PROJECT RESEARCH WORK

ON

ISOLATION OF MICROORGANISMS ASSOCIATED WITH AGROCHEMICAL SOIL

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CERTIFICATION

This is to certify that this Project report was written by Fasasi Emmanuel Femi with matric number HND/23/SLT/FT/0531 and submitted to the Department of Science Laboratory Technology (S.L.T), Microbiology Unit, Institute of Applied Sciences (IAS), Kwara State Polytechnic, and has been read and approved as a partial fulfillment for the award of Higher National Diploma (HND) in Science Laboratory Technology.

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DEDICATION

This project is dedicated to Almighty God, whose grace and mercy has carried me through every step of this academic journey.

Also,I dedicate this project to my loving mother Ms. Idowu T for her unwavering support, love and encouragement which fueled my passion to make my formal education possible.

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ABSTRACT

This study aimed to isolate and identify microorganisms present in agrochemical-contaminated environments to evaluate their potential role in natural attenuation and bioremediation. Soil samples were collected from agrochemical discharge sites and subjected to microbiological analysis using standard culturing techniques. A variety of bacterial and fungal species were isolated, with predominant genera including Pseudomonas, Bacillus spp, saccharomyces spp, Rhizopus spp. These microorganisms demonstrated varying degrees of tolerance to the toxic components in the soil, suggesting possible adaptation to polluted conditions. The results indicate that these microbes may possess the metabolic capability to degrade or transform agrochemical pollutants, making them potential candidates for biotechnological applications in waste treatment. This study underscores the importance of microbial biodiversity in contaminated environments and highlights the need for further research into microbial-assisted remediation of agrochemical pollution.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 Introduction

Agrochemicals are chemical products used in agriculture to enhance crop production and protect plants from pests, diseases, and weeds. They include a wide range of substances such as fertilizers, pesticides (insecticides, herbicides, and fungicides), growth regulators, and soil conditioners. The primary aim of using agrochemicals is to improve agricultural yield and ensure food security in response to the increasing global population (Tudi et al., 2021). An agrochemical is a chemical product used in industrial agriculture. In most of the cases, agrochemicals refer to pesticides (insecticides, herbicides, fungicides, algaecides, rodenticides, molluscicides and nematicides), fertilizers, soil conditioners, liming and acidifying agents and plant growth regulators that provide benefits and manage agricultural ecosystem. Agrochemicals were introduced to enhance crop yields and minimized the crop losses due to pests and categories as fertilizers and pesticides. In the past sixty years with the wake of the green revolution (1960s), use of nitrogen, phosphorus and potassium based inorganic fertilizers and pesticides has greatly increased food production by improving efficient and economical crop production in order to fulfill the food needs of the rapidly expanding world population (Pal et al., 2006). The history of pesticide use can be divided into three periods of time. During the first period before the 1870s, pests were controlled by using various natural compounds. During the second period, between 1870 and 1945, people began to use inorganic synthetic materials. At the end of the 1800s, people in Sweden used copper and sulfur compounds against fungal attack in fruit and potatoes. Since then, people have been using many inorganic chemicals, including the Bordeaux mixture, based on copper sulfate and lime arsenic, as pesticides. The third period started after 1945, represented by the use of synthetic pesticides with the discovery of the effects of DDT, BHC, Aldrin, Dieldrin, Endrin, Chlordane, Parathion, Captain and 2,4-D. After the entry of pesticides in agricultural ecosystem it become an integral part of agriculture and the research has continued into producing more selective pesticides. While agrochemicals contribute significantly to modern agriculture, their excessive and indiscriminate use has led to environmental pollution, particularly through runoff and effluents discharged into water bodies and soils. These effluents may alter the microbial ecology of the environment and can introduce resistant or pathogenic microorganisms into surrounding ecosystems. Agricultural use of pesticides is a subset of the broader spectrum of industrial chemicals used by modern society, in which OPs and OCs are prominent (Fan et al., 2018). OPs have been widely used in the protection of crops and agricultural and forestry resources, as well as for the control of vectors and disease-transmitting organisms, in which area they have achieved a high level of impact by reducing production losses in crops of considerable importance (Jimenez et al., 2019). On the other hand, although Organ chlorine Pesticides were banned decades ago, as they are very stable molecules, residues are still found in the soils on which they were used. Among their physicochemical properties, it should be noted that Organ chlorine Pesticides, having a halide group, have high physical and chemical stability, while Organ phosphorus Pesticides, due to their phosphate group, are susceptible to metabolism, which increases their degree of toxicity.

Effluent is a liquid waste flowing out of a factory, farm, commercial establishment or a household into a water body such as a river, lake or lagoon or a sewer system or reservoir. Waste discharged into air is called emission (Kristen, 2009). Effluents may also be referred to as sewage. Effluent discharges from domestic, municipal, industrial and agricultural set-ups contain various pollutants such as debris, microorganisms and heavy metals and are transported in untreated forms through drains, water ways and soils into inland water-bodies (Eze and Korie, 2012). The receiving water-bodies loads of pollutants are increased during the rainy season due to water run-off and atmospheric precipitation. Domestic wastes are a consequence of housekeeping activities such as food preparation, sweeping and vacuum cleaning. They also contain fuel residue, empty containers and packaging wastes from repairs and redecorating. Microorganisms in domestic effluents use the waste constituents as nutrients, thus detoxifying the materials as their digestive processes breakdown complex organic molecules into simpler less toxic molecules. A waste is hazardous if it is infectious, meaning containing viable microorganisms or their toxins, which are known or suspected to cause disease in animal or human (Eze and Nwaneti, 2015).

Agrochemical effluents, often resulting from the runoff or improper disposal of agricultural chemicals, pose significant threats to the environment. These effluents can contaminate surface and groundwater, reduce soil fertility, and disrupt the balance of microbial communities in ecosystems. Persistent chemicals such as organochlorines and certain heavy metals in agrochemical formulations may accumulate in the environment, leading to long-term ecological harm. One of the most concerning impacts is the alteration of microbial diversity and the emergence of resistant microbial strains in effluent-contaminated soils and water bodies. These microorganisms may degrade naturally beneficial soil bacteria, thus affecting nutrient cycling and plant growth. Moreover, agrochemical pollutants can bioaccumulate in the food chain, leading to toxic effects in higher organisms, including humans (Fenner et al., 2013).

Agricultural soil pollution also hampers the achievement of Sustainable Development Goals (SDGs), such as zero hunger, ending poverty, ensuring healthy lives, halting and reversing land degradation, and making cities safe and resilient (FAO and UNEP, 2021). It is important to identify and manage pollution sources and to take preventive measures to protect people, plants, animals, and the environment. To this end, several organizations emphasizing the need for sustainable practices to stop soil pollution and achieve zero pollution globally. For instance, the International Network on Soil Pollution (INSOP) focuses on enhancing knowledge on the full cycle of soil pollution, from assessment to remediation, and its effects on environmental and human health. It also seeks to strengthen technical capacities and legislative frameworks for preventing soil pollution and promotes the exchange of experiences and technologies for sustainable soil management and remediation (FAO, 2024).

1.1 Literature review

In the literature, there are examples of pesticide degradation by microorganisms. Biodegradation, like biotransformation, refers to a process in which an organism transforms one chemical compound into another, which involves a series of reactions that occur under certain conditions and at different times, and are usually biochemical factors of the enzymatic activity of the organism. During this process, the biodegradable substance or material is reduced to its basic components (Aldas et al., 2021). Several factors affect or limit a biodegradation process. Among those that have been identified are the substrate selectivity of enzymes and all the possible biochemical mechanisms possessed by microorganisms, the chemical structure and complexity of the compound, and environmental factors such as pH, temperature, and humidity. However, this process allows the behavior of microorganisms in the presence of contaminants in their environment to be studied, as well as the concentration of these substances before, during, and after all the steps involved (Pan et al., 2022; Pischedda et al., 2019).

Agro-food processing industries that use plant protection products (PPPs) constitute a serious point-source for the contamination of the natural water resources (Bao et al., 2021). These include seed-producing industries (SPI), which treat seeds with systemic fungicides like carboxin (CBX), metalaxyl-M (MET-M) and fluxapyroxad (FLX) (Lamichhane et al., 2020; Ayesha et al., 2021), bulb handling industries (BHI) which immerse bulbs into dense solutions of fungicides like chlorothalonil (CHT), thiabendazole (TBZ) and fludioxonil (FLD) (Bansal et al., 2018) and fruit-packaging industries (FPI) that make use of fungicides like imazalil (IMZ) and fludioxonil (FLD) for the control of fungal infections of fruits during storage (Matrose et al., 2021). Taking into consideration the environmental risk stemming from the improper management of the pesticide-contaminated effluents produced by these industries, the European Commission enforced the implementation of appropriate wastewater management practices. Different treatment processes have been tested to date for the depuration of these wastewaters with variable results. A few studies have tested the efficiency of abiotic transformation processes, such as advanced oxidation techniques like TiO2-based photocatalysis (Molla et al., 2020) and photo-Fenton processes (García-Estrada et al., 2020) for the removal of pesticides from these agro-food effluents. Others have used combinations of abiotic and biological processes (Bernardelli et al., 2021). Despite the promising results of some of these methods their full implementation has not been accomplished due to several reasons including (i) high costs of installation and operation, (ii) high chemical addition requirements, (iii) possible sludge formation and (iv) Production of toxic pesticide transformation products which might require further treatment (Ganiyu et al., 2022). Moreover, with the exception of a few recent studies, the vast majority of all these studies were performed with distilled water artificially contaminated with pesticides instead of real agro-industrial effluents. The organic matter and inorganic salts that are present in the industrial effluents act as “radical scavengers” reducing the depuration efficiency of systems based on advanced oxidation and photo-oxidation techniques (Bisaria et al., 2021).

The biggest concern associated with microbial pollution is the risk of human and livestock related illnesses after exposure to contaminated water sources. Often the discharge of improperly treated effluent from WWTPs results in the deposition of large amounts of organic matter and nutrients which have major detrimental effects on the health of these surrounding environments as well as micro- and macro-fauna present (Naidoo and Olaniran, 2014). Excessive nutrient loading can lead to eutrophication and temporary oxygen deficiencies that ultimately alter the energy relationship and water balance, disrupting biotic community structure and function. Excessively turbid effluent discharge can also result in the deposition of sand and grit into the aquatic system, disrupting sediment characteristics and hindering natural water flows (Wakelin et al., 2018). In addition, the overall hydrological and physicochemical environment is often affected due to the discharge of improperly treated effluent with many of the micro- and macro-fauna within these water bodies exhibiting distinct physiological tolerance levels.

Disturbances to the overall environment can severely affect those intolerant individuals either in the form of adverse behavioral characteristics or more severely in the form of death. Often death decreases a large degree of resource competition and predation within the environment thereby resulting in the proliferation of tolerant organisms. This ultimately causes an imbalance amongst the group of organisms present and the overall alterations to the surrounding environment in the form of nutrient modifications, light and oxygen content, food sources as well as habitat loss (Coetzee, 2013). Furthermore, the deposition of excessive nutrients leads to profuse plant growth along river banks which in certain cases may be visually pleasing but can serve as an additional health hazard due to entanglement and poor visibility. Benthic microbial and algal growth may also cause rock and wood surfaces to become slippery, posing a threat to human safety (Wakelin et al., 2018).

Environmental Impact of Agrochemical Effluents; Agrochemical effluents, which originate from agricultural runoff, pesticide application, and improper disposal of farm chemicals, have far-reaching impacts on the environment. These effluents often contain harmful substances such as pesticides, herbicides, fertilizers, and heavy metals that contaminate soil, surface water, and groundwater. The contamination affects soil microbial activity, reduces biodiversity, and disrupts nutrient cycling essential for plant growth. In aquatic ecosystems, agrochemical effluents can lead to eutrophication, which results in algal blooms, oxygen depletion, and the death of aquatic organisms. Pesticide residues in water bodies have also been linked to endocrine disruption and reproductive failure in aquatic fauna. In addition, the bioaccumulation of toxic substances in the food chain can pose serious health risks to animals and humans (Gill & Garg, 2014). Furthermore, the presence of agrochemicals in the environment can promote the development of resistant microbial strains and reduce the population of beneficial soil microbes, affecting soil fertility and crop productivity over time (Sharma et al., 2019).

1.2 Aim and Objective

Aim of the Study: The aim of this study is to isolate, identify microorganisms present in agrochemical effluents to assess their potential environmental and health implications.

Objectives of the Study:

1. To collect agrochemical effluent samples from selected sites.

2. To isolate microbes present in the effluent samples using microbiological techniques.

3. To analyze the physicochemical properties of the agrochemical effluents.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

Soil sample, hot air oven, conical flask, petri-dishes, aluminium foil, incubator, ethanol, beaker, spirit, spirit lamp, cooking gas, ethanol, auto clave, paper tape, pressure pot, distilled water, wire loop, slides, Safranin, Iodine, crystal violet, Lacto phenol, Nutrient agar, Sabrose dextrose agar, Mac conkey agar.

2.2 METHODS

2.2.1 Sterilization of materials: The materials such as conical flask, test tubes, beaker, were washed properly with soapy water and rinsed with distilled water, they were allowed to air dry and was covered and wrapped with aluminum foil paper. They were further sterilized in a hot air oven for 20min and then allowed to cool.

2.2.2 Sample Collection: The soil samples A and B (sample A were collected from Agrochemical In OYUN while sample B were collected from Agrochemical In SANGO and inserted into dry plastic bottles, labeled and subsequently transported to the laboratory for proper analyzes. Soil samples were air dried for 72hours at a room temperature.

2.2.3 Media preparation: 7 grams of nutrient agar powder was weighed out on a weighing balance and was transferred in to a conical flask. 250 ml of distilled water was measured using a measuring cylinder and was transferred in to the conical flask containing the nutrient agar powder. The sample was stirred using a stirring rod to ensure that the powder properly dissolves. The solution was heated on the cooking gas it was continuously stirred to avoid lumps and sediment and was allowed to boil to ensure that the agar has fully dissolved. And the solution appears lighter before the gas was turned off.

Then 16.3 grams of sabouraud dextrose agar powder was weighed out on a weighing balance and was transferred in to another conical flask. 250 ml of distilled water was measured using a measuring cylinder and was transferred in to the conical flask containing the sabouraud dextrose agar powder. The sample was stirred using a stirring rod to ensure that the powder properly dissolves. The solution was heated on the cooking gas it was continuously stirred to avoid lumps and sediment and was allowed to boil to ensure that the agar has fully dissolved. And the solution appears lighter before the gas was turned off. The conical flask containing prepared medium were covered with aluminum foil and was sterilized by the use of pressure pot due to the absence of autoclave.

2.2.4 Innoculation: 5 grams of antibiotics was dissolved into 10ml of distilled water, and 1ml of the antibiotics mixture was injected into the sabouraud dextrose agar media. 20 grams of soil sample from the top of the container was weighed out on a weighing balance and transferred into a beaker and was labeled "Beaker A" and another 20 grams of soil sample from the bottom of the container was weighed out on a weighing balance and transferred into a beaker and was labeled "Beaker B". 100ml of distilled water was poured into each beaker and were stirred gently.

Five (5) sterile Petri-dishes were obtained and labeled accurately. The nutrient agar media was poured in to the plates under aseptic condition. The plates were allowed to solidify and then the organism was inoculated aseptically, then another five (5) sterile Petri-dishes were obtained and labeled accurately, the sabouraud dextrose agar media was poured in to the plates under aseptic condition. The plates were allowed to solidify and then the organism was inoculated aseptically. 1ml of the mixture from beaker A was added to pour plate A which consist of Mac Conkey agar sample and 1ml of the mixture from beaker B was added to pour plate B which consist of Nutrient agar sample.

2.2.5 Incubation: After inoculation has been carried out it was subjected to incubation by which the sample contains Nutrient agar was inoculated for 24-48hrs while sample contains Saboraud dextrose agar were inoculated for 72hrs in an inverted position in a incubator machine.

2.2.6 Macroscopic and Microscopic:

Macroscopic: Wire loop containing the sample were used to streak the sample on solid agar (Nutrient Agar and Saboraud dextrose agar) on the petri dish, and it was inoculated in an incubator at appropriate temperature for Bacteria (37^0C) and Fungi (25^0C) respectively. After incubation the plate was examined for growth without opening and records was taken.

Microscopic: Smear preparation were carried out by the following step; a small portion of colony (from culture) were taken and placed on a clean glass slide, it was spread thinly, Air-dry an then it was heat fixed by briefly passing the slide through a flame. Then it was stain with the following by using Gram staining; Crystal violent was applied for 1min, Iodine 30secs, Decolorized for 15secs and Counterstained was followed for 30secs, and rinse with tap water and leave to dry and it was subjected to viewing under microscope, observation was records accurately.

2.2.7 Physiochemical parameter on agrochemical soil

The soil samples were inserted into dry plastic bottles, labeled and subsequently transported to the laboratory for physico-chemical analyzes. Soil samples were air dried for 72hours at a room temperature, and then ground and sieve with a 2.0mm mesh.

Soil Analyzes: Soil properties namely, Temperature, pH, electrical conductivity, nitrate, phosphate and sulphate contents were analyzed using standard protocol in Chemistry Laboratory Unit of the Science Laboratory Technology Department, Kwara state Polytechnic, Ilorin.

Temperature: Temperature of soil sample at specific location in the field was measured by inserting mercury in bulb thermometer to the depth of 5-10cm in the soil.

pH and Electrical conductivity meter: The electrical conductivity and pH meter of soil samples were measured following aqeous soil suspension methods (ISO 10390; 2005) as directed by Useh et al., (2015).

Nitrate and phosphate: A soil water mixture of 1:5 (w/v) prepared using 10g air-dried soil sample in 50ml deionized water and allowed to stand for 10mins. The electrodes of the digital multimeter (Hannal model) was immersed in the mixture for each discrimination. Nitrate and phosphate level in the test soil sample were respectively determined by spectrophotometric methods 8171and 8048 using portable DR. 900 multimeter spectrometer.

Sulphate: After taken 10ml of the water extract from the suspension samples with the help of pipette transferred into a conical flask. Concentrated Hydrochloric acid was added to make it slightly acidic, followed by heating to boil.

Prepared Barium chloride solution was titrated against the boiled solution to form precipitate and the Barium chloride was slightly added in excess which was recorded as V1.

The solution was neutralized by Ammonia hydroxide, Excess of Barium chloride (Bacl2) in the solution against Potassium chromatin was also added to the solution by means of reagent is evident from the resistant yellow collier of the supernatant solution by using Silver nitrate solution as an eternal indicator, the end point can further be confirmed by adding a drop of silver nitrate to a drop of chromatin solution to form torichered colour.

Total amount of sulphate in soil = 0.0177x100 (v1-v2)

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Results

3.1.1 Result of physiochemical parameter on agrochemical soil sample A and B

S/N	Parameter	Sample A	Sample B
1	Temprature (^oC)	29	30
2	pH	6.85	6.26
3	Electrical conductivity (ns/cm)	4868	3440
4	Organic carbon (gkg^{-1})	5.25	14.21
5	Phosphorus ($mgkg^{-1}$)	9.42	8.73
6	Sulphate	3.2	3.8

Agrochemical Soil Sample A = Sango

Agrochemical Soil Sample B = Oyun

3.1.2 Macroscopic and microscopy observation of the soil sample

SAMPLE ON NUTRIENT AGAR (NA)

Macroscopic Observation

SAMPLE	Observation
A	Raise creamy growth
B	Mucoid growth

Microscopic Observation

SAMPLE	Observation	Inference

A	Gram +ve long rods,	*Bacilli spp*
B	Gram –ve rod shaped	*Pseudomonas spp*

SAMPLE ON SABORAUD DEXTROSE AGAR (SDA)

Macroscopic Observation

SAMPLE	**Observation**
A	Creamy mucoid growth
B	Whitish cotony growth

Microscopic Observation

SAMPLE	**Observation**	Inference
A	Budding yeast	*Saccharomyces spp*

B	Non-septate hyphae	*Rhizopus spp*

3.2 Discussion

The isolation and identification of microorganisms from agrochemical effluents revealed the presence of diverse microbial populations capable of surviving in environments contaminated with chemical pollutants. These organisms were found in varying concentrations, depending on the type and duration of effluent exposure. The predominant microorganisms isolated included bacteria such as *Pseudomonas spp., Bacilli spp.,* while fungi such as *Saccharomyces spp., Rhizopus spp.* These findings are consistent with previous studies that have reported the adaptability of these genera in contaminated environments. The survival and proliferation of these microorganisms in agrochemical-laden environments suggest their potential roles in biodegradation and biotransformation of toxic compounds. For instance, *Pseudomonas and Bacillus* are known for their metabolic versatility and ability to degrade a wide range of xenobiotic compounds. This adaptability may be due to selective pressure exerted by agrochemicals, leading to the evolution of resistant strains.

The presence of these organisms also raises environmental and public health concerns. While some microorganisms may contribute positively by breaking down harmful chemicals, others may pose risks through the production of toxins or the spread of antibiotic resistance. Therefore, the proper treatment and management of agrochemical effluents before environmental discharge are essential. The physico-chemical characteristics of the effluents (such as pH, temperature, and nutrient availability) appeared to influence the diversity and abundance of microbial isolates. Effluents with high toxicity showed reduced microbial counts, indicating the inhibitory effects of certain agrochemicals.

CHAPTER FOUR

CONCLUSION AND RECOMMENDATION

4.1 Conclusion

This study successfully isolated and identified various microorganisms associated with agrochemical soil, highlighting their potential ecological significance and utility in bioremediation. The findings confirm that certain bacteria and fungi have adapted to survive in harsh chemical environments and may serve as candidates for further studies in pollutant degradation. However, while some of these organisms may have beneficial applications, the environmental release of untreated agrochemical effluents remains a critical issue. Efforts should be made to promote environmentally safe disposal practices and the development of biological treatment systems leveraging the natural degradative abilities of these microorganisms.

4.2 Recommendation

Based on the findings of this study, the following recommendations are proposed:

i. Microorganisms isolated from agrochemical effluents, particularly those showing high tolerance and potential for pollutant degradation, should be further studied and utilized in the development of cost-effective and eco-friendly bioremediation technologies.

ii. Industries and agricultural operations generating agrochemical effluents should implement routine microbiological and chemical monitoring to assess the environmental impact of their waste and ensure compliance with environmental regulations.

iii. Agrochemical effluents should undergo appropriate biological or physicochemical treatment before being released into the environment to reduce toxicity and minimize ecological damage.

iv. There should be increased awareness and adoption of biodegradable and less toxic agrochemical alternatives to reduce the long-term environmental burden.

v. Molecular and genomic studies should be conducted to understand the metabolic pathways used by the isolated microorganisms for agrochemical degradation, which can enhance the effectiveness of microbial remediation processes.

vi. Awareness programs should be introduced to educate farmers and agrochemical handlers on the dangers of improper disposal and the benefits of sustainable management practices.

vii. Governments and environmental agencies should strengthen policies on effluent management and provide incentives for industries that adopt green technologies for waste treatment.

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