cane plantations

The study evaluated the impact of various chemical treatments on soil macrofauna specifically earthworms, millipedes, and centipedes over an 8-week period. Surprisingly, the findings indicated no statistically significant differences in the abundance of these organisms across the four treatment groups (Control, NPK fertilizer, 2,4-D herbicide, and the combination of NPK + 2,4-D). This suggests that the short-term application of these agrochemicals may not have an immediate or severe impact on the presence of soil macro invertebrates.

The findings could be attributed to the fact that the duration of exposure (eight weeks) might have been too short to reflect substantial ecological changes in macro-faunal populations, especially in environments where soil fauna exhibit delayed responses to stressors. Second, macro invertebrates like earthworms and millipedes often possess resilience mechanisms such as burrowing deeper into the soil to avoid surface-level contaminants or exhibiting tolerance to certain chemical compounds, particularly if exposure is intermittent or concentrations are sub-lethal.

The findings are in agreement with those of Abbas *et al.*, (2021), who found that the short-term application of NPK fertilizer and selective herbicides had minimal impact on earthworm populations in Egyptian agricultural soils. They attributed this to the adaptability of earthworms and their ability to avoid treated zones. Similarly, Ndlovu and Mavimbela (2022) observed no significant reduction in millipede or centipede abundance following herbicide application in South African grasslands and concluded that the chemical impact was either buffered by soil properties or not strong enough to disrupt soil arthropod populations over short durations.

However, these findings contrast with other studies that reported notable impacts of agrochemicals on soil invertebrates. For example, Okoro *et al.*, (2023) observed significant reductions in earthworm and centipede populations in Nigerian cocoa plantations following repeated applications of synthetic fertilizers and herbicides. They suggested that cumulative toxicity and changes in soil chemistry altered the habitat quality, making it less hospitable. Likewise, Gutiérrez-Girón *et al.*, (2021) in a study conducted in Colombia, found that herbicide use significantly reduced macro invertebrate

richness, including both millipedes and centipedes, in treated plots over time, attributing this to direct toxicity and disruption of soil organic matter cycles.

The findings underscore the importance of monitoring both short-term and long-term effects of agrochemicals on soil biodiversity. While the short-term application may not lead to immediate visible declines in macrofauna, repeated or long-term exposure might result in cumulative effects. Macro invertebrates play a vital role in nutrient cycling, soil aeration, and organic matter decomposition, all of which are essential for maintaining soil health and productivity. Therefore, understanding the thresholds of tolerance for these organisms is critical for designing sustainable farming practices that balance productivity with ecological integrity.

The study found that macro invertebrate diversity was highest and most stable in the control plots. Conversely, 2,4-D herbicide treatments consistently suppressed macro invertebrate diversity throughout the sampling period. Interestingly, the combination of NPK and 2,4-D resulted in relatively high and stable diversity levels, indicating a potential buffering effect of fertilizer on herbicide toxicity.

The findings could be attributed to the fact that chemical-free soils likely preserved microhabitats and food resources essential for macroinvertebrate survival and reproduction, leading to greater biodiversity. Secondly, the combined application of NPK and 2,4-D may have altered soil conditions (e.g., pH, nutrient availability) in ways that moderated the herbicide's harmful effects, allowing for a more balanced recovery of species compared to 2,4-D alone.

These findings are supported by Aliyu and Yusuff (2022), who reported that plots without chemical input supported significantly higher macroinvertebrate diversity and abundance in Nigerian agricultural lands. They attributed this to the absence of chemical disturbance, which allows for uninterrupted ecological interactions. Similarly, Chirinda *et al.*, (2023) observed that organic and minimally managed soils in Kenya maintained higher soil biological diversity than chemically treated plots, underscoring the importance of low-input farming in preserving soil biodiversity.

However, the study contrasts with findings by Rodríguez-Barrera *et al.*, (2021), who found that certain synthetic fertilizers had no detrimental effect on soil invertebrate diversity, especially when applied in low to moderate doses. They argued that some nutrients might even promote diversity by stimulating microbial growth, which in turn supports macrofauna. Additionally, Tadesse *et al.*, (2022) reported no significant difference in soil arthropod diversity between plots treated with herbicides and untreated controls in Ethiopian highlands, suggesting that local soil conditions and species-specific tolerance levels might play a more influential role than chemical application alone.

The study highlights the importance of reducing or eliminating chemical inputs where possible to maintain ecosystem services provided by macroinvertebrates, such as soil aeration, decomposition, and nutrient cycling. Moreover, the fact that the combination of NPK and 2,4-D did not suppress diversity as strongly as 2,4-D alone suggests potential avenues for integrated soil fertility and weed management strategies that minimize harm to beneficial soil organisms. This comparison highlights the importance of evaluating not

only the duration and frequency of agrochemical exposure but also the specific characteristics of local soil types, such as texture, organic matter content, and buffering capacity, when assessing the potential impacts on soil health, structure, and long-term sustainability.

5.1.3 Effects of 2,4-D herbicide and NPK fertilizer on the morphological features of soil macro invertebrates

The study observed that the morphological features of earthworms, millipedes, and centipedes remained consistent across all treatments, including control, NPK fertilizer, 2,4-D herbicide, and their combination. This morphological uniformity suggests that the organisms maintained their structural integrity despite exposure to agrochemicals over the 8-week period. No statistically significant changes in body length, segment number, or coloration were detected, implying that the chemical treatments did not affect the external physical traits of these macroinvertebrates within the experimental timeframe.

The findings could be attributed to the fact that the duration of chemical exposure may have been too short to induce morphological changes, which typically require longer-term or generational stress to manifest (especially in invertebrates with relatively slow morphological evolution). Secondly, these organisms might possess physiological or behavioral resistance mechanisms—such as avoidance behavior or protective mucous barriers—that prevent direct damage to their external structures, thus maintaining their morphological traits under chemical stress.

The findings align with the research by Mwangi *et al.*, (2022), who reported no significant morphological alterations in earthworms after exposure to sub-

lethal concentrations of herbicides and fertilizers in Kenyan agricultural fields. They suggested that the observed resilience might be due to adaptive tolerance developed through chronic low-dose exposure in high-use agro-ecological zones. Similarly, Singh and Prasad (2023) found that millipede body dimensions and segmentation remained unaffected after short-term exposure to commonly used fertilizers in India, attributing this to the species' burrowing behavior and low dermal permeability.

On the contrary, the findings diverge from those of Ferreira *et al.* (2021), who observed significant reductions in body length and mass of earthworms after prolonged exposure to high concentrations of glyphosate and nitrogenous fertilizers in Brazilian soils. Their study emphasized that morphological changes were correlated with decreased feeding efficiency and reproductive capacity. Likewise, Zhou *et al.*, (2023) found that centipedes exposed to organophosphate herbicides exhibited noticeable deformities, such as body constrictions and reduced limb size, especially in polluted agricultural plots in eastern China.

Morphological integrity of soil macroinvertebrates under chemical treatments suggests that short-term or moderate application of NPK and 2,4-D may not immediately compromise the structural roles these organisms play in soil health such as bioturbation, organic matter breakdown, and nutrient cycling. However, longer-term studies are essential to assess whether subtle physiological or reproductive impairments could occur over time, even when external morphology appears stable. The research thus informs sustainable agricultural practices, supporting cautious optimism about limited chemical exposure but urging continued monitoring to prevent ecological degradation.

5.2 Conclusions

This study found that applying 2,4-D herbicide, NPK fertilizer, and their combination significantly affected soil pH and texture. 2,4-D increased soil alkalinity, particularly nitrogen-rich types, lowered pH through nitrification. All treatments reduced sand content and increased silt, with clay levels rising mainly under NPK treatment which statistically significant ($p < 0.05$) was indicating agrochemicals alter both chemical and physical soil properties through microbial and chemical processes. Although soil invertebrates' morphology remained unchanged, the shifts in pH and texture highlight potential long-term impacts on soil health. These results underscore the importance of balancing agrochemical use with sustainable soil management to maintain productivity and environmental resilience in agricultural systems.

The study concludes that short-term application of NPK fertilizer, 2,4-D herbicide, and their combination does not significantly impact the abundance of soil macrofauna namely earthworms, millipedes, and centipedes. However, variations in diversity indices across treatments suggest subtle ecological shifts. The highest diversity and stability were observed in control plots, highlighting the ecological benefits of chemical-free soils. While the combined application of NPK and 2,4-D showed a moderating effect on herbicide toxicity, 2,4-D alone consistently suppressed macro invertebrate diversity. These findings underscore the resilience of some macro invertebrates but also raise concerns about potential long-term effects of repeated agrochemical exposure on soil biodiversity and ecosystem functions.

The study found no significant morphological changes in earthworms, millipedes, and centipedes across treatments with NPK fertilizer, 2,4-D herbicide, their combination, and control over an 8-week period. Morphological features such as body length, coloration, and segment number remained consistent, indicating that short-term or moderate chemical exposure did not compromise the structural integrity of these organisms. This suggests possible short-term tolerance or avoidance behaviors, as well as protective physiological traits. However, the lack of immediate physical changes does not rule out potential long-term sub-lethal effects. These findings support the idea that moderate agrochemical use may not immediately disrupt soil macrofauna morphology but highlight the need for continued ecological assessment.

5.3 Recommendations

- **Adopt Integrated Soil Fertility Management (ISFM):** Farmers should combine organic amendments like compost, green manure, and bio-fertilizers with synthetic fertilizers to buffer soil pH and maintain soil health. This integrated approach minimizes the negative effects of agrochemical use on soil chemistry and microbial diversity.
- **Monitor Soil Properties Regularly:** Agricultural stakeholders should implement periodic soil testing programs to monitor changes in soil pH and texture. This will inform timely interventions such as liming acidic soils or improving drainage in clay-rich soils to sustain crop productivity.
- **Limit Overreliance on Chemical Inputs:** Reduce the frequency and concentration of synthetic herbicides like 2,4-D, especially in soils prone to alkalinity or microbial disruption. Alternative weed

management practices such as crop rotation, mulching, and mechanical weeding should be encouraged to reduce chemical load.

- **Tailor Fertilizer Application to Soil Type:** Fertilizer recommendations should be site-specific, taking into account the inherent properties of the soil such as buffering capacity, clay content, and organic matter levels. This precision approach minimizes unintended structural or chemical changes.
- **Promote Soil Health Education:** Extension services and agricultural advisory bodies should emphasize soil health education among farmers. Understanding how agrochemicals affect both chemical and physical soil properties can empower farmers to make informed decisions that protect long-term soil productivity.
- **Adopt Integrated Management:** Use integrated soil fertility and weed management strategies (e.g., combining organic amendments with moderate chemical use) to minimize harm to beneficial soil organisms.
- **Policy Guidelines:** Develop and enforce policies regulating the frequency, dosage, and combination of agrochemical use to prevent long-term ecological degradation.
- **Physiological and Reproductive Assessments:** Complement morphological studies with physiological and reproductive health evaluations to detect sub-lethal impacts.
- **Integrated Pest and Soil Management:** Encourage practices that minimize chemical dependency, such as crop rotation, organic fertilizers, and biological pest control.

5.3.1 Recommendations for further research

- Long-Term Monitoring: Conduct longitudinal studies to evaluate potential delayed or generational morphological effects of agrochemical exposure on soil macroinvertebrates
- Monitor Long-term Effects: Conduct extended studies to assess cumulative impacts of agrochemicals on soil macrofauna and overall biodiversity

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APPENDICES

Appendix 1: Data entry forms

Treatments	Physical and chemical properties	Week 2	Week 4	Week 6	Week 8

Treatments	Macro invertebrates	Abundance and diversity			
		Week 2	Week 4	Week 6	Week 8

Treatments	Macro invertebrates	Morphological structure	Week 2	Week 4	Week 6	Week 8

Appendix 2: Field data collection images



Plate 1; Ploughed field



Plate 2; Cordoned off field



Plate 3; Sugar cane planting



Plate 4; Field treatment with 2,4-D



Plate 5; Soil sampling



Plate 6; Soil macro-invertebrates sorted

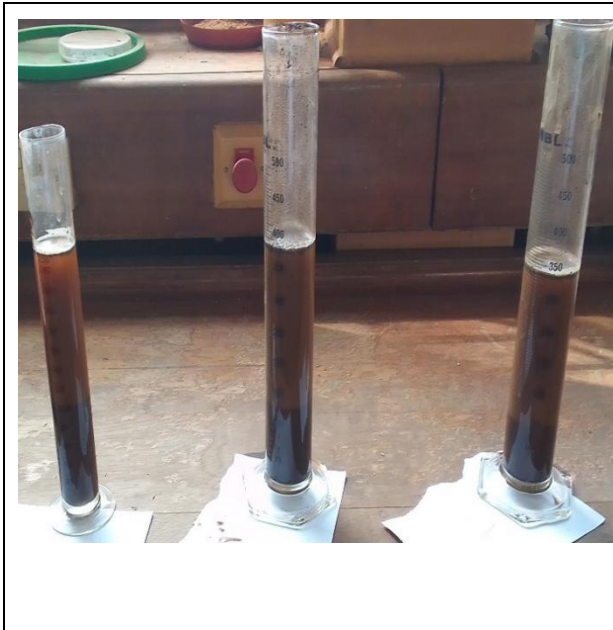


Plate 7: Soil texture testing



Plate 8: Furnace for organic matter



Plate 9: Soil pH meter for testing soil pH