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Effect of organochlorine pesticide consortium on the indigenous farm soil microorganisms

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Abstract
The soil samples were taken from the school farm at Rivers State University in Port Harcourt, Rivers State. A sterile soil auger was used to extract a soil sample from a depth of 0 to 20 cm. The material was delivered to the microbiology lab at Rivers State University for examination. The effects of organochlorine pesticide on the soil microorganisms were investigated using five (5) different plastic bowls. 100 grams of soil sample were weighed and placed in each plastic dish. For each plastic bowl containing the 100g soil sample, different amounts of the organochlorine pesticide (force-up, dieldrin) were added: 5%, 10%, 15%, 20%, and 25%. Bacterial and fungal population were analyzed interval of seven days at; 7days, 14days and 21adays, using standard procedures. The total heterotrophic bacteria counts (THBC) ranged from 1.0 X 10^5cfu/g to 9.0 X 10^5cfu/g while the fungi count ranged from 1.0x10^3cfu/g to 6.0x10^3cfu/g. A total of 166 bacteria and 73 fungi were isolated from the contaminated soil belonging to 9 and 7 genera of bacteria and fungi, respectively. The bacterial isolates included; *Flavobacterium* sp, *Enterobacter cloacae, Klebsiella* sp, *Staphylococcus* sp, *Pseudomonas* sp, *Serratia* sp., *Acinetobacter* sp and *Alcaligenes* sp. *The bacterial isolates with the highest percentage occurrence was Bacillus ((23.5%).* Whereas, the fungi isolates included; *Mucor vacernosus, Trichoderma* sp, *Aspergillus Niger, Penicillium* sp, *Fusarium* sp, *Candida* sp *and Verticillum.* The fungi isolate with the highest percentage occurrence is the *Aspergillus Niger* (27.4%). The findings of this study demonstrated that the impacts of the organochlorine pesticide (OCP) resulted in a reduction in the number of soil microorganisms present in the contaminated soil as compared to that of the uncontaminated soil. This demonstrated the pesticide's tested impact on soil microbes.

Keywords: Bacterial isolates, effect, fungal isolates, organochlorine pesticide, soil microorganisms

Introduction

Pesticides are being widely and inappropriately used to boost crop output in Port Harcourt as a result of the city's rapidly expanding human population (Husain *et al*., 2003) [15]. According to Miglioranza *et al*. (2003) [22], this has grown to be a significant environmental hazard. To evaluate how frequently used pesticides affect soil microbial numbers and activities (Doolotkeldieva *et al*., 2018) [8]. Sprays of pesticides may reach the soil directly or by dropping to the ground if they are not blocked by vegetation (Doolotkeldieva *et al*., 2018) [8]. Individual populations of microflora may suffer substantial consequences due to the exposure rate (Cycon *et al*., 2010) [7]. The specific pesticide, its concentration, and its duration all affect how the microbial community structure is affected by pesticides (Tien *et al*., 2012; Ajitha *et al*., 2022) [35, 1]. The current study looks on how commonly used pesticides affect the microbial populations in soil.

The soil top layer is typically where microbial activity and pesticide residue accumulations coexist. According to Subhani *et al*. (2000) [30], the types of pesticides employed, their volumes, and the soil's characteristics all affect the behavior of soil microorganisms. The effects of various pesticides on the development of soil microorganisms and their activities are currently a major source of concern because, regardless of the pesticides' use levels (low or high), they have an impact on the chemical and biological properties as well as the physical nature, activity, and soil microorganisms. The biodegradation of organic matter, recycling of nutrients, humus formation, soil structural stability, nitrogen fixation, stimulation of plant growth, disease biocontrol, and other biochemical transformations like ammonification, nitrification, and phosphorus solubilization are just a few of the important roles that beneficial soil microorganisms play in soil fertility and productivity (Barragan-Huerta *et al*., 2007) [3]. Alternative planting techniques are now advised in order to safeguard the fragile ecological makeup of agricultural soils.

As a result, the current work explores how organochlorine pesticides affect soil microorganisms as well as how soil microorganisms affect the quality of farm soil.

Bacteria, actinomycetes, fungi, and algae are among the soil microorganisms that are typically found near the plant roots in the top layer of soil (Fang *et al*., 2010) [10]. Soil microorganisms often increase the fertility of the soil by decomposing organic matter and contributing humus, hence enhancing soil quality (Egbe *et al*., 2020) [9]. They have a crucial role in the cycling of nutrients, the weathering of rocks and minerals, and the stabilization of soil aggregates (Husain *et al*., 2003) [15]. However, unneeded anthropogenic activities and excessive chemical use, particularly with organo-chorine pesticides that increase agricultural productivity, lead to environmental pollution and have a significant negative impact on both the healthy soil structure and these soil microorganisms (Gill *et al*., 2014; Jayaraj *et al*., 2016; Egbe *et al*., 2020) [13, 17, 9].

According to the EPA (2012), a pesticide is any substance or mixture of substances that is used to prevent, eliminate, repel, or mitigate any pest (insects, mites, nematodes, weeds, rodents, etc.). Pesticides can also be used to control pest. The term "pesticides" refers to any substance or combination of substances intended for preventing or controlling pests. This definition includes any substance or combination of substances intended for use as a defoliant, plant growth regulator, or administered to animals to promote growth or alter their reproductive behavior. According to Pandit and Sharma (2002) [28], pesticides continue to represent the greatest class of hazardous compounds that pose a serious threat to the environment and public health. An estimated one-third of the world's agricultural production is lost each year due to pest problems (Beena and Kathpal, 2010) [4]. Increased soil, water, and air pollution is a result of the increased usage of pesticides in modern agriculture. Even after many years of application, the nature of pesticides, such as lipophilicity and bioaccumulation, typically increases the likelihood of contamination of air, water, and soil (Miglioranza, *et al*., 2013) [23]. Pesticides are a crucial part of pest management techniques for food production, the environment, and public health, according to Manoranjan *et al*. (2020) [25]. Whatever the application method, pesticide eventually finds its way to the soil, which acts as a storage area for these chemicals and allows them to seep into the surrounding environment, including aquatic habitats (Husain *et al*., 2003) [15]. Plants absorb pesticide residues, which then enter the food chain and build up in both human and animal body fat. The synthetic insecticides known as organochlorine (OC) pesticides are widely utilized across the world. They are a member of a class of chlorinated hydrocarbon derivatives that are widely used in both agriculture and the chemical industry. Although pesticides are designed to attack a specific organism, non-target species are frequently adversely affected (Manoranjan *et al*., 2020) [25].

The majority of these substances are made to disrupt the physiological processes of the intended organism, which results in malfunction and decreased vitality. According to Odukkathil *et al*. (2016) [26], pesticide residues could be a substantial source of contamination for soil, water, and other environmental elements. The coexistence of plant and animal populations within ecosystems may be continuously threatened by this occurrence (Kumar *et al*., 2014; Kafilzadeh *et al*., 2015) [19, 18]. Newer pests and illnesses arise every year, which magnifies the situation. Growing soil, water, and air pollution has been caused by the growing usage of pesticides for enhanced agricultural production. The likelihood of pesticide contamination of the air, water, and soil has increased as a result of their properties, such as high lipophilicity, bioaccumulation, extended half-lives, and propensity for long distance transport. The main occupational risk for agriculturalists in underdeveloped nations is pesticide exposure. These consequences of pesticide use include health problems and environmental contamination. According to estimates, there are around one million deaths and chronic illnesses worldwide each year as a result of pesticide exposure (Shahid *et al*., 2021) [33]. Pesticide abuse or misuse has a negative impact on ecosystem services and the health of the environment. Numerous aquatic and terrestrial animals have reportedly been harmed by pesticides. Pesticides have a detrimental effect on aquatic ecosystem life, including microorganisms, invertebrates, plants, and fish.

Organochlorines are infamous for their extreme toxicity and persistence. These insecticides have negative acute and long-term health impacts, including brain damage and endocrine abnormalities. Due to their toxicological risks on the biomes of the impacted soil, organochlorine pesticides (OCPs) employed in agricultural activities are a cause for worry worldwide (Egbe *et al*., 2020) [9]. Organochlorine pesticide pollution of farm soil consequently has a significant impact on the ecosystem. Although farmers are considered to be the main risk group, formulations, loaders, mixers, production workers and agricultural farm workers are all extremely susceptible groups. The non-occupational hazard may be due to pollution of the ecosystem or habitat as a whole (Egbe *et al*., 2020) [9].

Indiscriminate use of banned pesticides in agricultural soil, along with their simultaneous toxicological effect on the microbial community and the processes they regulate, is of great concern. Microbial communities in soil ecosystems have vital roles in many essential soil processes; such as organic matter decomposition and nutrient cycling thereby maintaining a balance in soil ecosystems (Syed, *et al*., 2013; Weaver *et al*., 2019) [31, 39]. Any change in the composition of the soil's microbial population could have a substantial impact on the soil fertility needed for plant growth and the production of agricultural products. After spraying pesticides on crops, a considerable portion of the pesticides wash down the crop and pollute the soil. As a result, the contaminated pesticides reduce the functionality of microorganisms, which has an effect on soil fertility. This study thoroughly assesses the validity of the organochlorine pesticide (OCP) and its effects on the environment and microbial community (Weaver *et al*., 2019) [39].

Consequently, the goal of the current investigation is to extract and characterize bacteria and fungi from farm soil polluted by organochlorine pesticide consortia. To assess the impact of various organochlorine pesticide doses on farm soil microorganisms.

Materials and Methods

Study Analysis

The study analysis was carried out in Rivers State University, Microbiology Laboratory in Port Harcourt L.G.A, Rivers State.

Sample Collection

Soil sample was collected from Rivers State University School farm (Latitude-N4º47’44.64636”, longitude E-6º5-856.16876”) in Port Harcourt L.G.A, Rivers State. The soil sample was collected using sterile soil auger to make a depth of 0-20 cm of soil. The soil sample was aseptically put in a plastic bag and immediately stored in cold (ice pack) and transported to Microbiology Laboratory Rivers State University for microbiological analysis within 2 hours (Yang, *et al*., 2013) [40]. Processing and analyzing of soil samples commenced immediately using standard microbiology experimental protocols according to APHA, (2005) [2]; Prescott *et al*., (2005) [27]; Watanabe, (2010) [37].

Processing of soil sample/experimental setup layout

Six (6) different basin were set up and in each of the plastic basin/pot, 100g of soil sample was weighed and put in each of the pot. Different concentrations (5%, 10%, 15%, 20% and 25%) of the organochlorine herbicide (force up, dieldrin) were put in each of the basin/pot containing the 100g soil sample (pot 2 to pot 6), while the first pot (pot 1) which served as control was left uncontaminated with organochlorine herbicide (Plate 1.) (Mehjin, *et al*., 2019) [24]. Each set up experiments was done in duplicate Cycon, *et al*., 2010) [7]. Each set up sample was gently and homogenously mixed with separate spatulas and allowed to acclimatize with the new environment (Sofo, *et al*., 2012) [34]. The set up pots as shown in plate 1 were analysed at seven days interval, at day 7, day 14 and day 21 by culturing the microrganisms in the different soil set up samples (Castillo, *et al*., 2006; Syed, *et al*., 2013; Mehjin, *et al*., 2019) [5, 31, 24].

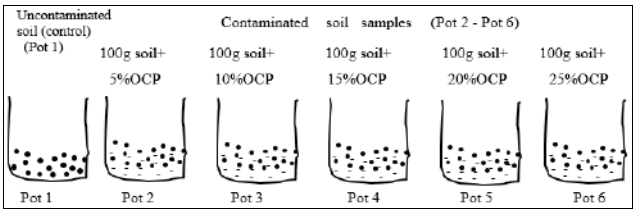

Plate 1: Experimental setup samples in duplicates

Microbial Analysis of Soil Samples

Serial dilution

1g of each soil sample were put into a test tube containing 9ml of normal saline and shaken vigorously to a homogenous suspension to form the stock solutions. 1ml of the soil suspension was aseptically transferred into 9ml of normal saline to give 10^{-1} dilution blank. The dilutions was made tube by tube upto 10^{-6} dilution factor (Prescott, *et al*., 2005; Tien, *et al*., 2012) [35]. This technique is known as ten-fold serial dilution (Prescott, *et al*., 2005).

Isolation and enumeration of Total Heterotrophic Bacteria (THB)

The counts of total heterotrophic bacteria in the organochlorine herbicide soil samples were determined by spread plate technique. Aliquot of 0.1ml was plated on already prepared Nutrient agar (NA) plates of 10^{-5} dilutions in duplicate and evenly spread with sterile bent glass rod. The plates were incubated at 37 °C for 24hours. After 24hours incubation, bacterial yielding 30-300 colonies were counted and reported as colony forming unit per gram(cfu/g) of soil samples samples (Prescott, *et al*., 2005; Cheesbrough, 2006).

Isolation and enumeration of Total Fungi Counts (TFC)

The total heterotrophic fungi counts (THFC) was determined by spread plate method. 0.1ml aliquot of the inoculum from 10^{-2} and 10^{-5} was in duplicate onto already prepared sterile SDA plated and was shaped evenly using bent glass rod. The inoculated plates were incubated aerobically at ambient room temperature (28-30ºC) for 3-5days. The developed colonies were counted after the incubation period and multiplied by the corresponding dilution factor to give total number of fungi population per gram of the analyzed soil samples (Prescott, *et al*., 2005; Watanabe, 2010) [37].

Sub-culture and maintenance of pure microbial isolates

Discrete Bacterial colonies were repeatedly transferred (sub-cultured) to freshly prepared Nutrient agar (NA) plates by the streak plate technique, and allowed to grow at 37C for 24hours before stocking (Cheesbrough, 2006) [6].

Similarly, distinct fungal colonies were sub-cultured repeatedly on freshly prepared Sabouraud Dextrose Agar (SDA) plates and incubated at 37ºC for 72 hours. Pure microbial isolates were maintained on agar slants in bijou bottle or McCartney bottle as stock culture, which were stored and preserved in the refrigerator at 4ºC for further analysis (Cheesebrough, 2006) Cheesbrough.

Identification and characterization of bacterial isolates

Isolates were analyzed based on morphological features, Gram staining and Biochemical characterization. Catalase, Motility, Citrate, Oxidase, Indole and Urease tests were carried out to verify the identity of the organisms. The bacterial isolates were identified and confirmatory of identities of bacteria were made using Bergy's manual of determinative Bacteriology (Holt, *et al*., 1994) [14].

Identification of fungal isolates

Fungal isolates were identified based on their colonial morphology and cell morphology using a procedure described by De-loop in Atlas of Clinical Fungi as a guide (Watanabe, 2010) [37].

Results and Discussion

Pesticides are being widely and inappropriately used to boost crop output in Port Harcourt due to the city's rapidly expanding human population. This has grown to be a significant environmental issue. To evaluate how commonly used pesticides affect the number of soil microbes and their activity. Sprays of pesticides can reach the soil directly or by dropping to the ground if they are not blocked by vegetation. Due to the high exposure rate, specific populations of micro flora may suffer significant harm. The specific pesticide, its concentration, and its duration all affect how the microbial community structure is affected by pesticides (Tien, *et al*., 2012) [35]. As a result, the current study examines how commonly used pesticides affect the microbial populations of soil.

The results of microbial counts of the farm soil

The results of the microbial counts of the farm soil contaminated with organochlorine pesticide consortium of (OCP) at different concentrations and also the control samples are presented in Table 1 and Table 2. The total heterotrophic bacterial counts (THBC) ranged from $1.2x10^5$cfu/g to $9.5x10^5$cfu/g while, the fungal counts (FC) ranged from $1.0x10^3$cfu/g to $7.1x10^3$cfy/g.

Bacterial counts

On Day 1, there was a decrease at 5%, 15%, 20% and 25%. While at Day 14, the concentration of the pesticide reduced the bacterial count, but as the percentage increased so did the count reduce. This indicates the effects of the organochlorine. On the 7th day, the bacterial count reduced at 15% and 20%. The presence of the pesticide decreased the bacterial count. The reduction of bacteria population for a long time weakens some biogeochemical reactions accomplished by these microorganisms.

Fungal counts

On Day 1, there was a decrease at 5%, 15% and 20%. While at Day 7 there was a reduction at 10% and 15%. Similar observation occurred on Days 14 and Day 21 which indicates that the presence of the organochlorine pesticide had reduced the fungal count but at different percentage. This is due to the sensitivity of the fungi to the pesticide's toxicities on the contaminated soil. For the Days that had increased fungal count, this was simply due to the presence of organochlorine degrading fungi.

Table 1: Effect of different concentrations OCP on bacterial counts at weekly interval

OCP Concentration	Day 1	Day 7	Day 14	Day 21
5%	$9.0x10^5$	$7.2\ x10^5$	$6.0\ x10^5$	$5.0\ x10^5$
10%	$7.8\ x10^5$	$7.0\ x10^5$	$5.6\ x10^5$	$3.9\ x10^5$
15%	$6.5\ x10^5$	$5.9\ x10^5$	$5.1\ x10^5$	$2.4\ x10^5$
20%	$5.4\ x10^5$	$4.8\ x10^5$	$3.7\ x10^5$	$2.0\ x10^5$
25%	$3.9\ x10^5$	$3.1\ x10^5$	$2.5\ x10^5$	$1.2\ x10^5$
Control	$9.5\ x10^5$	$8.9\ x10^5$	$8.2\ x10^5$	$7.8\ x10^5$

Table 2: Effect of different concentrations OCP on fungal counts at weekly interval

OCP Concentration	Day 1	Day 7	Day 14	Day 21
5%	$6.0x10^3$	$5.8\ x10^3$	$4.0\ x10^3$	$3.6\ x10^3$
10%	$5.5\ x10^3$	$4.4\ x10^3$	$3.1\ x10^3$	$2.0\ x10^3$
15%	$4.3\ x10^3$	$3.0\ x10^3$	$2.2\ x10^3$	$1.7\ x10^3$
20%	$3.6\ x10^3$	$2.7\ x10^3$	$1.8\ x10^3$	$1.4\ x10^3$
25%	$2.2\ x10^3$	$1.8\ x10^3$	$1.2\ x10^3$	$1.0\ x10^3$
Control	$7.1\ x10^3$	$6.6\ x10^3$	$6.0\ x10^3$	$5.3\ x10^3$

The Results of the frequency and percentage occurrence of bacterial isolates

The results of bacteria isolated from both uncontaminated and contaminated soil samples are presented in Table 3. A number of Bacteria isolates including; *Flavobacterium* sp, *Enterobacter cloacae, Klebsiella* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Alcaligens* sp., *Acinetobacter* sp., *Bacillus* sp. and *Serratia* sp. were identified as shown in Table 3. Also, it can be seen from Table 5. That the bacteria isolate with the highest percentage of occurrence is *Bacillus* sp. (23.5%) with number of isolates (39). While, *Acinetobacter* sp. had the lowest percentage occurrence of 4.2% and occurred lesser in the contaminated soil with a number of 7 isolates as presented in Table 5.

The results of the frequency and percentage occurrence of fungal isolates

The results of bacteria isolated from both uncontaminated and contaminated soil samples are presented in Table 4. A number of Bacteria isolates including; *Verticillium* sp., *Trichoderma* sp, *Aspergillus niger*, *Penicillium* sp, *Mucor* sp. and *Candida* sp specie were identified as shown in Table 4. On the other hand, it can be seen from Table 6. That the fungi isolate with the highest percentage of occurrence is *Aspergillus Niger*, (27.4%) with number of isolates (20). While, *Verticillium* sp. had the lowest percentage occurrence of 5.5% and occurred lesser in the contaminated soil with a number of 4 isolates as presented in Table 6.

Table 3: Bacterial Identified from the Soil Samples

Bacterial isolates	Contaminated soil	Uncontaminated soil
Flavobacterium sp.	+	-
Enterobacter sp.	+	+
Bacillus sp.	+	+
Serratia sp.	+	-
Pseudomonas sp.	+	+
Alcaligenes sp.	+	-
Klebsiella sp.	-	+
Staphylococcus sp.	+	+
Acinetobacter sp.	+	+

Table 4: Fungi Identified from the Soil Samples

Bacterial isolates	Contaminated soil	Uncontaminated soil
Penicillium sp.	+	+
Mucor sp.	+	+
Verticillium sp.	-	+
Aspergillus sp.	+	+
Trichoderma sp.	-	+
Candida sp.	+	+

Table 5: Percentage Occurrence of Bacterial Isolates

Bacterial Isolates	Freq. occurrence	Percentage occurrence (%)
Flavobacterium sp.	10	6.0
Enterobacter cloacae	15	9.0
Klebsiella sp.	21	12.7
Bacillus sp.	39	23.5
Acinetobacter sp.	7	4.2
Staphylococcus sp.	25	15.1
Pseudomonas sp.	29	17.5
Alcaligeres sp.	8	4.8
Serratia sp.	12	7.2
Total	166	100

Table 6: Percentage Occurrence of Fungal Isolates

Fungal Isolates	No. of Isolates	Percentage Occurrence (%)
Mucor vacernosus	5	6.9
Trichoderma sp.	8	10.9
Aspergillus niger	20	27.4
Penicillium sp.	12	16.5
fusarium sp.	15	20.5
Candida sp.	9	12.3
Verticillium	4	5.5
Total	73	100

Discussion

The indiscriminate use of banned pesticides in agricultural soils, as well as their concurrent toxicological effects on the native microbial ecology and the processes they govern, are major causes for worry. After being applied, these substances go into the soil and interact negatively with soil microorganisms (Kumar *et al.*, 2014; Kafilzadeh *et al.*, 2015; Wang *et al.*, 2019) [18]. Such chemical pesticides can destroy soil physicochemical processes, as well as the microbiological composition and structure of the soil, when used erratically and without consideration (Wang *et al*., 2019). Due to a lack of degradation processes, repeated applications of pesticides in agricultural farmlands cause a buildup of pesticides in the soil layers (Shari *et al*., 2013). This research is comparable to that of Leonardo *et al*. (2016), who examined the behavior of organochlorine pesticides (OCPs), including DDTs, endosulfans, HCHs, heptachlors, drins, and chlordanes, in the agricultural areas along the Quequén Grande River and came to the conclusion that the high OCP levels found in the agricultural soils pose a threat to the food web because soil organisms are one of the first steps in. This result suggested the importance of using terrestrial biota for studying recalcitrant pesticides and also strongly advised that a continuous monitoring should be employed in minimizing environmental risks (Leonardo *et al.*, 2016).

According to the findings of the current investigation, soil microorganisms retrieved from soil polluted with organochlorine pesticide had fewer counts than soil microorganisms present in the uncontaminated soil (control). The reduction in the quantity of soil microorganisms in the contaminated soil utilized in this study indicates that the organochlorine pesticide is effective at eliminating the microorganisms present in agricultural soil, according to this result. Additionally, several soil microorganisms that were isolated and reported previously by Birolli *et al*. (2019) and Fragoeiro Megan (2005) agree with the bacterial and fungal microorganisms that were collected from the contaminated soil in the current investigation.

The current study's findings are remarkably similar to those of Mehjin *et al*.'s study from 2019 [24] which showed that the addition of the three pesticides in question considerably reduced the microbial activity and counts of soil bacteria, fungus, and actinomycetes. They concluded that the investigated pesticides adversely affected microbial counts and activity in soil, which confirms and supports previously reported environmental studies. They also stressed that the observed effect depended on the type and amount of pesticide as well as the length of incubation period.

In the present studies, the bacteria isolated from the farm soils included; *Flavobacterium* sp, *Enterobacter cloacae, Klebsiella* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Alcaligens* sp., *Acinetobacter* sp., *Bacillus* sp. and *Serratia* sp. Several researchers including; Kumar, *et al*., (2014); Kafilzadeh, *et al*., (2015) [18] also isolated similar species of bacteria in their research works. *Bacillus* sp. was observed to be the most frequently occurring bacteria species in both contaminated and uncontaminated farm soils. This could be as a result of their cability to degrade endosulfan components in the toxic substance present in the chemical pesticide (Kumar, *et al*., 2014). Whereas, on the other hand *Acinetobacter* sp. was the least frequently occurred bacteria.

In a research carried out by Chen *et al.* (2004), the bacteria isolate with the highest percentage occurrence was the *Bacillus* sp. designated DG-02, which could degrade 95.6% of 50mg L-B- Phenoxylbenzene acid within 72hours at a pH of 7.7 and 30.9 °C. This indicated that the *Bacillus* sp. Was able to degrade a higher percentage of the pesticide (Chen *et al.*, 2004).

Furthermore, the report from work carried out by Birolli *et al.,* (2019) revealed that, *Pseudomonas* sp. was effective in degrading a variety of pesticides such as dieldrin, Aldrin and heptachloride in water. They also stated clearly that, the emergence of some organisms to mineralize or tolerate organochlorine pesticides may be due to long term exposure to organochlorine pesticide's pollution, leading to the evolution of resistant strains with organochlorine pesticide's degrading genes. For example, specie of Klebsiella were reportedly among the dominant bacteria in soils containing pesticide residues. These findings corroborate previous studies including studies done by Anode *et al.,* (2018) who isolated bacterial communities with potential to degrade pesticides in agricultural soil. Examples of species isolated in these previous studies included; *Arthrobacter* sp, *Enterobacter* sp, *Alcaligenes* sp, *Serratia* sp, *Bacillus* sp and *Pseudonas* sp (Anode *et al.*, 2018). All except the first two genera were identified in this study. Therefore, it is possible that, this could be as a result of the use of the organochlorine pesticide which had drastically eliminated the bacteria species: *Arthrobacter* sp. and the *Enterobacter* sp.

In the present studies, the fungi isolate with the highest percentage occurrence is the *Aspergillus* Niger. Many fungi have been tested for their ability to degrade endosulfan, including *Aspergillus Niger* and *Aspergillus terreus* (Lee *et al.,* 2003). Several *penicillium* sp. have been reported to bioremediate organochlorine pesticides. For example a marine derive *Penicillium miczynkskii* strain degraded insecticide dieldrin. (Birolli *et al.*, 2015), and some isolates of *Penicillium* sp degraded endosulfan (Ahmad, 2020). Fungi are actually more tolerant to high concentrations of contaminant chemical than bacteria. Till date, several genera of fungi have been investigated for their degradation potential of xenobiotics like dieldrin. (Fragoeiro and Megan, 2005). The low abundance of fungi species; *Verticillium* and *Mucor vacernosus* in the contaminated soil is an indication of possible sensitivity of these phyla to organochlorine pesticide's toxicities on the contaminated soil. A host of fungi including; *Mucor vacernosus*, *Trichoderma* sp, *Aspergillus Niger*, *Penicillium* sp, *Fusarium* sp, *Candida* sp and *Verticillum* were identified.

In a review by Weber, *et al*., (2010) [38] whose research investigates the fate and behaviour of endosulfan, in a current application of the organochlorine pesticide, at temperate environments and the Arctic. Where usage data and patterns, physical-chemical properties, environmental partitioning and degradation, environmental levels, global distribution and temporal trends were evaluated and discussed in the context of criteria that designate a substance as a persistent organic pollutant. They experimented, observed and documented that; Endosulfan is one of the most abundant OC pesticides in the global world and is capable of undergoing long range transport systems in the degradation of the two isomers, alpha- and beta-endosulfan, that usually occur in temperate/tropical soil and aquatic systems, both by abiotic and biotic processes. They further explained that the extent of environmental pollution as a result of the use of the organochlorine pesticide is highly dependent on the prevailing environmental conditions (Weber, *et al*., 2010) [38].

In a work on: Organochlorine pesticides in soil under irrigated cotton farming systems in vertisoils of the Naomi Valley, North Western Wales, Australia carried out by Weaver, *et al.*, (2012), who investigated on the influence of Organochlorine pesticides (OCPs) and detected Organochlorine pesticides (OCPs) including; DDT and DDE in the surface 0.2m of sols in the lower valley of North

(149°18'E, 30°12'S) in the Western New South Wales, Australia, even though they have not been applied to crops since about thirty years between 1982 to 2012. However, their presence in the deeper soil horizons has not been investigated. The OCPs detected and their metabolites were α-endosulfan, β-endosulfan, endosulfan sulphate, DDD, DDE, DDT and endrin. They explained the persistence of DDT and DDE in the majority of soil samples that the movement of these OCPs into the sub-soils may have occured when irrigation or rain transports soil colloids and organic matter via preferential flow systems into the deeper layers of a soil profile. Persistence of OCPs was closely correlated to soil organic carbon concentrations. The persistence in soil of OCP's applied to some crops grown more than two decades ago suggested that they could enter the food chain and that their presence at depths of 1.2m suggested that they could move into groundwater that may eventually be used for domestic and stock consumption (Weber, *et al.,* 2012), contaminating the vegetations, waterbodies and environment, thereby resulting to environmental and public health implications affecting the population at large.

The anthropogenic activities of the OCPs-impacted soil via pesticide spraying must have caused the disappearance of 33,397 fungal and 61,005 bacterial species exclusively found in the pristine soil. Their study provided current and useful data on rarely reported microbial structure of an agricultural soil impacted by a mixture of OCPs. They also recommended that future work should focus on investigating the functionality of the microbial community in relation to self-recovery process of the impacted agricultural soil (Mehjin, *et al.,* 2019) [24].

This work corroborate work done by Rasul, *et al.,* (2001) [29] who measured the number of bacteria and fungi in all soils after 42 days of incubation using the dilution plate technique and reported that the pattern of response of bacteria to various pesticide treatments was similar in 100/g soil in various pesticide treatment and that a smaller population of soil bacteria colony-forming unit was observed in the control treatment followed by soil in other treatment. A smaller population of bacteria in the remaining four pesticide treatments indicated a strong inhibitory effect on bacterial growth. Thus, the inhibitory effect of pesticides on bacterial growth was higher in the soils treated with the various pesticides compared to the soil used as control without any treatment. Similarly, reports from the present work observed, like bacteria to varying degrees of concentrations, the pesticides also suppressed the fungal population.

Rasul, *et al.,* (2001) [29] further emphasized that, the effect of pesticides varied greatly with the type, rate, and time after application of pesticides. Efforts must be made to determine the proper type and dosage of pesticide for agricultural crops in order to prevent their adverse effect on the environment in general and on useful soil organisms in particular. The authors' investigations show that insecticide coded; B had no harmful influence on soil microbes if used as recommended whereas, the insecticides coded; D and E were highly toxic to the soil microbes and their effect was rapid. Also, pesticides coded; A and C were also toxic, but their effect on soil microbes was more gradual, becoming severe 5-7 days after application. Thus concluded that, all pesticides investigated, with the exception of B, were persistent and remain toxic even after 42 days in the soil hence, may have the potential to cause environmental pollution (Rasul, *et al.,* 2001) [29].

A study by Smith and Jong, (2001) showed that only 0.3% of applied pesticides goes to target pest and 99.7% goes into the environment. This may be due to contamination as a result of improper legislation, improper market regulation and ignorance exhibited by individuals and agricultural workers Beena, and Kathpal, (2010) [4].

Conclusion and Recommendation

In addition to affecting the crop, the use of pesticides in agriculture has changed the ecosystem and the food chain. In addition to having a negative impact on the crops, animals, and birds in a particular area, these chemicals also have a negative impact on the ecosystem's equilibrium and cause health problems. The findings of this study demonstrate that the soil microorganisms were impacted by the organochlorine pesticide (OCP). Observing that there were more soil microorganisms in the control (uncontaminated) soil samples than in the contaminated soil samples. According to the findings of the current study, the amount of microorganisms in the agricultural soil had decreased due to the use of organochlorine pesticides (OCP). This might be because organochlorine pesticides contain harmful ingredients. High death rates can be brought on by these insecticides. Therefore, it is important to limit the use of conventional pesticides while increasing the usage of biopesticides. In order to lessen the impact of pesticides on the environment, numerous alternatives are thus suggested and made available. Among these options are manual removal, providing heat, weeds are covered with plastic. Setting up lures and traps, eradicating pest breeding grounds, maintaining wholesome soils that produce wholesome, more resilient plants, cultivation of native plant species, which are by nature more resistant to local pests, and encouragement of biocontrol agents like birds and other pest predators. In conclusion, the general public should be informed about: being aware of regarding the long-term harm caused by pesticides.

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