MUTAGENIC POTENTIAL OF SELECTED INDUSTRIAL EFFLUENTS AND THEIR EFFECTS ON GERMINATION, GROWTH AND YIELD PARAMETERS OF COWPEAS *(VIGNA UNGUICULATA* L*)*

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19/U/GMSM/18958/PD

A DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN CONSERVATION AND NATURAL RESOURCE MANAGEMENT OF KYAMBOGO UNIVERSITY

NOVEMBER, 2024

DECLARATION

This dissertation is my original work and has not been presented for the award of any degree in any other University.

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DEDICATION

This study is dedicated to my late mum Mrs Turufisa Aliobe Bako, my wife and children.

ACKNOWLEDGEMENT

Sincere thanks to almighty God for seeing me throughout the study period. I am deeply thankful to my supervisors Dr. Grace Ssanyu Asiyo and Dr. Abubaker Muwonge for their guidance, advice, support and encouragement. Thank you for not giving up on me. I also appreciate the teaching staff in the Department of Biology, Kyambogo University (KyU) for their valuable guidance offered during the study period. My class mates especially kwiyocwinye Emmanuel for the team work exhibited. I thank my employer National Water and Sewerage Corporation (NWSC) especially the Senior Director, Directorate of Business and Scientific Services Dr. Rose Kaggwa, Senior Manager Water Quality Management Department Dr. Irene Nansubuga, Manager Research Mr. Christopher Kanyesigwe for providing an enabling environment for employees to achieve their dreams. In a special way, am great full to my direct supervisor Dr. Babu Muhammed, Manager Central Laboratory Services for the professional guidance. Madam Sharon T Gabamwoyo for the effort you rendered, thank you. To Julius Wanialla quality control officer Ggabba lab for the memorable trip we took up to Namulonge agricultural research farm in search for research space, cowpeas seeds and sampling of various industrial effluent, your effort did not go to waste. I am grateful to Araa Kennedy Principal Analyst Biology Section for the technical guidance offered during the Ames test. The Chemistry team; Principal Analyst Madam Muhairwe Robinah, Senior Analyst Madam Kisalu Phyllis and Analysts Agatha Nabaterega and Sandra Namulinda for the continued support you rendered me during laboratory work. Special thanks go to my family members, my wife Annet and children; Johnson, Shephard and Augustus. For the encouragement and patience especially when I had to take time off during the study period.

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B O D	Biological Oxygen Demand
BFRs	Brominated Flame Retardants
BPA	Bisphenol A
C O D	Chemical Oxygen Demand,
CSO	Civil Society Organisations
DES	Diethyl Sulphate
DNA	Deoxyribose Nucleic Acid
EMS	Ethyl Methane Sulphonate
IARC	International Agency for Research on Cancer (IARC)
ION	Iron Oxide Nano Particles
NEMA	National Environmental Management Authority
NGO	Non – Governmental Organisations
NWSC	National Water and Sewerage Corporation
OCP	Organo-chlorine Pesticides
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyl
PhACs	Pharmaceutical Active Substances
PO	Propylene Oxide
PVC	Polyvinylchloride (PVC)
SA	Sodium Azide
TDI	Toluene Diisocyanate
UWASNET	Uganda Water and Sanitation NGO Network
WWTWs	Effluent Water Treatment Works

ABSTRACT

Increasing industrialization in the developing world especially Uganda has been inevitable leading to build up of industrial effluents. The Industrial effluent composition varies depending on activity in the manufacturing process at a specific time. These industrial effluents possess temporal toxicity of unknown magnitude due to the chemical reactions that take place and this has possible mutagenic risk to the ecosystem. The study assessed the mutagenic potential of selected industrial effluents in Kinawataka Area, Kampala Uganda and their effects on germination, growth and yield parameters of Cowpeas (*Vigna unguiculata* L). The selected industrial effluents were assessed for mutagenicity using Ame's test, the effluents were then used to intoxicate the seeds of cowpeas and thereafter, the germination, growth and yield parameters were determined on the plants in randomised experimental design. The industrial effluent from the plastic and mattress industry had the highest mutagenic potential with undiluted effluent having 32.0 (1.41) and 27.0 (1.14) revartants respectively. Aluminium products industry had the lowest mutagenicity with 12 (1.41) revartants. Regarding the germination and seedling parameters, the undiluted effluent from mattress industry caused the highest reduction in germination rate by 50 %, shoot length by 75 %, root length by 55 % and wet weight by 47 % of the seedling. The dry weight of the seedling was reduced by 22 % by effluent from pharmaceutical industry. The 25 % dilution plastic industry effluent increased shoot significantly by 38 %. The undiluted mattress effluent registered a significant reduction effect on growth and yield parameters as follows; root nodules by 26 %, number of seeds per pod by 28 % and dry weight of seeds by 18 %. The paint effluent also significantly reduced number of root nodule per plant and dry weight of seeds at dilutions of 75 % and above. Aluminium waste also registered significant effect on number of root nodules per plant and number of seeds per plant. The other effluents had no significant effect on growth and yield parameters of cowpeas at $p \leq 5$ %. It was therefore established that all the industrial effluent assessed were mutagenic with varying potential. These observations were due to the fact that industrial effluents contain toxicants with varying chemicals that interact to cause such mutagenic effects, such as propylene oxide in mattress effluent which interfere with the DNA bases in order of Guanine > Adenine > Cytosine > Thymine. The government should therefore, increase surveillance of industrial effluents so as to enhance compliance and protect the environment.

CHAPTER ONE: INTRODUCTION

1.1 Background to the study

Globally, out of many forms of pollution, water pollution is ancient issue and is being made worse by increase in industrialization (Wasi *et al*., 2013). The recent decades have manifested increase in global population and industrialisation hence continued increase in industrial effluent generation, some of which, unfortunately, are mutagenic (Chinaza *et al*., 2020). The earth's ecological systems therefore can no longer sustain industrial pollution because more than 80 % of waste water is discharged without treatment which is getting out of hand (Harding *et al*., 2020 ; Achim and Newman, 2015). For instance, the industrial wastewater in China's amounted to more than 180 billion tons in 2015 which is quite threatening to the ecosystem (P. Zhang *et al*., 2020). Developing countries with poor effluent control strategies will be more affected due to the fact that many industries are shifting to emerging economies (Achim and Newman, 2015). In African countries, the crisis of industrial effluent management is caused by absence or weak policies on environmental management (Bamuwamye *et al*., 2017). Apparently, quality of treated wastewater mainly depends on the efficiency of the Waste Water Treatment Works (WWTWs) and influent qualities. However, most African countries have waste treatment plants with uncontrolled waste loads, power cuts, increasing wastewater flows, expensive energy and poor operation and maintenance (Josiane *et al*., 2013). More so, due to highly toxic industrial influents, most waste water treatment plants fail to produce effluent water of the conforming quality (Adewumi *et al*., 2010).

The industrial effluent discharge mixture contain many toxicants some of which are known mutagens (Tarso *et al*., 2013; Sharif *et al*., 2015; Zhang *et al*., 2013). For example, two compounds used in the mattress industry Propylene Oxide and Toluene Diisocyanate for

polyurethane foams production are known to be mutagenic (Pottenger *et al*., 2018). For plastics the problem is with the additives like Phthalates, Bisphenol A (BPA) and Brominated Flame Retardants (BFRs) which exhibit mutagenicity (Okunola *et al*., 2019). Whereas the paint industry uses Ion Oxide nanoparticles which are also mutagenic (Touati *et al*., 1995). Pharmaceutical industry contains pharmaceutical active substances (PhACs), metabolites, heavy metals, solvents, phenolics, synthesis reagents and halogenated compounds which also exhibit mutagenicity (Tarso *et al*., 2013 ; Zhang *et al*., 2013). Others toxicants include polycyclic aromatic hydrocarbons, trace radioactive substances among others. The challenging bit with mutagenicity of industrial effluent is that toxicity of the pure compounds may not represent the toxicity of the mixture in that mixtures may present toxicity of unknown magnitude (Rehana *et al*., 1995). For example, chromium alone is toxic but when combined with other elements in the tannery effluent it's even more toxic (Saviex *et al*., 2008). However regulatory authorities focus on Geno-toxicity tests so as to classify an agent as mutagenic or not, with genetic disease of concern being cancer (Macgregor *et al*., 2014).

These Industrial effluent contain toxicants that have high effect on plants (Wasi *et al*., 2013). Phthalate cause disruption in soil leading to reduced soil nitrogen availability and decreased plant vegetative properties (Xiong *et al*., 2019 ; Ma *et al*., 2018). As for Pharmaceuticals (Ibuprofen, diclofenac Antibiotics Chloroquine) exposure negatively affected vegetative properties of plants (Wijaya *et al*., 2020 ; Copolovici *et al*., 2017 ; Pedrosa *et al*., 2019 ; Bhatt, 2018). Whereas Cipro accelerated germination by promoting ROS accumulation in seeds of maize (Pedrosa *et al.*, 2019). Propylene Oxide positively affected the shoot and root elongation in tomato. Iron oxide nanoparticles hinders vegetative properties because of the high availability of iron and unavailability of other essential nutrients (Zia-ur-rehman *et al.*, 2018) and was found highly toxic to *Lemna minor*

plant and killed all the leaves within 7 days. Heavy metals affect plants in many ways for example, mercury level more than 2 mg/l, affected yield by more than the 50 % Vergara-fl *et al.,* (2020), cadmium toxicity leads to decline in vegetative parameters of cowpea seedling Basu and Rao, (2013) and aluminium stress reduced crop yield due to impairment in nutrient and water uptake (Ologundudu *et al.*, 2018).

In animals and humans, Toluene Diisocyanate exhibits it's toxicity by causing occupational asthma and chromosomal damage (Bolognesi *et al*., 2001). Propylene oxide reacts with DNA bases in order of Guanine > Adenine > Cytosine > Thymine hence changing DNA sequence making it mutagenic and carcinogenic (Solomon *et al*., 1988). Phthalates interfere with transcriptional mechanism of lipid and carbohydrate metabolism, testosterone hormone and sperm motility. (Okunola *et al*., 2019 ; Elisabet *et al*., 2021). Bisphenol A disrupts endocrine which acts like oestrogen in females causing polycyclic ovarian syndrome (PCOS), endometrial hyperplasia and recurrent miscarriages (Kehinde *et al*., 2019). Brominated Flame Retardants affects neural growth mechanisms, like thyroid or sex hormone levels and sex steroids (Dong *et al*., 2021). Iron Oxide nanoparticles possess mutagenic activity without chromosomal abnormalities and their interaction with cells can induce mutagenic effects (Gonc *et al*., 2020). Trace heavy metals leads to reduced growth, increased mortality and induce mutagenicity especially in animals (Dala-Paula *et al*., 2018). Pharmaceutical industrial effluent affects eukaryotic organisms (Zhang *et al*., 2013), and causes cardiac troubles, foetal-maternal death, diabetes mellitus and some neurobehavioral effect (Sharif *et al*., 2015). Exposure to trace radioactive substances like radon, uranium and thorium can lead to development of lung, liver, hepatic, pancreas, colon, bone, kidney and blood cancers (El *et al*., 2019).

Out of all known carcinogenic agents (viruses, radiations, and chemicals) in humans, chemicals were the most important in the induction of cancer (Domenico *et al*., 2016). Earlier research shows that there is high correlation value for carcinogenicity and chemical mutagenicity, which is estimated to be around 90% (Mortelmans and Zeiger, 2000). There exists a serious threat of cancer with global projections showing more than 25 million new cancer cases and more than 16 million cancer deaths each year (Jatho *et al*., 2020). More than 60% of new cancers were detected in middle income countries which is anticipated to increase by two folds by 2030 (Jatho *et al*., 2020). For example, in Uganda according to statistics in 2018, more than 30,000 new cases and more than 20,000 deaths occurred due to cancer and approximately 56,000 people were living with at least one form of cancer (Jatho *et al*., 2020).

Uganda's 2030 vision so as to industrialize for economic prosperity has presented many challenges in the aspect of environmental pollution (Angiro *et al*., 2020). This has led to establishment of industries in Kinawataka area like; factories of cosmetics, mattresses, paint, plastics, pharmaceuticals, aluminium products and breweries (Muwanga and Barifaijo, 2010). According to the Uganda Revenue Authority, in 1994, 1995 and 1996 Uganda imported 8704, 11160 and 190668 tonnes of chemicals respectively mostly for use in chemical industries located in Kampala (Matagi, 2001). Currently, Uganda is faced with challenge of having less than two thirds of industrial facilities in Kampala connected to less efficient sewage system, industries using out dated manufacturing technologies, most lack functional effluent treatment plants and there is poor compliance to legislation (Angiro *et al*., 2020 ; L. Emerton, L. Iyango, 1999; Ssempebwa and Carpenter, 2009 ; Chinaza *et al*., 2020). There is also rapid increase in industrialization in Kinawataka area which requires attention before it's too late (Wanasolo and Kansiime, 2018). The Papyrus swamps in Kinawataka have now failed to perform their detoxification function due to

degradation and discharge of too much toxic industrial effluent (Matagi, 2001; Kakuba and Kanyamurwa, 2021) and this has led to increasing pollution of Lake Victoria possibly with mutagenic effect (Banadda *et al*., 2009).

Therefore, as per the literature review and available knowledge, no data on mutagenicity of industrial effluents in Uganda has been documented and so little has been done on the effect of industrial effluent especially on plant. However, cowpeas *(Vigna unguiculata* L*)* has been widely used in mutagenicity studies mostly for plant breeding by Gaikwad and More, (2016), Olasupo *et al*., (2016), Ikhajiagbe, (2012) and (Namasaka *et al*., 2017). Previous research on mutagenicity of waters points to a direct connection between mutagenicity and the toxicants in water bodies (Siddiqui and Ahmad, 2003). There is alarming evidence by scholars on toxic effluents generated by industries in Kinawataka and Cancer levels in the country that necessitates urgent need for such study (Birikadde, 2017). This study therefore focused on assessment of the mutagenic potential of selected industrial effluents in Kinawataka Area, Kampala Uganda and their effects on germination, growth and yield parameters of Cowpeas.

1.2 Statement of the problem

Uganda is a developing country which is focusing on industrialization as per the National Development Plan (NDP) III. As a result, many industries have been established within the Kinawataka Drainage Area in Kampala and most of these industries discharge waste water without treatment. The industrial effluent contain toxicants such as Propylene Oxide, Toluene Diisocyanate, Bisphenol, Heavy metals among others, most of which are known to be mutagenic (Tarso *et al*., 2013; Sharif *et al*., 2015; Zhang *et al*., 2013). However, the mutagenic potential and effect on germination, growth and yield Cowpeas (*V. unguiculata*) of the industrial effluent discharged into Kinawataka wetland is unknown. Cancer being

one of the diseases associated with mutagenicity in Uganda (Macgregor *et al*., 2014). According to statistics in 2018, more than 30,000 new cases and more than 20,000 deaths occurred due to cancer and approximately 56,000 people were living with at least one form cancer (Jatho *et al*., 2020) in Uganda. Accordingly, the focus of regulatory authorities has basically been on Geno-toxicity tests to classify an agent as mutagenic or not. Therefore, there is limited information on the mutagenic properties of most effluent from industries and their effect on plants. This study therefore assessed the mutagenic potential of selected industrial effluents in Kinawataka Area, Kampala Uganda and their effects on germination, growth and yield parameters of Cowpeas (*V. unguiculata*). The mutagenic effects of industrial effluents will continue to increase if it is not addressed.

1.3 General Objective

To assess the mutagenic potential of selected industrial effluents in Kinawataka Area, Kampala Uganda and their effects on germination, growth and yield parameters of Cowpea (*V. unguiculata*) so as to establish their toxicity levels.

1.3.1 Specific Objectives

1. To establish the mutagenic potential of selected industrial effluents along Kinawataka wetland.
2. To determine the germination and seedling parameters of cowpeas exposed to different industrial effluents.
3. To determine the growth and yield parameters of cowpeas exposed to different industrial effluents.

1.4 Research Questions

1. What is the mutagenic potential of the selected industrial effluent in Kinawataka area?

2. How does germination and seedling growth parameters of cowpeas (*V. unguiculata*) vary with exposure to different industrial effluent?

3. How does growth and yield parameters of cowpeas (*V. unguiculata*) vary with exposure to different industrial effluent?

1.5 Scope of the study

The effluent water sample were collected from five industries from Kinawataka drainage area named and coded as follows; B = Pharmaceuticals Industry, C = Aluminium Industry, D = Mattress Industry, E = Paint Industry and F = Plastic Industry. The industrial effluents were used as samples for the study. The parameter of mutagenicity was directly analysed on the sample at the Central Laboratory of National Water and Sewerage Corporation at Bugolobi, Kampala Uganda. The germination, growth and yield experiments were done in a green house in Bulaya, Namata Subcounty, Mukono District. For the effect on phenotypic parameters of cowpeas seedlings the parameters of percentage germination, shoot length, root length, wet weight dry weight was assessed. As for the growth and yield parameters of cowpeas; leaf area, root nodules per plant, dry nodule weight, shoot length, number of pods per plant, number of seeds per pod, dry weight of seeds, days to maturity and dry weight of roots were assessed. The study was carried out in a period of six months, from January to June 2023.

1.6 Justification of the study

The study assessed mutagenic potential of industrial effluent and their effects on plant growth using cowpea as a model plant. The concept arose from my MSc specialization in ecotoxicology, industrial effluents being the main source of chemical toxicants of water bodies and the need for professionalism in this area. The government of Uganda is now promoting Buy Uganda and Build Uganda, where locally manufactured products attract

less taxes and efforts are geared towards export promotion (Kyambadde, 2014). This has led to establishment of many chemical-based industries in the country. The most industrialized drainage area in Uganda is Kinawataka drainage area. The drainage feeds the part of Lake Victoria, that is near the point for raw water obstruction for potable water treatment plant that supplies the city, this means that the city is drawing water from its own effluent dump (Matagi, 2001). It should also be noted that there exist gaps in the regulatory system. This is because most of the industries were established without proper guidelines; in wetlands and without effluent treatment facilities. National Environment Management Authority (NEMA) focuses on basic parameters like E.C, pH, Biological and Chemical Oxygen Demand, Nutrients and Salts to permit industrial effluent discharge without focussing on the toxicants themselves and the synergic effects of these effluents mixtures (NEMA, 1995). Currently, there is high level of pollution in Kinawataka Drainage area and Inner Murchison Bay which has direct links to cancer levels in the country. Most of the studies on industrial effluent focus on single sector and chemical toxicants like heavy metals, however the synergic effects of industrial effluents especially on plant growth has been neglected (P. Zhang *et al*., 2020).

1.7 Significance of the study

The study is expected to inform policy making by government agencies involved in monitoring and issuing of license for these industries. Furthermore, this study will help Non – Governmental Organisations (NGOs) and the civil society organisations (CSO) to raise awareness among the population on effects of these toxicants. This could be done through the Uganda Water and Sanitation NGO Network (UWASNET). The information generated will help trigger policy dialogue and inform policy change on monitoring of industrial effluents, trade issues and public health. In addition, it will also explore the possibility of using plants as bio indicators of environmental pollution especially plants

like cowpeas and soybeans since chemical analysis methods are quite expensive. Finally, the data will also help proprietors of industries to focus on devising means to mitigate these challenges so as to promote effluent treatments at the source. Some organisations are willing to address the impact of their activity through cooperate social responsibility but evidence of the impact of their activity is lacking.

CHAPTER TWO: LITERATURE REVIEW

2.1 Industrial Effluent Issues

2.1.1 Global effluent challenges

World over out of all types of pollution, water pollution is an ancient issue and is being made worse because of improper waste management (Wasi *et al*., 2013). This is as a result of increasing rates of urbanization, industrialisation and effluent generation from industries (Chinaza *et al*., 2020). This is caused by the fact that over recent decades, many industries are shifting to emerging economies which means the generation of hazardous effluent is increasing (Achim & Newman, 2015). The fact that avenues for recycling require sophisticated treatment technology and in addition, toxic pollutants such as heavy metals cause limited reuse public acceptability (Mateo-sagasta *et al*., 2015). Consequently, the release of industrial effluent globally cannot be sustained within the limits of the Earth ecological systems (Achim & Newman, 2015). For instance, globally over more than 80 % of effluent water is discharged untreated which is definitely going to worsen due to the trend of events in recent years (Harding *et al*., 2020). Recent statistics show that by December 2015, industrial wastewater discharge in china amounted to over 180 billion tons which threatens growth and development of fisheries and agriculture (P. Zhang *et al*., 2020). In 2001, chemicals worth 100,000 and 760,000 metric tonnes were released into water bodies and atmosphere respectively by industrial in the United States of America (USA) alone (Ohe *et al*., 2004).

2.1.2 Challenges of Industrial effluent in Africa

African countries are faced with challenges of industrial effluent management due to political, financial, infrastructural and human resource factors which has led to absence or weak policies on environmental management (Bamuwamye *et al*., 2017). Overtime it has

caused issues such as lack of data on effluent water management. For instance, 32 of 48 countries mainly in Sub-Saharan Africa had no data available on effluent water management with exception of Senegal, Seychelles and South Africa (Harding *et al*., 2020). Furthermore, adoption of effluent water reuse most especially in industrial reuse, irrigation use, toilets and urinary flashing is lacking (Adewumi *et al*., 2010). The challenge is projected to worsen especially as urban populations continue to increase (Harding *et al*., 2020).

The treated industrial effluent quality solely depends on the effectiveness of the Waste Water Treatment Plant (WWT) and influent qualities. In the continent most countries have treatment plants with high wastewater flow rates, operation and maintenance challenges, and lack of resources (Josiane *et al*., 2013). Furthermore, due to highly toxic industrial influents most WWTWs mostly cannot produce wastewater of non-hazardous quality hence preventing its reusability especially as fertilizers due to concerns with potentially toxic compounds (Adewumi *et al*., 2010). To make matters worse, the coverage of improved sanitation is as low as 34 % in most African countries (Josiane *et al*., 2013).

2.1.3 Uganda's industrial effluent challenge

Uganda's vision 2030 which is geared towards industrialisation has presented many environmental challenges (Angiro *et al*., 2020). Uganda is a developing country which is focusing on industrialization as per the National Development Plan (NDP) III, (Campenhout *et al.*, 2024). This has led to establishment of industries in Kinawataka drainage area like factories of cosmetics, mattresses, paint, plastics, pharmaceuticals, aluminium products and breweries (Muwanga and Barifaijo, 2010). According to the Uganda Revenue Authority, in 1994, 1995 and 1996 Uganda imported 8704, 11160 and 190668 tonnes of chemicals respectively mostly for use in chemical industries located in

Kampala (Nyakaana, 2012; Banadda *et al*., 2009). Less than two thirds of industrial facilities in Kampala were connected to sewage system, the effluent is partially treated at Bugolobi Sewage (L. Emerton, L. Iyango, 1999), industries use out-dated manufacturing technologies, most lack functional effluent treatment plants (Angiro *et al*., 2020). In addition, poor effluent management practices i.e., shipping back to the supplier and recycling accounts for only 9% of the effluent disposal practices. The government mainly focuses on public health, hence the management of hazardous materials is neglected (Ssempebwa and Carpenter, 2009) and there is poor compliance to legislation (Chinaza *et al*., 2020). In the end this has now led to increased pollution of Lake Victoria as a result of discharge of untreated wastewater by many of these industries (Banadda *et al*., 2009).

Lake Victoria is highly polluted due to discharge of untreated effluent by many industries (Banadda *et al*., 2009). The highly polluted water in Lake Victoria effects aquatic life, the health of communities close to the channel, and the quality of drinking water for the city since the discharge point is close to raw water abstraction point for the city (Kayima *et al*., 2008). Such waters have impact on soil properties like pH, buffering capacity, salinity, toxicants, and essential nutrients for plant growth, affecting soil microbial environment (Liang *et al*., 2014). Hence contaminants accumulate in soils, groundwater and plants (Anyakora *et al*., 2013). Consequently, this leads to environmental pollution and thereby affecting the ecosystem, and accelerating extinction of species of organisms in water bodies (Walakira and Okot-okumu, 2011). This is made worse due to the fact that toxicants bio accumulative and magnify in the food chain affecting plants and animals (Anyakora *et al*., 2013). It therefore implies that even trace concentrations of these pollutants are not safe.

It should also be noted that, some of the hazardous chemicals used in industrial processes are persistent and not biodegradable (Iloms *et al*., 2020). These effluents contains drugs, metabolites, solvents, heavy metals, phenolic, synthesis reagents and halogenated compounds, some of which are known mutagens (Tarso *et al*., 2013 ; Sharif *et al*., 2015 ; Zhang *et al*., 2013). The pollutants that were reported at high levels include, heavy metals especially Lead, Pharmaceutical Active Substances (PhACs) and Mercury levels reported mostly above the Ugandan National Standards for effluent discharge (Dietler *et al*., 2019).

From the literature reviewed, it is evident that their exists global crisis in industrial effluent management (Wasi *et al*., 2013 ; Achim & Newman, 2015). This is caused by a number of factors which include; urbanisation (Achim & Newman, 2015), political influence (Bamuwamye *et al*., 2017), and poor effluent management practices (Ssempebwa and Carpenter, 2009 ; Chinaza *et al*., 2020). From the general outlook, there exists laxity in efforts to address the industrial effluent challenges.

2.2 Effects of specific industrial effluent on fauna and flora

2.2.1 Heavy metals

The heavy metals with high eco toxicological effects include; chromium, cadmium, lead, mercury and arsenic (Tchounwou *et al*., 2012). It occurs mainly due to anthropogenic activities, that explains why Anyakora *et al*., (2013) found that, direct correlation exists between industrialization and heavy metal concentration. In animals, consumption of trace metals, can reduce growth, increase mortality and may also induce mutagenic effects especially in animals (Dala-Paula *et al*., 2018). In one study, analysis for heavy metals within the dumpsite for mercury, copper and cadmium were 0.047, 0.096 and 4.94 mg/l respectively (Hamdy, 2013). In another study the levels of heavy metals namely; cadmium, nickel, manganese, chromium, cobalt in cowpeas were as follows; Cd (0.26± 0.10),

Ni(2.45±0.87), Mn (9.11±1.80), and Co (1.67 ± 0.20 mg/L (Akande *et al*., 2019). This was considered as a major health risk (Girija and Dhanavel, 2013). The chromium alone was toxic and when it is combined with other elements in the tannery effluent makes it more toxic (Saviex *et al*., 2008).

Heavy metals affect plants in many ways for example, mercury level more than 2 mg/l, affected yield by more than the 50 % (Vergara-fl *et al*., 2020). Treatment at low concentrations of mercury affected root length adversely compared to shoot length (Devi *et al*., 2007). Mercury inhibited vegetative properties of cowpea *Vigna unguiculata* L, like root elongation by 66 % (Umadevi *et al*., 2009 ; Iqbql and Siddiqui, 2015). Cadmium toxicity leads to decline in fresh weight, dry weight and germination rates of cowpea seedling. (Basu and Rao, 2013). Aluminium is not an essential nutrient for plants, levels more than 2 mg/l causes root growth inhibition by impairment of nutrient and water uptake (Ologundudu *et al*., 2018). This is made worse by the fact that heavy metals bio accumulate especially in *V. unguiculata.* above minimum permissible limits Increasing Cadmium concentrations resulted in an increase of Cd uptake by seedling, reaching a maximum 39.50 mg/ plant at 1.2 mg/l (Hamdy, 2013).

2.2.2 Polycyclic Aromatic Hydrocarbons (PAHs)

These are several chemically-related, recalcitrant compounds with various structures and exhibiting varying toxicity (Rengarajan *et al*., 2015). Examples include Naphthalene, Anthracene, Fluorene, Pyrene and Benzo(a)pyrene (Gavrilescu, 2014). The industrial PAH emissions were from burning of fuels such as oil, gas and coal, production of primary aluminium, rubber tires, and manufacture of cement and asphalt (Rengarajan *et al*., 2015). However, PAHs exhibit mutagenicity effects in animals and plants (Abdel-shafy and Mansour, 2018) which makes it a public health concern (Arinaitwe *et al*., 2012; Teixeira *et*

al., 2017). According to Ishizuka, (2015), PAHs are common problems in urban area of African countries with 84.7 % of PAHs from the city centres.

Soil microbial community responds to PAH contamination with changes in the structure and activity even with short time PAH exposure (Picariello *et al*., 2020). For instance, PAHs increased abundance of the bacterial Acidobacteriota and Gemmatimonadota, while decreased the relative abundance of Bacteroidota, Desulfobacterota, and Firmicutes (Qi *et al*., 2024). In plants, PAHs causes shorter root length, lower root weight and chlorophyll content of the seedlings (Lázaro & Ana, 2021). In one study by Yahaya *et al*., (2021), they discovered that the length of shoots and roots of cowpeas were highly reduced by more than half of the control by PAHs. This may be caused by disruption of anti-oxidant system and damages DNA (Yang *et al*., 2022). In higher plants, it also lead to decreased photosynthetic pigment contents, stomata conductance and growth (Jajoo *et al*., 2014).

2.2.3 Industrial Effluent with radioactive effects

Radiation is present everywhere on earth and some human activities release radiations which has made the plants and animals to be exposed to these radiations (Avwiri and Olatubosun, 2014). It is caused by, dumping of municipal, industrial, commercial and domestic effluent – such as polyethene, scrap metals, textiles, plastic, insecticides containers, bulbs and paints that produce some radioactivity is on increase and very common (Avwiri and Olatubosun, 2014). Many of the industries like pharmaceuticals and petrochemicals employ radioactive materials in their process line and these end up in the effluents (Farai *et al*., 2009).

Human body is constantly exposed to terrestrial and extra-terrestrial sources of natural radiations though it's affects depend on the radiation types, received doses, exposure times and exposure routes (El *et al*., 2019). According to the International Agency for Research

on Cancer (IARC), any compound that produces ionizing radiations are classified as carcinogenic to animals though its effect may take years (El *et al*., 2019). 226Ra when in body of animals, the radioisotopes mimic calcium and are deposited in the bones. For instance, 226Ra has been detected in high levels in bones due to exposure. The levels of 226Ra found in human bone range from 0.03 Bq Kg^{-1} to values above 0.36 Bq Kg^{-1} (Avwiri and Olatubosun, 2014). Radionuclides like 238 U, 232 Th series and 40 K were found in environment (Otansev *et al*., 2016). For example, exposer to radon inhalation is associated with development of lung cancer and radium inhalation causes cranial and bone tumours. Exposure to thorium are historically known to cause development of hepatic, pancreatic, kidney, bone, and blood cancers (El *et al*., 2019). Radioactive substance and industrial chemicals are toxic themselves but their total effects when they react in mixtures can be greater than effect of individual compounds (Magagi, 2012).

2.2.4 Pharmaceuticals

Pharmaceutical industry effluent contains drugs, metabolites, heavy metals, solvents, phenolics, synthesis reagents and halogenated compounds which have been detected in natural surface waters, effluents from Sewage Treatment Plant (STP), soil and fish (Tarso *et al*., 2013). For example, antibiotics, psychiatric drugs and analgesics were found in the water bodies of South Asia (Khan *et al*., 2020). One common drug, Ibuprofen for treatment pain and fever and had a total of about 44,000 metric tons used in 2019 however, it has now contaminated surface waters and sediments globally (Wijaya *et al*., 2020). In Uganda Trimethoprim, sulfamethoxazole was the most common PhACs in the water bodies (Victoria *et al*., 2020).

Pharmaceutical industry effluents discharge may contains heavy metals that include; cadmium, lead, chromium, nickel and mercury (Sharif *et al*., 2015). The lead may be from

chemicals that may contain lead compounds used in manufacture of drugs (Muwanga and Barifaijo, 2010). Arsenic-based drugs ware used to treat diseases like sleeping sickness, amoebic dysentery in animals and blackhead in birds (Tchounwou *et al*., 2012). Mercury-based drugs are also a major source of mercury exposure (WHO, 2014). In one study, the chemical analysis of effluent water from pharmaceutical industry revealed higher levels of Fe 4.96 mg L^{-1}, Cr 0.43 mg L^{-1}, Lead (Pb) 0.21 mg L^{-1}, Arsenic (As), 0.83 mg L^{-1}. Cadmium (Cd) 0.02 mg L^{-1} (Sharif *et al*., 2016).

The mutagenicity of pharmaceutical industrial effluent has adverse effect on any eukaryotic organism (Chang *et al*., 2013). This may explain why people near pharmaceutical industries suffer waterborne diseases such as cardiac troubles, hypertension, foetus-maternal death, diabetes mellitus and some neurobehavioral effects (Sharif *et al*., 2015). These effects are bound to become prominent because pharmaceuticals were being partially removed in wastewater treatment plants (Mydlarczyk *et al*., 2022). Therefore, the effects on living organisms health should be investigated through eco toxicological research (Kapoor, 2015).

Pharmaceuticals affect plants in many ways, Ibuprofen (IBU) exposure reduced the leaf area, shoot and root lengths, fresh and dry weights and chlorophyll a contents (Wijaya *et al*., 2020). As for diclofenac it reduced the seedlings growth and lead to increase in the enzymatic activity of leguminous plants (Copolovici *et al*., 2017). Antibiotics affected negatively seed germination, root development and oxidative enzyme activity of tomato, cucumber and carrots (Pedrosa *et al*., 2019). Chloroquine antimalarial decreased plant growth (Bhatt, 2018). On the other side, Cipro accelerated germination by promoting ROS accumulation in seeds of maize (Pedrosa *et al*., 2019). Tetracycline increased radish yields

in maize plants, but not in pinto beans hence the effects vary from plant to plant (F. Liu *et al*., 2009).

2.2.5 Mattress industrial effluent

Propylene Oxide (PO) and Toluene Diisocyanate (TDI) are the main chemicals for production of polyurethane foams (Bootman, 1979) which exhibit toxicity. Mattress industry also uses heavy metal based pigments Cd, Cr, Ni and organics (Birikadde, 2017). Due to the highly reactive nature, TDI exhibits it's toxicity by causing occupational asthma, irritation and toxic reactions and chromosomal damage and induces mutation in *Salmonella typhimurium* strains TA98, TA100 under S9 liver mix activation (Bolognesi *et al*., 2001). This is due to the fact that TDI hydrolyses in the body of animals to form toluene di-amine (TDA), a mutagenic carcinogen (Hoffmann, 1995). As for propylene oxide it reacts with the DNA bases in order of Guanine > Adenine > Cytosine > Thymine, hence making it mutagenic and carcinogenic (Solomon *et al*., 1988).

As for plants, no studies using radiolabelled propylene oxide and TDI are available, though propylene oxide reacts with water to produce propylene glycol which still has insufficient data (Credland *et al*., 2002). Shorobi *et al*., (2023) showed that polypropylene did not significantly affect seed germination, but positively affected the shoot and root elongation in tomato. Antibodies to TDI was detected in polyurethane foam manufacturing plant, indicating that environmental releases are causing exposures to this toxicant (Environmental Protection Agency, 2011).

2.2.6 Paint industrial effluent

The materials used in production of emulsion paint include; titanium iv oxide, kaolin, biocide P.V.A, ammonia, kerosene, iron oxide nano particles (IONPs) and heavy metals (Birikadde, 2017). The ION particles include, hematite, maghemite and magnetite with

size from 1 to 100 nm and which has properties different from of the original materials (Valdiglesias *et al*., 2015). It's phytotoxic threshold is 50 ppm as plant growth increased by 8.0–24.0 %, above 50 ppm plant growth and biomass were decreased by 7.0–16.0 % (Shafqat *et al*., 2023). However, they exhibit mutagenicity activity with no chromosomal abnormalities at concentration of 70 ppm (Touati *et al*., 1995). Hence their interaction with cells can induce mutagenic effects (Gonc *et al*., 2020).

IONPs are toxic to soil microbes and affected carbon and N mineralization of manure, and hence the nitrogen cycling from the manure-soil-plant system (Kamran *et al*., 2020). Iron oxide nanoparticles hinders vegetative properties because of the high availability of iron and unavailability of other essential nutrients (Zia-ur-rehman *et al*., 2018). The chlorophyll content decreased at high IONP concentrations, which disrupted the light absorption mechanism (Rodrigues *et al*., 2019). The IONPs were highly toxic to *Lemna minor* plant and killed all the leaves within 7 days, irrespective of IONPs concentration (Rodrigues *et al*., 2019). On the other side, IONPs enhance plant growth by improving photosynthetic performance and the availability of Fe and P (Feng, Kreslavskii, *et al*., 2022). Nutrient stress tolerance in plants by provision of cofactors that are the constituents of many antioxidant (Zia-ur-rehman *et al*., 2018). It also enhances photosynthesis and elevated contents of essential elements promoting leaf growth (Feng, Kreslavski, *et al*., 2022).

2.2.7 Plastics industrial effluent

Plastics are being used as constituents in the manufacture of products like food packaging materials, medical devices and water bottles (Elisabet *et al*., 2021). Its degradation forms micro plastic fibers which is more detrimental than plastics yet has been less considered by researchers and legislators (Kehinde *et al*., 2019). Plastic industrial effluent are toxic to animals especially when the population is exposed to the additives (Meeker *et al*., 2009).

Some of which include, Bisphenol A, Phthalates, Dioxins, heavy metals, brominated flame retardants, nonylphenol, poly-chlorinated biphenyl ethers, polycyclic aromatic hydrocarbon, Poly-chlorinated biphenyls (Kehinde *et al*., 2019). For instance, In the manufacture of poly-vinyl chloride plasticizers, phthalates and bisphenol A must be added so as to minimize thermal and photo-degradation (J. Li *et al*., 2018). The most ecologically toxic additive especially to humans health are Bisphenol A, Brominated Flame Retardants and Phthalates (Okunolaa *et al*., 2019).

Phthalates make up to 50 % of polyvinyl chloride (PVC) plastics by weight (Hahladakis *et al*., 2018). These additives are not chemically part of the polymer which makes them easily leach out into the water bodies and food chain of aquatic organisms (Elisabet *et al*., 2021). Di-butyl phthalate (DBP) and Di-(2-ethylhexyl) phthalate (DEHP) were the most common Phthalates with levels of about 1.5 mg/L of DBP and 4.4 mg/L of DEHP detected in fresh waters (Elisabet *et al*., 2021). The routes of entry into the population include; ingestion, inhalation and through the dermal tissue (Meeker *et al*., 2009). In Animals, phthalates acts through the activation of peroxisome proliferator activated receptors (PPARs), which are involved in transcriptional control of the metabolism of lipids and carbohydrates (Elisabet *et al*., 2021). Phthalates interfere with testosterone, sperm motility (Kehinde *et al*., 2019). In one study, phthalates have been found to bio accumulates in zebra fish causing mitotic arrest in spermatogenesis process (Elisabet *et al*., 2021).

When in soil, it persists over long time, leading to potentially harmful effects on soil microorganisms (Xiong *et al*., 2019). Disruption in soil occurs at higher concentrations, greatly affected by soil pH, organic matter content and the water content (Ma *et al*., 2018). Which causes reduced soil nitrogen availability, leading to decreased plant vegetative properties (Xiong *et al*., 2019). For example, it accumulates in testa pores and delays seed

germination of Lepidium sativum by blocking the pores in the seed capsule (Chen *et al*., 2024). In one study, phthalate effected elongation of roots, biomass and growth of seedlings in all the test plant species of wheat, alfalfa, perennial ryegrass, radish, cucumber, oat, and onion hence high toxicity (Sharma & Kaur, 2020).

Bisphenol A (BPA) is a xenoestrogen used in the manufacture of plastics (Blackadar, 2016). Bisphenol A is one of chemicals highly produced worldwide; about 4,000 metric tons were used in 2006 (Unuofin, 2020). BPA exists in polymeric form, though it disintegrates into monomeric form with time especially due to high temperatures, this form can easily leach into food chain (Unuofin, 2020). In animals, BPA bio accumulates and participates in dys-regulation of Calcium homeostasis whereby it inhibits calcium channels such as SPCA1/2 meaning BPA can induce disease morphology and mutagenicity (Jalal *et al*., 2018). Several research discovered measureable levels of BPA in packaged foods especially those where wrapping of BPA were used (Meeker *et al*., 2009). Research done in more than 120 waste water treatment plants globally found high levels of bisphenol A in waste water implying it's not completely removed by effluent water treatment plants (Unuofin, 2020). BPA is an endocrine disruptor and mimics estrogen in females with adverse effects like miscarriages, poly-cyclic ovarian syndrome and endometrial hyperplasia (Kehinde *et al*., 2019). In one study, rats uterus were exposed to BPA, at lowest levels according to EPA and it caused mammary gland ductal hyperplasia and prostatic intra-epithelial neoplasia in the rats (Blackadar, 2016).

Bisphenol A affected the micro-biological and bio-chemical balance of soil as well as the growth and development of plants. BPA significantly suppressed the activity of acid phosphatase, dehydrogenases and urease enzymes (Zaborowska *et al*., 2021). However, there is enough evidence that BPA can adversely affect plant growth and development by

interfering with key physiological and metabolic processes (Ali *et al*., 2018). Consequently, it was established that, BPA at concentrations higher than 17.2 mg/l inhibit root growth in soybeans, above 120 mg/l decreased stem dry weight of corn, cabbages and oatsand concentrations ranging from 10 to 50 mg/l were identified to inhibited pollen tube formation and elongation in kiwis (X. Li *et al*., 2018). This is achieved through inhibition of production of plants' hormones, affecting photosynthesis by inhibiting carbon assimilation and induces oxidative stress by increasing production of reactive oxygen species (Geri *et al*., 2023).

The third toxicant is brominated flame retardant which is a raw material used to increase safety of the product. The main BFR used in plastic manufacture are tetra-bromobisphenol A (TBBPA) and poly-brominated di-phenyl ethers (PBDEs) (Kehinde *et al*., 2019). In the year 2017, the amount of BFR used in China alone was about 500 metric tons which is anticipated to grow as demand for plastics increases (Dong *et al*., 2021). Several BFRs accumulate in animals and wildlife especially those found in the aquatic environment like whales and fatty fish. BFRs levels in human breast milk and adipose tissues have been on the increase of recent (Darnerud, 2003). BFRs, are linked to impaired T4 metabolism, and cancer development (Feiteiro *et al*., 2021). NBFRs affect neural development mechanisms, causing abnormalities in thyroid or sex hormone intensities and non-receptor mediated sex steroids (Dong *et al*., 2021). In plants, BFRs were found associated with inhibition of seed germination and seedling growth and reduction in plant biomass, chlorophyll, and protein content (Q. Zhang *et al*., 2021). In one study in China, the average concentrations of BFRs in roots and shoots were 28.8 and 30.0 ng/g respectively.

From the literature review, we established that industrial activities generate effluents that contain chemical toxicants like heavy metals (Anyakora *et al*., 2013), pharmaceuticals

(Tarso *et al*., 2013), Plastic additives like Bisphenol A, Brominated Flame Retardants and Phthalates (Okunolaa *et al*., 2019) among others. These toxic compounds exhibit mutagenic characteristics (Sharif *et al*., 2015 ; Touati *et al*., 1995) and exhibit significant effects on plant yield (Vergara-fl *et al*., 2020), fresh weight, dry weight and germination rates (Basu and Rao, 2013). However, though the literature shows that these industry specific toxicants exist in the effluents, little is being done to treat them, with treatment plants unable to remove these toxicants and some increasing toxicity especially during biological treatment of waste water (Mydlarczyk *et al*., 2022 ; Mathur, 2007).

2.3 Mutagenicity

2.3.1 Introduction

Mutagenesis is defined as changes that occur in cell genetic material in cells caused by physical or chemical agents with later generations differing in a heritable and permanent manner from their predecessors (Rao *et al*., 2004). Genetic mutations can be caused by mis-pair displacement between bases in the process DNA replication. However, the regularity of displacement changes substantially as a consequence of chemical modification of the naturally occurring DNA bases (Privat and Sowers, 1996), this is because mutagens change DNA. All organisms have double helical structure and four nuc1eotides, and hence organisms can be used as an indicator for mutagenicity study (Ames and Yanofsky, 1971). However, Mutation is viewed as a shortcut breeding procedure, which has created new varieties through heritable changes in genetic characters in crops (Oki, 2014).

2.3.2 Ames test

The Ames test, a *Salmonella typhimurium* mutagenicity assay or reversion assay, is globally accepted mutagenicity test (Kauffmann *et al*., 2020). The test is a vital preliminary

screening tool for testing new chemicals like environmental mixtures, body fluids, drugs physical agents and food contact materials for mutagenicity (Kauffmann *et al*., 2020 ; Claxton *et al*., 2010). In addition, it is used to test, cosmetics, pharmaceutical and agro-products as requirement by regulatory agencies so as to authorise marketing of these products (Levy *et al*., 2019). The test is well established and validated for reliable general use (Rehana *et al*., 1995).

The TA98 and TA 100 *S. typhimurium* strains has specific mutation at the histidine biosynthesis pathway (Mortelmans and Zeiger, 2000). The genotype of TA 98 strain shows that mutation of TA98 strain is caused by damage in the DNA of a frameshift-type affecting the GCGCGCGC base pairs of the hisD3052, rfa cell wall gene and uvrB gene of *S. typhimurium* (Mortelmans and Zeiger, 2000). As for the orb TA 100 strain, its mutation mechanism is a base-pair replacement of GGG loci which is in the hisG46, a removal of the rfa gene (Tejs, 2008). In both TA98 and TA100 *S. typhimurium* strains, the uvrB DNA repair arrangement is affected, causing the elimination of excision-repair arrangement (Szalay and Tátrai, 2012). In addition, mutation of rfa leads to loss of part of the lipo-polysaccharide coating on the surface of the bacteria which makes it to become more susceptible to toxic substances (Tejs, 2008). The results from the different strains of *S. typhimurium* was not the same because each strain is characterised by a unique mutation in the gene required in the bio synthesis of amino acid needed for growth (Levy, *et al*., 2019).

2.3.3 Use of cow peas

Cowpeas (*V. unguiculata*) has been widely used in mutational breeding experiments where radioactive particles or chemical compounds are exposed to plant materials which change genetic constitution of plants (Roy *et al*., 2019). In the case of Africa, this has been carried out on cowpeas so as to increase disease and insects resistance, achieve early maturity,

desired seed quality, day length insensitivity, improve growth habits and best fitting to intercropping (Boukar *et al*., 2018). Some of the aspects studied include; Genet *et al*., (2006) effect of SA, MMS and EMS, Dhanavel, (2019) worked on cowpea (*V. unguiculata*) variety CO7 use of gamma rays, ethyl-methane sulphonate (EMS) and performance of combination of Gamma rays, Electron beam and chemical (EMS) mutagens was also done by (Priyadharshn *et al*, 2020). All these chemicals or combination showed significant mutations that have been used for decades to select better varieties. This experiment therefore used Cowpeas as a model organism to ascertain the level of toxicity in industrial effluent.

The review shows that the ames test is widely used and accepted for mutagenicity tests of industrial effluents (Kauffmann *et al*., 2020 ; Claxton *et al*., 2010). The salmonella strains of TA 98 and TA 100 indeed mutate when exposed to mutagenic compounds (Mortelmans and Zeiger, 2000; Cowpeas *V. unguiculata* has been widely used in plant breeding using mutagens by Gaikwad and More, (2016), Olasupo *et al*., (2016), Ikhajiagbe, (2012) and (Namasaka *et al*., 2017). However, the use of the plant especially for environmental toxicological studies is lacking. Furthermore, bio toxicity tests are expensive and there is limited local capacity to conduct such them.

2.4 Research on mutagenicity

2.4.1 Studies on the effect of standard mutagens on cowpeas *(V. unguiculata)*

In one study in Nigeria, sensitivity of cowpea varieties to gamma rays was assessed on eight varieties of cowpeas (*V. unguiculata*). The seeds of cowpea varieties were irradiated using gamma rays within a dose range of 100 to 500 Gy. Pots were used to assess the aspects of germination of seeds, seedling survival rate and growth habits of first generation. Low Seed Germination rate (10 % - 45 %) were achieved at high doses (500 -

400 Gy) in variety of Ife Brown (IB), whereas high Seed Germination rates (74 % - 94 %) were observed in IT90K-284-2 treatments. The general seedling survival rate was inversely proportional to gamma dosage. Gamma irradiation at low dosage of (100 Gy) increased the vegetative vigour of M1 seedlings in the parameters of leaf area, leaflet area, seedling height and plant height at 42 days. Doses of 200 Gy caused reduction in vegetative strength of cowpea. The researchers concluded that, radio-sensitivity treatment (100 Gy) can be used to improve cowpea at M1 generation (Olasupo *et al*., 2016).

The genotypic response of cowpeas to two different mutagens of gamma rays and sodium azide was carried out in India. This was done by the use of LD50 values using parameters of seed germination index and plant survival rate. The increase in the irradiation of mutagens caused increase in germination inhibition. The variety Pusa 578 was more sensitive in comparison with the variety Gomati VU-89. The results of this study revealed that, 0.04 % of SA and 400 Gy of gamma radiations were the highest non-lethal dose of mutagen for efficient mutation in the cowpea varieties of Gomati VU-89 and Pusa 578 respectively (Raina *et al*., 2018).

In Nigeria, an assessment of mutagenic effects of sodium azide on some selected vegetative parameters of 5 varieties of cowpea was studied. At lower doses (0.016 % and 0.031 %) significant changes in vegetative parameters were obtained. Higher doses (0.125 % and 0.25 % NaN3) were lethal. The study found significant decrease in percentage germination compared to the control. Shoot length reduced in the 0.031 % NaN3 treatment but improved in 0.016 % NaN3 treatment in cultivars TVU-3574 and TVU-3541 respectively. Lower levels (0.016 % and 0.013 %) of NaN3 also significantly lead to higher leaf area in TVU-3615, TVU-2521, TVU-3541, and TVU-3485. There was no significant effect recorded in number of root nodules in the cowpea varieties studied. The

total dry weight of mutant plants decreased significantly. The number of pods per plant increased in all the cultivars. The 0.031 % NaN3, TVU-3541 showed the highest number of pod/plant. Therefore, the yield parameters signified that the changes observed were due to sodium azide treatment were genetic other than environmental (Ikhajiagbe, 2012).

An experiment was done to assess induced chemical mutagenesis by EMS, MMS and SA on two cowpeas (*V. unguiculata)* varieties of, RC19 and RC101. The first generation was grown from higher doses of the mutagens, in second generation; many macro-mutations were discovered in the off springs of the varieties. The MMS treatment emerged as the best of all. Most of the second and third generation varieties had significantly superior yields than their parents (Genet *et al*., 2006).

In one study, mutagenicity was applied using NaN_2 and gamma rays to yield mutant lines at the M4 generation using cowpea cultivars of Gomati VU - 89 and Pusa - 578. The characterization was done using sodium dodecyl sulphate poly-acrylamide electrophoresis gel, cAAt box derived polymorphism markers and simple sequence repeats was applied to assess induced genetic divergence. The nitrate reductase and chlorophyll concentration, carotenoid, protein and minerals were generally high in the mutant lines in comparison with parent genotypes. Physiological, biochemical and molecular results revealed Gomati VU-89-G and Pusa-578-C as the most genetically diverse higher yielding genotypes that exhibited a significantly higher levels of proteins and nutrients, which was in line with growers requirements for improved varieties (Raina *et al*., 2020).

Cowpea (*V. unguiculata*) variety CO7 was irradiated with different concentrations of gamma rays to quantify parameters like plant height, number of branches, clusters, and seeds per plant, and seed yield and weight. In M1 population, the results showed more reduction due to higher concentrations in relation to lower concentrations for all

characteristics studied. The results clearly showed that different doses of gamma rays were effective in creating variability for various traits of cowpeas (*V. unguiculata*) crop (Girija and Dhanavel, 2013).

In one investigation in India, cowpea variety Pusa Komal was irradiated with physical mutagen, gamma rays at doses 100 through to 500 Gy and chemical mutagen, EMS in the ranges from 0.25 % through to 0.45 % with the sole aim of determining genetic variations in M2 and M3 population. Analysis using ANOVA at the M2 and M3 generations revealed significant differences in the treatments for all other attributing characters apart from protein percentage. For all characters, phenotypic coefficient of variation (PCV) measured higher than genotypic coefficient of variation (GCV). High GCV as well as PCV was obtained in M2 and M3 generation for parameters of branches, protein content, seed weight and plants yield. High heritability and high genetic advance was obtained in protein percentage in M2 as well as M3 generations (Var and Komal, 2019).

2.4.2 Studies on the mutagenicity of industrial effluent water

In one study in Spain in 2003, mutagenicity of sewage sludge material from two wastewater treatment plants (WWTP), Crude solutions of sludge were extracted using ultrasonic method with dichloromethane, and column fractionation to yield fraction containing mutagenic polycyclic aromatic hydrocarbons (PAHs). The tests done identified 16 PAHs that were classified as significant toxicants by the U.S. Environmental Protection Agency. The mutagenicity test done using *Salmonella* strains TA98 and TA100 correlated directly with the levels of mutagenic PAH and the initiation of genetic mutations. In general, the crude extracts and the PAH-fractions induced positive responses in the assay with both bacterial strains on metabolic activation by S9 rat-liver homogenate, whereas direct-acting mutagens were not detectable. TA98 proved to be more sensitive than

TA100; however, similar sensitivities of the tester strains were also observed for two other reference sewage sludge materials of the same origin (Pérez *et al*., 2003).

The assessment of textile industry waste water for mutagenicity using bacterial bioassays was carried out in India. The Textile effluent from the Panki near Kanpur city, India was used. So as to assess the toxicity of contaminants existing in the effluent, a number of biological assays were done which include; DNA repair defective mutation, Ames and Allium cepa chromosomal aberration test methods. Effluent samples were extracted using XAD-4/8 resins. The findings showed that, XAD- extracted samples exhibited more mutagenicity than the ones obtained by liquid-liquid extraction method. Ames test was the most responsive strains (S. Khan *et al*., 2019).

Another research was also done in Pakistan, to assess the mutagenicity of pharmaceutical industrial effluent. The heavy metals and pollutants were analysed using AAS and GC-MS, respectively. Mutagenicity extend of the pharmaceutical effluent water was investigated using ames test at dilutions of 6.25, 12.5, 25, 50, 100 % v/v effluent to distilled water. Chromium, lead, arsenic and cadmium were more than WHO- and EPA-recommended highest limits. In addition, GC-MS revealed lignocaine, vitamin E digitoxin, caffeine and trimethoprim in effluent water. *Salmonella* strains TA98 was more sensitive than strains TA102. The study reveals that pharmaceutical effluent contains many toxicants and has potential mutagenic effect when exposed to living organisms (Sharif *et al*., 2015).

One researcher studied sludge from treatment plant treating textile industries waste water in Pali, India for mutagenicity potential using Ames test. *Salmonella typhimurium* tester strains TA98 and TA100 detected positive mutagenic pollutants in the sludge. The mutagenic potential of biological sludge was more than chemical sludge effluent. The

results suggest that some mutagenic compounds become more stable during biological treatment process of the wastewater (Mathur, 2007).

Researchers did investigate the industrial, ground and surface water of Aligarh area of India for mutagenicity. All samples were assessed for mutagenicity using Ames assay. The ground and river water samples were obtained through XAD concentration prior to the mutagenicity testing and the industrial effluent water was assessed without XAD concentration. The samples recorded mutagenicity in the two testing systems. The *Salmonella* strains of TA98 and TA100 strains were the most responsive compared to TA 102 and TA 104 (Siddiqui and Ahmad, 2003).

A study to find the types of mutations induced by lead and mercury in AS52 cells of gpt gene was carried out in United States of America. This was done by southern blot analyses and multiplex polymerase chain reaction, 138 lead, 192 of mercury, 29 reactive oxygen radical induces and 20 spontaneously arising mutants for point and deletion mutations in the gpt gene were characterised. Some point mutations were seen in lead and mercury induced populaces (47 and 54, respectively). It was much less than the ones occurring in the spontaneously arising and reactive oxygen intermediate-induced mutants. Point mutations were revealed at metal concentrations less than 0.4 lM, while at more concentrated solutions, deletion was the most common type of mutation. It was concluded that lead and mercury mutations in eukaryotic cells were caused by two mechanisms (Ariza and Williams, 1999).

In Turkey a study was done to determine the mutagenic effects of waste from hospital origin so as to assess the efficiency of wastewater treatment plants (WTP). The mutagenicity and cytotoxicity effects of three different samples from Istanbul were assessed using AMES, lactate dehydrogenase (LDH) and XTT methods. Mutagenicity was

discovered in all samples. No cytotoxic potential was found in fibroblasts for XTT and LDH methods but high cytotoxicines were found in the samples by XTT assay. They concluded that, even though advanced technology was used for treatment, genotoxic effects had persisted, and hence there was need for some improvement in the present technologies (Cevik *et al*., 2020).

In another study in Illinois City, five municipal sewage sludge samples were analysed using multi organism testing system to assess mutagenicity. The sewage sludge samples were assessed by us of the following assays; *Salmonela* mutation, locus *Zea mays*, micronucleus in Tradescantia and sister chromatid exchange induction. Mutagenic potential was found in sludge from non-domestic reiterating the fact that industrial effluent possess mutagenic potential (Namiki, 1988).

This particular study assessed the mutagenic level of soil leachate from automobile workshop and tobacco wastewater using the *Escherichia coli* PQ37 SOS chromo-test and the ames test. The physico-chemical tests were also analysed concurrently. Ames test showed significantly positive for all samples. The SOS chromo-test results agreed with the ones of Ames *Salmonella* Fluctuation Assay. However, Ames assay was more accurate than *E. coli* PQ37 assay. The mutagenicity was as a result of cadmium, Iron, copper, manganese, chromium, zinc, arsenic and lead contents analysed in the samples. In a nut shell, it was concluded that, automobile workshop and tobacco wastewater pose high mutagenic risk and genotoxic risk (Okunola *et al*., 2014).

In another study wastewater from two cities in India were compered by using Ames plate and Ames fluctuation test. The TA98 and TA100 strains of *S. typhimurium* exhibited the highest significant sensitivity towards the Saharanpur sample (SWW) in the plate incorporation assay and the strain more sensitive towards Aligarh (AWW) was TA98.

However, TA100 and TA98 strains recorded the highest mutagenicity in the samples from Saharanpur in the fluctuation test. TA102 and TA100 exhibited maximum response to AWW in this test. Both samples generated different types of predominant reactive oxygen species (ROS). The samples from SWW had a high level of superoxide radicals and hydrogen peroxide, though the hydroxyl radicals were more in AWW. From their study, in conclusion, both water samples were highly mutagenic (Tabrez and Ahmad, 2011).

The potential of gamma radiation on textile effluents detoxification was carried out in Pakistan. The tests conducted on the effluent pre and post gamma radiation treatment include; Brine shrimp, allium cepa, heamolytic and Ames assay. The wastewater samples were irradiated with concentrations of 5k Gy, 10k Gy, and 15k Gy, and then tested. Gamma rays were more effective in detoxification of contaminants in industrial effluent. Microbial pollution was completely eliminated, and root count (RC) and root length (RL) increased to about 30 % and 38 % respectively, at 5 k Gy. Cytotoxicity reduced to 39.6 %, and 79.6 % for brine shrimp and red blood cells (RBC) respectively. Mutagenicity was eliminated after treatment. Hence, gamma rays was efficient in decontamination of the toxicants in effluents water (Iqbal *et al*., 2015).

A study was done to estimate the possibility of causing chromosomal abnormalities in *Allium cepa* species to ascertain the presence of toxicants in Sewage Sludge samples. They exposed different dilutions of the extract of the samples from five Waste Water Treatment Plants (WWTPs), with different features and treatment technologies. It was found out that Sewage Sludge (SS) lead to significant mutagenic changes, even in minute dilutions. Furthermore, samples from WWTPs that were treated with activated sludge method and received sewage of mostly industrial source caused higher toxico-genenic alterations in *A.*

cepa. Hence the use of SS in agriculture needs effective treatment methods so as to prevent detrimental effect on organisms in the environment (Caritá *et al*., 2019).

Pharmaceutical effluent was assessed using in vivo micronucleus method on mice, to assess mutagenicity and geno-toxicity of pharmaceutical waste from Anápolis - Goiás. Of all samples, only the phenolics had levels that were non-compliant according to CONAMA Agreement 430/2011. The level of phenols and reactions with possible heavy metals found in the wastewater was the cause of the mutagenic and geno-toxic findings. This shows that even at low concentrations of pollutants industrial effluent can still exhibit mutagenic potential (Tarso *et al*., 2013).

A study was carried out on metallurgy, industrial effluent, waste water treatment plants and incinerators for geno-toxicity potential using *Salmonella* assay in France. For effective assessment of the environmental health, the water soluble portion of effluent was analysed. Water soluble micro-pollutants were obtained using liquid to liquid removal before geno-toxicity assay was done. No aqueous fractions showed mutagenicity but concentrates from chemical industry and organo-phospherus effluent water had positive response. Chemical analysis of the positive extracts did not show presence of known mutagens, therefore mutagenicity could have been due to interaction between the micro pollutants (Bessi and Colin, 1992).

In India, *Salmonella* reversion assay was used for assessing the pollutants of three effluent treatment plants of Pali textile industrial area, Mandia road industrial area and Rajasthan. The samples from these areas were assessed using Ames test. The samples from surface water, underground water and CETP influent and effluent had mutagens hence treatment method had failed in these circumstances to remove mutagens from effluent water. The

research revealed that the Ames test was more sensitive and relatively rapid screening tool for assessment of mutagenicity in the environment (Nupur *et al*., 2012).

Researchers in China studied, the geno-toxicity of iron oxide nanoparticles (IONPs) of two particle sizes 10 and 30 nm and of two different surface layers of PEI and PEG using geno-toxicity assays of Ames, the in vivo micronucleus and in vitro mammalian chromosome. Altogether, the results showed that, IONPs with PEG coating exhibited mutagenic potential without clastogenic and chromosomal abnormalities and smaller IONPs (SMG-10) exhibited higher mutagenic potential than larger sized ones (SMG-30). This shows that mutagenicity of IONPs was caused by size of particles and the surface layer types (Y. Liu *et al*., 2014).

In one of the first in Africa, high volumes (24h) of Polycyclic Aromatic Hydrocarbons (PAHs) were analysed in the samples collected from the air from Kakira (KAK) and Entebbe (ENT) in the Lake Victoria shores of Uganda, so as to examine source contributions and set target for East Africa. Samples were collected twice from 2000 to 2004 for KAK and ENT1 and 2008 to 2010 for ENT2. The samples were further processed using accelerated solvent extraction method and 30 PAHs were analysed using GC−MS. The mean overall PAH levels recorded (ng/m3) were found to be 74.3 for Kakira, 56.8 for Entebbe1 and 33.1 for Entebbe2. The three ring structured PAHs was the most common group with mean levels of 36ng/m^3(ENT1), 31 ng/m^3(KAK) and 23 ng/m^3(ENT2). Naphthalene exhibited a uniquely high mean concentration (21.9 ng/m3) for KAK compared to 0.44 and 0.39 ng/m3 in ENT1 and ENT2 respectively, this is attributed to the agricultural operations at KAK (Arinaitwe *et al*., 2012).

2.4.3 Studies on the effect of industrial effluent mutagens on animals and plants

Plastic factory workers in Slovenia were exposed to toluene-diisocyanates (TDI) in plastic manufacture process were tested for mutagenicity. The research had 26 participants that were exposed to TDI during the manufacture of plastics and 21 people, not exposed served as control. The level of TDI recorded was from 0.007 mg/m^3 to 0.016 mg/m^3. The sister chromatid exchange (SCE) were found in about 50 cells, with a mean of 8.13 and micronucleus (MN) in 500 CB, with an average of 12.07. The averages of the control group were 1.89 (SCA), 5.52 (SCE), and 4.38 (MN). The found a statistical significance difference between the groups. The study confirmed mutagenicity of iso-cyanates or their metabolic products (Bilban, 2004).

Occupational combustion exposure emissions of polycyclic Aromatic Hydrocarbons (PAHs) and other organic mutagens were assessed in the personnel of Ottawa Fire Service (OFS) in Canada. The method of Paired urine and dermal wipe was used. Samples from 27 male OFS fire fighters and 18 OFS non-fire fighters were used as controls. The method used was the measurement of the urinary PAH metabolite concentrations, PAHs levels in dermal wipes and mutagenicity of urine using the *Salmonella* mutagenicity test. They recorded significance of about 3.0 to 5.0 times increase in urinary PAH metabolites. The average urinary levels also had 4 times mutagenicity significant increase. The study concluded that, on-shift fire suppression causes significant exposures to the combustion toxicants (Keir *et al*., 2017).

The effect of toxicants from wastewater effluents from textile industries on seasonal vegetables irrigated using water from Amani Shah Nallah drainage, India was studied for mutagenicity. Both mutations types, frame shift and base pair mechanisms were found in vegetable samples with frame shift type of mutation being more dominant. Results of

Ames test thus showed that both *Lageneria siceraria* and *Abelmoscus esculentus* have compounds that were mutagenic. Therefore, in conclusion, use of industrial effluent in irrigation of plants can induce genotoxic response in vegetables (Mathur *et al*., 2006).

Iron oxide nanoparticle (IONPs) was investigated for geno-toxic and mutagenic potential using female guppy. The fish were treated with IONPs at iron concentrations of 0.3 mg L^{-1} for three weeks and the fish were then assessed at the start, and then in three, seven, fourteen days' time and then in three weeks period of exposure. The mutagenicity and geno-toxicity were assessed using the tests of; comet, 3 micronucleus (MN) and also erythrocyte nuclear abnormalities (ENA). The IONP caused DNA damage in *Poecilia reticulate* after three and seven days, meanwhile mutagenicity was seen after long time of exposure. There was a direct correlation between DNA damage and exposure time. They concluded that, IONPs can induce mutagenicity and geno-toxicity in *P. reticulata* erythrocytes (Qualhato *et al*., 2017).

In Nigeria, the effect of 0.1 % solutions of colchicine for 0, 2, 4, and 6 hours on vegetative and yield parameters in Cowpea (*V. unguiculata*.) in first generation was tested. Lethal dose recorded was 59 % after treatment for about two hours. The treatments were significant for percentage germination, plant height, number of nodes, number of leaves, percentage of survival and days to first flowering. However, treatments were not significant for all other characteristics investigated. The study concluded that, colchicine can cause mutagenicity in cowpea and hence destructive to the ecosystem (Oki, 2014).

In this particular study, the effects of iron oxide nanoparticles (IONPs) were studied in male rats in Hungary. In the in vivo experiment, IONPs were studied in adult male Wistar rats by morphological methods after intratracheal instillation. Control was given 1ml of physiological saline, and the treatment group was given 5mg/kg of the body weight IONPs.

Internal organs and lungs were examined histo - pathologically after 1, 3, 7, 14 and 30 days. The mutagenic potential was evaluated using *Salmonella typhimurium* different strains, and Escherichia coli WP2uvrA strain. The in vitro geno-toxicity of IONPs in Vero cells was assessed after four hours and twenty-four hours. In their findings, there were no significant pathological variations seen in the organs, except some weak pulmonary fibrosis after one month. The in vitro MTT study showed a moderate geno-toxicity and however, IONPs did not exhibit any mutagenicity in the bacterial systems (Szalay and Tátrai, 2012).

This particular study assessed electro-plating industry wastewater effect on vegetative parameters of cowpea (*V. unguiculata*) seedlings at dilutions of 1 %, 2.5 %, 5 %, 10 %, 20 %, 30 %, 40 %, 50 %, 75 % and 100 %). The cowpea (*V. unguiculata*) seeds were treated with chrome plating industry effluent for seven days in Petri dishes. The records revealed that the effluent reduced germination, root and shoot growth and leaf pigment of chlorophyll and carotenoids. This was caused by high concentrations of chromium in the effluent water (Basu and Rao, 2013).

A research was done in China to assess the effect of colchicine on yield and growth characteristics of cowpeas. Cowpea seeds were exposed to colchicine at concentrations in the range of 0.05 to 0.20 g/dl. The phenotypic characteristics such as percentage germination, height of the plant, number of leaves/branches, and length of branches, pods and seed yield were assessed. There was a significant statistical difference in leave numbers, branches and seed on each plant due to colchicine treatment according to findings of the study. Implying that pharmaceutical waste that contains colchicine were toxic to cowpeas (Essel *et al*., 2015).

In Saudi Arabia, a study was done to ascertain the toxicological impact of pharmaceutical contaminant Ibrufen (IBU) on vegetative characteristics of cowpeas (*V. unguiculata*). Morphological and physic-chemical plant growth parameters were assessed under a number of IBU levels in a range from 400 up to 2000 ppm IBU. The IBU toxicity decreased in the shoot and root lengths, leaf area, fresh and dry weights, chlorophyll, potassium, magnesium and soluble protein contents. However, it concurrently, increased calcium and magnesium contents, sodium translocation to shoots from roots, hydrogen peroxide level, malondialdehyde, IBU uptake and catalase were observed. The level of bio accumulated IBU was 8 %. The findings suggests that ibuprofen is indeed toxic on cowpea plants (Wijaya *et al*., 2020).

Researchers in Finland studied Toluene Diisocyanate (TDI) and 4,4'-methyl enediphenyl diisocyanate (MDI) for sister chromatid exchanges and chromosome aberrations induction in cultured cells of humans. The chromosome aberrations were induced after 24 h treatment in cultures of human blood lymphocyte, MD1 dose was from 0.5 - 4.3 per ml whereas TDI (0.02 - 0.15 per ml). Both mixtures significantly increased level of mutagenicity. MD1 also greatly increased sister chromatid exchanges at concentration of (2.17 per ml). In addition, both TDI and MD1 formed polymers, however at higher doses those polymers in the samples hindered metaphase testing (Norppa and Makipaakkanen, 1987).

Another work in Egypt focussed on effects of Cadmium (Cd^{+2}) toxicity on vegetative parameters of cowpea seedling and biochemical and physiological changes. It was established that treating cowpeas seedlings with Cd^{+2} lead to decrease in fresh weight, dry weight and growth rates. The Cd^{+2} treatments also caused changes in chloroplast structure and the chlorophyll levels. The Cd^{+2} stress decreased leaves water content, cell membrane

stability index and total proteins. Cd^{+2} also affected antioxidants such as glutathione S-transferase, catalase and glutathione reductase activity. Therefore, parameters measured can act as bio-indicators of the negative effect of Cd^{+2} stress (El-Kafafi *et al*., 2017).

One study was also done to assess the production oxidative stress due to exposure to pharmaceutical effluent water in rats and their response to vitamin E treatment. The rats were placed into five groups with negative control. Pharmaceutical effluent water (PEW) was treated as follows; PEW 100% + Vitamin E, PEW 100 %, PEW 10 %, PEW 1 %. The Oxidative stress in rats was assessed using the levels of catalase (CAT), superoxide dismutase (SOD) and hydrogen peroxide (H_2O_2) in the body organs. The exposure significantly reduced the SOD, CAT and H_2O_2 levels in the body. However, increased levels of H_2O_2 were found in vitamin E exposed rats. In conclusion, pharmaceutical effluent water caused serious oxidative stress in the Wistar rats and some histo-pathological lesions the in vital body organs meaning it's highly toxic (Sharif *et al*., 2016).

A study was done to investigate, heavy metal levels in vegetables grown on Nakivubo wetland area of Lake Victoria, in Uganda. The heavy metals contents in vegetables from farm lands was analysed using wet acid digestion method with a recovery of 90 %. The results revealed that though heavy metal concentrations of cadmium, zinc, manganese, and lead were mostly higher than the ones in similar vegetables collected from rural areas that were used as control sites, the metals of Cadmium and Lead were above the maximum permissible levels provided for by World Health Organisation (WHO). Thus lead and Cadmium levels were a threat to human health and therefore it was recommended that pollution control measures be employed (Mbabazi *et al*., 2010).

In India, plastic ware materials for babies were assessed for mutagenicity using *S. typhimurium*, viz., Ames bacterial method. They also went further to investigate element

migration in baby plastic material so as to demonstration evidence leaching of Lead, Bisphenol A (BPA) and Di-2 ethyl hexyl phthalate (DEHP) from these materials. Their findings showed that these materials have a highly significant ability to initiate mutagenicity. It was then concluded that, plastic ware material for babies can have mutagenic effect on the infants and should be removed as materials for babies (Nepalia *et al*., 2018).

From available research, no work has been done on mutagenicity and geno-toxicity of industrial wastewater in Uganda and East Africa. Furthermore, limited studies exist in literature reporting the mutagenic properties of industrial effluent and their ability to form toxic by-products (Cevik *et al*., 2020 ; Mathur, 2007) and mutagenic impact of the effluent on especially plants or animals themselves (Claxton *et al*., 1998). Furthermore, most study done in these areas focused on industrial wastewater of one sector in a particular area and on specific toxicants with synergic effects missing (P. Zhang *et al*., 2020). This study focused specifically on assessment of the mutagenic effect of selected industrial effluent from Kinawataka drainage area on germination, growth and yield parameters of cowpeas.

CHAPTER THREE: MATERIALS AND METHODS

3.1 The study area

Kinawataka wetland is 5 kms east of Kampala City with map coordinate grid system of 457 800, 39750 to 462 500, 33500 (Mafabi *et al*., 1995). According to Ministry of Lands, Housing and Urban Development in 1993 kinawataka had 187 hectares marked as industrial areas which continues to increase (Matagi, 2001). It protects the inner Murchison bay from pollution due to catchments of Ntinda, Kyambogo, Kireka, Nakawa, Naguru, Mbuya, Luzira, Mutungo, and Butabika (Paul, 2011). The wetland has more than 4000 households, which makes it as the most populated wetland in Uganda (Kakuba and Kanyamurwa, 2021). Emphasis are directed to the fact that by 1993, Nakawa Ntinda, West Bugolobi, Kinawataka had 499 hectare covered by industries (Matagi, 2001). The industries established at the drainage area include; plastics, pharmaceuticals, aluminium products and paints (Walakira and Okot-okumu, 2011).

Kinawataka wetland decreased by 46 % from 1992 to 2013 with recent records of land area estimated at 1.5 km^2 (Kakuba and Kanyamurwa, 2021). However, its' being degraded through marram filling, infrastructure development, direct toxic industrial effluents dumping, and cultivation of crops such as yams, sweet potatoes, banana plantations and sugar cane. The pollution resulting from industrial development is unquestionable especially given the current infringement by industries increasing from 9 % to 12 % from 2015 to 2018 (Kakuba and Kanyamurwa, 2021). Hence, out of all wetlands in Uganda, Kinawataka has faced the greatest degradation, limiting its ability to perform its detoxification functions; this therefore it was very crucial to carry the current study at this location.

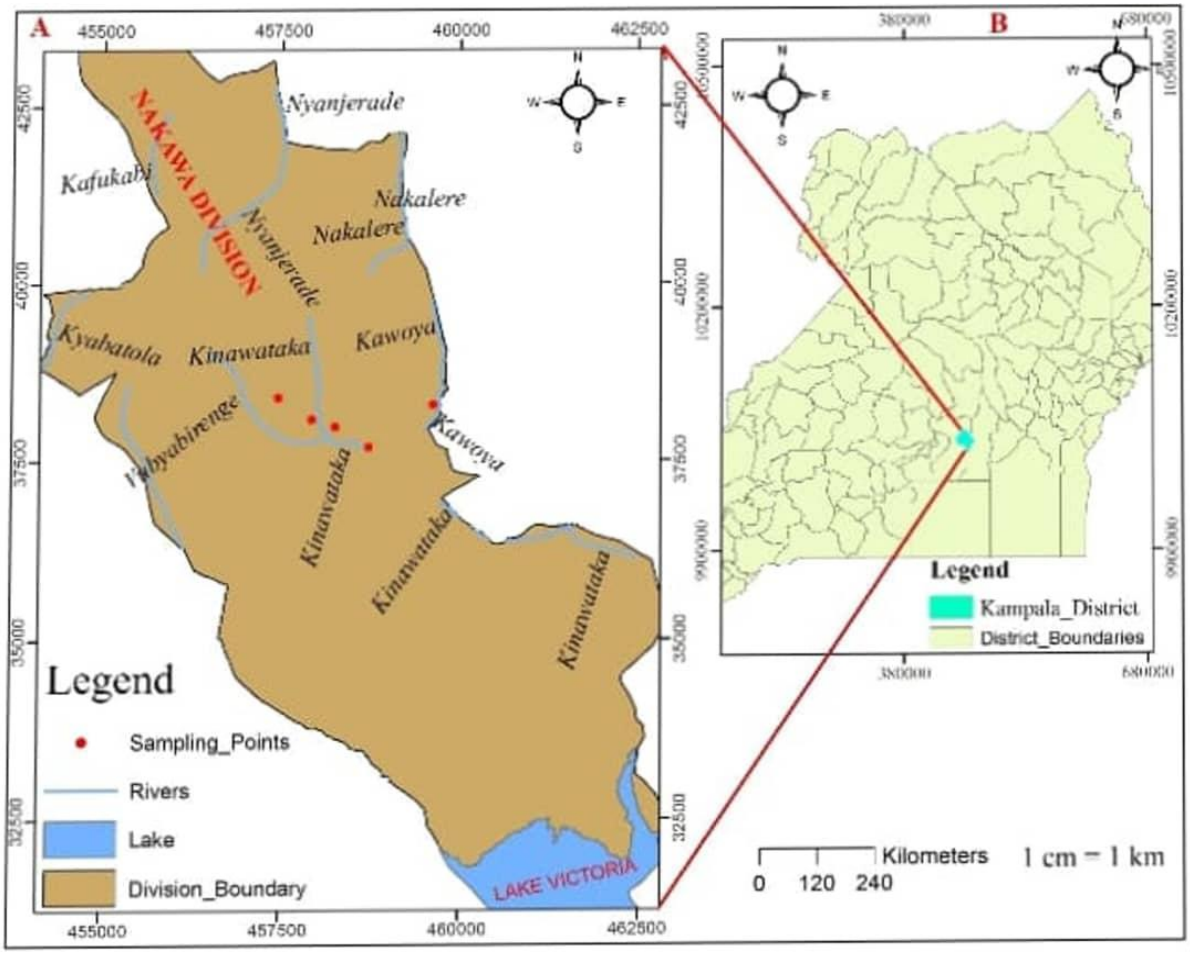


Figure 3.1: Map of the study area (A: Nakawa Division and sampling points; B: Location of Nakawa Division on the Map of Uganda)

3.2 Research Design

The research applied descriptive and experimental research design approach. For objective one, a simple experimental research design was used. The samples were analysed with negative, positive controls in triplicates to ascertain reproducibility and credibility. For objective two and three, a completely randomized design (CRD) experimental research design was used to cater for homogeneity. Treatments were randomized over the whole experimental plot with each treatment consisting of 3 replicates. Treatments bags were properly labelled according to their name and replicate number. Treatment was then randomized over the whole plot, each bearing an identification tag as in

Table 3.1: Experimental layout of completely randomised research design for planting cowpeas seeds**.** Field experiments were done in a greenhouse at Namataba Town Council, Mukono district and the laboratory analysis was done at National Water and Sewerage Central Laboratory at Bugolobi, Kampala.

Table 3.1: Experimental layout of completely randomised research design for planting cowpeas seeds

1	A0a	E4a	B1c	G2c	D4b	C1a
2	F1b	C4b	D1b	G1a	B4b	E1a
3	Aob	E4c	B1b	G4a	D2b	C2a
4	C1c	D3b	G1b	B2c	E3a	F4a
5	F1c	Aob	E2b	B3b	G2c	D2a
6	B2a	E3c	Aoc	F2b	C2c	C3b
7	G1c	D3c	C3a	F3a	E2a	B3c
8	E1b	Aoc	F2c	C2b	D2b	G4a
9	B2b	D3a	F3b	F2a	G3b	G1a
10	F3c	D4c	G2a	B3a	C3c	E2c
11	AOb	E3b	C4a	B4c	G3c	D1a
12	G2b	B1b	C1b	E4b	AOC	F4b
13	D4a	F1a	Aoa	E1c	C4C	B4a
14			F4c	D1c	G3a	

3.3 Industrial Effluent Samples and Sampling

3.3.1 Industrial Effluent Samples

Five industrial effluent were collected from those industries categorised as “Class 1” industries by Birikadde (2017) basing on high risk of toxicity from Kinawataka drainage area. These industries are shown in table 3.2 below.

Table 3.2: GPS coordinates of the sources of industrial effluents used from the study

CODE	INDUSTRY TYPE	EASTINGS	NORTHINGS
Ph	Pharmaceuticals	0.3404533	32.618415
Al	Aluminium products	0.3366683	32.621822
Ma	Mattress	0.3415433	32.617087
Pa	Paint	0.3434350	32.639333
Pl	Plastics	0.3412467	32.617648

The plastic industry mostly deals in High Density Polyethene (HDPE), Low Density Polyethene (LDPE), Polypropylene (PP) and Polyvinyl Chloride (PVC) (Birikadde, 2017).

3.3.2 Industrial Effluent Sample Preparation

The industrial effluent treated samples were picked using clean acid washed and distilled water rinsed dry bottles, and kept in cold cool box. The samples immediately transported to the laboratory and kept at 4°C inside a refrigerator.

The samples were diluted with distilled water to four levels 25 % (1:3), 50 % (1:1), 75 % (3:1) and 100 % (1:0). The values in appendices depict ratio of effluent to distilled water. These were labelled in the field as $B_1$25 %, $B_2$50 %, $B_3$75 % and $B_4$100 % for the first industrial effluent and others followed a similar pattern. The negative control was distilled water, designated as dilution water (DW). The mutagen or positive control was sodium azide solution of the following concentrations; 0.016 %, 0.031 %, 0.125 %, and 0.250 % w/w NaN_3 in solution of distilled water.

Table 3.3: The different dilutions of industrial effluents

A0 = (CP + DW)	C2 = (CP + Al 50%)	D4=(CP + Ma 100%)	F2= (CP + Pl 50%)
B1 = (CP + Ph 25%)	C3 = (CP + Al 75%)	E1= (CP + Pa 25%)	F3= (CP + Pl 75%)
B2 = (CP + Ph 50%)	C4 = (CP + Al 100%)	E2= (CP + Pa 50%)	F4 = (CP + Pl 100%)
B3 = (CP + Ph 75%)	D1 = (CP + Ma 25%)	E3 = (CP + Pa 75%)	G1=(CP + NaN_2 0.016 w/w)
B4 = (CP+ Ph 100%)	D2 = (CP + Ma 50%)	E4= (CP + Pa 100%)	G2=(CP + NaN_2 0.031 w/w)
C1 = (CP+Al 25%)	D3 = (CP + Ma 75%)	F1= (CP + Pl 25%)	G3 = (CP + NaN_2 0.125 w/w)
			G4=(CP+NaN_2 0.250 w/w)

The effluents samples were as follows; Negative Control or Distilled Water (A), industrial effluent from Pharmaceuticals (B), Aluminium Products (C), Mattresses Manufacture (D), Paint Industry (E), Plastics Industry (F) and Positive Control or Sodium Azide (G), as shown in table 3.3 the different dilutions of industrial effluents.

Where CP = Cowpea, A to G were the different treatments, Ph, Al, Ma, Pa, Pl and represent industrial effluent of Pharmaceuticals, Aluminium Products, Mattresses Manufacture, Paint, Plastics and Sodium Azide with their dilutions in relation to distilled water.

3.4 Determination of mutagenicity of Selected Industrial Effluent

The mutagenicity test was done using Ame's test kit for testing of water and effluent waters for presence of possible mutagenic compounds. The Ames – 384 ISO procedure version 1.1 method was used. The different concentrations of samples in Table 3.3: The different dilutions of industrial effluents were analysed in triplicates using *Salmonella typhimirium* strains of TA98 and TA100.

During the Ames mutagenicity test, the *S. typhimirium* strains TA98 and TA100 were exposed to different industrial effluents. The first 24 wall plates were used for exposure and the second 24 well plate were used for reversion. 24 well plate shown in appendix VI Photography Objective I. Mutagenicity (4). These were then scored in the 384 well plates the one shown in plate 4.1 below. First two rows consist of 48 wells was filled by one sample. The yellow wells were scored as positive whereas the purple wells were scored as negative. The scoring and results interpretation is better illustrated in Appendix IV (b) results interpretation.

For instance, in the figure 4.1 below, the first 48 wells had 3 (three) yellow wells which were counted as positive revertants and 45 purple wells which were counted as negative wells. The fourth 48 wells below had 47 yellow revertant wells which were counted as positive revertants and one purple as negative revertant. Therefore, for each effluent in Table 3.2, the different dilutions of samples were pipetted in 48 wells and the revartants counted manually.

Yellow Revertant Positive Well

Negative Purple non Revertant well

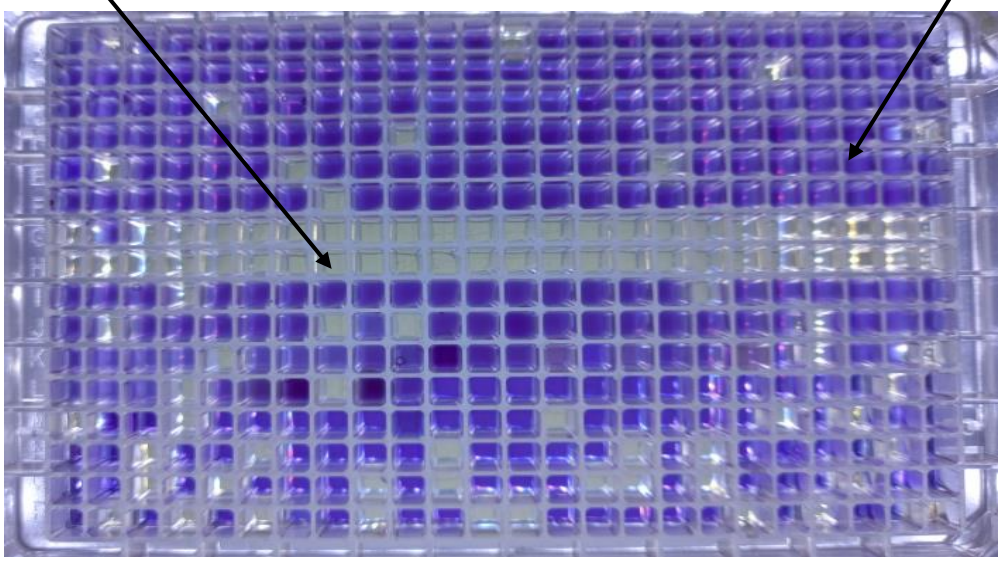


Figure 3.2: 384 – well plate showing negative wells in purple and positive wells in yellow.

Procedure for determination of mutagenicity using Ame's test kit:

The *S. typhimurium* bacterial strains TA98 and TA100 was mixed with Reagent V and Nutrient Broth and incubated overnight. The overnight bacteria were diluted using exposure media. The exposure solution made using (D-Biotin, L-histine and 40 % D-Glucose) was then transferred to 24-well plate. Negative, positive and sterile controls and samples were also transferred to their respective wells. The Bacteria Strains were also transferred to the 24-well plate. The mixture was then incubated at 37 ^{o}C for 100 minutes. Reversion solution (40 % D-Glucose, Bromocresol Purple, D-Biotin) was prepared and filled in 24-wall Reversion Plate. Bacteria were then transferred from exposure plate to 24-well reversion plate. The bacteria-reversion mix was then transferred to 384 well plates. The 384 well plates were incubated at 37 ^{o}C for 2 days. The plates were simply scored visually by counting, yellow and partial yellow wells as positive and purple wells as negative revertants. The schematic summary of the procedure is summarised in Appendix IV (a); Ames – 384 ISO Procedure Version 1.1

3.5 Assessment of the effect of industrial effluent on germination and Seedling growth of cowpeas

3.5.1 Preparation of the soil

Predominantly loam top soil less than 10 cm deep was collected for use in the study. The soil was sun dried, and 5 kgs were measured into palm nursery polybags 30 cm and 23 cm in height and diameter respectively. Holes were made at the bottom and left for one week before sowing of the seeds. The bags were buried three-quarters into the soil in the green house. The bags were irrigated with 200 ml water daily in evening, and cleared of any unwanted weeds during the period under fallow. Fallowing was done to let the soil recover

production potential and minimise the level of pests. The bags were placed on the field at a spacing of 60 cm x 30 cm, as proposed by Ikhajiagbe, (2012).

3.5.2 Pre-treatment of Seeds with Selected Industrial Effluent

The cowpeas *Vigna unguiculata* L variety SECOW 2W named by National Agricultural Research Organization (NARO) was purchased from Pearl Seeds. This particular species was used because it is the most common grown in the area of the study (Afutu *et al*., 2016). For chemical treatment, the seeds were pre-soaked in distilled water for 9 hours and transferred to Ph25 %, Ph50 %, Ph75 % and Ph100 % for the first industrial effluent then others followed a similar pattern for 6 hours at room temperature of 25 ± 2 ^{0}C (Raina *et al*., 2018). This was followed by washing of seeds in running water for one minute to remove excess chemicals.

3.5.3 Sowing of Seeds

The pre-soaked seeds were sown directly into soil in potted bags. Planting was carried out in the evening, towards sunset so as to give the seedling time to acclimatise with the soil overnight. Three seeds were sown per bag at a depth of 3cm following a method used by Njoku *et al*., (2009). Weeding was done depending on need by hand picking the weeds. 200ml tap water was applied per bag beyond sunset to cater for water needs since planting was done under screen house.

3.5.4 Measurement of germination and seedling growth parameters of cowpeas (*V. unquiculata L*)

The following parameters were assessed after seven days of planting as proposed by (Ikhajiagbe, 2012);

Percentage germination (%)

Three seeds of *V. unguiculata* were sown into each bag and the percentage germination was calculated seven days after sowing using method adopted from (Njoku *et al*., 2009). The mean percentage germination for three bags was found using the formula below;

$$\text{Percentage germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total Number of seeds planted}} \text{X100 \%}$$

Radical length (cm)

The Three seedlings were uprooted after seven days from the bag and the plant was stretched straight, the total root length of sprouted seedling was measured using a ruler and mean value for the three bags recorded (Maity *et al*., 2018).

Fresh weight of sprouted seedlings (g)

The Three seedlings were uprooted from the bag, then the fresh weight of the seedlings was directly measured using analytical balance following a method used by (Giridhar *et al*., 2020). The weighing dish was placed on the analytical balance, zeroed and the seedlings placed inside the dish. The wet weight was taken directly. The mean weight for three bags was recorded.

Dry weight of seedlings (g)

The dry weight of seedlings was established by method used by Iqbql and Siddiqui (2015). Aluminium foils were cut into small sizes, dried in oven for 1 hour and weight recorded as W1, the seedling were place in them and dried at 80 ^{0}C for 24 hours, cooled to room temperature in a desiccator the total dry weight recorded as W2. The dry weight of sprouted seedlings calculated using formula below. The mean dry weight from the

recorded results for number (n) seedlings analysed was calculated using the equation below;

$$\text{Mean Dry Weight} = \frac{\sum \text{W2} - W1}{n}$$

The specifications of the research instruments for assessment of the parameters above are highlighted in Appendix I.

3.6 Determination of the effect of industrial effluent on growth and yield parameters of cowpeas

3.6.1 Preparation of the soil

Predominantly loam top soil below 10 cm deep was collected for use in the study. The soil was sun-dried, and then 5 kg of soil measured into palm nursery polybags 30 cm in height and 23 cm in diameter. Holes made at the bottom and left for one week before sowing of the seeds. The bags were buried three-quarters into the soil in the green House. Bags irrigated with 200 ml water daily in evening, and were cleared of any unwanted weeds promptly during the period under fallow. Fallowing was done to let the soil recover production potential and minimise level of pests. The bags were placed on the field at a spacing of 60 cm x 30 cm, as proposed by Ikhajiagbe, (2012).

3.6.2 Pre-treatment of Seeds with Selected Industrial Effluent

For chemical treatment, the seeds were pre-soaked in distilled water for 9 hours and transferred to B_1 25 %, B_2 50 %, B_3 75 % and B_4 100 % for the first industrial effluent then others followed a similar pattern for 6 hours at room temperature of 25 ± 2 ^{0}C (Raina *et al*., 2018). Then this was followed by washing of seeds in running water for one minute to remove excess chemicals.

3.6.3 Sowing of Seeds

The pre-soaked seeds were sown directly into the soil. Planting was carried out in the evening, towards sunset. Three seeds were sown per bag at a depth of 3 cm. Weeding was done depending on need. 200 ml tap water was applied per bag beyond sunset to cater for water needs since planting was done under screen house (Ikhajiagbe, 2012).

3.6.4 Measurement of germination and seedling growth parameters of cowpeas (*V. unquiculata*)

During growth and after harvesting, the parameters of the growth and yield; leaf area, number of root nodules, dry nodule weight, shoot length, number of pods, number of seeds, dry weight of seeds, days to maturity and dry weight of roots made were assessed as proposed by Ikhajiagbe, (2012).

Shoot Length (cm)

Shoot length was established by a method used by Smith *et al*. (1998). After harvesting the Three cowpeas' plants after maturity, the shoot was stretched straight on a board and the shoot length measured from main primary stem, base to apex using a ruler and the mean results for the three bags considered.

Leaf Area (cm^2)

Leaf area of five different leaves of harvested mature plant was measured independently. This was done by measuring maximum length and maximum width of the leaf and mean for three bags recorded using a method used by (Gowthami and Suchandranath, 2022) as follows;

Leaf Area in cm^2 = Maximum Length X Maximum Width X 0.747

No of root nodules per plant

The polybags were torn, and the loam soil on the root was carefully removed care taken to avoid the rood nodules from breaking off. The total number of root nodules was then counted directly from the harvested plants. The mean for three bags was recorded (Labbo *et al*., 2023).

10 dry nodule weight (g)

Ten (10) root nodules from mature cowpeas plant were dried at 80 ^{o}C for 24 hours and the weight measured using analytical weighing balance. The mean for plants in three bags were recorded (Iqbql and Siddiqui, 2015).

Number of pods per plant

The number of pods per plant were counted directly from the mature cowpeas plant and the mean result for three bags recorded (Giridhar *et al*., 2020).

100 dry seeds weight (g)

The dry seeds weight was determined by drying the seeds from mature cowpeas plants in an oven at 80 ^{0}C for 24 hours (Iqbql and Siddiqui, 2015). The mean value for seedlings in three bags was recorded. Aluminium foils were cut into small sizes, dried in oven for 1 hour and weight taken as W1, the 100 seeds were placed in it and then dried in the oven at 80 ^{0}C for 24 hours, cooled to room temperature in a desiccator the weight taken as W2. The dry weight of sprouted seedling calculated using the formula below;

$$\text{Dry Weight} = \text{W2} - W1$$

Number of seeds per pod

The seeds in mature pods on the plant were counted and averaged for that particular plant. Then the mean for the three cowpeas in a bag recorded, and the mean for the three triplicate bags was calculated as described by (Angelotti *et al*., 2020).

The specifications of the research instruments used for assessment of the parameters above are highlighted in Appendix I.

3.7 Data Analysis

Data was compiled in Microsoft Excel and statistically analyzed with IBM SPSS Statistic 27.

For objective one, mutagenicity as proposed by Levy *et al*., (2019), results were considered positive if; there was a concentration related rise in revertants in at least one concentration compared to the concurrent solvent control or otherwise considered negative. Significance of increase was assessed using Table 5.1: The statistical results significance as provided for by the Ames 384 test kit at (P>0.05) provided for by the Ames 384 test kit at (P>0.05) in appendix V.

For objective two and three, differences between means of germination, growth and yields parameters for different treatment were analyzed using two way ANOVA in R – Program. Two way ANOVA was used because the data collected had two independent variable of industrial effluent and the different dilutions. Lenene's test of equality passed normality test as in Table 5.2: Shows results for levene's test of equality for normality test. Null hypothesis was rejected if $p > 0.05$. The significant effect of industrial effluent was shown using superscripts.

3.8 Ethical considerations

No ethical issues exist in this study. Authorization was sort from Kyambogo University department of Biological Sciences to proceed with this project. The introduction letter is attached in appendix II. Permission to conduct the research was sort from research and development manager of National Water and Sewerage Corporation. Authorization latter attached in Appendix III.

CHAPTER FOUR: RESEARCH FINDINGS AND DISCUSSIONS

4.1 Research Findings

4.1.1 Mutagenicity of Selected industrial effluent

The figures 4.2 to 4.6 below shows the positive revertant *Salmonella typhimurium* wells for TA98 and TA100 Strains when treated using industrial effluent with +S9 Activation. The percentages show the quantity of the effluent in the solution in relation to distilled water. For example, 50 % concentration means ratio of 1:1 of distilled water to industrial effluent. The revertants were not uniform because the sensitivity of *S. typhimurium* TA98 and TA100 Strains vary depending on level of mutagenicity of industrial effluent. The white stars in the bars indicate significant revertants using quick reference chart in Table 5.1: The statistical results significance as provided for by the Ames 384 test kit at (P>0.05). The pharmaceutical effluent exhibited medium mutagenicity compared to other effluents with significant revertants at dilutions of 75 % above. However, the *S. typhimurium* TA100 Strain was more sensitive with 19 (1.41) revertants compared to 13 (2.12) for *S. typhimurium* TA98 Strain for 100 % of the effluent out of 48 revertants.

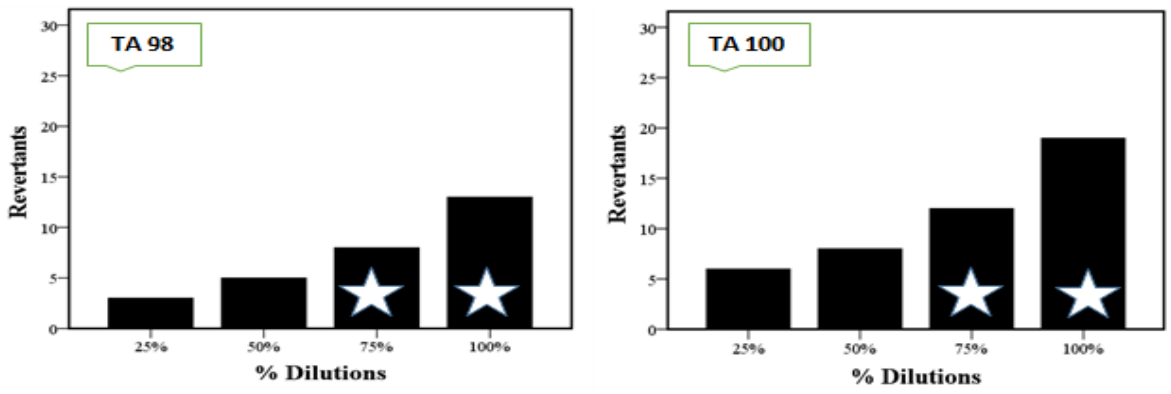


Figure 4.1: Number of positive revertants of *Salmonella typhimurium* strains exposed to pharmaceutical effluent.

The aluminium products industry effluent exhibited the lowest mutagenicity with significant revertants at only undiluted effluent. The *S. typhimurium* TA100 Strain was

however, more sensitive with 12(1.41) revertants compared to 8.5 (0.00) for *Salmonella typhimurium* TA98 Strain for undiluted form of the effluent.

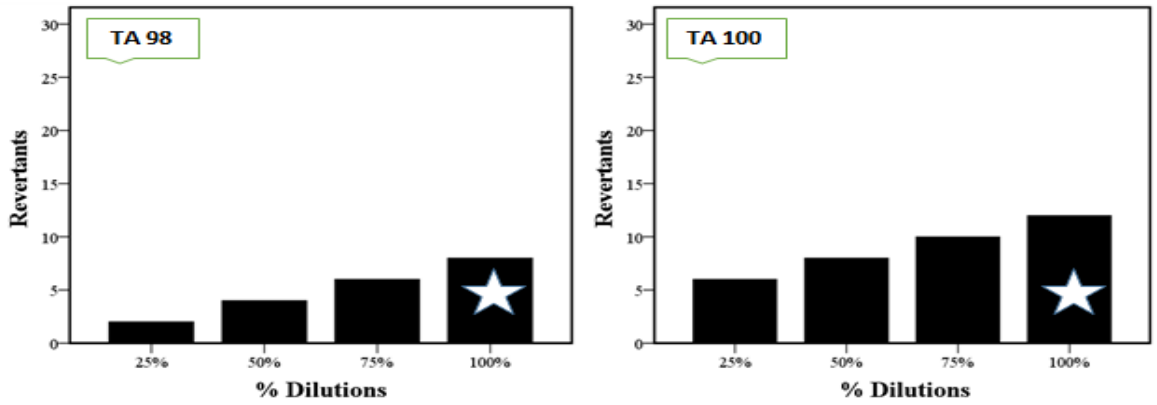


Figure 4.2: Number of positive revertants of *Salmonella typhimurium* strains exposed to aluminium products industry Effluent

The mattress industry effluent exhibited the high mutagenicity with significant revertants at dilutions of 50 % and above. However, the *S. typhimurium* TA100 Strain was more sensitive with 32(1.41) revertants compared to 24.0 (1.41) for *S. typhimurium* TA98 Strain for pure form of the effluent.

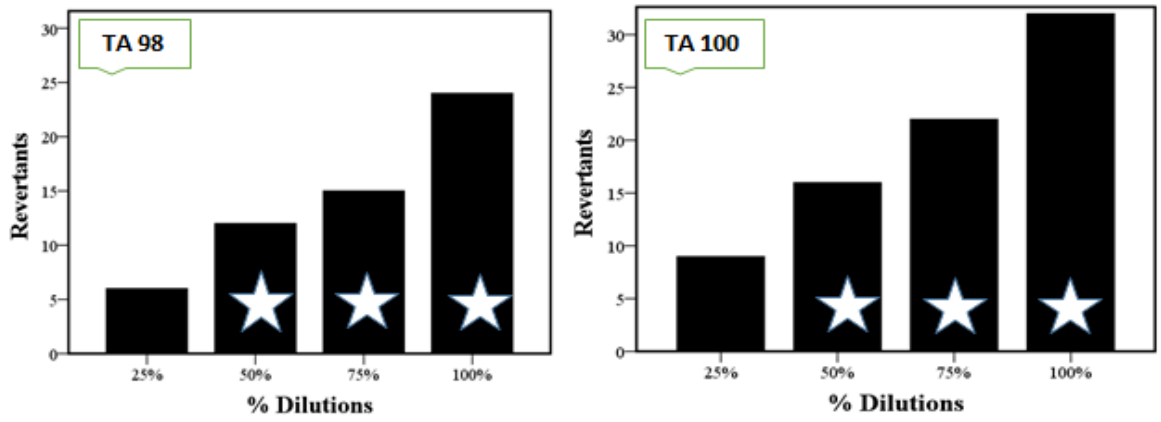


Figure 4.3: Number of positive revertants of *Salmonella typhimurium* strains exposed to mattress industrial effluent

The paint industry effluent exhibited above average mutagenicity with significant revertants at dilutions of 50 % or more for *S. typhimurium* TA98 and 75 % or more for *S. typhimurium* TA100 Strains. The *S. typhimurium* TA100 Strain was however, more sensitive with 18(1.41) revertants compared to 15.0 (1.41) for *S. typhimurium* TA98 Strain for pure form of the effluent.

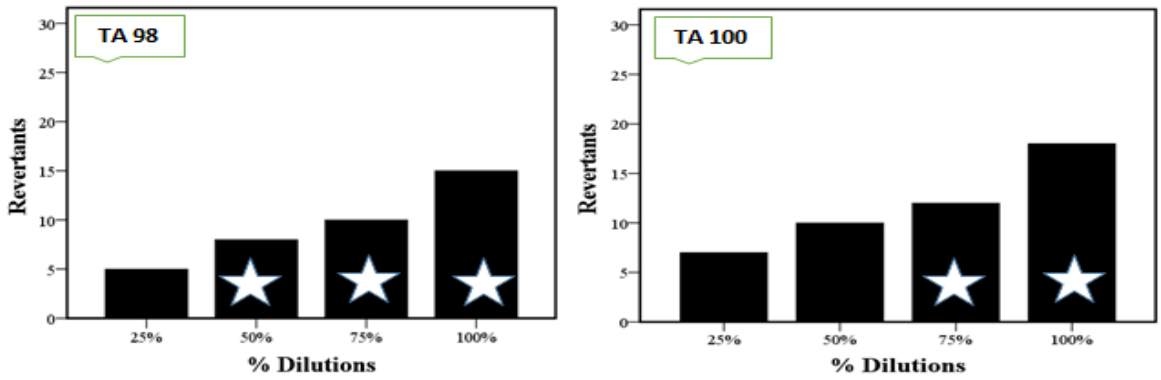


Figure 4.4: Number of positive revertants of *Salmonella typhimurium* strains exposed to paint industry effluent

The plastic industry effluent exhibited high mutagenicity with significant revertants at dilutions of 50 % and above for *S. typhimurium* TA98 and TA100 Strains. However, the *S. typhimurium* TA100 Strain was more sensitive with 27.0 (1.41) revertants compared to 20.5 (1.41) for *S. typhimurium* TA98 Strain for pure form of the effluent.

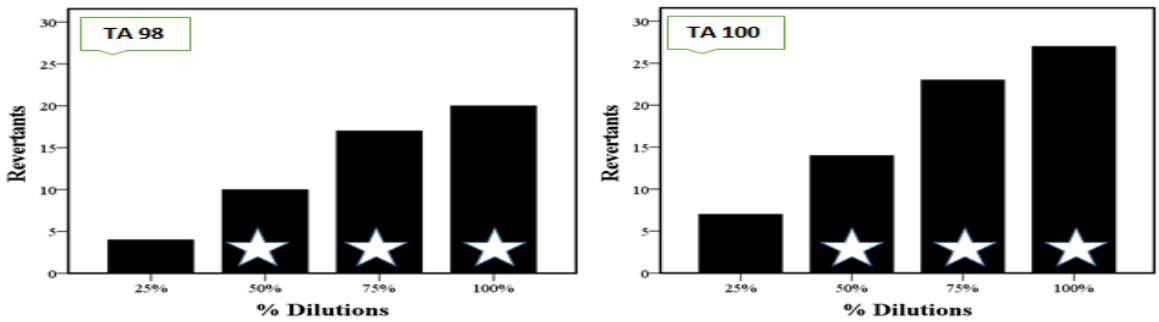


Figure 4.5: Number of positive revertants with *Salmonella typhimurium* strains exposed to plastic industry effluent

Table 4.1 shows the summary of observations made of the revertants when the *S. tyhimurium* TA98 and TA100 Strains were exposed to four different dilutions of industrial effluent. The positive control of 2-Nitroflourene (NF) for *S. tyhimurium TA 98* had 46 (0.0) revertants and nitroquinoline-N-Oxide (4NQO) for *S. tyhimurium* TA 100 had 45.5 (0.71) revertant. The distilled water which was used as negative control had 1.75 (0.5) and 3.75 (0.5) revertants *S. tyhimurium TA 98 and TA 100* respectively. The Asterix (*) show the statistically significant results at 95 % confidence interval as per Table 5.1: The statistical results significance as provided for by the Ames 384 test kit at (P>0.05), from the Ames test kit

Table 4.1: Mean (SDV) number of revertant wells after treatment of TA 98 and TA 100 *Salmonella typhimirium* strains with the different industrial effluents

Sample	Identity	Dilutions	Number of Revertant Wells	
			TA 98	**TA 100**
Distilled Water	A_0	0	1.75 (0.5)	3.75 (0.5)
Pharmaceuticals (B)	B1	25 %	3 (0.71)	5.5(0.71)
	B2	50 %	5 (1.41)	8.5(0.71)
	B3	75 %	8 (0.71) *	12 (0.00) *
	B4	100 %	13 (2.12) *	19 (1.41) *
Aluminum Products (C)	C1	25 %	2.5 (0.71)	5.5 (0.71)
	C2	50 %	4.5 (2.12)	7.5 (0.71)
	C3	75 %	6.5 (0.71)	9.5 (0.71)
	C4	100 %	8.5 (0.00) *	12 (1.41) *
Mattresses (D)	D1	25 %	6.0 (1.41)	9.0 (1.41)
	D2	50 %	11.5(0.71) *	16.0(1.41) *
	D3	75 %	15.0 (1.41) *	22.5(1.41) *
	D4	100 %	24.0 (1.41) *	32.0(1.41) *
Paint (E)	E1	25 %	5.0 (1.41)	7 (1.41)
	E2	50 %	8 (1.41) *	9.5 (3.54)
	E3	75 %	10.5 (0.71) *	12.0(4.24) *
	E4	100 %	15.0 (1.41) *	18.0(1.41) *
Plastics (E)	F1	25 %	4 (1.41)	7.0 (1.41)
	F2	50 %	10 (1.41) *	14.5(0.71) *
	F3	75 %	17 (1.41) *	23 (23.0) *
	F4	100 %	20.5 (0.71) *	27.0(1.41) *
Positive Controls	2NF		46 (0.00)	
	4NQO			45.5(0.71)

The results in the table above show the mean and standard deviations (in parentheses).

The Asterix (*) indicate the means were significance at $p \leq 5\%$.

4.1.2 The effect of industrial effluents on germination and seedling growth of cowpeas

Figure 4.7 to 4.10, the effect of different industrial waste on; shoot length, root length, wet weight of seedlings and dry weight of seedlings were assessed. The percentages show the quantity of the effluent in the solution in relation to distilled water for example 50 % stands for a ratio of 1:1 of distilled water to industrial effluent. The bars were a representation of the magnitude of the effect of industrial effluent on germination and seedling growth parameters of cowpeas.

The shoot length was highly affected by the mattress industry effluent reducing it to 1.55 (0.71) cm for undiluted effluent close to 1.45 (0.35) cm for positive control, Sodium Azide followed by pharmaceutical industry effluent at 2.75 (0.67) cm. The plastic industry waste had the least effect on shoot length with the 25 % effluent increasing the shoot length to 8.52 (2.09) cm significantly higher than negative control of 6.19 (0.97) cm.

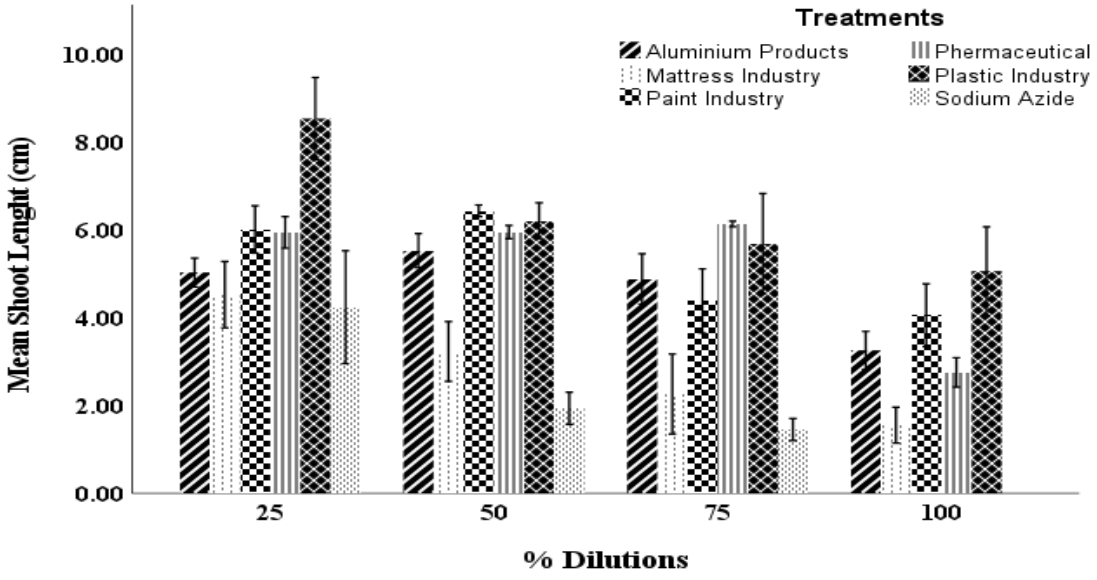


Figure 4.6: Shoot length of one week old cowpeas seedlings in different treatments of industrial effluents

The root length ranged from 13.35 (1.87) in the blank control and 2.05 in 0.125 % w/w sodium azide. It was highly affected by the mattress industry effluent reducing it to 6.02 (1.42) cm in pure waste, followed by pharmaceutical industry effluent at 7.55 (1.23) cm. On the contrary the plastic industry effluent led to an increase in root length with increasing dilution the 25 % effluent increasing the root length to 8.7 (3.24) and pure effluent increasing to 12.33 (0.61).

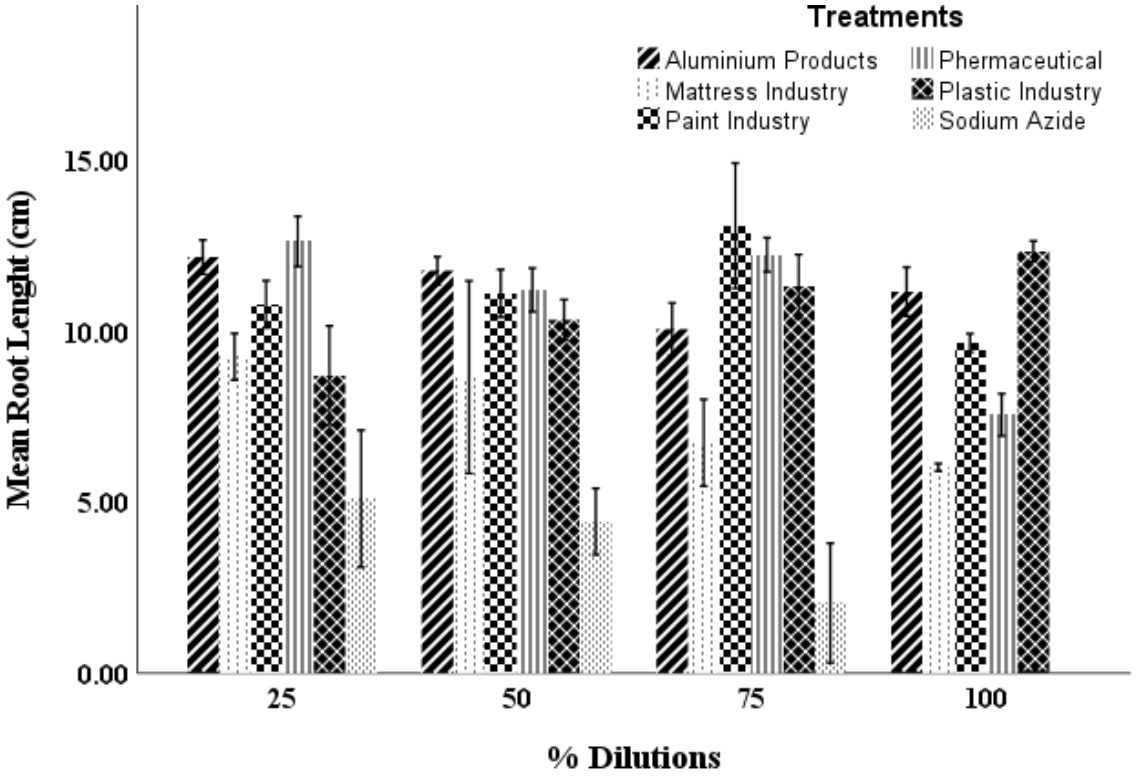


Figure 4.7: Root length of one week old cowpeas seedlings in different treatments of industrial effluents

The wet weight ranged between 1.41 (0.24) in plastic effluent and 0.69 (0.24) I mattress effluent. A higher significance was registered in mattress industrial waste where the pure form reduced the wet weight to 0.69 (0.24) followed by 0.99 (0.04) in the paint industry waste. Compared to 1.29 (0.19) for negative control and 0.58 (0.09) for 0.125 % sodium azide.

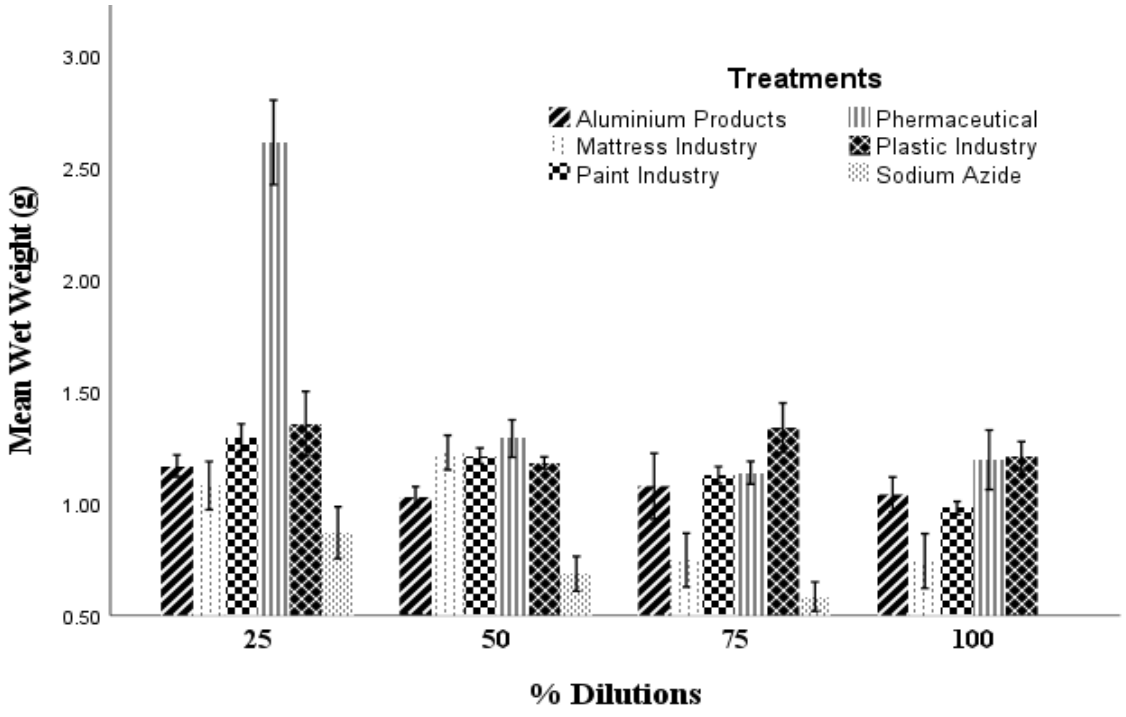


Figure 4.8: Wet weight of one week old cowpeas seedlings in different treatments of industrial effluents

The dry weight reduced in pharmaceutical effluent to 0.14 (0.03) grams compared to 0.18 (0.03) for negative control and paint industry effluent to 0.16 (0.03) grams. The plastic waste increased the wet weight to 0.23 and 0.25 in the 25 % and 50 % dilutions of the effluent.

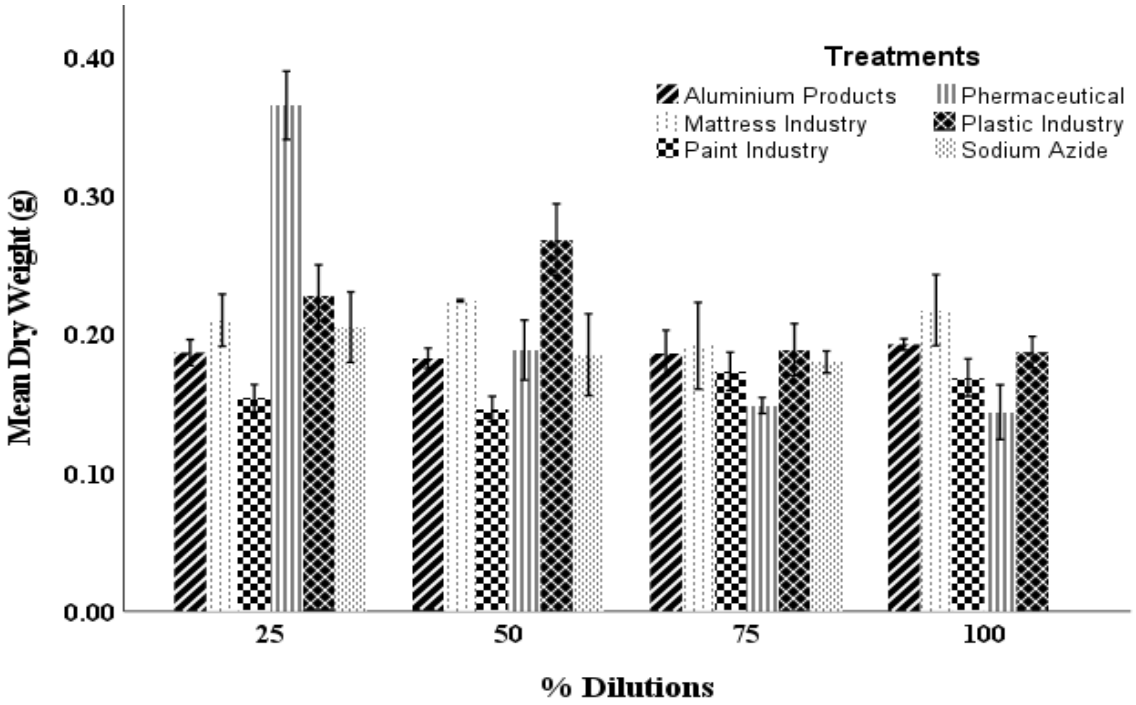


Figure 4.9: Dry weight of one week old cowpeas seedlings in different treatments of industrial effluent

Table 4.2 shows the summary of the characteristics of percentage germination, shoot length, root length, wet weight and dry weight made when treated with four different dilutions of industrial effluent from pharmaceuticals (B), aluminum products (C), mattresses (D), paint (E) and Plastics (E). Sodium Azide (G) a known mutagen was used as a positive control which was lethal at highest concentrations of 0.25 % w/w. The negative control distilled water had shoot length 6.19 (0.97), root length 13.35 (1.87), wet weight 1.29 (0.19) and dry weight 0.18 (0.03).

Table 4.2: The effect of industrial effluent on percentage germination and phenotypic characteristics of sprouted cowpeas seedlings

ID	Percentage Germination(%)	Shoot Length (cm)	Root Length (cm)	Wet Weight (g)	Dry Weight (g)
Ao	100.00	6.19 (0.97)	13.35 (1.87)	1.29 (0.19)	0.18 (0.03)
B1	100.00^{a}	5.93 (0.88)^{a}	12.62 (1.80)^{ab}	1.31 (0.41)^{cdef}	0.18 (0.05)^{abcd}
B2	83.33^{abc}	5.94 (0.34)^{a}	11.2 (1.43)^{bcdef}	1.25 (0.18)^{a}	0.18 (0.06)^{f}
B3	66.66^{cde}	6.13 (0.13)^{a}	12.23 (0.99)^{abcd}	1.15 (0.12)^{cdef}	0.15 (0.01)^{def}
B4	66.67^{cde}	2.75 (0.67)^{cde}	7.55 (1.23)^{ef}	1.15 (0.15)^{abcdef}	0.14 (0.03)^{ef}
C1	83.33^{abc}	5.02 (0.73)^{a}	12.16 (1.11) ^{abcde}	1.20 (0.12)^{bcdef}	0.19 (0.02)^{ab}
C2	83.33^{abc}	5.52 (0.87)^{a}	11.76 (0.91) ^{abcdef}	1.011 (0.11)^{ef}	0.19 (0.02)^{abcd}
C3	71.43^{abcde}	4.88 (1.14)^{a}	10.05 (1.54) ^{def}	1.14 (0.31)^{def}	0.19 (0.03)^{ab}
C4	66.67^{ab}	3.25 (0.87)^{bcde}	11.15 (1.42) ^{bcdef}	1.04 (0.18)^{ef}	0.19 (0.00)^{abc}
D1	85.71^{abcd}	4.52 (1.85)^{abc}	9.25 (1.67)^{ef}	1.08 (0.21)^{ef}	0.21 (0.03)^{bcdef}
D2	66.67^{cde}	3.23 (1.36)^{bcde}	8.65 (5.62)^{ef}	1.23 (0.19)^{abcd}	0.22 (0.00)^{cdef}
D3	66.67^{ab}	2.26 (1.82)^{e}	6.74 (2.53)^{ef}	0.75 (0.12)^{f}	0.19 (0.03)^{abc}
D4	50.00^{bcde}	1.55 (0.71)^{e}	6.02 (0.19)^{ef}	0.69 (0.24)^{f}	0.21 (0.05)^{bcdef}
E1	83.33^{abc}	6.00 (1.20)^{a}	10.78 (1.54)^{cdef}	1.30 (0.06)^{a}	0.15 (0.02)^{cdef}
E2	66.67^{cde}	6.43 (0.26)^{a}	11.10 (1.39)^{bcdef}	1.20 (0.06)^{abcde}	0.14 (0.02)^{def}
E3	66.67^{cde}	4.38 (1.46)^{ab}	13.08 (3.66)^{a}	1.12 (0.09)^{cdef}	0.18 (0.03)^{abcde}
E4	66.67^{cde}	4.05 (1.43)^{abcd}	9.65 (0.54)^{def}	0.99 (0.04)^{ef}	0.16 (0.03)^{abcdef}
F1	83.33^{abc}	8.52 (2.09)^{abcde}	8.7 (3.24)^{ef}	1.41 (0.24)^{abc}	0.23 (0.02)^{def}
F2	83.33^{abc}	6.20 (0.91)^{a}	10.32 (1.34)^{def}	1.19 (0.04)^{bcdef}	0.25 (0.07)^{f}
F3	83.33^{abc}	5.68 (2.54)^{a}	11.30 (2.08)^{bcdef}	1.29 (0.24)^{ab}	0.18 (0.04)^{a}
F4	66.67^{cde}	5.05 (2.02)^{a}	12.33 (0.61)^{abc}	1.18 (0.14)^{abcde}	0.19 (0.03)^{a}
G1	50.00^{e}	4.90 (2.02)^{a}	6.10 (3.46)^{ef}	0.92 (0.26)^{f}	0.21 (0.06)^{abcde}
G2	50.00^{e}	1.93 (0.64)^{e}	4.43 (1.68)^{ef}	0.71 (0.13)^{f}	0.19 (0.05)^{abc}
G3	33.33^{de}	1.45 (0.35)^{de}	2.05 (2.47)^{ef}	0.58 (0.09)^{f}	0.18 (0.01)^{abcde}
G4					
Mean	71.83	4.66	9.69	1.09	0.19
CV	31.59	26.93	20.67	14.73	17.35
LSD	5.0	2.01	3.75	0.38	0.064

The results in the table above were the mean and standard deviations (in parentheses) for 6 determinations. The superscripts show significant differences. The symbols representation was as follows; Negative control or Distilled Water (A), pharmaceuticals (B), aluminum products (C), mattresses (D), paint (E), Plastics (F) and Sodium Azide or Positive Control (G).

4.1.3 The effect of industrial effluents on growth and yield parameters of cowpeas

Figure 4.7 to 4.10 shows the effect of different industrial effluent on dry leaf area, shoot length, number of seeds pods per plant, dry weight of 100 seeds, days of maturity, weight of roots on cowpeas seedlings. The percentages show the quantity of the effluent in the solution in relation to distilled water for example 50 % means ratio of 1:1 of distilled water to industrial effluent. The bars are the representation of the magnitude of the effect of industrial effluent on the growth and yield parameters of cowpeas.

The leaf area was moderately negatively affected by the mattress industry effluent reducing it to 6.42 (0.11) cm^2 for undiluted effluent compared to negative control of 7.46 (0.71). This was closely followed by Aluminum products effluent at 6.33 (0.84) cm^2. The plastic industry waste had the least effect on leaf area with the undiluted effluent reducing the leaf area to 7.14 (0.19). Meanwhile the positive control of sodium azide reduced the leaf area to 6.48 (1.40) cm^2 at 0.031 % w/w concentration.

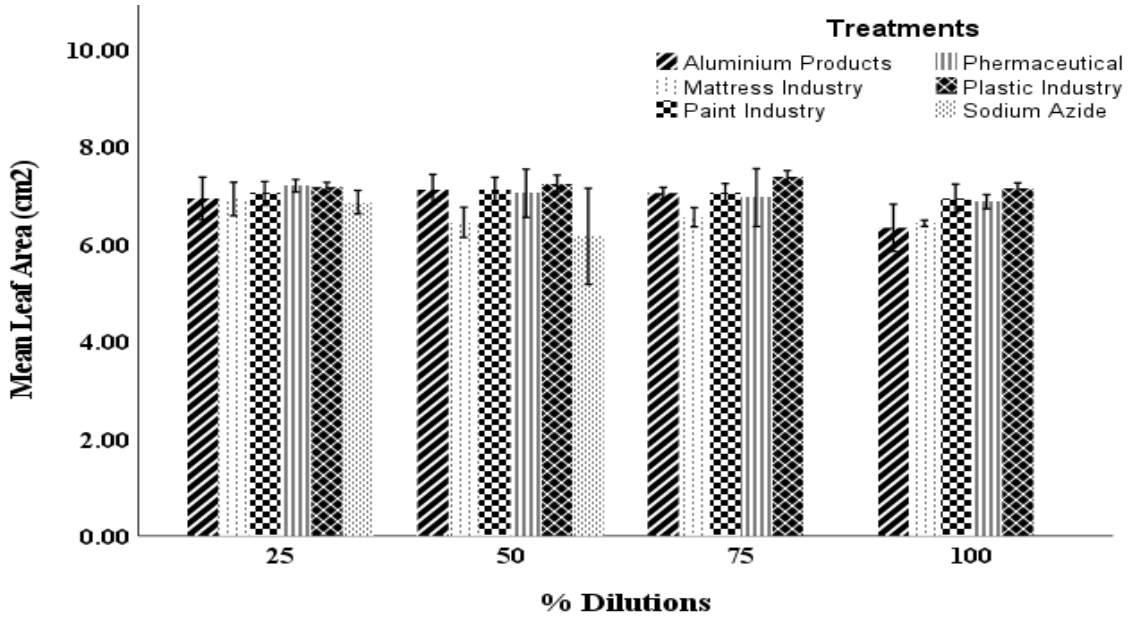


Figure 4.10: The leaf area of mature cowpeas plants exposed to different treatments of industrial effluent

The shoot length ranged between 88.67 (2.52) cm in 25 % dilution of plastic effluent and 67 (4.24) for positive control at 0.025 % w/w. The mattress industry effluent reduced it to 66.33 (2.52) and 72.00 (4.58) mm for 75 % and 100 % effluent respectively. This was followed by paint effluent at 72.00 (9.54) Aluminum products effluent at 6.33 (0.84) cm^2. The plastic industry waste had the least effect on leaf area with the pure waste reducing the shoot length to 80.33 (0.19) mm. The negative control had 82.33 (3.00) mm.

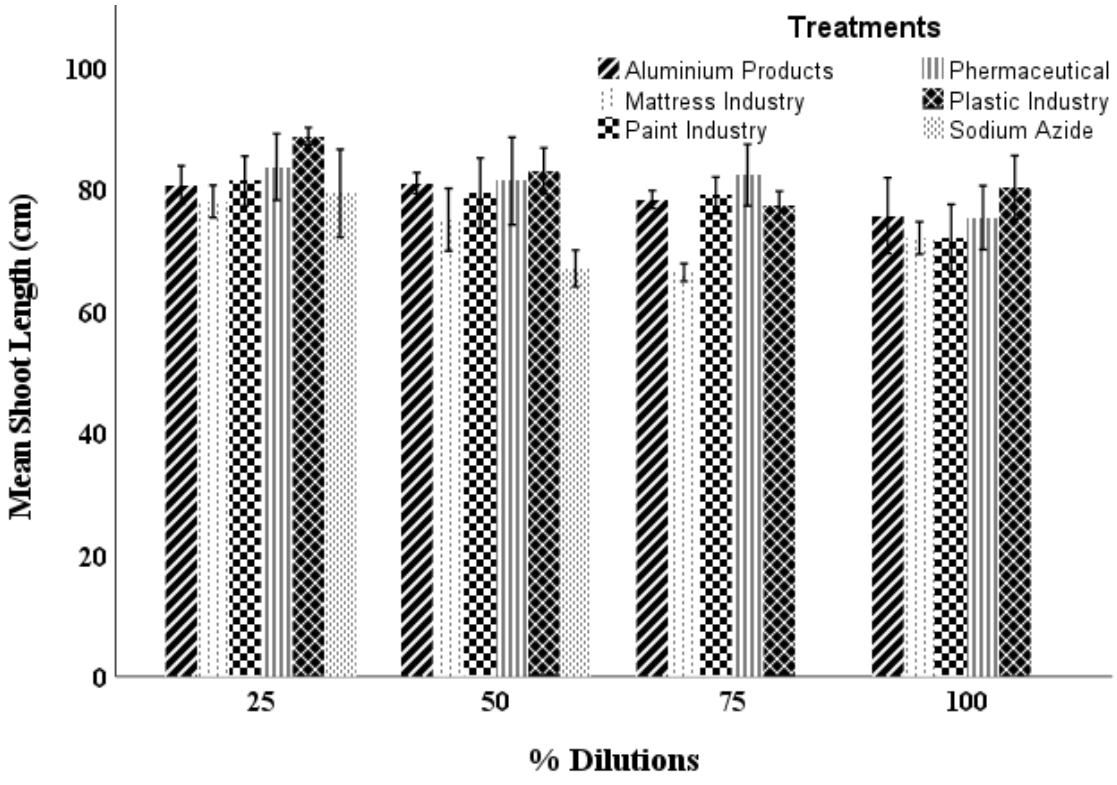


Figure 4.11: The shoot length of mature cowpeas plants exposed to different treatments of industrial effluent

The number of seeds per pod ranged from 5.15 (0.16) in the negative control and 3.73 (0.49) in the positive control of 0.031 w/w sodium azide. The mattress effluent was significant at all dilutions with the undiluted reducing to 3.67 (0.05). This was followed by paint at 4.32 (0.29) and Aluminum waste at 4.43 (0.93). Other effluents were less significant at $p \leq 5$ %.

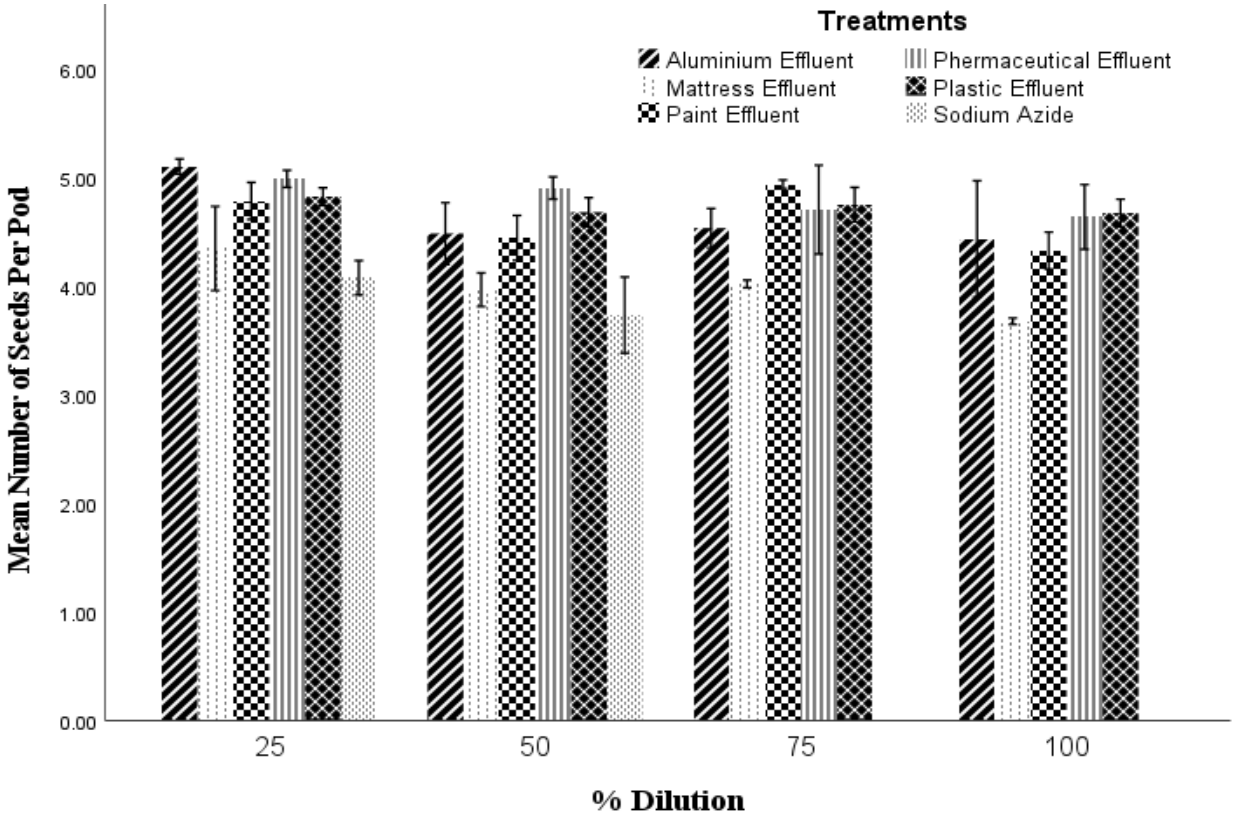


Figure 4.12: The number of seeds per pod of cowpeas exposed to different treatments of industrial effluent

The dry weight of 100 seeds was negatively affected by the mattress industry effluent with all four dilutions registering significant effect, the undiluted form reducing the weight to 11.87 (0.53) grams. This was followed by paint effluent at 12.75 (0.34). Aluminum products effluent had the least effect on the dry weight of 100 seeds with the undiluted effluent reducing the dry weight to 13.24 (1.42) grams. The negative control had 14.58 (1.21) grams and the positive control had 10.05 (0.15) grams for 0.031 w/w solution of sodium azide.

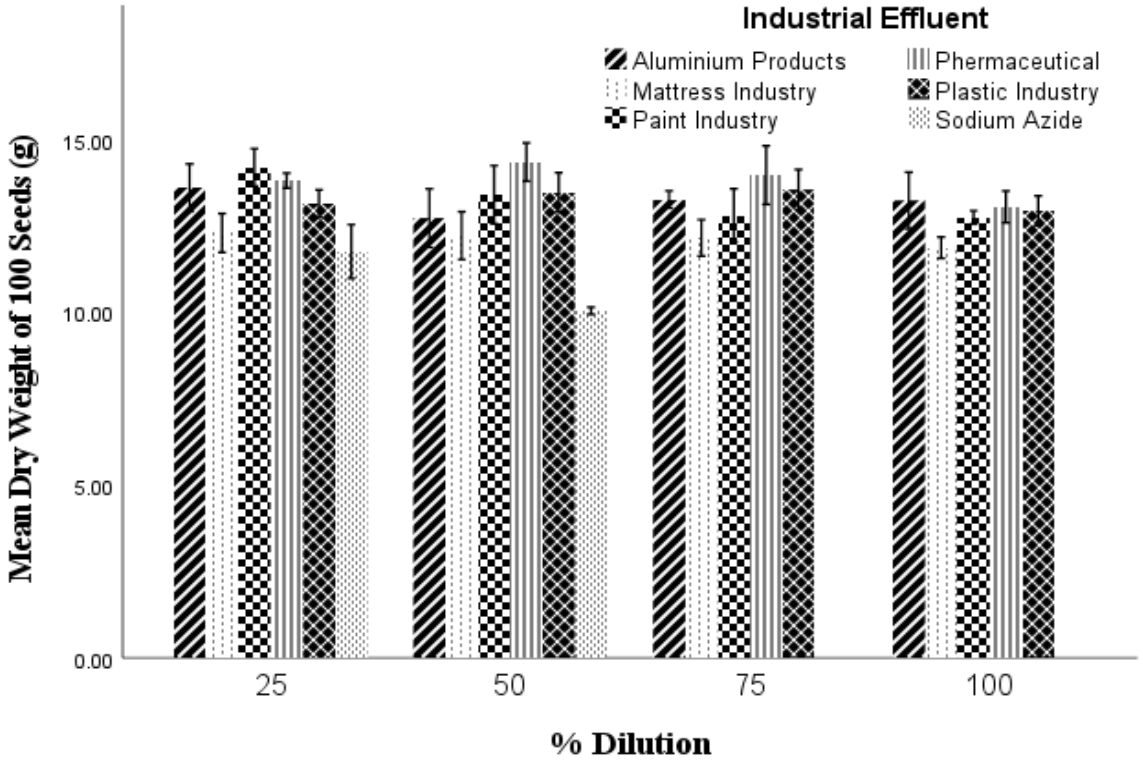


Figure 4.13: The Dry weight of 100 seeds of mature cowpeas exposed to different treatments of industrial effluent

The dry weight of roots ranged from 4.01 (0.28) in aluminum effluent and 3.07 (0.17) in 75 % dilution mattresses effluent. The dry weight of the roots was negatively affected by the mattress industry effluent reducing the weight to 3.17 (0.06) grams in undiluted effluent. This was followed by pharmaceutical effluent at 3.40 (0.30). Plastics effluent had the least effect on the dry weight of roots with the undiluted increasing the dry weight to 3.78 (0.26) grams compared to 3.75 (0.12) grams for negative control.

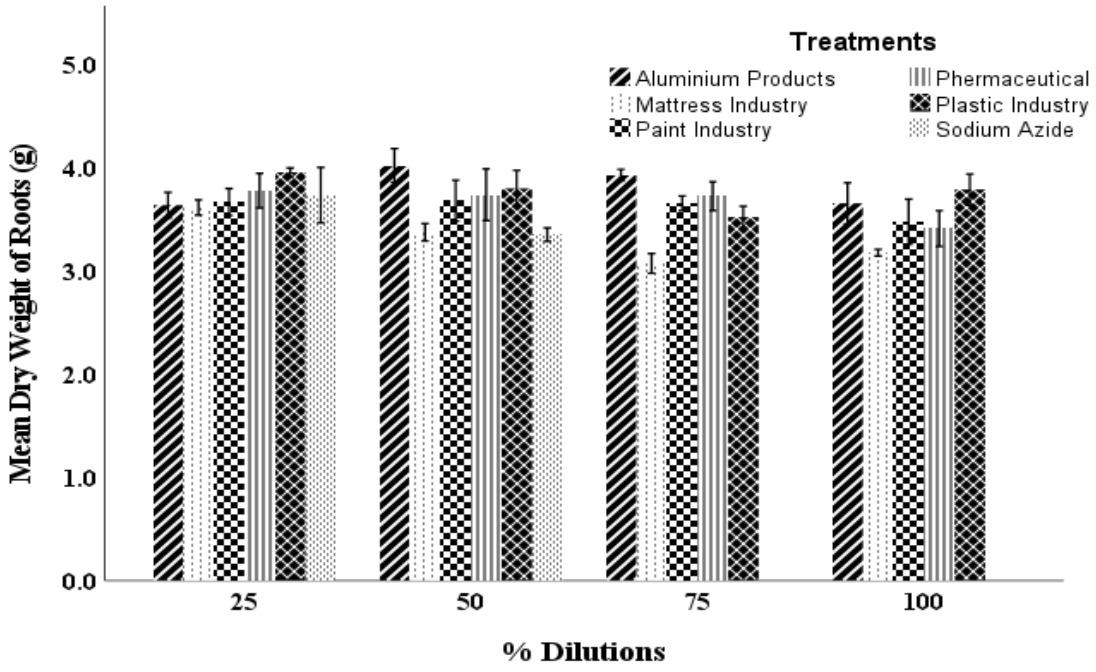


Figure 4.14: The dry weight of roots of mature cowpeas plants exposed to different treatments of industrial effluent

Table 4.3 shows the summary of observations made on the growth and yield characteristics of leaf area, number of root nodules/ plant, dry nodule weight, shoot length, number of pods / plants, number of Seeds / Pod, dry weight of seeds, days to maturity and dry weight of roots made when treated with four different dilutions of industrial effluent from pharmaceuticals (B), aluminum products (C), mattresses (D), paint (E) and Plastics (F). Sodium Azide (G) a known mutagen was used as a positive control. The Asterix (*) shows the results at 95% confidence interval when analyzed using R-Program.

Table 4.3: Effect of different dilutions of industrial effluents on growth and yield parameters of cowpeas

ID	Leaf Area (cm2)	No of Root Nodules/ Plant	10 Dry Nodule Weight (Grams)	Shoot Length (cm)
Ao	7.46 (0.71)	23.87 (0.64)	1.23 (0.15)	82.33 (3.06)
B1	7.19(0.22)^{abc}	22.23 (2.35)^{abcde}	1.47 (0.15)^{cde}	83.67 (9.45)abc
B2	7.04 (0.86)^{abcde}	21.70 (3.48)^{abcdef}	1.23 (0.21)^{a}	81.33 (12.42)ab
B3	6.95 (1.03)^{abcdef}	22.7 (2.20)^{abc}	1.33 (0.15)^{abcd}	82.23 (8.74)a
B4	6.86 (0.25)^{bcdef}	20.73 (1.21)^{bcdef}	1.47(0.34)^{cde}	75.33 (9.07)def
C1	6.93 (0.76)^{abcdef}	21.00 (2.35)^{bcdef}	1.37 (0.15)^{abcde}	80.67 (5.51)abcd
C2	7.12 (0.53)^{abcd}	21.17 (1.19) ^{bcdef}	1.37 (0.15)^{abcde}	81.00 (3.00)abc
C3	7.04 (0.20)^{abcde}	23.17 (2.46)^{ab}	1.33 (0.06)^{abcd}	78.33 (2.52)abcdef
C4	6.33 (0.84)^{ef}	19.83 (2.14)^{ef}	1.37 (0.12)^{abcde}	75.67 (10.79)cdef
D1	6.92 (0.59)^{abcdef}	19.00 (1.87)^{ef}	1.23 (0.21)^{a}	78.00 (4.58)abcdef
D2	6.44 (0.54)^{def}	19.47 (1.86)^{ef}	1.40 (0.10)^{bcde}	75.00 (8.89)def
D3	6.54 (0.34)^{cdef}	18.6 (1.56)^{f}	1.13 (0.06)^{abcd}	66.33 (2.52)f
D4	6.42 (0.11)^{def}	17.67 (1.75)^{f}	1.13 (0.21)^{abcd}	72.00 (4.58)ef
E1	7.04 (0.42)^{abcde}	22.60 (1.79)^{abcd}	1.40 (0.20)^{bcde}	81.33 (7.09)ab
E2	7.10 (0.45)^{abcd}	21.10 (2.21)^{bcdef}	1.40 (0.10)^{bcde}	79.33 (10.02)abcde
E3	7.04 (0.35)^{abcde}	20.1 (2.52)^{def}	1.1 (0.20)^{abcde}	79 (5.20)abcde
E4	6.93 (0.50)^{abcdef}	20.33 (1.62)^{cdef}	1.27 (0.29)^{ab}	72.00 (9.54)ef
F1	7.16 (0.17)^{abc}	21.10 (1.87)^{bcdef}	1.30 (0.2)^{abc}	88.67 (2.52)cdef
F2	7.24 (0.31)^{ab}	21.6 (2.61)^{abcdef}	1.20 (0.10)^{ab}	83.00 (6.56)ab
F3	7.37 (0.23)^{a}	23.43 (2.86)^{a}	1.30 (0.3)^{abc}	77.33 (4.04)bcdef
F4	7.14 (0.19)^{abcd}	21.47 (2.06)^{abcdef}	1.20 (0.20)^{ab}	80.33 (8.96)abcd
G1	6.86 (0.42)^{bcdef}	19.37 (0.80)^{ef}	1.0 (0.2)^{cde}	79.33 (12.5)abcde
G2	6.48 (1.40)^{ef}	19.75(2.9)^{def}	0.75 (0.07)^{de}	67 (4.24)f
G3				
G4				
Mean	6.94	20.99	1.26	78.28
CV	8.05	9.98	14.66	9.55
LSD	0.92	3.44	0.31	12.23

The results in the table above were the mean and standard deviations (in parentheses) for 6 determinations. The superscripts show significant differences. The symbols representation was as follows; Negative control or Distilled Water (A), pharmaceuticals (B), aluminum products (C), mattresses (D), paint (E), Plastics (F) and Sodium Azide or Positive Control (G).

Table 4.3 Continuation…

ID	No of Seeds / Pod	Dry Weight of 100 Seeds (grams)	Days to Maturity (Days)	Dry Weight of Roots (g)
Ao	5.15 (0.16)	14.58 (1.21)	76.64 (3.89)	3.75 (0.12)
B1	4.98 (0.14)^{ab}	13.81 (0.37) ^{abcd}	73.54 (4.35)^{abcde}	3.77 (0.29)^{a}
B2	4.90 (0.18)^{abc}	14.35 (0.96)^{a}	80.59(3.5)^{abcdef}	3.73 (0.43)^{a}
B3	4.70 (0.71)^{abcde}	13.97 (1.47)^{abc}	74.61 (9.05)^{abc}	3.72 (0.24)^{ab}
B4	4.63 (0.51)^{bcde}	13.05 (0.79)^{cdef}	73.2 (3.95)^{abcdef}	3.40 (0.30)^{cdef}
C1	5.10 (0.12)^{a}	13.61 (1.19)^{abcde}	81.15 (4.77)^{bcdef}	3.63 (0.22)^{abcde}
C2	4.48 (0.49)^{de}	12.73 (1.47)^{def}	80.4 (2.57)^{abcdef}	4.01 (0.28)^{bcdef}
C3	4.53 (0.31)^{cde}	13.27 (0.43)^{bcdef}	80.50 (3.57)^{abcdef}	3.92 (0.10)^{abcdef}
C4	4.43 (0.93)^{de}	13.24 (1.42)^{bcdef}	72.50 (5.05)^{bcdef}	3.65 (0.35)^{abcd}
D1	4.34 (0.67)^{e}	12.30 (0.97)^{ef}	82.21 (3.3)^{cdef}	3.61 (0.13)^{abcde}
D2	3.96 (0.27)^{e}	12.22 (1.20)^{ef}	82.54 (4.34)^{def}	3.37 (0.15)^{def}
D3	4.02 (0.06)^{e}	12.16 (0.91)^{ef}	78.99 (3.10)^{abcde}	3.07 (0.17)^{ef}
D4	3.67 (0.05)^{e}	11.87 (0.53)^{f}	78.65 (2.87)^{abcde}	3.17 (0.06)^{ef}
E1	4.78 (0.30)^{abcde}	14.19 (0.95)^{ab}	80.84 (2.73)^{bcdef}	3.66 (0.23)^{abc}
E2	4.44 (0.36)^{de}	13.40 (1.46)^{abcdef}	81.5 (3.13)^{cdef}	3.67 (0.34)^{abc}
E3	4.93 (0.07)^{abc}	12.81 (1.33)^{def}	75.14 (7.59)^{ab}	3.65 (0.12)^{abcd}
E4	4.32 (0.29)^{e}	12.75 (0.34)^{def}	78.54 (2.79)^{abc}	3.47 (0.38)^{bcdef}
F1	4.82 (0.14)^{abcd}	13.14 (0.69)^{bcdef}	80.97 (3.54)^{bcdef}	3.95 (0.07)^{abcdef}
F2	4.68 (0.23)^{bcde}	13.47 (0.99)^{abcdef}	77.19 (6.05)^{a}	3.79 (0.31)^{ab}
F3	4.75 (0.27)^{abcde}	13.55 (1.01)^{abcde}	86.26 (0.87)^{f}	3.51 (0.18)^{bcdef}
F4	4.67 (0.22)^{bcde}	12.94 (0.75)^{cdef}	78.23 (1.54)^{ab}	3.78 (0.26)^{ab}
G1	4.07 (0.27)^{e}	11.76 (1.35)^{f}	75.27 (0.67)^{ab}	3.73 (0.46)^{a}
G2	3.73 (0.49)^{e}	10.05 (0.15)^{f}	68.03 (3.66)^{ef}	3.35 (0.09)^{def}
G3				
G4				
Mean	4.53	13.01	78.23	3.62
CV	8.34	7.91	5.35	7.05
LSD	0.63	1.69	6.88	0.42

The results in the table above were the mean and standard deviations (in parentheses) for 6 determinations. The superscripts show significant differences. The symbols representation was as follows; Negative control or Distilled Water (A), pharmaceuticals (B), aluminum products (C), mattresses (D), paint (E), Plastics (F) and Sodium Azide or Positive Control (G).

4.2 Discussions

4.2.1 Mutagenicity effect of selected industrial effluent

All the industrial effluents assessed had mutagenicity of varying magnitude. This is because they contain one or more toxicants that was able to cause frame shift type mutation and base-pair replacement in gene of *Salmonella typhimurium* TA98 and TA 100 strain respectively (Mortelmans and Zeiger, 2000; Tejs, 2008). However, industrial effluent from mattress industry and plastics had the most statistically significant results. The mutagenicity in the mattress effluent may be attributed to the use of Propylene Oxide (PO) and Toluene Diisocyanate(TDI) in the manufacture of polyurethane foams (Bootman *et al*., 1979). TDI induces mutation in *S. typhimurium* TA98 and TA100 in S9 liver activation (Bolognesi *et al*., 2001). As for propylene oxide it reacts with the DNA bases in order of Guanine > Adenine > Cytosine > Thymine (Solomon *et al*., 1988). Our findings agree with the work done by Norppa and Makipaakkanen, (1987) where TDI significantly induced mutagenicity in Ames test. For plastics, the mutagenicity may be due to ecologically toxic additives like Bisphenol A (BPA), Phthalates and Brominated Flame Retardants (BFRs) (Okunolaa *et al*., 2019). According to Jalal *et al*., (2018), BPA bio accumulates and is involved in dys-regulation of Calcium homeostasis of the cell by inhibiting calcium channels such as SPCA1/2, this means BPA can induce mutagenicity. Phthalates exert their effects through the activation of peroxisome proliferator activated receptors (PPARs), which play a key role in the transcriptional control of lipids and carbohydrate metabolism (Elisabet *et al*., 2021). BFRs cause impaired T4 metabolism and cancer development, Feiteiro *et al*. (2021), reported defects on DNA, by phosphorylation of histone due to brominated flame retardants. The findings of the current study agree with

the work done by Nepalia *et al*. (2018) which showed evidence of migration of ecologically toxic additives from plastics with potential to cause mutagenicity.

The paint industrial effluent followed suit with dilutions of 50 % and 75 % exhibiting significant mutagenicity at $p \leq 5\%$ in Ame's test for *S. typhimurium* strains TA98 and TA100 respectively. In the paint industrial effluent, the mutagenicity is likely due to the use of iron oxide nano particles that possess mutagenic activity without chromosomal abnormalities but still able to induce mutagenic effects (Touati *et al*., 1995 ; Oxide *et al*., 2015; Gonc *et al*., 2020). This is in line with work done by Liu *et al*., (2014), where iron oxide nano particles induced significant mutagenicity in Ames test.

The pharmaceutical industry had significant mutagenicity at 75 % and 100 % for both stains of *S. typhimurium.* This may be due to the presence of pharmaceutical active ingredients like Ibrufen (IBU), phenols, colchicine and heavy metals which may induce mutagenicity (Oki, 2014; Wijaya *et al*., 2020 ; Tarso *et al*., 2013). This is in line with work done by Tarso *et al*., (2013) that reported mutagenicity in pharmaceutical industrial waste water even at small concentrations. Alluminium products produced the least significant result, exhibiting mutagenicity for only the undiluted effluent. Though aluminium product effluent is known to contain Aluminium (Al), Zinc (Zn) and lead (Pb), the results were probably due to low levels of these heavy metals (Birikadde, 2017).

The experimental controls employed included the negative control, duplicate analysis, the positive control 2-nitrfluorene (2-NF) for the *S. typhimurium* strain TA98 and 4-nitroquinoline (4NQO) for *S. typhimurium* Strain TA100 had expected results implying that the experiment was successful and is reproducible globally. In summary, all the industrial effluent examined exhibited dose related increase in mutagenicity however there was increasing significance in mutagenic activity from aluminium products,

pharmaceuticals, plastics, paint and mattress industry effluent was the most mutagenic. Therefore, this study has shown that industrial effluent mixtures can exhibit mutagenicity or toxicity of unknown magnitude which is a risk to the ecosystem.

4.2.2 The effects of industrial effluents on germination and seedling growth characteristics

According to the findings, all the industrial effluent affected germination significantly at all dilutions except 25 % pharmaceutical waste and below. The effluent from the mattress inhibited germination most with undiluted effluent causing 50 % decline in germination rate. This may be attributed to the use of toxic chemicals like cadmium, chromium which are common in the pharmaceutical industry effluent and are known to cause inhibition of growth (Birikadde, 2017). Our findings agree with work done by (El-Kafafi *et al*., 2017), where Cd^{+2} toxicity led to decline in fresh weight, dry weight and germination rates of cowpea seedling. Basu and Rao, 2013 also reported decline in germination rates due to chromium toxicity. This was closely followed by paint with dilutions above 50% having percentage germination of 66.67 %, this was probable due to toxicity of iron oxide nano particles that exhibits mutagenicity at the concentration of 70 ppm as reported by Touati *et al*. (1995). Pharmaceutical effluent were moderately significant, possible as a result of colchicine where Essel *et al*. (2015) found that 0.1 % solutions registered a lethal dose of 59% after 2 hours of exposure. As for plastics, according to Feiteiro *et al.* (2021), brominated flame retardants can cause seed germination inhibition.

The shoot length was highly reduced by the effluent from the mattress industry with dilutions 50 % exhibiting significant effect, this could be attributed to the cadmium, chromium and Nickel used in the in the mattress industry (Birikadde, 2017). According to Martin *et al*. (2007), Nickel concentration of 1.4 µM was found to reduce vegetative

properties by 10 %. Our study is also in agreement with the work done by Basu and Rao (2013) where chromium lead to decrease in shoot length. This was closely followed by the effluent from the pharmaceutical industry, possibly due to drugs like Ibuprofen (IBU) that had demonstrated reduction in the shoot length according to Wijaya *et al*. (2020). Aluminium products and paint effluent had significant effect on shoot length at the undiluted form of the effluent. According to Ologundudu *et al*. (2018), Aluminium stress is known to reduce shoot growth due to impairment in nutrient and water uptake. For paint, this is probable due to iron oxide nano particles that inhibit shoot growth at high concentrations (Zia-ur-rehman *et al*., 2018). Plastic waste initially increased the shoot length but then reduced as the dilution ratio increased, this may be because initially absorption was low and other chemicals that increase vegetative properties were more absorbed and as the concentration increased toxicants like brominated flame retardants increased which lead to inhibition of shoot growth (Feiteiro *et al*., 2021).

On the other hand, the root length also reported interesting results like the shoot length. Highest significant reduction in root length was recorded in effluent from the mattress industry with all dilutions showing significant reduction in the root length. This could be attributed to the chromium, nickel and cadmium used in the paint industry. This finding is similar to the one by Basu and Rao (2013) in which chromium in the effluent was found to reduce root length. Nickel activities are toxic to the roots leading to reduced growth and prohibition of lateral root formation as reported by Martin *et al*. (2007). The pharmaceutical effluent was significantly mutagenic at undiluted form only. This agrees with work done by Wijaya *et al*. (2020) where Ibuprofin toxicity reduced significantly root lengths in cowpeas. However, the 25 % dilution waste from plastics industry had a significant increase in the root length to 8.52 (2.09) cm compared to the control (6.19) cm

and Aluminium products waste did not exhibit any significant effect on the root length. This could be attributed to low levels of these toxicants (Birikadde, 2017).

The results of the wet weight still had the mattress effluent showing the more significant effect. This may be attributed to Propylene Oxide (PO) and Toluene Diisocyanate (TDI) used in the manufacture of mattress. PO causes chromosomal damage and induces reverse mutation (Bolognesi *et al*., 2001) and reacts with bases in DNA causing mutation (Solomon *et al*., 1988). This is in line with work done by Norppa and Makipaakkanen, (1987) where TDI significantly increased mutation. The other industrial effluents did not show any significant effect.

The dry weight was slightly affected by the pharmaceutical waste and the paint industry effluent. In the pharmaceutical effluent it may be due to heavy metals like cadmium which causes decline in dry weight of cowpea seedling. (Basu and Rao, 2013). This translates well with the work done by El-Kafafi *et al*., (2017), where the researchers reported reduction in dry weight due to cadmium toxicity at levels as low as 0.3 mgL^{-1}. The same explanation applies for paint industry effluent since Birikadde, (2017) reported heavy metals of lead, cadmium and chromium as possible heavy metals contaminants in paint industry effluent. In other effluents there was no effect.

The negative control (Distilled Water), did not significantly affect any parameters assessed and was used as a reference for assessing the effect of the industrial effluent on cowpeas. The positive control Sodium Azide, was the most significant at ($P>0.05$). At the concentration of 0.25 w/w, it was lethal to cowpeas. This is in agreement with the work done by Ikhajiagbe, (2012) who had similar results with concentrations of 0.25 % w/w NaN_3. This therefore implied that the controls employed played their role and the experiment can be replicated globally.

In summary, industrial effluent from mattress industry affected germination and seedling growth parameters the most, followed by pharmaceuticals then aluminium products and paint and plastics industry had the least effect on seed germination and seedling characteristics.

4.2.3 Effect of industrial effluents on growth and yield parameters of cowpeas

The mattress industrial effluent exhibited significant reduction effect on number of root nodules, number of seeds, dry weight of seeds, this was registered at all levels of dilution evidence of toxicity on these parameters. However, leaf area, shoot length and dry weight of roots were significantly reduced at dilutions of 75 % and above. This is attributed to Propylene Oxide (PO) which reacts with bases in DNA and can lead to these characteristics (Solomon *et al*., 1988) and Toluene Diisocyanate (TDI) causes chromosomal damage and induces reverse mutation (Bolognesi *et al*., 2001). Researchers have evidence of toxicity of TDI causing DNA mutations in animals though studies on plants was still limited due to the volatility of these compounds in both aquatic and terrestrial environment (Gharehbakhsh *et al*., 2020).

The paint industry effluent had significant effect on number of root nodules, number of seeds and dry weight of seeds at ($P>0.05$). This was only at higher dilutions above 75 %. This could be due to lead toxicity which is what Kopittke *et al*., (2007) reported in their study whereby it caused reduced root growth, lack of apical dominance, and affected some root tips. Aluminium effluent exhibited significant effect on leaf area, number of root nodules and seeds and dry weight of seeds at dilutions of above 75 %. This is in line with the findings of De Manzi and Cartwright (1984) where Aluminium ions affected the root system, causing a reduction in total root length and fresh weight. No significant change was recorded due to treatment with pharmaceutical effluent and plastics effluent as

compared with negative control experiment. For plastics, it's probably due to low concentrations of the additives, since they leach in small amounts though are still mutagenic (Elisabet *et al*., 2021).

The negative control (Distilled Water), did not significantly affect parameters assessed and was used as a reference for assessing the effect of the industrial effluent on cowpeas. The positive control Sodium Azide, was the most significant at ($P>0.05$). At the concentration of 0.125 w/w and 0.25 w/w, it was lethal to cowpeas. This is in agreement with the work done by Ikhajiagbe, (2012) who had similar results with concentrations of 0.250 % w/w NaN_3. This therefore implied that the controls employed played their role and the experiment can be replicated globally.

In conclusion, industrial effluent from aluminium products, mattress and paint industry had significant effects on the growth and yield parameters of cowpeas. This therefore means, industrial effluent was being discharged into the environment with devastating effect on the ecosystem. This should be a matter of concern because mutagenic effects may not be realised immediately but persists in generations and cannot be reversed hence the need for environmental protection agencies to advocate for policy to protect the environment.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

All the industrial effluent assessed were mutagenic, with plastic and mattress effluents exhibiting the highest while the lowest result in the aluminium products industry effluent. This was attributed to mutagenicity of the effluent due to presence of phthalates, propylene oxide, toluene dioxide and heavy metals in it. The *Salmonella typhimurium strain* TA100 was more susceptible to effluent treatment; hence prominent mutation was base pair replacement of the GGG loci in the gene.

The germination and seedling growth characteristics of cowpeas were mostly affected by the mattress effluent followed by pharmaceutical effluent however the rest had low effect. This was attributed to the toxicity of the effluent due to presence of toxicants causing long term effects on cowpeas. This effects correlate highly with level of cancer infection in the country.

The growth and yield parameters of cowpeas were mostly affected by mattress industrial effluent and least affected by the aluminium effluent. This was attributed to the toxicity of the effluent due to presence of toxicants causing long term effects on cowpeas. This effects correlate highly with level of cancer infection in the country.

5.2 Recommendations

Recommendations for government and general public

a) Support the National Environment Management Authority to increase capacity especially in conducting bio-toxicity test on industrial effluents.

b) The stake holders in the environmental sector should look at the policy in line with the protection of the environment so as to include bio toxicity measurements of industrial effluents.

c) The general public should desist from degrading the wetlands especially by farming because this reduces their detoxification potential as well as introduce the toxicants into the food chain.

d) The environmental bodies should take action since mutagens persist in the environment and are not reversible.

Recommendations for further research in regard to the study

i) The Ames test should be supplemented by other tests like *allium cepa* test for better confirmation of mutagenicity of industrial effluent.

ii) Other plants like Soybeans should also be used so as to ascertain the toxic effect of the industrial effluent across plants.

iii) Similar study should be conducted to detect the effect of mutagenicity at genotypic level in plants.

iv) Chemical analysis to ascertain the concentration of mutagenic pollutants in the effluents should be conducted.

REFERENCES

Abdel-shafy, H. I., & Mansour, M. S. M. (2018). Phytoremediation for the Elimination of Metals, Pesticides, PAHs, and Other Pollutants from Wastewater and Soil. In *Phytobiont and Ecosystem Restitution*. Springer Nature Singapore Pte Ltd. https://doi.org/10.1007/978-981-13-1187-1

Achim, S., & Newman, D. (2015). *Global Waste Management Outlook*. International Solid Waste Association General Secretariat.

Adewumi, J. R., Ilemobade, A. A., & Zyl, J. E. Van. (2010). Treated wastewater reuse in South Africa: Overview, potential and challenges. *Resources, Conservation and Recycling*, *55*(2), 221–231. https://doi.org/10.1016/j.resconrec.2010.09.012

Afutu, E., Mohammed, K. E., Odong, T. L., Biruma, M., & Rubaihayo, P. R. (2016). Evaluation of Ugandan Cowpea Germplasm for Yield and Resistance to Scab Disease. *American Journal of Experimental Agriculture*, *12*(2), 1–18. https://doi.org/10.9734/AJEA/2016/25138

Akande, M. G., Sanni, F. S., & Enefe, N. G. (2019). Assessment of the concentrations and health risk of some heavy metals in cowpea (Vignus unguiculata) in Gwagwalada , Nigeria. *Drug and Chemical Toxicology*, *0*(0), 1–6. https://doi.org/10.1080/01480545.2019.1621334

Ali, I., Wakeel, A., Upreti, S., Liu, D., Azizullah, A., Jan, M., Ullah, W., Liu, B., Ali, A., Daud, M. K., & Gan, Y. (2018). *Effect of Bisphenol A-induced Oxidative Stress on the Ultra Structure and Antioxidant Defence System of Arabidopsis thialiana Leaves*. *27*(3), 967–978. https://doi.org/10.15244/pjoes/76038

Ames, B. N., & Yanofsky, C. (1971). The Detection of Chemical Mutagens with Enteric Bacteria. *Chemical Mutagens*, *156*, 267–282.

Angelotti, F., Barbosa, L. G., Barros, J. R. A., & Dos Santos, C. A. F. (2020). Cowpea development under different temperatures and carbon dioxide concentrations. *Pesquisa Agropecuaria Tropical*, *50*, 1–7. https://doi.org/10.1590/1983-40632020V5059377

Angiro, C., Abila, P. P. O., & Omara, T. (2020). Effects of industrial effluents on the quality of water in Namanve stream , Kampala Industrial and Business Park , Uganda. *BMC Research Notes*, 4–9. https://doi.org/10.1186/s13104-020-05061-x

Anyakora, C., Ehianeta, T., & Umukoro, O. (2013). Heavy metal levels in soil samples from highly industrialized Lagos environment. *African Journal of Environmental Science and Technology*, *7*(9), 917–924. https://doi.org/10.5897/AJEST2013.1543

Arinaitwe, K., Kiremire, B. T., Muir, D. C. G., Fellin, P., Li, H., Teixeira, C., & Mubiru, D. N. (2012). Atmospheric Concentrations of Polycyclic Aromatic Hydrocarbons in the Watershed of Lake Victoria, East Africa. *Environmental Science and Technology*, *46*, 11524–11531. https://doi.org/dx.doi.org/10.1021/es302238w |

Ariza, M. E., & Williams, M. V. (1999). Lead and mercury mutagenesis: Type of mutation dependent upon metal concentration. *Journal of Biochemical and Molecular Toxicology*, *13*(2), 107–112. https://doi.org/10.1002/(SICI)1099-0461(1999)13:2<107::AID-JBT6>3.0.CO;2-0

Avwiri, G. O., & Olatubosun, S. A. (2014). Assessment Of Environmental Radioactivity In Selected Dumpsites In Port Harcourt , Rivers. *International Journal of Scientific and Technological Research*, *3*(4), 263–269.

Bamuwamye, M., Ogwok, P., Tumuhairwe, V., Eragu, R., & Nakisozi, H. (2017). Human Health Risk Assessment of Heavy Metals in Kampala (Uganda) Drinking Water. *Journal of Food Research*, *6*(4), 6–16. https://doi.org/10.5539/jfr.v6n4p6

Banadda, E. N., Kansiime, F., Kigobe, M., Kizza, M., & Nhapi, I. (2009). Landuse-based nonpoint source pollution: A threat to water quality in Murchison Bay, Uganda. *Water Policy*, *11*(SUPPL. 1), 94–105. https://doi.org/10.2166/wp.2009.106

Basu, S., & Rao, P. V. V. P. (2013). Effect of chrome plating industry effluent on cowpea. *An International Quaterly Journal of Environmental Sciences*, *III*(Special Issue), 241–246. www.theecoscan.in

Bessi, H., & Colin, F. (1992). Genotoxicity of Hazardous Leachates from Solid Wastes Evaluated for Environmental Impact with the Ames Test. *Environmental Toxicology and Water Quality*, *7*, 71–86.

Bhatt, E. (2018). *Review Article Impact of Antibiotics on Plants*. *52*(09), 49–53.

Bilban, M. (2004). Mutagenic Testing of Workers Exposed to Toluene-Diisocyanates During Plastics Production Process. *American Journal of Industrial Medicine*, *474*, 468–474. https://doi.org/10.1002/ajim.10365.

Birikadde, G. K. (2017). Water Quality Assessment for industrial pollution hotspots in kinawataka drainage sub catchments of kampala city. In *Kampala Pollution Control Task Force*.

Blackadar, C. B. (2016). Historical review of the causes of cancer. *World Journal of Clinical Oncology*, *7*(1), 54–86. https://doi.org/10.5306/wjco.v7.i1.54

Bolognesi, C., Baur, X., Marczynski, B., Norppa, H., Sepai, O., & Sabbioni, G. (2001). Carcinogenic Risk of Toluene Diisocyanate and Epidemiological and Experimental Evidence. *Critical Reviews in Toxicology*, *31*(6), 737–772.

Bootman, J., Lodge, D. C., & Whalley, H. E. (1979). Mutagenic activity of propylene oxide in bacterial and mammalian systems. *Mutation Research*, *67*, 101–112.

Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., Haruna, M., Diallo, S., Lawan, M., Olusoji, U., & Christian, O. (2018). Cowpea (Vigna unguiculata): Genetics, genomics and breeding. *Plant Breeding*, 1–10. https://doi.org/10.1111/pbr.12589

Campenhout, B. Van, Nabwire, L., Minten, B., & Ariong, R. M. (2024). *Institutional and technological innovations to foster agro-industrialization in Uganda Insights from the dairy value chain* (Issue April 2020).

Caritá, R., Mazzeo, D. E. C., & Marin-Morales, M. A. (2019). Comparison of the toxicogenetic potential of sewage sludges from different treatment processes focusing agricultural use. *Environmental Science and Pollution Research*, *26*(21), 21475–21483. https://doi.org/10.1007/s11356-019-05453-y

Cevik, M., Dartan, G., Ulker, M., Bezci, K., Deliorman, G., Cagatay, P., Lacin, T., Cinel, I. H., Aksu, B., Keskin, Y., Can, Z. S., Yurdun, T., & Susleyici, B. (2020). Evaluation of Cytotoxicity and Mutagenicity of Wastewater from Istanbul: Data from Hospitals and Advanced Wastewater Treatment Plant. *Bulletin of Environmental Contamination and Toxicology*, *104*(6), 852–857. https://doi.org/10.1007/s00128-020-02853-6

Chang, V. W. C., Zhang, J., Giannis, A., & Wang, J. (2013). Science of the Total Environment Removal of cytostatic drugs from aquatic environment : A review. *Science of*

the Total Environment, The, *445–446*, 281–298. https://doi.org/10.1016/j.scitotenv.2012.12.061

Chen, Y., Li, Y., Liang, X., Lu, S., Ren, J., Zhang, Y., Han, Z., Gao, B., & Sun, K. (2024). Effects of microplastics on soil carbon pool and terrestrial plant performance. *Carbon Research*, 1–23. https://doi.org/10.1007/s44246-024-00124-1

Chinaza, G., Gospel, C., & Somtochukwu, V. (2020). Industrial and Community Waste Management: Global Perspective. *Amercan Journal of Physical Science*, *1*(1), 1–16.

Claxton, L. D., De Umbuzeiro, G. A., DeMarini, D. M., Umbuzeiro, G. D. A., DeMarini, D. M., De Umbuzeiro, G. A., & DeMarini, D. M. (2010). The Salmonella Mutagenicity Assay: The Stethoscope of Genetic Toxicology for the 21st Century. *Environmental Health Perspectives*, *118*(11), 1515–1522. https://doi.org/10.1289/ehp.1002336

Claxton, L. D., Houk, V. S., & Hughes, T. J. (1998). *Genotoxicity of industrial wastes and effluents*. 237–243.

Copolovici, L., Timis, D., Taschina, M., Copolovici, D., Cioca, G., & Bungau, S. (2017). *Diclofenac Influence on Photosynthetic Parameters and Volatile Organic Compounds Emission from Phaseolus vulgaris L . Plants*. *February 2019*, 1–4. https://doi.org/10.37358/RC.17.9.5826

Credland, P., Armitage, D., Bell, C., Cogan, P., & Highley, E. (2002). *In Advances in stored product protection. Proceedings of the 8th International Working Conference on Stored Product Protection*. *250*, 921–924.

Dala-Paula, B. M., Custódio, F. B., Knupp, E. A. N., Palmieri, H. E. L., Silva, J. B. B., &

Glória, M. B. A. (2018). Cadmium, copper and lead levels in different cultivars of lettuce and soil from urban agriculture. *Environmental Pollution*, *242*, 383–389. https://doi.org/10.1016/j.envpol.2018.04.101

Darnerud, P. O. (2003). Toxic effects of brominated flame retardants in man and in wildlife. *Environmental International*, *29*, 841–853. https://doi.org/10.1016/S0160-4120(03)00107-7

De Manzi, J. M., & Cartwright, P. M. (1984). The effects of pH and aluminium toxicity on the growth and symbiotic development of cowpeas (Vigna unguiculata (L.) Walp). *Plant and Soil*, *80*(3), 423–430. https://doi.org/10.1007/BF02140049

Devi, P. U., Murugan, S., Akilapriyadharasini, S., Suja, S., & Chinnaswamy, P. (2007). Effect of mercury and effluent on seed germination, root-shoot lenght, amylase activity and phenolic compounds in Vigna Unguiculata. *Nature Environment and Pollution Technology*, *6*(3), 457–462.

Dietler, D., Babu, M., Fuhrimann, S., Cissé, G., Halage, A. A., Malambala, E., & Fuhrimann, S. (2019). Daily variation of heavy metal contamination and its potential sources along the major urban wastewater channel in Kampala, Uganda. *Environmental Monitoring and Assessment*, *191*(2). https://doi.org/10.1007/s10661-018-7175-4

Domenico, A., Serena, G., Alberto, M., Nicola, M., & Emilio, P. (2016). A knowledge-based expert rule system for predicting mutagenicity (Ames test) of aromatic amines and azo compounds. *Toxicology*. https://doi.org/10.1016/j.tox.2016.09.008

Dong, L., Wang, S., Qu, J., You, H., & Liu, D. (2021). Ecotoxicology and Environmental Safety New understanding of novel brominated flame retardants (NBFRs): Neuro (

endocrine) toxicity. *Ecotoxicology and Environmental Safety*, *208*. https://doi.org/https://doi.org/10.1016/j.ecoenv.2020.111570

El-Kafafi, S. H., El-Demerdash, F. M., & Taha, A. A. (2017). Toxic Effect of Cadmium Stress on Rooting , Physiological and Biochemical Perturbations in Cowpea Seedling. *Middle East Journal of Agriculture Research.*, *6*(3), 646–661.

El, R., Beek, P. Van, Castet, S., Souhaut, M., Grégoire, M., & Courjault-radé, P. (2019). Natural radioactivity and radiation hazard assessment of industrial wastes from the coastal phosphate treatment plants of Gabes (Tunisia , Southern Mediterranean Sea). *Marine Pollution Bulletin*, *146*(June), 454–461. https://doi.org/10.1016/j.marpolbul.2019.06.075

Elisabet, P., Solís, A., Bani, I., & Porte, C. (2021). Ecotoxicology and Environmental Safety PLHC-1 topminnow liver cells : An alternative model to investigate the toxicity of plastic additives in the aquatic environment. *Ecotoxicology and Environmental Safety*, *208*. https://doi.org/10.1016/j.ecoenv.2020.111746

Environmental Protection Agency, U. S. (2011). *U.S. Environmental Protection Agency April 2011*. *April*, 1–16.

Essel, E., Asante, I. K., Laing, E., & Accra, L.-. (2015). Effect of colchicine treatment on seed germination, plant growth and yield traits of cowpea (vigna unguiculata (L.) walp). *Canadian Journal of Pure and Applied Sciences*, *9*(3), 3573–3576.

Farai, I. P., Adewole, O. O., Isinkaye, M. O., & Jibiri, N. N. (2009). Radiological effects of some industrial wastes and by-products generated in Lagos , Nigeria. *Jurnal Fizik Malaysia*, *30*(1).

Feiteiro, J., Mariana, M., & Cairr, E. (2021). Health toxicity effects of brominated flame retardants: From environmental to human exposure. *Environmental Pollution*, *285*(April). https://doi.org/10.1016/j.envpol.2021.117475

Feng, Y., Kreslavski, V. D., Shmarev, A. N., Ivanov, A. A., Zharmukhamedov, S. K., Kosobryukhov, A., Yu, M., Allakhverdiev, S. I., & Shabala, S. (2022). *Photosynthesis , Antioxidant Activity and Distribution of Mineral Elements in Wheat (Triticum aestivum) Plants*.

Feng, Y., Kreslavskii, V., Shmarev, A., Ivanov, A., Zharmukhamedov, S., Kosobryukhov, A., Yu, M., Allakhverdiev, S., & Shabala, S. (2022). *Effects of Iron Oxide Nanoparticles (Fe3O4) on Growth, Photosynthesis, Antioxidant Activity and Distribution of Mineral Elements in Wheat (Triticum aestivum) Plants.*

Gaikwad, B. S., & More, A. D. (2016). Effects of mutagens on chlorophyll content of morphological mutants of cowpea [Vigna unguiculata (L .) Walp .]. *International Journal of Researches in Biosciences, Agriculture and Technology*, *4*(1), 130–132.

Gavrilescu, M. (2014). Environmental Biotechnology : Achievements , Opportunities and Challenges Environmental Biotechnology : Achievements , Opportunities and Challenges. *Dynamic Biochemistry, Process Biotechnology and Molecular Biology*, *4*(1), 1–36. https://www.researchgate.net/publication/260322506

Genet, J., Singh, V. V, Ramkrishna, K., & Arya, R. K. (2006). Induced chemical mutagenesis in cowpea [Vigna unguiculata (L.) Walp. *ResearchGate*, *66*(4), 312–315. https://www.researchgate.net/publication/327177933

Geri, M., Gajski, G., & Gagi, S. (2023). *Phytotoxicity of Bisphenol A to Allium cepa Root*

Cells Is Mediated through Growth Hormone Gibberellic Acid and.

Gharehbakhsh, H., Panahi, H. A., Toosi, M. R., Hassani, A. H., & Moniri, E. (2020). Application of polyamide thin-film composite layered on polysulfone-GO/TiO2 mixed matrix membranes for removal of nitrotoluene derivatives from petrochemical wastewaters. *Environmental Science and Pollution Research*, *27*(34), 42481–42494. https://doi.org/10.1007/s11356-020-10210-7

Giridhar, K., Raju, P. S., Pushpalatha, G., & Patra, C. (2020). Effects of plant density on yield parameters of cowpea (Vigna unguiculata L.). *International Journal of Chemical Studies*, *8*(4), 344–347. https://doi.org/10.22271/chemi.2020.v8.i4f.10090

Girija, M., & Dhanavel, D. (2013). Effect of Gamma Rays on quantitative traits of cowpeas in m1 generation. *International Journal of Research in Biological Sciences*, *3*(2), 84–87.

Gonc, B. B., Dias, F. C., & Rocha, T. L. (2020). Co-exposure of iron oxide nanoparticles and glyphosate-based herbicide induces DNA damage and mutagenic effects in the guppy (Poecilia reticulata). *Environmental Toxicology and Pharmacology*. https://doi.org/10.1016/j.etap.2020.103521

Gowthami, C., & Suchandranath, B. (2022). Oryza nivara allele of a major effect QTL qFLA1.1 increases ag leaf area in rice. *ResearchSquare*, 1–23. https://doi.org/https://doi.org/10.21203/rs.3.rs-1353745/v1

Hamdy, A. (2013). Effects of Cadmium and Combined Cadmium-Zinc Concentrations on Rooting and Nutrient Uptake of Cowpea Seedlings Grown in Hydroponic. *American-Eurasian J. Agric. & Environ. Sci.*, *13*(8), 1050–1056.

https://doi.org/10.5829/idosi.aejaes.2013.13.08.11023

Harding, G., Chivavava, J., & Lewis, A. E. (2020). Challenges and shortcomings in current South African industrial wastewater quality characterisation. *Water SA*, *46*(2), 267–277. https://doi.org/https://doi.org/10.17159/wsa/2020.v46.i2.8242

Hoffmann, J. E. D. A. H. D. (1995). Toluene Diisocyanate: Carcinogenic risk following oral and inhalation exposure. *Toxicology and Industrial Health*, *11*, 13–32.

Ikhajiagbe, B. (2012). Comparative assessment of the mutagenic effects of sodium azide on some selected growth and yield parameters of five accessions of cowpea. *ResearchGate*, *July 2020*. www.scholarsresearchlibrary.com

Iloms, E., Ololade, O. O., & Ogola, H. J. O. (2020). Investigating Industrial Effluent Impact on Municipal Wastewater Treatment Plant in Vaal , South Africa. *International Journal of Environmental Research and Public Health*, 1–18. https://doi.org/doi:10.3390/ijerph17031096

Iqbal, M., Abbas, M., Arshad, M., Hussain, T., Khan, A. U., Masood, N., Tahir, M. A., Hussain, S. M., Bokhari, T. H., & Khera, R. A. (2015). Gamma Radiation Treatment for Reducing Cytotoxicity and Mutagenicity in Industrial Wastewater. *Pol. J. Environ. Stud. Vol. 24, No. 6 (2015), 2745-2750*, *24*(6), 2745–2750. https://doi.org/10.15244/pjoes/59233

Iqbql, Z., & Siddiqui, K. (2015). Effect of Mercury on Seed Germination and Seedling Growth of Mungbean (Vigna radiata (L.) Wilczek). *J. Appl. Sci. Environ. Manage*, *19*(2), 191–199. https://doi.org/http://dx.doi.org/10.4314/jasem.v19i2.4 Introduction

Ishizuka, M. (2015). environmental Pollution in Africa. *WHOsymposium*, 1–15.

http://hdl.handle.net/2115/61515

Jajoo, A., Rao, N., Singh, R., Grieco, M., Tikkanen, M., & Aro, E. (2014). Journal of Photochemistry and Photobiology B : Biology Inhibitory effects of polycyclic aromatic hydrocarbons (PAHs) on photosynthetic performance are not related to their aromaticity. *JOURNAL OF PHOTOCHEMISTRY & PHOTOBIOLOGY, B: BIOLOGY*, 8–12. https://doi.org/10.1016/j.jphotobiol.2014.03.011

Jalal, N., Surendranath, A. R., Pathak, J. L., Yu, S., & Chung, C. Y. (2018). Bisphenol A (BPA) the mighty and the mutagenic. *Toxicology Reports*, *5*(June 2017), 76–84. https://doi.org/10.1016/j.toxrep.2017.12.013

Jatho, A., Tran, B. T., Cambia, J. M., & Nanyingi, M. (2020). Cancer Risk Studies and Priority Areas for Cancer Risk Appraisal in Uganda. *Annals of Global Health*, *86*(1), 1–24. https://doi.org/https://doi.org/10.5334/aogh.2873

Josiane, N., Figoli, A., National, I., & Vienna, L. S. (2013). Wastewater treatment practices in Africa - Experiences from seven countries. *ResearchGate*, *January*.

Kakuba, S. J., & Kanyamurwa, J. M. (2021). Management of wetlands and livelihood opportunities in Kinawataka wetland, Kampala-Uganda. *Environmental Challenges Journal*, *2*(October 2020). https://doi.org/https://doi.org/10.1016/j.envc.2020.100021

Kamran, M., Ali, H., Farhan, M., Faiq, H., Hassan, Z., Tahir, M., Abbas, G., Asif, M., Imtiaz, M., & Mustafa, G. (2020). Ecotoxicology and Environmental Safety Unraveling the toxic effects of iron oxide nanoparticles on nitrogen cycling through manure-soil-plant continuum. *Ecotoxicology and Environmental Safety*, *205*(August), 111099. https://doi.org/10.1016/j.ecoenv.2020.111099

Kapoor, D. (2015). Impact of Pharmaceutical Industries on Environment, Health and Safety. *Journal of Critical Reviews ISSN-*, *2*(4).

Kauffmann, K., Gremm, L., Brendt, J., Schiwy, A., Bluhm, K., Hollert, H., & Büchs, J. (2020). Alternative type of Ames test allows for dynamic mutagenicity detection by online monitoring of respiration activity. *Science of the Total Environment*, *726*, 137862. https://doi.org/10.1016/j.scitotenv.2020.137862

Kayima, J., Kyakula, M., Komakech, W., & Echimu, S. (2008). A study of the degree of Pollution in Nakivubo Channel, Kampala, Uganda. *Journal of Applied Science and Environmental Management*, *12*(2), 93–98. www.bioline.org.br/ja%0AJ.

Kehinde, O., Oluwaseun, A., Olufiropo, A., & Okunola, A. (2019). Public and Environmental Health Effects of Plastic Wastes Disposal: A Review. *Journal of Toxicology and Risk Assessment*, *5*(2). https://doi.org/10.23937/2572-4061.1510021

Keir, J. L. A., Akhtar, U. S., Matschke, D. M. J., Kirkham, T. L., Chan, H. M., Ayotte, P., White, P. A., & Blais, J. M. (2017). Elevated Exposures to Polycyclic Aromatic Hydrocarbons and Other Organic Mutagens in Ottawa Fire fighters Participating in Emergency, On-Shift Fire Suppression. *Environmental Science and Technology*. https://doi.org/10.1021/acs.est.7b02850

Khan, H. K., Yasir, M., Rehman, A., & Malik, R. N. (2020). Fate and toxicity of pharmaceuticals in water environment : An insight on their occurrence in South Asia. *Journal of Environmental Management*, *271*(March), 111030. https://doi.org/10.1016/j.jenvman.2020.111030

Khan, S., Anas, M., & Malik, A. (2019). Mutagenicity and genotoxicity evaluation of

textile industry wastewater using bacterial and plant bioassays. *Toxicology Reports*, *6*(October 2018), 193–201. https://doi.org/10.1016/j.toxrep.2019.02.002

Kim, D., Kwak, J. Il, & An, Y. (2018). Chemosphere Effects of bisphenol A in soil on growth , photosynthesis activity , and genistein levels in crop plants (Vigna radiata). *Chemosphere*, *209*, 875–882. https://doi.org/10.1016/j.chemosphere.2018.06.146

Kopittke, P. M., Asher, C. J., Kopittke, R. A., & Menzies, N. W. (2007). Toxic effects of lead on growth of cowpea (Vigna unguiculata). *Environmental Pollution*, *150*, 280–287. https://doi.org/10.1016/j.envpol.2007.01.011

Kyambadde, A. (2014). Buy Uganda and Build Uganda Policy. *Ministry of Trade, Industry and Cooperatives*, *September*. www.mtic.go.ug

L. Emerton, L. Iyango, P. L. and A. M. (1999). The Present Economic Value of Nakivubo Urban Wetland, Uganda. *National Wetlands Conservation and Management Programme*, *September*.

Labbo, Z., Sidi, D., Egbulefu, C. S., Suleiman, R. O., Polytechnic, K., Agency, B. D., Abuja, Z.-, & State, K. (2023). Influence of two glomus species on root nodule number of cowpeas (Vigna unguiculata (L) walp) varieties aletra Vogelii Inoculated Soil. *Science World Journal*, *18*(1), 90–96.

Lázaro, M., & Ana, S. (2021). *Biochemical and Metabolic Plant Responses toward Polycyclic*.

Levy, D. D., Zeiger, E., Escobar, P. A., Hakura, A., Leede, B. M. Van Der, Kato, M., Moore, M. M., & Sugiyama, K. (2019). Recommended criteria for the evaluation of

bacterial mutagenicity data (Ames test). *Mutat Res Gen Tox En*, *July*, 403074. https://doi.org/10.1016/j.mrgentox.2019.07.004

Levy, D. D., Zeiger, E., Escobar, P. A., Hakura, A., van der Leede, B. jan M., Kato, M., Moore, M. M., Sugiyama, K. ichi, Leede, B. M. Van Der, Kato, M., Moore, M. M., Sugiyama, K. ichi, van der Leede, B. jan M., Kato, M., Moore, M. M., & Sugiyama, K. ichi. (2019). Recommended criteria for the evaluation of bacterial mutagenicity data (Ames test). *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *848*(July), 403074. https://doi.org/10.1016/j.mrgentox.2019.07.004

Li, J., Liu, H., & Paul Chen, J. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Research*, *137*, 362–374. https://doi.org/10.1016/j.watres.2017.12.056

Li, X., Wang, L., Wang, S., Yang, Q., Zhou, Q., & Huang, X. (2018). Ecotoxicology and Environmental Safety A preliminary analysis of the e ff ects of bisphenol A on the plant root growth via changes in endogenous plant hormones. *Ecotoxicology and Environmental Safety*, *150*(December 2017), 152–158. https://doi.org/10.1016/j.ecoenv.2017.12.031

Li, Y., Liang, Y., Li, Y., Che, X., Zhao, S., Zhang, Z., & Gao, H. (2018). Mechanisms by which Bisphenol A affect the photosynthetic apparatus in cucumber (Cucumis sativus L .) leaves. *Scientific Reports*, *June 2017*, 1–9. https://doi.org/10.1038/s41598-018-22486-4

Liang, Q., Gao, R., Xi, B., Zhang, Y., & Zhang, H. (2014). Long-term effects of irrigation using water from the river receiving treated industrial wastewater on soil organic carbon fractions and enzyme activities. *Agricultural Water Management*, *135*, 100–108. https://doi.org/10.1016/j.agwat.2014.01.003

Liu, F., Ying, G., Tao, R., Zhao, J., Yang, J., & Zhao, L. (2009). Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. *Environmental Pollution*, *157*(5), 1636–1642. https://doi.org/10.1016/j.envpol.2008.12.021

Liu, Y., Xia, Q., Liu, Y., Zhang, S., & Cheng, F. (2014). Genotoxicity assessment of magnetic iron oxide nanoparticles with different particle sizes and surface coatings. *IOP Publishing Ltd*, *425101*(25). https://doi.org/10.1088/0957-4484/25/42/425101

Ma, T., Id, W. Z., Chen, L., Wu, L., Christie, P., & Liu, W. (2018). *Toxicity of phthalate esters to lettuce (Lactuca sativa) and the soil microbial community under different soil conditions*. 1–17.

Macedo, F. C. (2019). *Transfer and effects of brominated flame retardants (BFRs) on three plant species and one earthworm species in anthroposoils To cite this version : HAL Id : tel-02420943 Transfert et effets des retardateurs de flamme bromés (RFBs) sur trois espèces végétales et une espèce de lombric dans des anthroposols*.

Macgregor, J. T., Frötschl, R., White, P. A., Crump, K. S., Eastmond, D. A., Fukushima, S., Guérard, M., Hayashi, M., Soeteman-hernandez, L., Kasamatsu, T., Levy, D. D., & Morita, T. (2014). IWGT report on quantitative approaches to genotoxicity risk assessment I. Methods and metrics for defining exposure–response relationships and points of departure (PoDs). *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, 1–10. https://doi.org/10.1016/j.mrgentox.2014.09.011

Mafabi, P., Malinga, A., Kyambadde, R., & Division, W. I. (1995). *Buffering capacity studies in a rural and an urban wetland in Lake*.

Magagi, J. Y. (2012). Effects of waste dump on the quality of plants cultivated around

mpape dumpsite fct abuja, nigeria. *Ethiopian Journal of Environmental Studies and Management EJESM*, *5*(4), 567–573. https://doi.org/http://dx.doi.org/10.4314/ejesm.v5i4.S17 Received

Maity, A., Natarajan, N., Pastor, M., Vijay, D., Gupta, C. K., & Wasnik, V. K. (2018). *Nanoparticles influence seed germination traits and seed pathogen infection rate in forage sorghum (Sorghum bicolour) and cowpea (Vigna unguiculata)*. *56*(June), 363–372.

Matagi, S. V. (2001). Some issues of environmental concern in Kampala, The capital city of Uganda. *Environmental Monitoring and Assessment*, *77*, 121–138.

Mateo-sagasta, J., Raschid-sally, L., & Thebo, A. (2015). Global Wastewater and Sludge Production , Treatment and Use. *Springer Science and Business Media*, 15–38. https://doi.org/10.1007/978-94-017-9545-6

Mathur, N. (2007). *Mutagenicity evaluation of industrial sludge from common effluent treatment plant*. *67*, 1229–1235. https://doi.org/10.1016/j.chemosphere.2006.10.073

Mathur, N., Bhatnagar, P., & Verma, H. (2006). Genotoxicity of vegetables irrigated. *Journal of Environmental Sciences*, *18*, 964–968.

Mbabazi, J., Wasswa, J., Kwetegyeka, J., & Bakyaita, G. K. (2010). Heavy metal contamination in vegetables cultivated on a major Urban wetland inlet drainage system of Lake Victoria, Uganda. *International Journal of Environmental Studies*, *67*(3), 333–348. https://doi.org/10.1080/00207231003612613

Meeker, J. D., Sathyanarayana, S., & Swan, S. H. (2009). *Phthalates and other additives in plastics : human exposure and associated health outcomes*. 2097–2113.

https://doi.org/10.1098/rstb.2008.0268

Mikula, P. (2017). *Brominated Flame Retardants in the Environment : their Sources and Effects (A Review). June*. https://doi.org/10.2754/avb200675040587

Mortelmans, K., & Zeiger, E. (2000). The Ames Salmonella/microsome mutagenicity assay. In *Mutation Research* (Vol. 455).

Muwanga, A., & Barifaijo, E. (2010). Impact of industrial activities on heavy metal loading and their physico-chemical effects on wetlands of lake Victoria basin (Uganda). *African Journal of Science and Technology*, *7*(1), 51–63. https://doi.org/10.4314/ajst.v7i1.55197

Mydlarczyk, A., Abdullah, H., Aba, A., & Esmaeel, A. (2022). Investigations on pharmaceuticals and radioactive elements in wastewater from hospitals in Kuwait. *14th Gulf Water Conference: Water in the GCC…*, *263*(February), 145–151. https://doi.org/10.5004/dwt.2022.28219

Namasaka, R. W., Tusiime, G., Orawu, M., Gibson, P., Nyiramugisha, J., & Edema, R. (2017). Evaluation of Cowpea Genotypes for Resistance to Fusarium redolens in Uganda. *American Journal of Plant Sciences*, *8*, 2296–2314. https://doi.org/10.4236/ajps.2017.89154

Namiki, M. (1988). Chemistry of Maillard Reactions: Recent studies on the browning reactions mechanism and the development of ntioxidants and mutagens. *Advances in Food Research*, *32*, 115–184.

NEMA. (1995). Standards for Discharge of Effluent into Water or on Land. *The National*

Environmental Act, Cap 153., *20*.

Nepalia, A., Singh, A., Mathur, N., Kamath, R., & Pareek, S. (2018). Ecotoxicology and Environmental Safety Assessment of mutagenicity caused by popular baby foods and baby plastic- ware products : An imperative study using microbial bioassays and migration analysis. *Ecotoxicology and Environmental Safety*, *162*(March), 391–399. https://doi.org/10.1016/j.ecoenv.2018.07.002

Njoku, K. L., Akinola, M. O., & Taiwo, B. G. (2009). Effect of gasoline diesel fuel mixture on the germination and the growth of Vigna unguiculata (Cowpea). *African Journal of Environmental Science and Technology*, *3*(12), 466–471. http://www.academicjournals.org/AJEST

Norppa, H., & Makipaakkanen, J. (1987). Chromosome aberrations and sister chromatid exchanges induced by technical grad toluene diisoctanate and methylenediphenyl diisocyanate in culture human lymphocytes. *Toxicology Letters*, *36*, 37–43.

Nupur, M., Pradeep, B., & Prakash, B. (2012). Use of Salmonella / microsome reversion bioassay for monitoring industrial wastewater treatment plants in Rajasthan , India. *Journal of Environmental Biology*, *May*, 2012.

Nyakaana, J. B. (2012). *Solid waste management in urban centers : The case of Kampala - Uganda*. *7961*. https://doi.org/10.1080/00707961.1997.9756235

Ohe, T., Watanabe, T., & Wakabayashi, K. (2004). Mutagens in surface waters : a review. *Science Direct*, *567*, 109–149. https://doi.org/10.1016/j.mrrev.2004.08.003

Oki, O. C. (2014). Influence of Colchicine Treatments on Character Expression and Yield

Traits in Cowpea (Vigna. *Global Journal of Science Frontier Research*, *14*(5).

Okunola, A., Babatunde, E. E., Chinwe, D., Pelumi, O., & Ramatu, S. G. (2014). Mutagenicity of automobile workshop soil leachate and tobacco industry wastewater using the Ames Salmonella fluctuation and the SOS chromotests. *Toxicology and Industrial Health*. https://doi.org/10.1177/0748233714547535

Okunolaa, A., Sorungbe, A., & Adeoluwa, Y. (2019). In vitro mutagenicity and genotoxicity of raw and simulated leachate from plastic waste dumpsite. *Toxicology Mechanisms and Methods*, *0*(0), 000. https://doi.org/10.1080/15376516.2019.1566426

Olasupo, F. O., Ilori, C. O., Forster, B. P., & Bado, S. (2016). Mutagenic Effects of Gamma Radiation on Eight Accessions of Cowpea (Vigna unguiculata [L .] Walp .). *American Journal of Plant Sciences*, *7*, 339–351. https://doi.org/http://dx.doi.org/10.4236/ajps.2016.72034 Mutagenic

Ologundudu, F., Ajayi, O., Ajayi, O., Ajani, I., & Oladipupo, S. (2018). Aluminium tolerance: a determinant factor to cowpea Vigna unguiculata (L.) Walp. (Fabales: Fabaceae) productivity. *Brazilian Journal of Biological Sciences*, *5*(9), 105–113. https://doi.org/10.21472/bjbs.050911

Otansev, P., Tas, H., & Bas, A. (2016). Distribution and environmental impacts of heavy metals and radioactivity in sediment and seawater samples of the Marmara Sea. *Chemosphere*, *154*, 266–275. https://doi.org/10.1016/j.chemosphere.2016.03.122

Pan, W., Xiong, C., Wu, Q., Liu, J., Liao, H., Chen, W., & Liu, Y. (2013). Chemosphere Effect of BPA on the germination , root development , seedling growth and leaf differentiation under different light conditions in Arabidopsis thaliana. *Chemosphere*,

93(10), 2585–2592. https://doi.org/10.1016/j.chemosphere.2013.09.081

Paul, W. (2011). *Impact Of Industrial Effluent on Water Quality of Receiving Streams in Nakawa-ntinda, Uganda*. Makerere University.

Pedrosa, M., Sobrinho, V., Monteze, E., Cristina, D., Navarro-silva, M. A., Sof, P., Souza, Q., Francisco, B., & Anna-santos, S. (2019). *Science of the Total Environment Effects of Cipro fl oxacin and Roundup on seed germination and root development of maize*. *651*(October 2018), 2671–2678. https://doi.org/10.1016/j.scitotenv.2018.09.365

Pérez, S., Reifferscheid, G., Eichhorn, P., & Barceló, D. (2003). Assessment of the mutagenic potency of sewage sludges contaminated with polycyclic aromatic hydrocarbons by an Ames fluctuation assay. *Environmental Toxicology and Chemistry*, *22*(11), 2576–2584. https://doi.org/10.1897/02-416

Picariello, E., Baldantoni, D., & Nicola, F. De. (2020). Acute effects of PAH contamination on microbial community of different forest soils *. *Environmental Pollution*, *262*, 114378. https://doi.org/10.1016/j.envpol.2020.114378

Pottenger *et al*. (2018). Understanding the Importance of Low-Molecular Weight (Ethylene Oxide- and Propylene Oxide-induced) DNA Adducts and Mutations Collaborative Discussions. *Environmental AndMolecularMutagenesis (2018) Review*, *August*. https://doi.org/10.1002/em.22248

Privat, E. J., & Sowers, L. C. (1996). A proposed mechanism for the mutagenicity of Sformyluracil. *Elsevier Science B.V. All*, *354*, 151–156.

Priyadharshni1, S., Saravanan, K., Elanchezyan, A., Kavitha, P., & Arumugam, M. (2020).

Breeding Efficiency and effectiveness of physical and mutagens in cowpea (Vigna ungiculata (L.) Walp). *Journal of Plant Breeding*, *11*(3), 803–808. https://doi.org/https://doi.org/10.37992/2020.1103.132

Qi, Y., Wu, Y., Zhi, Q., Zhang, Z., Zhao, Y., & Fu, G. (2024). *Effects of Polycyclic Aromatic Hydrocarbons on the Composition of the Soil Bacterial Communities in the Tidal Flat Wetlands of the Yellow River Delta of China*.

Qualhato, G., Rocha, T. L., Celma, E., Lima, D. O., Melo, D., Cardoso, J. R., Grisolia, C. K., & De, S. M. T. (2017). Genotoxic and mutagenic assessment of iron oxide (maghemite-γ-Fe2O3) nanoparticle in the guppy Poecilia reticulata 3. *Chemosphere*. https://doi.org/10.1016/j.chemosphere.2017.05.061

Raina, A., Khursheed, S., & Khan, S. (2018). Optimisation of Mutagen Doses for Gamma Rays and Sodium Azide in Cowpea Genotypes. In *Trends in Biosciences* (Vol. 13, Issue 11). https://www.researchgate.net/publication/324691911%0AOptimisation

Raina, A., Laskar, R. A., Tantray, Y. R., & Khursheed, S. (2020). *Characterization of Induced High Yielding Cowpea Mutant Lines Using Physiological , Biochemical and Molecular Markers*. 1–22. https://doi.org/10.1038/s41598-020-60601-6

Rao, K., Xu, Y., Shaw, E., & Parton, J. W. (2004). Mutagenicity Testing Applied for Regulation of Developing Products. *Current Separations*, *4*(20), 141–144.

Rehana, Z., Malik, A., & Ahmad, M. (1995). Mutagenic activity of the ganges water with special reference to the pesticide pollution in the river between Kachla to Kannauj (U.P.), India. *Mutation Research/Genetic Toxicology*, *343*(2–3), 137–144. https://doi.org/10.1016/0165-1218(95)90079-9

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., & Nishigaki, I. (2015). Asian Pacific Journal of Tropical Biomedicine. *Asian Pacific Journal of Tropical Biomedicine*, *5*(3), 182–189. https://doi.org/10.1016/S2221-1691(15)30003-4

Rodrigues, L., Souza, R., Bernardes, L. E., Felipe, M., Barbetta, S., Andreia, M., & Silva, M. (2019). *Iron oxide nanoparticle phytotoxicity to the aquatic plant Lemna minor : effect on reactive oxygen species (ROS) production and chlorophyll a / chlorophyll b ratio*. *Escobar 2015*.

Roy, U., Basak, D., & Nath, S. (2019). Mutagenic sensitivity analysis of gamma irradiations in Cowpea (Vigna unguiculata L . Walp). *Emergent Life Sciences Research*, *5*, 12–16. https://doi.org/https://doi.org/10.31783/elsr.2019.521216

Saviex, L., Nath, K., Singh, D., Shyam, S., & Sharma, Y. K. (2008). Effect of chromium and tannery effluent toxicity on metabolism and growth in cowpea (Vigna sinensis L . Saviex Hassk) seedling. *ResearchGate*, *June*, 91–94. https://www.researchgate.net/publication/207896797%0AEffect

Shafqat, U., Hussain, S., Shahzad, T., Shahid, M., & Mahmood, F. (2023). Elucidating the phytotoxicity thresholds of various biosynthesized nanoparticles on physical and biochemical attributes of cotton. *Chemical and Biological Technologies in Agriculture*, 1–15. https://doi.org/10.1186/s40538-023-00402-x

Sharif, A., Ashraf, M., Anjum, A. A., Javeed, A., Altaf, I., Akhtar, M. F., Abbas, M., & Akhtar, B. (2015). Pharmaceutical wastewater being composite mixture of environmental pollutants may be associated with mutagenicity and genotoxicity. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-015-5478-3

Sharif, A., Ashraf, M., Javeed, A., Anjum, A. A., Akhtar, M. F., Akhtar, B., & Saleem, A. (2016). Oxidative stress responses in Wistar rats on subacute exposure to pharmaceutical wastewater. *Environmental Science and Pollution Research*, *23*, 24158–24165. https://doi.org/10.1007/s11356-016-7717-7

Sharma, R., & Kaur, R. (2020). Physiological and metabolic alterations induced by phthalates in plants : possible mechanisms of their uptake and degradation. *Environmental Sustainability*, *November*. https://doi.org/10.1007/s42398-020-00141-x

Sheta, M. H., El-wahed, A. H. M. A., Elshaer, M. A., & Bayomy, H. M. (2024). *Green Synthesis of Zinc and Iron Nanoparticles Using Psidium guajava Leaf Extract Stimulates Cowpea Growth , Yield , and Tolerance to Saline Water Irrigation*.

Shorobi, Fauzia & Vyavahare, Mr. Govind & Seok, Yeong & Park, J. H. (2023). *Effect of polypropylene microplastics on seed germination and nutrient uptake of tomato and cherry tomato plants.*

Siddiqui, A. H., & Ahmad, M. (2003). The Salmonella mutagenicity of industrial, surface and ground water samples of Aligarh region of India. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *541*(1–2), 21–29. https://doi.org/10.1016/S1383-5718(03)00176-1

Smith, S. E., Kuehl, R. O., Ray, I. M., Hui, R., & Soleri, D. (1998). Evaluation of Simple Methods for Estimating Broad-Sense Heritability in Stands of Randomly Planted Genotypes. *Crop Science*, *37*, 1125–1129. https://doi.org/10.2135/cropsci1998.0011183X003800050003x

Solomon, J. J., Mukai, F., Fedyk, J., & Segal, A. (1988). Reactions of Propylene Oxide

With 2 Deoxynucleosides and in Vitro With Calf Thymus DNA. *Chem.-BioL Interactions, 67*, *67*, 275–294.

Ssempebwa, J. C., & Carpenter, D. O. (2009). The generation ,use and disposal of waste crankcase oil in developing countries : A case for Kampala district , Uganda. *Journal of Hazardous Materials Journal*, *161*, 835–841. https://doi.org/10.1016/j.jhazmat.2008.04.028

Szalay, B., & Tátrai, E. (2012). *Potential toxic effects of iron oxide nanoparticles in in vivo and in vitro experiments*. *October 2011*, 446–453. https://doi.org/10.1002/jat.1779

Tabrez, S., & Ahmad, M. (2011). Mutagenicity of industrial wastewaters collected from two different stations in northern India. *Journal of Applied Toxicology*, *31*(8), 783–789. https://doi.org/10.1002/jat.1635

Tarso, P. De, Sales, F., Oliveira, D. B. De, Schimidt, F., & Campos, L. C. (2013). Characterization and genotoxicity evaluation of effluent from a pharmacy industry. *Ambiente & Água - An Interdisciplinary Journal of Applied Science*, *8*(12), 34–45. https://doi.org/10.4136/1980-993X

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Molecular, clinical and environmental toxicicology Volume 3: Environmental Toxicology. In *Molecular, Clinical and Environmental Toxicology* (Vol. 101). https://doi.org/10.1007/978-3-7643-8340-4

Teixeira, E. C., Schneider, I. L., Agudelo-casta, D. M., Silva, L. F. O., & Rinc, S. (2017). *Exposure to polycyclic aromatic hydrocarbons in atmospheric PM 1 . 0 of urban environments : Carcinogenic and mutagenic respiratory health risk by age groups *.*

https://doi.org/10.1016/j.envpol.2017.01.075

Tejs, S. (2008). The Ames test : a methodological short review. *Environmental Biotechnolgy*, *4*(1), 7–14.

Touati, L. E., Jacques, M., Tardat, B., Bouchard, L., Despied, S., Monod, I. J., National, C., & Recherche, D. (1995). *Lethal Oxidative Damage and Mutagenesis Are Generated by Iron in ◌ fur Mutants of Escherichia coli : Protective Role of Superoxide Dismutase*. *177*(9), 2305–2314.

Umadevi, P., Kannikaparameswari, N., Selvi, S., & Murugan, S. (2009). Effect of Mercury Exposure on Vigna unguiculata (Cowpea) Seeds. *Nature Environment and Pollution Technology*, *8*.

Unuofin, J. O. (2020). Garbage in garbage out: the contribution of our industrial advancement to wastewater degeneration. *Environmental Science and Pollution Research*, *27*(18), 22319–22335. https://doi.org/10.1007/s11356-020-08944-5

Valdiglesias, V., Carla, C., Natalia, F.-B., Pasaro, E., & Blanca, P. (2015). Effects of IronOxideNanoparticles: Cytotoxicity, Genotoxicity, Developmental Toxicity, and Neurotoxicity. *Environmental and Molecular Mutagenesis*, *56*(September 2014), 125–148. https://doi.org/10.1002/em

Var, L. W., & Komal, P. (2019). Induced Genetic Variability In Cowpeas [Vigna Unguiculata (L .) Walp] Var. Pusa Komal. *ResearchGate*, *December*. https://doi.org/10.13140/RG.2.2.10095.38569

Vergara-fl, V., Bravo, A. G., & Díez, S. (2020). *Chemosphere Transfer and*

bioaccumulation of mercury from soil in cowpea in gold mining sites Durango-Hern a. *250*. https://doi.org/10.1016/j.chemosphere.2020.126142

Victoria, L., Dalahmeh, S., Björnberg, E., Elenström, A., Niwagaba, C. B., & John, A. (2020). Pharmaceutical pollution of water resources in Nakivubo wetlands and. *Science of the Total Environment*, *710*, 136347. https://doi.org/10.1016/j.scitotenv.2019.136347

Walakira, P., & Okot-okumu, J. (2011). *Impact of Industrial Effluents on Water Quality of Streams in Nakawa-Ntinda , Uganda*. *15*(2), 289–296. www.bioline.org.br/ja

Wanasolo, W., & Kansiime, F. (2018). Evaluation of Industrial Effluent Levels in Kinawataka Stream, Its Tributaries and Kinawataka Swamp, Prior to Discharge into Lake Victoria. *American Journal of Chemistry and Materials Science*, *5*(4), 49–56. http://www.openscienceonline.com/journal/ajcms

Wang, W., Craig, Z. R., Basavarajappa, M. S., Hafner, K. S., & Flaws, J. A. (2012). *Mono-(2-Ethylhexyl) Phthalate Induces Oxidative Stress and Inhibits Growth of Mouse Ovarian Antral Follicles 1*. *87*(October), 1–10. https://doi.org/10.1095/biolreprod.112.102467

Wasi, S., Tabrez, S., & Ahmad, M. (2013). Toxicological effects of major environmental pollutants: An overview. *Environmental Monitoring and Assessment*, *185*(3), 2585–2593. https://doi.org/10.1007/s10661-012-2732-8

WHO. (2014). *Chemicals of Public Health Concern* (Issue July). Regional Assesment Report.

Wijaya, L., Alyemeni, M., Ahmad, P., Alfarhan, A., Barcelo, D., El-Sheikh, M. A., & Pico, Y. (2020). Ecotoxicological effects of ibuprofen on plant growth of Vigna unguiculata L.

Plants, *9*(11), 1–14. https://doi.org/10.3390/plants9111473

Xiong, P., Yan, X., Zhu, Q., Qu, G., Shi, J., Liao, C., & Jiang, G. (2019). *A Review of Environmental Occurrence , Fate , and Toxicity of Novel Brominated Flame Retardants*. https://doi.org/10.1021/acs.est.9b03159

Yahaya, H. I., Farouq, A. A., Rabah, A. B., Muhammad, A. B., & Aliyu, R. U. (2021). Effect of Soil Contamination with Crude Petroleum on Cowpea: An Insight into the Prospects of Crop Production in Nigerian Frontier Basins. In *Journal of Environmental and Agricultural Studies* (Vol. 2, Issue 2, pp. 50–62). https://doi.org/10.32996/jeas.2021.2.2.5

Yang, X., Hu, Z., Liu, Y., Xie, X., Huang, L., Zhang, R., & Dong, B. (2022). Effect of pyrene - induced changes in root activity on growth of Chinese cabbage (Brassica campestris L .), and the health risks caused by pyrene in Chinese cabbage at different growth stages. *Chemical and Biological Technologies in Agriculture*, 1–15. https://doi.org/10.1186/s40538-021-00280-1

Zaborowska, M., Wyszkowska, J., Borowik, A., & Kucharski, J. (2021). *Bisphenol A — A Dangerous Pollutant Distorting the Biological Properties of Soil*.

Zhang, P., Yang, D., Zhang, Y., Li, Y., Liu, Y., & Cen, Y. (2020). Re-examining the drive forces of China ’ s industrial wastewater pollution based on GWR model at provincial level. *Journal of Cleaner Production*, *262*, 121309. https://doi.org/10.1016/j.jclepro.2020.121309

Zhang, Q., Yao, Y., Wang, Y., Zhang, Q., Cheng, Z., Li, Y., Yang, X., Wang, L., & Sun, H. (2021). Plant accumulation and transformation of brominated and organophosphate

flame retardants : A review ☆. *Environmental Pollution*, *288*(July), 117742. https://doi.org/10.1016/j.envpol.2021.117742

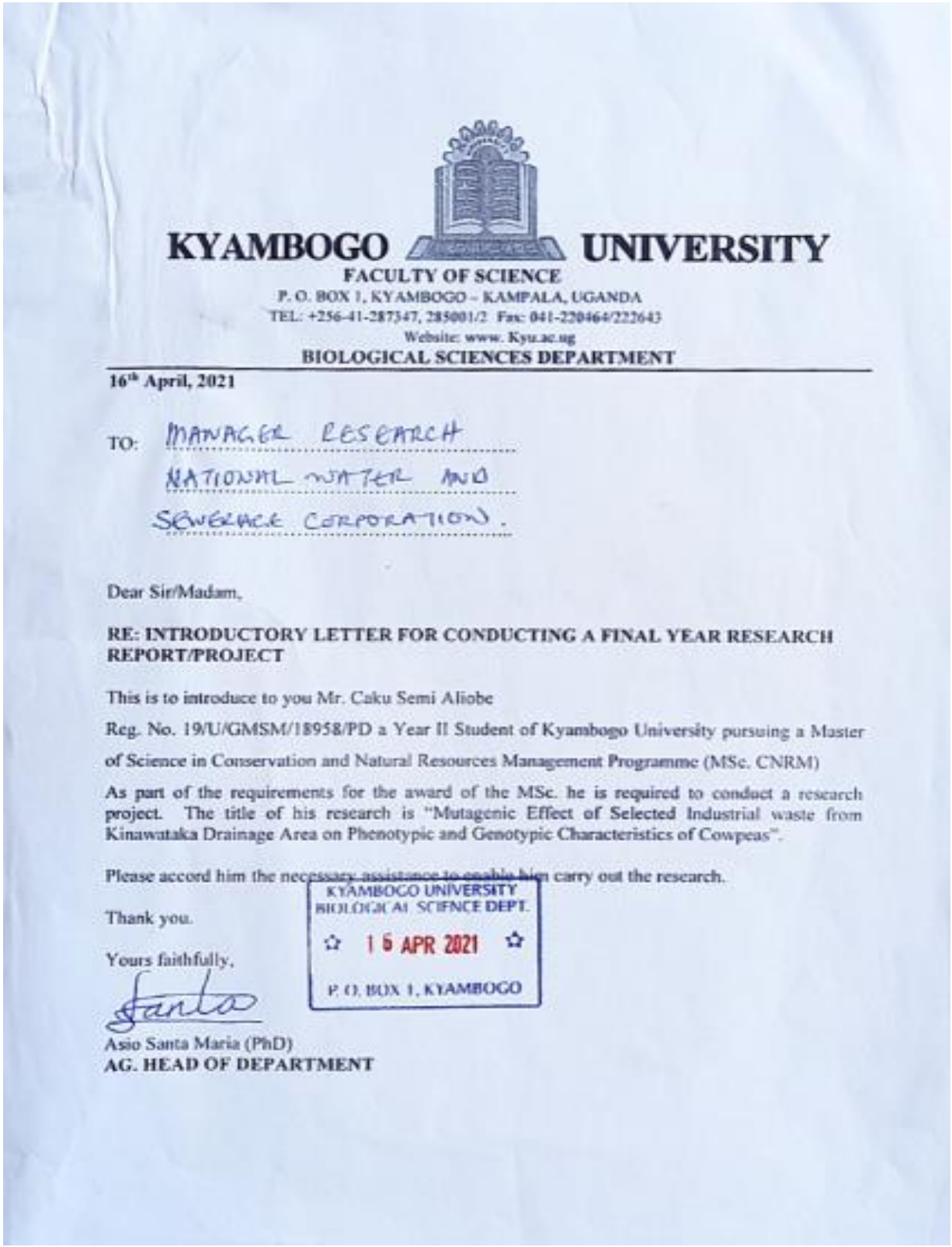
Zia-ur-rehman, M., Naeem, A., Khalid, H., Rizwan, M., Ali, S., & Azhar, M. (2018). Responses of Plants to Iron Oxide. In *Nanomaterials in Plants, Algae, and Microorganisms*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-811487-2.00010-4

APPENDICES

APPENDIX I: RESEARCH INSTRUMENTS

- EBPI's Ames ISO Test Kit – 384 Well Format with all accessories.
- Constructed Green House for planting cowpeas.
- Weighing Scale, Mettler Tolledo with precision of 0.01 mg and max weight of 220 g
- Drying Oven, P- Selector with precision of 0.1 ^{0}C and Max temperature of 250 ^{0}C
- Incubator Set at 37 ^{0}C with precision of 0.1 ^{0}C
- Measuring tape for measurement of length with precision of 0.1cm
- Sterility biosafety cabinet.
- Precision pipette 20 µL, 100 µL, 200 µL, 500 µL, 1000 µL and 10 mL.

APPENDIX II: LETTERS OF INTRODUCTION



KYAMBOGO UNIVERSITY

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Website: www. Kyu.ac.ug

BIOLOGICAL SCIENCES DEPARTMENT

16th April, 2021

TO: MANAGER RESEARCH
NATIONAL WATER AND
SEWERAGE CORPORATION.

Dear Sir/Madam,

RE: INTRODUCTORY LETTER FOR CONDUCTING A FINAL YEAR RESEARCH REPORT/PROJECT

This is to introduce to you Mr. Caku Semi Aliobe

Reg. No. 19/U/GMSM/18958/PD a Year II Student of Kyambogo University pursuing a Master of Science in Conservation and Natural Resources Management Programme (MSc. CNRM)

As part of the requirements for the award of the MSc. he is required to conduct a research project. The title of his research is "Mutagenic Effect of Selected Industrial waste from Kinawataka Drainage Area on Phenotypic and Genotypic Characteristics of Cowpeas".

Please accord him the necessary assistance to enable him carry out the research.

KYAMBOGO UNIVERSITY
BIOLOGICAL SCIENCE DEPT.
16 APR 2021
P. O. BOX 1, KYAMBOGO

Thank you.

Yours faithfully,

Asio Santa Maria (PhD)
AG. HEAD OF DEPARTMENT

NATIONAL WATER & SEWERAGE CORPORATION

HEAD OFFICE

TELEGRAMS WATERS KAMPALA
Telephone : 256 - 313 - 315000/100
Twitter : @nwscug
Facebook : www.facebook.com/waterug
Email : info@nwsc.co.ug

P.O. Box 7053
Plot 3 Nakasero
KAMPALA

Our Ref: Ref: BSS/R&D/21-10

October 18, 2021

TO WHOM IT MAY CONCERN

Dear Sir/Madam,

RE: INTRODUCTION LETTER FOR COLLECTION OF SAMPLES FOR RESEARCH PURPOSES

This is to introduce to you Mr. Caku Semi Aliobe a Student of Kyambogo University pursuing a Master of Science in Conservation and Natural Resources Management Programme (MSC. CNRM).

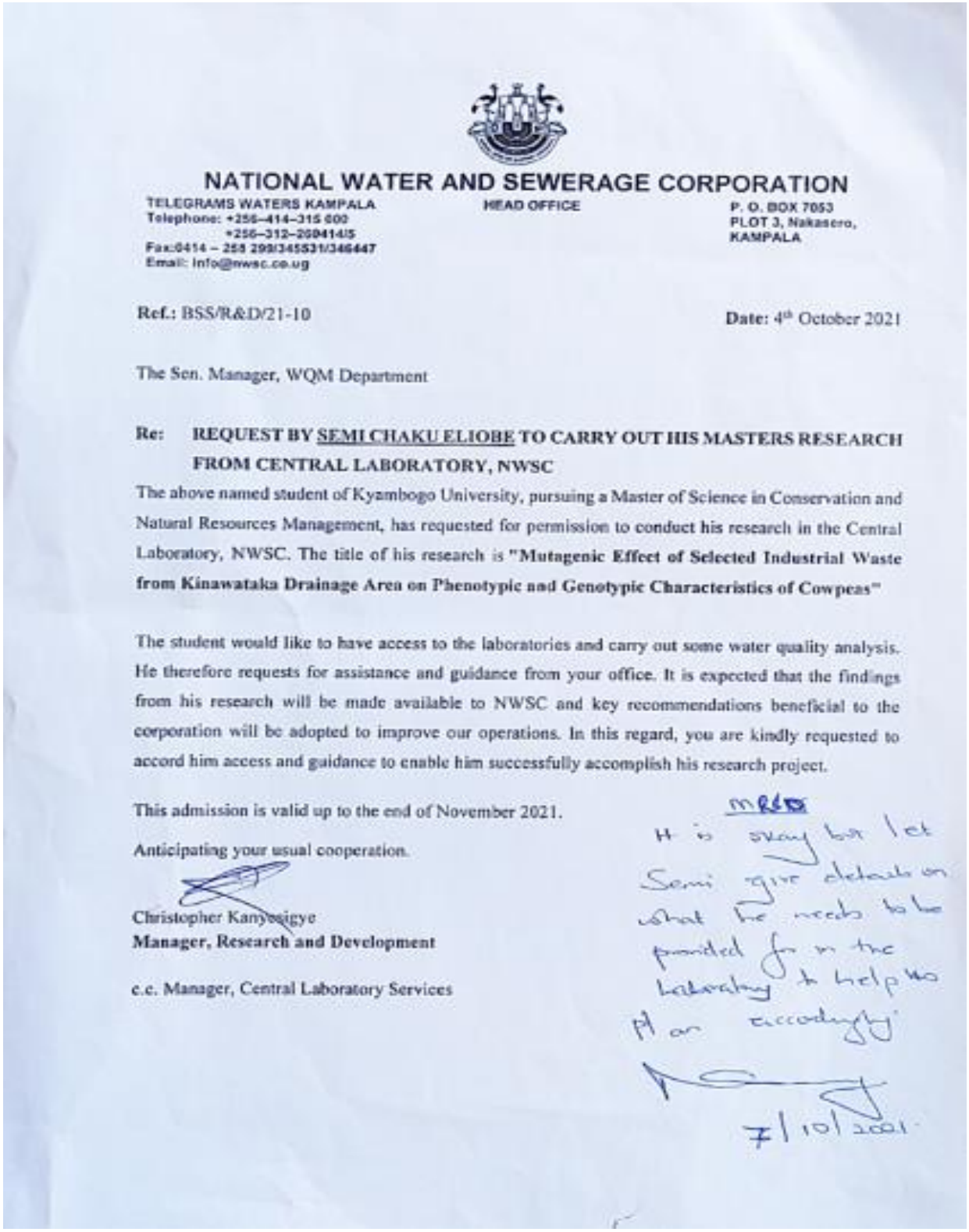
As part of the requirements for the award of the MSc, he is required to conduct a research project. The title of his research is "Mutagenic Effects of selected Industrial Waste from Kinawataka Drainage Area on Phenotypic and Genotypic Characteristics of Cowpeas".

Please accord him the necessary information to enable him carry out the research.

Yours faithfully,

Mr. Christopher Kanyesigye
MANAGER RESEARCH AND DEVELOPMENT

APPENDIX III: AUTHORIZATION LETTER



NATIONAL WATER AND SEWERAGE CORPORATION

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HEAD OFFICE

P. O. BOX 7053
PLOT 3, Nakasero,
KAMPALA

Ref.: BSS/R&D/21-10

Date: 4th October 2021

The Sen. Manager, WQM Department

Re: REQUEST BY SEMI CHAKU ELIOBE TO CARRY OUT HIS MASTERS RESEARCH FROM CENTRAL LABORATORY, NWSC

The above named student of Kyambogo University, pursuing a Master of Science in Conservation and Natural Resources Management, has requested for permission to conduct his research in the Central Laboratory, NWSC. The title of his research is **"Mutagenic Effect of Selected Industrial Waste from Kinawataka Drainage Area on Phenotypic and Genotypic Characteristics of Cowpeas"**

The student would like to have access to the laboratories and carry out some water quality analysis. He therefore requests for assistance and guidance from your office. It is expected that the findings from his research will be made available to NWSC and key recommendations beneficial to the corporation will be adopted to improve our operations. In this regard, you are kindly requested to accord him access and guidance to enable him successfully accomplish his research project.

This admission is valid up to the end of November 2021.

Anticipating your usual cooperation.

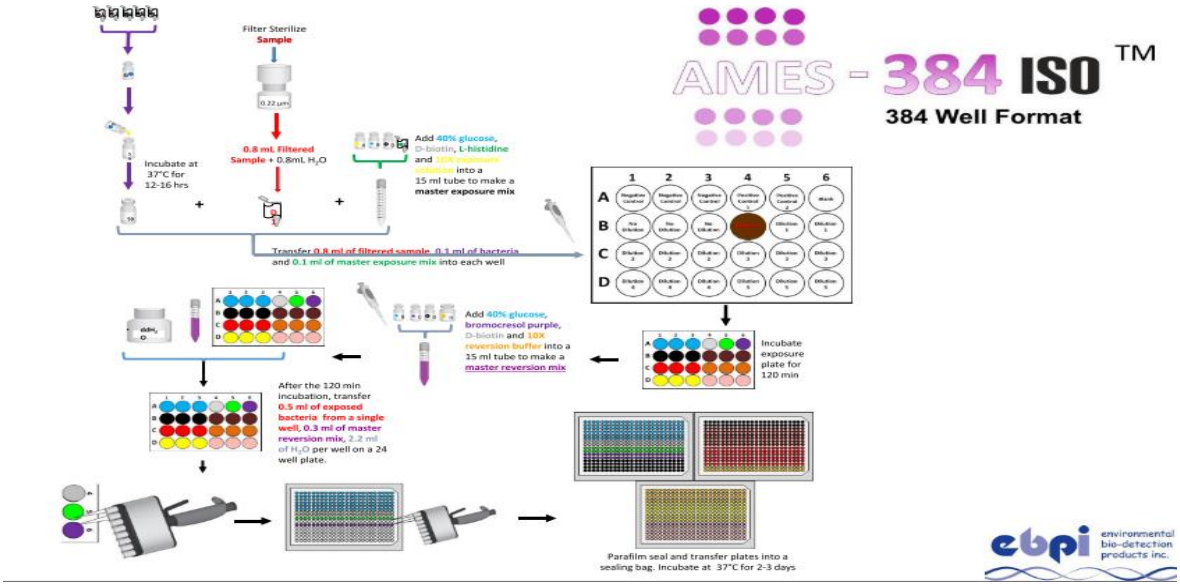
Christopher Kanyesigye
Manager, Research and Development

c.c. Manager, Central Laboratory Services

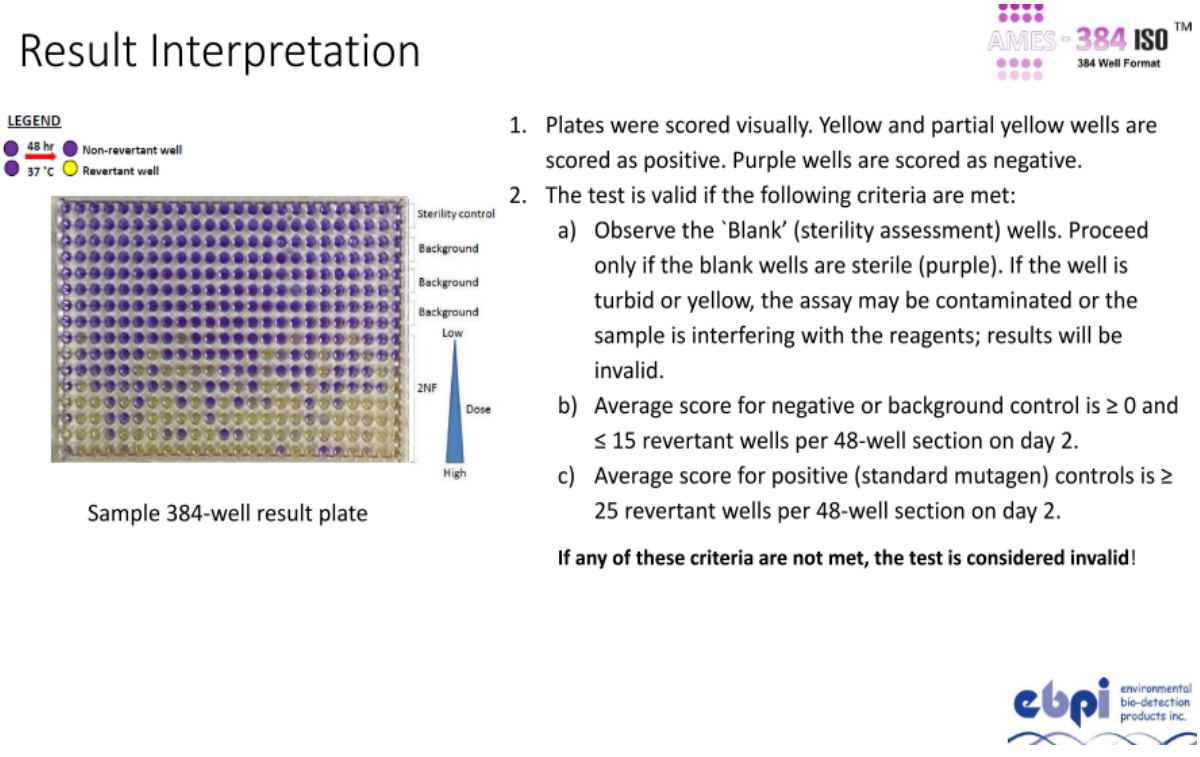
MRDS
He is okay by let Semi give details on what he needs to be provided for in the laboratory to help him accordingly
7/10/2021

APPENDIX IV: AMES TEST PROCEDURE

a) SUMMARY AMES – 384 ISO PROCEDURE VERSON 1.1



b) RESULTS INTERPRETATION



APPENDIX V: DATA ANALYSIS

Objective One

Table 5.1: The statistical results significance as provided for by the Ames 384 test kit at (P>0.05)

Result Significance

AMES - 384 ISO™
384 Well Format

No. Wells Positive in Background Plate	No. Wells Positive in Treatment Plate			No. Wells Positive in Background Plate	No. Wells Positive in Treatment Plate		
	0.05	0.01	0.001		0.05	0.01	0.001
0	3	6	9	24	33	36	40
1	5	8	12	25	34	37	40
2	7	10	14	26	35	38	41
3	9	12	15	27	36	39	42
4	10	13	17	28	36	40	43
5	12	15	19	29	37	40	43
6	13	16	20	30	38	41	44
7	14	18	21	31	39	42	45
8	16	19	23	32	40	43	46
9	17	20	24	33	41	44	46
10	18	21	25	34	42	44	47
11	19	23	27	35	43	45	47
12	20	24	28	36	43	46	48
13	21	25	29	37	44	46	49
14	22	26	30	38	45	47	49
15	24	27	31	39	46	48	50
16	25	28	32	40	46	48	50
17	26	29	33	41	47	49	50

- Depending of amounts of revertant wells in tested samples and revertant wells in negative controls, different levels of significance can be assigned
- Use the quick reference chart included with your procedure (left) or more advanced statistical methods to assign significance to mutagenicity results

In general, for mutagenicity to be suggested, there must be greater than double the amount of revertant wells compared to controls and evidence of a concentration-dependant dose-response relationship. (lower doses produce lower amounts of revertants)

ebpi environmental bio-detection products inc.

Objective Two and Three

Data for objective two and three were analysed using R-Programming language at $p \geq 5\%$ and Mean, CV and LSD. Superscripts were assigned to the significant differences.

Levene's Test of Equality of Error Variances

Table 5.2: Shows results for levene's test of equality for normality test.

		Levene Statistic	df1	df2	Sig.
No of Pods / Plant	Based on Mean	1.224	22	45	0.277
	Based on Median	0.295	22	45	0.999
	Based on Median and with adjusted df	0.295	22	31	0.998
	Based on trimmed mean	1.118	22	45	0.365
No of Root Nodules/ Plant	Based on Mean	0.744	22	45	0.771
	Based on Median	0.278	22	45	0.999
	Based on Median and with adjusted df	0.278	22	32	0.999
	Based on trimmed mean	0.702	22	45	0.813
10 Dry Nodule Weight (Grams)	Based on Mean	1.011	22	45	0.471
	Based on Median	0.405	22	45	0.988
	Based on Median and with adjusted df	0.405	22	21	0.980
	Based on trimmed mean	0.963	22	45	0.523
Dry Weight of 100 Seeds (grams)	Based on Mean	1.052	22	45	0.429
	Based on Median	0.354	22	45	0.995
	Based on Median and with adjusted df	0.354	22	30	0.993
	Based on trimmed mean	0.989	22	45	0.495
Dry Weight of Roots (g)	Based on Mean	1.783	22	45	0.050
	Based on Median	0.467	22	45	0.972
	Based on Median and with adjusted df	0.467	22	24	0.962
	Based on trimmed mean	1.647	22	45	0.078
Dry Weight of Seedlings	Based on Mean	1.658	23	83	0.050
	Based on Median	0.832	23	83	0.682
	Based on Median and with adjusted df	0.832	23	40	0.675
	Based on trimmed mean	1.528	23	83	0.084

APPENDIX VI: PHOTOGRAPHY

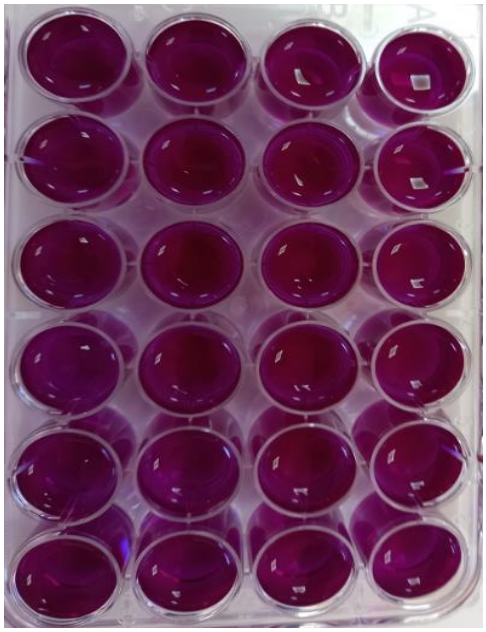
Objective 1. mutagenicity

1. Ames test kit set

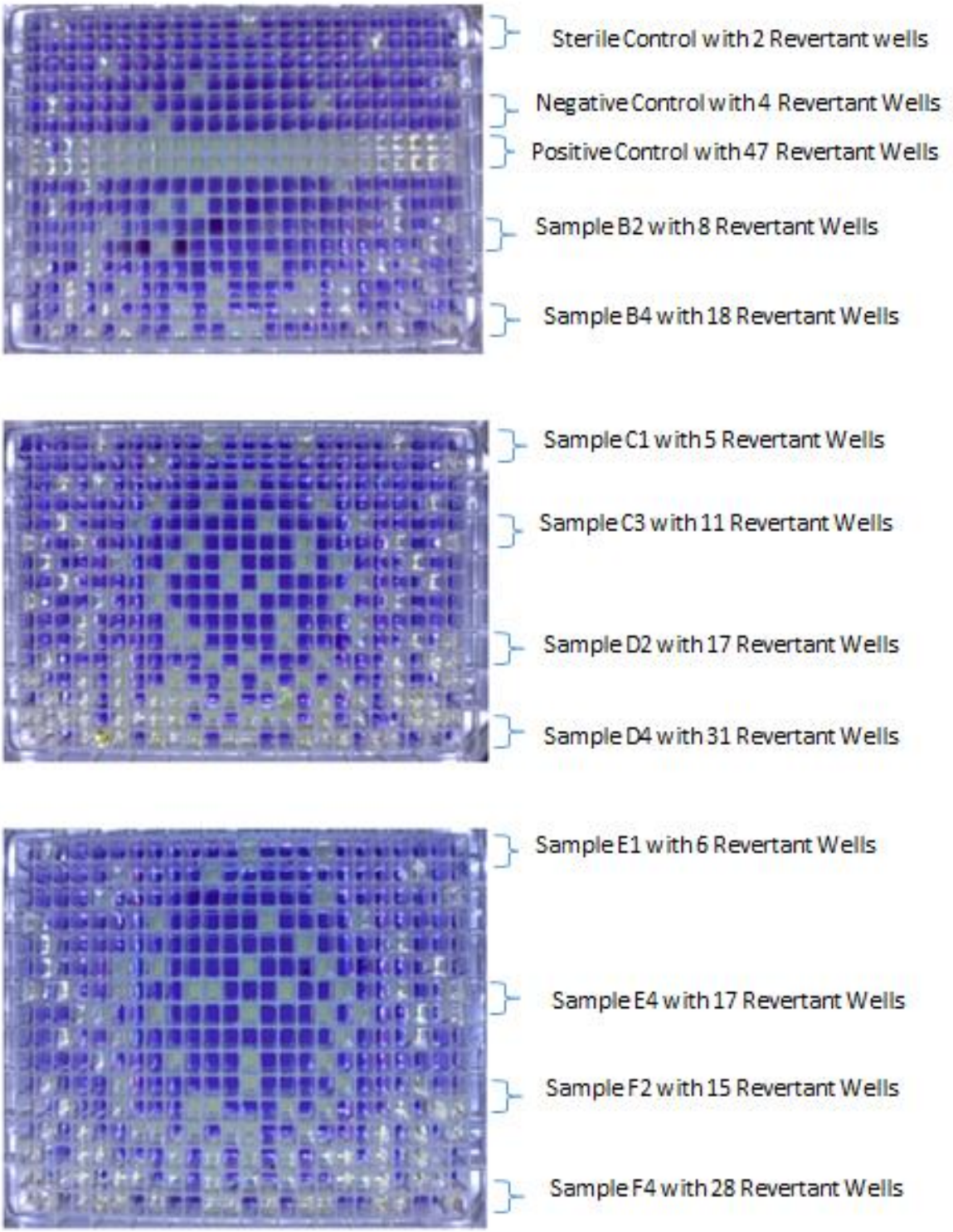
2. Researcher Performing Ames Test

3. Incubation of Samples at 37^{o}C

4. 24 Well Reversion Plate



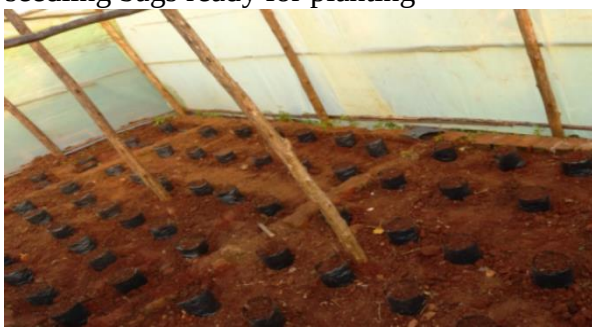
5. Experiment III using *Salmonella typhimurium* TA 100 Results showing yellow and purple Revertant Wells



Objective Two: Seedling Growth

1. Top Soil collected for filling polyethene Bags

2. Green House with poly-ethane seedling bags ready for planting



3. Seedlings from treatment with Distilled Water

4. Seedlings from treatment with Mattress Effluent

5. Seedling 1 (AO c)

6. Seedlings 2 (D4 a)

7. Oven Drying of Seedlings

8. Cowpeas Seedling

Objective Three: Growth and Yield Characteristics Cowpeas.

1. Completely Randomized Plot Layout

BI MONTHLY COWPEAS GROWTH OBSERVATION						
1	A0a	E4a	B1c	G2c	D4b	C1a
2	F1b	C4b	D1b	G1a	B4b	E1a
3	Aob	E4c	B1b	G4a	D2b	C2a
4	C1c	D3b	G1b	B2c	E3a	F4a
5	F1c	Aob	E2b	B3b	G2c	D2a
6	B2a	E3c	Aoc	F2b	C2c	C3b
7	G1c	D3c	C3a	F3a	E2a	B3c
8	E1b	Aoc	F2c	C2b	D2b	G4a
9	B2b	D3a	F3b	F2a	G3b	G1a
10	F3c	D4c	G2a	B3a	C3c	E2c
11	AOb	E3b	C4a	B4c	G3c	D1a
12	G2b	B1b	C1b	E4b	AOC	F4b
13	D4a	F1a	Aoa	E1c	C4C	B4a
14			F4c	D1c	G3a	

2. Cowpeas Seedlings at seven weeks

3. Experiment plot

4. Harvesting of ready seeds

5. Harvested Plant of Cowpeas

6. Shoot ready for measurement.