Identification of Carbofuran and Paraquat Degrading Microorganisms from Soil

T. L. Ataikiru1, P. O. Okerentugba2 and G. C. Okpokwasili2

1Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria.
2Department of Microbiology, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. Author GCO designed the study and wrote the protocol. Author TLA performed the laboratory analyses, statistical analysis and wrote the first draft of the manuscript. Author POO managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Introduction: Increased rates of pesticide misapplication and follow-on concerns on public health have become subjects of countless distress. The occurrence of pesticides in soils could result in modifications in soil physical, chemical as well as biological properties hence the need for ways to reduce such impacts.

Research Gap: Insufficient literatures on extensive identification of pesticides’ degraders from non-impacted soils. Existing literatures are restricted to a particular microbial group (bacteria or fungi).

Aim: The study aimed at isolating, characterizing and testing bacteria, moulds, yeasts and actinomycyes from soil for the biodegradation of pesticides.

Place and Duration of Study: The study was carried out at the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun and Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria/ four months.

Methodology: Carbofuran and Paraquat degrading microorganisms were isolated from a non-pesticides impacted soil using mineral salt medium (MSM). The MSM composed in grams per liter:
1. INTRODUCTION

Carbofuran (2, 3 – dihydro - 2, 2 – dimethylbenzofuran – 7 – yl methylcarbamate, C13H15NO3, CAS registry no. 1563-66-2) is one of the broad-spectrum, highly toxic carbamate (highly toxic pesticide “lb”) and a nerotoxic. Carbamates are inhibitors of acetylcholinesterase. The toxic action is a result of its ability to inhibit cholinesterase in the central and peripheral nerve systems where it functions in transmitting nerve impulses [1,2]. It is a wide-range N-methyl carbamate, applied as an insecticide, miticide and acaricide.

Carbofuran is the most commonly used carbamate insecticide in insect control on lawns, home gardens, citrus, fruit, forage and field (rice, corn) crops and potatoes. Agricultural crops with the highest annual use of Carbofuran include rice, corn and potatoes [3,4]. Carbofuran is banned in many countries including Canada and European nations. Environmental Protection Agency (EPA) initiated the ban on all granular formulation in certain ecologically sensitive areas. The ban was initiated to protect birds and not related to human health concerns. Bird kills have occurred when ingested Carbofuran granules that resemble grain seeds or when predatory or scavenging birds ingest small birds or mammals that have ingested Carbofuran granules [5,6,7].

Additionally, there is no ban on the liquid formulation but is grouped as a restricted use pesticides (RUP) due to their acute oral and inhalation toxic impact to man. Carbofuran is a systemic insecticide in plants and contact in insects. It is highly toxic by inhalation and ingestion and moderately toxic by dermal absorption. It disrupts an insect’s nervous system and could be lethal if touched or eaten. The impacts of Carbofuran on human and environmental health depend on its concentration, the length and frequency of contact. Effects are also dependent on the healthiness of an individual and/or certain environmental factors. It possesses one of the greatest acute toxicities to humans [8,9].

At low concentrations, it causes alteration of the concentration of hormones. Risks from exposure are especially high for persons with asthma, diabetes, cardiovascular disease, mechanical obstruction of the gastrointestinal or urogastal tracts. The symptoms on human associated with acute Carbofuran exposure may include vomiting, blurred vision, excessive salivation, imbalance, breathing difficulty, muscle weakness, nausea, abdominal cramps, sweating, increased blood pressure, restlessness, lack of controlled urine or faeces release (incontinence), drowsiness, lethargy, inability to concentrate and others. Death may result from respiratory system failure associated with Carbofuran exposure. In contact, burns the skin and irritate eyes. Chronic exposures can damage the nervous and reproductive systems [10,11]. The EPA has proposed 40 part per billion (ppb) as the maximum contaminant level goal.

Paraquat (N, N’ - dimethyl - 4, 4’ - bipyridinium dichloride, C12H4Cl2N2, CAS registry no.4685-14-7) is classified in category 111 as “slightly toxic”. Paraquat, a bipyridinium compound is an example of quaternary ammonium herbicides with the trade name, Gramoxone. Paraquat is known to act on the photosystem I within the photosystem II reaction centres.

K3HPO4, 4.8; KH2PO4, 1.2; NH4NO3, 1.0; MgSO4 7H2O, 0.2; Ca(NO3)2 .4H2O, 0.4, and Fe(SO4)3, 0.001 supplemented with 2 mM Carbofuran or Paraquat as the only carbon source. The ability of the microbial isolates to utilize Carbofuran and Paraquat was screened on MSM containing 150 part per million of the pesticides as the only carbon source. The isolates were identified using the analytical profile index (API), microscopic and macroscopic characteristics.

**Results:** Bacterial species identified were Bacillus, Pseudomonas, Kocuria, Enterobacter, Chryseobacterium, Corynebacterium, Acinetobacter, Paenibacillus, Lerceria and Proteus. Actinomycyes were Actinomyces israelli, Actinomyces naeslundi, Actinomyces viscosus 1, Actinomyces meyeri and Actinomyces viscosus 2. Yeast isolates were Candida stellatoidea, Candida krusei and Saccharomyces cerevisiae while moulds were Talaromyces, Cladosporium carionii and Curvularia species.

**Conclusion:** These findings indicated that Carbofuran and Paraquat degrading organisms are readily extant in soils and can be used to facilitate the removal of these pesticides from such polluted environments.

**Keywords:** Carbofuran; Paraquat; pesticide degraders; actinomyces; xenobiotics; soil.
photosynthetic membrane [12]. It inhibits carbon dioxide fixation through inhibiting the variable chlorophyll fluorescence by decreasing oxygen evolution and its activity is irreversible. European Union (EU) proposed a limit of less than 0.1 µg for pesticides and herbicides. Water polluted with Paraquat is a risk factor for liver, lung, kidney and heart illnesses. It is currently banned in 32 countries including European nations based on human concerns [13], but still registered and used in over 90 countries [14]. Several researches targeted at its toxicity in mammals including humans [15,16] at occupational and non-occupational exposures have been conducted [17]. Paraquat is applied post emergence to leaf tissues. It is non selective to plants, quick and lethal to man and animals. It is more acutely lethal to people compared to other herbicide in widespread commercial use and is connected to Parkinson’s disease [18,19]. Paraquat is termed a chemical flame gun, in so much as it kills the above-ground parts of plants whilst not damaging the roots. Such effects will usually kill annual weeds, but deeper-rooted and rhizomatous weeds will resprout; agricultural systems have been established to utilize these potentials [20]. Paraquat is a non- selective herbicide used to control most yearly weeds and several broad leaf weeds in field corn, potatoes, rice, cotton, soybeans, tobacco, peanuts and sunflowers. Paraquat is used for no till burn down and in aerial distribution of marijuana, almonds, cotton, grapes, alfalfa and cocoa plantings [21]. It is administered in pre-emergence and early post-emergence weed control.

Although, these pesticides play important roles in protecting agricultural crops from insect pests and weeds, and in controlling disease-transmitting vectors, they cause serious environmental pollution problems [22,23]. Slight losses of Paraquat can result from photodecomposition and volatilization [24]. Paraquat soil half-life is 36 days – 2.6 years [25,26,27]. Carbofuran has a half-life of 30-150 days [28] and degradation could be via chemical hydrolysis, photodegradation and microbial action [7]. The adsorption/desorption to/from soils results to the pollution of ground and surface water.

However, due to these pesticides stability and long half-life, residues in farm products and the natural environs are potential hazards to human health and other biotic life [29,30]. According to [31,32] pesticides may harm non-target organisms possibly threatening human health through the food chain. Similarly, it causes harm to immune-related genes and membrane proteins of non-target organisms [33,34,35]. For the past decades, overuse of these chemicals have severely polluted surface water, soil, food and biota. For that reason, it is imperative to raise fear about its environmental impacts and to advance an operational/effective and possible approach for eliminating their residues [36]. Studies on microbial isolation, identification and degradation are expedient in the development of schemes for the decontamination of pesticides by microorganisms [37].

The restoration of pesticide-contaminated sites is recognized as a cost-effective and reliable method. Microbes are key players during the degradation of these compounds thus, reducing their toxicity. Many bacteria capable of degrading different xenobiotics including pesticides have been isolated and identified [38]. These studies mainly focused on the role of some bacterial genera, such as Bacillus, Pseudomonas, Flavobacter, Arthrobacter, Diaphorobacter, Klebsiella, Ochrobactrum, Agrobacterium and others [33,39,40,41,42] in degradation of pesticides. However, till to date few data are available in the literature on the isolation of different microbial groups (bacteria, fungi and actinomycetes) involved in pesticides biodegradation. Hence, the present work aimed to isolate and characterize pesticides degrading microbial isolates from farmyard soils.

2. MATERIALS AND METHODS

2.1 Site Description

The study area was located in the Federal University of Petroleum Resources, Effurun, Delta State, Nigeria (7° 23' N; 3° 51'E and 26.7 m above mean sea level). The Niger Delta experiences tropical climate with distinct wet and dry seasons having a bimodal rainfall pattern with rainfall peaks mostly in June to September and average temperature of 25.2°C (78.8°F) - 28°C (82.4°F). The soils were mostly sandy loam at the top, to brown loamy sand sub soil and well drained. Four different representative locations having similar ecological conditions were chosen for this study. The locations had no history of pesticides applications.
### 2.2 Sample Collection

The surface soil samples were collected from 0 to 15 cm and mixed to form a composite sample at each location. Soil samples were sorted to remove stones, plant and root debris. All soil samples from the four locations were pooled together and transferred to the laboratory in a cooler with ice packs for analysis.

Pesticides (Carbofuran and Paraquat) used in the study were purchased from local retailers in Warri, Delta State.

### 2.3 Physicochemical Analysis

Fresh soil sample was characterized to assess the physicochemical properties. Soil pH, soil texture, electrical conductivity, moisture content, nitrogen, total organic carbon and phosphates were analyzed following the standard methods by American Public Health Association (APHA) [43].

Calcium, magnesium, potassium, sodium, mercury, arsenic, cadmium and lead were detected by flame analysis method using the atomic adsorption spectrophotometer Model AA500 (PG instruments) following digestion of samples according to the protocol described by APHA [43]. Microbial counts were determined using standard plate counts. Serial dilution was done using one (1) gram of soil sample suspended in 9 ml of sterile physiological saline. Aliquots (0.1 ml) of the dilutions were plated out using appropriate media for the enumeration of microorganisms. Rose-Bengal chloramphenicol agar was used for the enumeration of fungi [44] and plate count agar (PCA) was used for the enumeration of total heterotrophic bacteria [45]. Actinomycetes were enumerated using starch-casein agar [46] and Pikovskaya’s medium for phosphate solubilizing microbes [47]. Ashby agar was used to enumerate nitrogen fixers [44] and individual colonies were recorded as colony forming units (CFU).

### 2.4 Enrichment of Pesticide Degrading Microorganisms

The method described by Omolo et al. [48] was adopted for the enrichment of Carbofuran-degrading and Paraquat-degrading microorganisms. One gram each of soil sample was suspended in 10 ml of phosphate buffered mineral salt medium (MSM). The MSM composed in grams per liter: $K_2HPO_4$, 4.8; $KH_2PO_4$, 1.2; $NH_4NO_3$, 1.0; MgSO$_4$.7H$_2$O, 0.2; Ca(NO$_3$)$_2$.4H$_2$O, 0.4, and Fe(SO$_4$)$_3$, 0.001. The media was supplemented with 2 mM Carbofuran or Paraquat as the sole carbon source. Cultures were grown in 100 ml culture flasks under aseptic conditions at 30°C with shaking in a rotary shaker at 100 rpm for 18 days. Cultures were then streaked onto agar plates containing mineral salt medium supplemented with 2 mM of the pesticides. Single colonies obtained were re-suspended in basal medium (MSM) containing 2 mM of pesticides for 14 days to confirm the ability of the isolates to utilize pesticides. All the solutions, cultures and media were prepared and maintained using aerobic techniques which included covering media with cotton wool and shaking to allow air circulation in the cultures.

### 2.5 Laboratory Isolation of Pesticide Degrading Microorganisms using Cultural Methods

Serial dilution agar plating method was carried out for the isolation of microorganisms. Luria-Bertani medium containing 0.1 g/liter cycloheximide (Sigma Aldrich, Steinheim, Germany) to suppress fungal growth was used to determine bacterial population [48] while Rose - Bengal agar and starch casein agar were used for fungal and actinomycetes population, respectively. Streptomycin 40 μl/ml and griseofulvin 50 μl/ml were used to prevent bacterial and fungal contaminants [46] in starch casein agar while Rose Bengal contained chloramphenicol [44]. Selected pesticides degrading organisms were characterized by physiological, morphological and biochemical characters [3,4,49] using the analytical profile index (API).

### 3. RESULTS AND DISCUSSION

#### 3.1 Soil Characteristics

The soil physical, chemical and microbiological properties of the pooled soil are presented on Table 1. The pH of the soil was 6.7. The value of nitrates, phosphates (mg/kg), exchangeable cations (sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca)) present in the soil were 36.28 meq/100 g, 22.15 meq/100 g,
26.32 meq/100 g, 85.98 meq/100 g soil, respectively. The total heterotrophic bacterial, fungal, actinomycetes, nitrifiers and phosphate solubilizers counts in the fresh soil were $6.3 \times 10^7$ CFU/g, $1.41 \times 10^5$ CFU/g, $1.99 \times 10^4$ CFU/g, $1.45 \times 10^4$ CFU/g and $1.52 \times 10^4$ CFU/g, respectively. The physicochemical properties of the soil showed that it was a sandy (97.192%) silt (2.65%) soil from the particle size distribution with a close to neutral pH value of 6.70. According to Kyverga et al. [50] most agricultural soils have a pH in the range of 5.5 to 8.0 but under different agricultural practices soils pH values may increase or reduce [51]. The solubility of soil macronutrients, micronutrients or essential trace elements are influenced by soil pH [52,53]. These macronutrients and micronutrients are highly solubilized in soils between the pH range of 5.5 to 7.0 such that these high pH levels in the soil result in leaching of nutrients and releases aluminium in solubilized forms from its insoluble state [54]. Furthermore, it prevents cell division and growth in the roots; affects the plant’s uptake of cations and stimulates organic acid secretion [55]. Heavy metal analysis showed that soil had a higher lead content (19.25) relative to arsenic (0.215), mercury (<0.001) and cadmium (0.725). Heavy metals examination indicated soil had very low levels of the metals hence, large numbers of microorganisms could thrive in the soil. In addition, the soil had appreciable amount of nitrates and phosphates essential for the growth and proper functioning of the microbial communities present in soil.

### 3.2 Isolation of Pesticides Degraders

Microorganisms are the main degraders of pollutants in the environment. Various organisms were isolated by enrichment to isolate pesticides degraders using Carbofuran and Paraquat, respectively as the sole carbon and energy source from soil. Microorganisms are focal degraders of contaminants in various environment. The taxonomic classification of isolates performed using the API platforms placed the isolates into seventeen bacterial, three yeast and five actinomycetes species. Microscopic and macroscopic characteristics of the isolates also supports mould genus assignments.

| Parameters | Value |
|------------|-------|
| Physicochemical |       |
| Electrical conductivity (µs/cm) | 144   |
| pH | 6.70 |
| Total organic carbon (TOC %) | 3.316 |
| Total nitrogen (%) | 0.3029 |
| Nitrates (mg/kg) | 36.28 |
| Phosphates (mg/kg) | 26.32 |
| Moisture content (%) | 19.41 |
| Calcium (meq/100g) | 61.83 |
| Magnesium (meq/100g) | 22.15 |
| Sodium (meq/100g) | 0.97 |
| Potassium (meq/100g) | 1.03 |
| Arsenic (mg/kg) | 0.215 |
| Mercury (mg/kg) | <0.001 |
| Lead (mg/kg) | 19.25 |
| Cadmium (mg/kg) | 0.725 |
| Soil particle size distribution (%) |       |
| Silt | 2.65 |
| Sand | 97.192 |
| Clay | 0.158 |
| Microbiological |       |
| Microbial counts (CFU/g) |       |
| Total heterotrophic bacteria | $6.30 \times 10^7$ |
| Fungi | $1.41 \times 10^5$ |
| Actinomycetes | $1.99 \times 10^4$ |
| Nitrifying bacteria | $1.45 \times 10^4$ |
| Phosphate solubilizers | $1.52 \times 10^4$ |
Thirty five bacterial, fifteen fungal and nine actinomycetes isolates were obtained after pesticides enrichment. Of the bacterial isolates; nineteen were Gram positive and sixteen were Gram negative. The bacterial isolates identified were *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa*, *Bacillus circulans*, *Bacillus basidium*, *Bacillus megaterium*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Proteus penneri*, *Lercleria adcarboxylata* and *Enterobacter* species from Carbofuran (Table 2) while *Bacillus amyloliquefaciens*, *Bacillus basidium*, *Bacillus mycoides*, *Bacillus megaterium*, *Kocuria varians*, *Corynebacterium capsicum*, *Chryseobacterium indologenes*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Enterobacter* species, *Acinetobacter baumannii* and *Enterobacter sakazaki* were identified from Paraoquat as shown on Table 3. The results of our study were in confirmation with the outcomes of other researches.

Omolo et al. [48] isolated bacterial isolates closely associated to members of *Pseudomonas* from Carbofuran enriched soil using the partial 16S rDNA sequence analysis. Three unalike species of *Bacillus* were isolated by Nisha et al. [4] from Carbofuran enriched soil. Obuotor et al. [37] isolated and identified species of *Pseudomonas* and *Enterobacter* from Paraoquat contaminated soil using the API. These reports are in corroboration with our findings. Again, Desaint et al. [56], equally isolated *Pseudomonas* and *Bacillus* species using amplified ribosomal DNA restriction analysis (ARDRA) analysis of 16S rDNA as Carbofuran-degrading bacteria from soil. Report has it that *Burkholderia*, *Pseudomonas*, *Cupriavidus*, *Corynebacterium*, *Arthrobacter*, *Micrococcus*, and *Sphingomonas* species have been identified as pesticide degraders using the culture-dependent studies, a number of which use the compound as an exclusive carbon source [57].

Table 4 shows the yeast isolates obtained during the experiment. The yeast species identified were *Candida stellatoidea*, *Candida krusei* and *Saccharomyces cerevisiae*. Again, Obuotor et al. [37], isolated *Saccharomyces cerevisiae* and *Candida* species from their research which was also true from this present study. Martani [58] reported *Rhodotorula*, *Cryptococcus* and *Sporobolomyces* as Paraoquat degrading yeasts from fibric and saphric peat soils. Actinomycetes species isolated during the study are as presented on Table 5. The actinomycetes identified were *Actinomyces israelii*, *Actinomyces naeslundi*, *Actinomyces viscosus* 1 (Carbofuran) and *Actinomyces meyeri*, *Actinomyces naeslundi*, *Actinomyces viscosus* 2 from Paraquat. De Schrivjer & De Mot [59], equally isolated different members of *Actinomyces* during their studies. Fuentes et al. [60], isolated several actinomycetes in the genus *Streptomyces* and a member of *Micromonospora* which was in contrast to our findings. Furthermore, Chougale and Deshmuukh [61] reported that seven actinomycetal isolates were isolated and identified as *Streptomyces alanosicus*, *Streptomyces atratus*, *Strptoverticillium album*, *Nocardia farcinica*, *Norcadia vaccini*, *Norcadia amarae* and *Micromonospora chalcea* from soils. These actinomycetes when further tested for biodegradation of Carbofuran pesticide by cometabolism and as a sole carbon source were active degraders of the chemical.

*Talaromyces*, *Cladosporium carionii* and *Curvularia* were the mould species identified in this research as shown on Table 6. Our results were contrary to other researchers’ reports that isolated *Aspergillus*, *Penicillium*, *Mucor*, *Trichotherium* and *Rhizopus* suggesting that the soil type could determine the type of fungi present [62,63]. Also, Martani [58] isolated the moulds; *Penicillium* and *Aspergillus* based on their ability to degrade Paraoquat.

It is exciting to note that microbial isolates normally present in soil; a non-pesticide impacted environment, has the capability to utilize Carbofuran and Paraoquat as sole source of carbon and energy. Several researchers have reported in their work microbial populations capable of utilising these pesticides as sole source of carbon from soil [37,44,48,64]. There are reports that microbial communities exposed to xenobiotics adapt to this exposure through selective enrichment and genetic changes resulting in an increase in xenobiotic-degradation [65,66]. This pre-exposure of microorganisms make them better suited to degrade the pollutant through higher growth, reproduction and more efficient metabolism thus maximizing the rate of these pesticides removal from the soil.
Table 2. Gram positive bacterial isolates from the study

| Code | Glucose | Glycine | H₂S | Urease | VP | Indole | ONPG | Gelatine | Citrate | L-Arabinose | Fructose | Sorbitol | Inositol | Ribose | D-xylene | Esculin | Maltose | ADH | LDC | % ID | Identified Isolate |
|------|---------|---------|-----|--------|----|--------|------|----------|---------|-------------|----------|----------|----------|--------|----------|--------|--------|-----|-----|------|-------------------|
| Carbofuran |         |         |     |        |    |        |      |          |         |             |          |          |          |        |          |        |        |    |    |     |                   |
| B1   | +       | +       | -   | -      | +  | +      | +    | +        | +       | +           | +        | +        | +        | +      | +        | -      | -      |    |    | 92.5 | Bacillus amyloliquefaciens |
| B2   | +       | +       | -   | -      | +  | +      | +    | +        | +       | +           | +        | +        | +        | +      | +        | -      | -      |    |    | 94.5 | Bacillus subtilis |
| B3   | +       | +       | -   | -      | +  | +      | +    | -        | +       | + N         | +        | +        | +        | +      | +        | -      | -      |    |    | 99.9 | Paenibacillus polymyxa |
| B4   | +       | +       | -   | -      | -  | +      | -    | +        | + N     | N           | +        | +        | +        | +      | +        | -      | -      |    |    | 61.8 | Bacillus circulans |
| B5   | +       | +       | -   | -      | +  | +      | +    | +        | +       | +           | +        | +        | +        | +      | +        | -      | -      |    |    | 99.5 | Bacillus megaterium |
| B6   | +       | +       | -   | -      | +  | +      | +    | +        | +       | +           | +        | +        | +        | +      | +        | -      | -      |    |    | 92.5 | Bacillus badius |
| Paraquat |       |         |     |        |    |        |      |          |         |             |          |          |          |        |          |        |        |    |    |     |                   |
| B7   | +       | N       | N   | +      | N  | N      | N    | N        | N       | N           | N        | -        | N        | -      | -        | -      | -      |    |    | 99.0 | Kocuria varians |
| B8   | +       | -       | N   | N      | N  | N      | N    | N        | N       | N           | N        | -        | -        | -      | -        | -      | N      |    |    | 92.9 | Corynebacterium capsium |
| B9   | +       | +       | -   | -      | +  | +      | +    | +        | +       | +           | +        | +        | +        | +      | +        | -      | -      |    |    | 99.5 | Bacillus megaterium |
| B10  | +       | +       | -   | -      | +  | +      | +    | +        | +       | +           | +        | +        | +        | +      | +        | -      | -      |    |    | 92.5 | Bacillus badius |
| B11  | +       | +       | -   | -      | +  | +      | +    | +        | +       | +           | +        | +        | +        | +      | +        | -      | -      |    |    | 90.0 | Bacillus mycoides |
| B12  | +       | +       | -   | -      | +  | +      | +    | +        | +       | +           | +        | +        | +        | +      | +        | -      | -      |    |    | 89.5 | Bacillus amyloliquefaciens |

Key: H₂S - hydrogen sulphide, VP – Voges Proskauer, ONPG – O-nitrophenyl-β-galactopyranoside, ADH- arginine dehydrolase, LDC- lysine decarboxylase, N – Not applicable
| Code | Glucose | ONPG | ADH | LDC | Citrate | H₂S | Urease | Indole | VP | Gelatin | Mannose | Inositol | Sorbitol | % ID | Identified isolate |
|------|---------|------|-----|-----|---------|-----|-------|--------|----|--------|---------|----------|----------|-----|-------------------|
| Carbofuran | | | | | | | | | | | | | | | |
| B13 | + | + | - | N | + | - | - | - | - | + | - | + | 89.5 | Enterobacter species |
| B14 | + | N | N | N | N | N | + | - | - | N | + | N | N | 92.8 | Pseudomonas putida |
| B15 | + | + | - | N | + | - | - | - | - | - | + | - | + | 89.0 | Lerclercia adecarboxylata |
| B16 | + | - | - | - | + | + | + | + | + | - | - | - | + | 93.0 | Proteus penneri |
| B17 | + | N | - | N | + | N | - | - | - | + | + | N | N | 89.8 | Pseudomonas aeruginosa |
| Paraquat | | | | | | | | | | | | | | | |
| B18 | - | - | - | - | - | + | + | - | - | + | - | - | - | 99.6 | Chryseobacterium indologenes |
| B19 | + | N | N | N | N | N | + | - | - | N | + | N | N | 89.8 | Pseudomonas putida |
| B20 | + | + | - | N | + | - | - | - | - | + | - | + | + | 89.5 | Enterobacter species |
| B21 | + | N | - | N | + | N | - | - | - | + | + | N | N | 89.8 | Pseudomonas aeruginosa |
| B22 | + | - | - | - | + | - | - | - | - | - | - | - | - | 99.9 | Acinetobacter baumannii |
| B23 | + | + | + | - | + | - | - | - | - | + | + | + | - | 98.4 | Enterobacter sakazaki |

Key: H₂S - hydrogen sulphide, VP – Voges Proskauer, ONPG – O-nitropheryl-β-galactopyranoside, ADH- arginine dehydrase, LDC- lysine decarboxylase, N – not applicable
### Table 4. Yeast isolates from the study

| Code | Glucose | Glycine | Arabinose | Galactose | Inositol | Sorbitol | MDG | NAG | Lactose | Maltose | Trehalose | % ID | Identified isolate |
|------|---------|---------|-----------|-----------|----------|-----------|-----|-----|---------|---------|-----------|------|-------------------|
| **Carbofuran** |         |         |           |           |          |           |     |     |         |         |           |      |                   |
| CY1  | +       | +       | -         | -         | +        | +         | +   | +   | -       | +       | +         | 73.0 | Candida krusei     |
| CY2  | +       | +       | -         | +         | -        | -         | -   | -   | -       | +       | -         | 71.0 | Saccharomyces cerevisiae |
| **Paraquat** |         |         |           |           |          |           |     |     |         |         |           |      |                   |
| PY1  | +       | +       | -         | +         | -        | -         | -   | -   | -       | +       | -         | 71.0 | Saccharomyces cerevisiae |
| PY2  | +       | +       | -         | -         | -        | +         | +   | +   | -       | +       | +         | 73.0 | Candida stellatoidea |
| PY3  | +       | +       | -         | -         | -        | +         | +   | +   | -       | +       | +         | 73.0 | Candida krusei     |

Key: MDG – methyl-α-D-glucopyranoside, NAG – N-acetyl-glucosamine

### Table 5. Actinomycetes isolated from the study

| Code | Grams reaction | Glucose | Indole | Urease | Mannose | Lactose | Maltose | Xylose | Arabinose | Gelatin | Esculin | Raffinose | Sorbitol | % ID | Identified isolate |
|------|----------------|---------|--------|--------|---------|---------|---------|--------|-----------|---------|---------|-----------|----------|------|-------------------|
| **Carbofuran** |         |         |        |        |         |         |         |        |           |         |         |           |          |      |                   |
| A1   | +              | +       | -      | -      | +       | -       | +       | +      | -         | +       | -       | -         | 94.8     |      | Actinomyces isrealii |
| A2   | +              | +       | -      | -      | +       | -       | +       | -      | +         | -       | +       | -         | 94.2     |      | Actinomyces naeslundii |
| A3   | +              | +       | -      | -      | +       | -       | -       | -      | -         | +       | -       | -         | 98.4     |      | Actinomyces viscosus 1 |
| **Paraquat** |         |         |        |        |         |         |         |        |           |         |         |           |          |      |                   |
| A4   | +              | +       | -      | -      | +       | +       | +       | -      | -         | -       | -       | -         | 99.9     |      | Actinomyces meyeri |
| A5   | +              | +       | -      | -      | +       | -       | +       | -      | +         | -       | +       | -         | 94.2     |      | Actinomyces naeslundii |
| A6   | +              | +       | -      | -      | -       | -       | -       | -      | -         | -       | -       | -         | 99.3     |      | Actinomyces viscosus 2 |
Table 6. Mould isolates from Carbofuran and Paraquat during the study

| Reference | Culture characteristics                                                                 | Microscopic characteristics                                                                 | Probable genera          |
|-----------|----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|--------------------------|
| I         | Light grey-yellowish colonies which developed within 4 days. Reverse was yellowish with | Stripes bearing terminal biverticillate or less commonly monoverticillate                     | Talaromyces species      |
|           | furrows. There was dark red pigmentation when culture was old.                           |                                                                                               |                          |
| J         | Slow-growing white colonies with rug-like aerial mycelia and furrows on surface and     | Septate hyphae with dark, branched conidiophores with two or more conidial chains. The conidia  | Cladosporium carioni      |
|           | reverse side. Reverse was light brown with furrows.                                      | formed branching tree-like chains, which as oval and easily dislodged showing dark spots at the |                          |
| H         | White to brownish wooly surface with raised or higher mycelium colonies. Reverse side of | Branched conidiophores, septate hyphae. The macroconidia was large, slightly curved but had no | Curvularia sp.            |
|           | the plate was fairly blue-black.                                                        | rostrum                                                                                       |                          |

4. CONCLUSION

In Nigeria, farmers desire the use of pesticides mixture in the same farm. The isolation of Carbofuran and Paraquat degrading microbes from soils indicated that farm soils harbour pesticide degrading microbial populations and have a widespread genetical diversity and geographical distribution hence, are conceivably worthwhile in environmental biorestoration. The API analysis of the isolated species clustered them into nine different bacterial genera; *Pseudomonas*, *Proteus*, *Chryseobacterium*, *Enterobacter*, *Kocuria*, *Bacillus*, *Lerclercia*, *Acinetobacter*, *Paenibacillus*, *Corynebacterium*. Yeast genera were *Candida*, *Saccharomyces* and mould genera were *Talaromyces*, *Cladosporium carioni* and *Curvularia*, respectively. *Actinomycyes israelii*, *Actinomycyes naeslundii*, *Actinomycyes viscosus* 1, *Actinomycyes meyeri* and *Actinomycyes viscosus* 2 were the few actinomycyes isolated and identified. All microbial species were pesticides utilizers as observed in the screening which were indicated by increased turbidity of the culture medium. The microbes isolated from the unpolluted soil were capable of growing in the presence of these chemicals suggesting that isolates were potential pesticides degraders.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Orji, F.A. (PhD) and staff of Federal Institute of Industrial Research Oshodi, Lagos, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Goad RT, Goad JT, Atieh BH, Gupta RC. Carbofuran-induced endocrine disruption in adult male rats. Toxicol Mech Methods. 2004;14(4):233–239.
2. Lau TK., Chu W, Graham N. Degradation of the endocrine disruptor Carbofuran by UV, O3 and O3/UV. Water Sci Technol. 2007;55(12):275-280.
3. Mujeeb KA, Riaz A, Mushtaq S, Abbasi MH, Ali SS. Carbofuran degrading bacteria isolated from different areas of Punjab as potentila bioremediator of agricultural soil. Science International (Lahore). 2012;24(3):273-280.
4. Nisha P, Eldho PG, Priya P. Biodegradation of Carbofuran using bacteria from pretreated plantain field. J E xp Biol. 2016;6(2):30-35.
5. United States Environmental Protection Agency (USEPA). A Framework for assessing and reporting on ecological conditions. EPA-SAB-EPEC-02-009; 2002.
6. Pashu DC. 22 substances pesticides dangereuses bannies sur les 500 existantes; 2009. Available:http://www.developpementdurable.com (Access date: December 15, 2019)
7. Benicha M, Mrabet R, Azmani A. Dissipation processes of 14C-Carbofuran in soil from Northwest Morocco as influenced by soil water content, temperature and microbial activity. J Environ Chem Ecotoxicol. 2013;5(5):119-128.

8. Benicha M, Bensajjay Y, Oumchih A, Mrabet R. Monitoring of plant protection products in potato from the Loukkos perimeter and their impact on the environment. A Communication Presented at the 3rd International Congress on the Improvement of the Agricultural Production, (APA3), 17-18 March, Faculty of Science, Settat-Morocco; 2011a.

9. Salghi R, Luis G, Rubio C, Hormatallah A, Bazzi L, Gutierrez AJ, Hardisson A. Pesticide residues in tomatoes from greenhouses in Souss Massa Valley, Morocco. Bull Environ Contam Toxicol. 2012;88:358-361.

10. Benicha M, Mrabet R, Azmani A. Behavior of 14C Carbopar in sugar beet crop under field conditions. Communication presented at 7th Congress of AMPP, Mai, Rabat-Morocco; 2010.

11. Benicha M, Mrabet R, Azmani A. Biodegradation and dissipation of 14C-Carbofuran in clay soil from Loukkos perimeter, Northwestern Morocco. JSSEM. 2011b;2(12):404-410.

12. Ikpesu TO Ariyo AB. Health implication of excessive use and abuse of pesticides by the rural dwellers in developing countries: The need for awareness. GJEMPS. 2013;2(5):180-188.

13. United States Environmental Protection Agency (USEPA). From Marianne Mannix to Yu-Ting Guijaran: Amendment to Paraquat dichloride human health mitigation decision. Washington, D.C.: U.S. Environmental Protection Agency. Memorandum Dated February, 6; 2018.

14. Fortenberry GZ, Beckman J, Schwartz A, Prado JB, Graham LS, Higgins S, Lackovic M. Magnitude and characteristics of acute Paraquat-and diquat-related illnesses in the US: 1998–2013. Environ Res. 2016;146:191–199.

15. Lock EA, Wilks MF. Paraquat. In: Krieger RI (Ed.). Hayes' Handbook of Pesticide Toxicology. United States, New York: Academic Press. 2010;1771-1827.

16. Xie L, Zhou D, Xiong J, You J, Zeng Y, Peng L. Paraquat induce pulmonary epithelial-mesenchymal transition through transforming growth factor-β1-dependent mechanism. Exp Toxicol Pathol. 2016;68(1):69-76.

17. Tsai WT. A review on environmental exposure and health risks of herbicide Paraquat. Toxicol Environ Chem. 2013;95(2):197–206.

18. Firestone JA, Smith-Weller T, Franklin G, Swanson A, Checkowary H. Pesticides and risk off Parkinson disease: A population-based case control study. Arch Neurol. 2005;62(1):91-95.

19. Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, Scott BL, Vance JM, Scott WK. Pesticide exposure and risk of Parkinson's disease: A family-based case control study. BMC Neurology. 2008;8(6):46-55.

20. Bromilow RH. Paraquat and sustainable agriculture. Pest Manag Sci. 2004;60(4):340–349.

21. Connell JH, Colbert F, Krueger W, Cudney D, Gast R, Bettner T, Dallman S. Vegetation management options in almond orchards. Horttechnology. 2001;11(2):254-257.

22. Katovai E, Burley AL, Mayfield MM. Understory plant species and functional diversity in the degraded wet tropical forests of Kolombangara Island, Solomon Islands, Biol. Conserv. 2012;145(1):214–224.

23. Zheng Y, Long L, Fan Y, Gan J, Fang J, Jin W. A review on the detoxification of organophosphorus compounds by microorganisms. Afr J Microbiol Res. 2013;7(20):2127-2134.

24. Moretti ML, Shrestha A, Hembree KJ, Hanson BD. Post emergence control of glyphosate/Paraquat-resistant hairy fleabane (Conyza bonariensis) in tree nut orchards in the Central Valley of California. Weed Technol. 2015;29(3):501–508.

25. Amondham W, Parkpian P, Polprasert C, DeLaune RD, Jugsujinda A. Paraquat adsorption, degradation and remobilization in tropical soils of Thailand. J Environ Sci Heal B. 2006;41(5):485–507.

26. Ismail BS, Sameni M, Halimah M. Kinetics of the microbial degradation of 2,4-D and 14C-labeled Paraquat in two types of tropical agricultural soil. World Appl Sci J. 2011;14(2):324–333.

27. Sartori F, Vidrio E. Environmental fate and ecotoxicology of Paraquat: A California perspective. Toxicol Environ Chem. 2018;100(5-7):479-517.
28. Yen JH, Hsiao FL, Wang YS. Assessment of the insecticide Carbofuran’s potential to contaminate groundwater through soils in the Subtropics. Ecotox Environ Safe. 1997;38:260-265.
29. Dhameja SK. Environmental Science. Delhi: S.K. Kataria & Sons; 2006.
30. Larson SJ, Capel PD, Majewski M. Pesticides in surface waters: Distribution, trends and governing factors (No. 3). CRC Press; 2010.
31. Ojo J. Pesticides use and health in Nigeria. IJS. 2016;18(4):981-991.
32. Gill HK, Garg H. Pesticides: Environmental impacts and management strategies. In: Solenski S, Larramenday ML (Eds.). Pesticides - toxic aspects. InTech, Croatia. 2014;187-230.
33. Tang M, You M. Isolation, identification and characterization of a novel triazophos-degrading Bacillus sp. (TAP-1). Microbiol Res. 2012;167:299-305.
34. Mostafalou S, Abdollahi M. Concerns of environmental persistence of pesticides and human chronic diseases. Clin Exp Pharmacol. 2012;51(2):129.
35. Cocco P, Satta G, Dubois S, Pili C, Pilleri M, Zucca M, Mannetje AM, Becker N, Benavente Y, Sanjose SD, Foretova L, Tesina S, Maynadie M, Nieter A, Brennan P, Miligi L, Ennis MG, Boffetta P. Lymphoma risk and occupational exposure to pesticides: Results of the Epilymph study. Occup Environ Med. 2013;70:91-98.
36. Wesley B, Ajugwo GC, Adeleye SA, Ibegbulem CR, Azuike PA. Effects of agrochemicals (insecticides) on microbial population. EC Microbiol. 2017;8(4):211-221.
37. Obuotor TM, Yahaya PB, Akinloye OA, Sakariyau AO. Biodegradation of Paraquat dichloride contaminated soil with fermented corn step. UJS. 2016;1(1):67-73.
38. Horne I, Sutherland TD, Harcourt RL. Identification of an opd (organophosphate degradation) gene in an Agrobacterium isolate. Appl Environ Microbiol. 2002a;68(7):3371-43376.
39. Liu J, Xie J, Chu Y, Sun C, Chen C, Wang Q. Combined effect of cypermethrin and copper on catalase activity in soil. J Soil Sediment. 2005;8:327-332.
40. Guo QX, Li R, Lin DQ, Zhu B, Li SP, Jiang JD. Isolation and characterization of a triazophos-degrading strain GS-1 and its degrading characteristics. Microbiol. 2009;36(8):1143-1149.
41. Wang LH, Zhang L, Chen HL. Isolation of a triazophos-degrading strain Klebsiella sp E6 effectively utilizing triazophos as sole nitrogen source. FEMS Microbiol Lett. 2005;253(2):259-65.
42. Xiao HP, Cheng SP, Wu ZB. Microbial community variation in phytoremediation of triazophos by Canna indica Linn in a hydroponic system. J Environ Sci. 2010;22(8):1225-31.
43. American Public Health Association (APHA). Standard methods for the examination of water and waste water. Standard Methods. 2012;541.
44. Chikere CB, Ekwuabu CB. Culture dependent characterization of hydrocarbon utilizing bacteria in selected crude oil impacted sites in Bodo, Ogoniland, Niger Delta, Nigeria. Afr J Environ Sci Biotechnol. 2014;8(6):401-406.
45. Alharbi SA, Arunachalam C, Murugan AM, Wainwright M. Antibacterial activity of actinomycetes isolated from terrestrial soil of Saudi Arabia. JFAE. 2012;10(2):1093-1097.
46. Lone AH, Raverkar KP, Pareek N. In-vitro effects of herbicides on soil microbial communities. The Bioscan. 2014;9(1):11-16.
47. Baboo M, Pasayat M, Samal A, Kujur M, Maharana JK, Pate AK. Effect of four herbicides on soil organic carbon, microbial biomass - C, enzyme activity and microbial populations in agricultural soil. IJREST. 2013;3(4):100-112.
48. Omolo KM, Magoma G, Ngamau K, Muniru T. Characterization of methomyl and Carbofuran degrading bacteria from soils of horticultural farms in Rift Valley and Central Kenya. AJEST. 2012;6(2):104-114.
49. Devashree Y, Dutta BK, Paul SB, Choudhury S. The effect of Paraquat and fipronil on the soil and rhizosphere microflora of tea (Camellia sinensis). IJIAS. 2014;7(4):1534-1543.
50. Kyverga PM, Blackmer AM, Ellsworth JW, Isla R. Soil pH effects on nitrification of fall-applied anhydrous ammonia. Soil Sci Soc Am J. 2004;68:545-551.
51. Ayansina ADV, Oso BA. Effect of two commonly used herbicides on soil microflora at two different concentrations. Afr J Biotech. 2008;5(2):129-132.
52

acidity as main obstacles to the transfer of basidiomycetous ground fungi into (organically or heavy-metal contaminated) soils. J Basic Microbiol. 2007;47:309-316.

53. Naramabuye FX, Haynes RJ. The liming effect of five organic manures when incubated with an acid soil. JPNSS. 2007;170:615-622.

54. Marschner H. Mineral nutrition of higher plants. Second Edition. Academic Press, London. 1995;313-404.

55. Minocha R, Minocha SC. Effects of soil pH and aluminium on plant respiration. In: Lambers H, Ribas-Carbo M, (Eds.). Plant respiration. Springer, Netherlands. 2005;159-176.

56. Desaint S, Hartmann A, Parekh NH, Fournier J-C. Genetic diversity of Carbofuran-degrading soil bacteria. FEMS Microbiol Ecol. 2010;34:173-180.

57. Itoh H, Navarro R, Takeshita K, Tago K, Hayatsu M, Hori T, Kikuchi M. Bacterial population succession and adaptation affected by insecticide application and soil spraying history. Front Microbiol. 2014;5(457):1-12.

58. Martani E.Paraquat biodegradation in peat soil by fungi and yeast. AGRIS. 2008;9(18):171-180.

59. De Schrijver A, De Mot R. Degradation of pesticides by actinomycetes. Crit Rev Microbiol. 2008;25(2):85-119.

60. Fuentes MS, Benimeli CS, Cuozzo A, Amoroso MJ. Isolation of pesticide degrading actinomycetes from a contaminated site: Bacterial growth, removal and dechlorination of organochlorine pesticides. Int Biodeterior Biodegr. 2010;64(6):434-441.

61. Chougale VV, Deshmukh AM. Biodegradation of Carbofuran pesticide by saline soil actinomycetes. AJMBES. 2007;9(4):1057-1061.

62. Andy IE, Edu GS, Bassey IU, Markson AA, Umana EI, Udo SE. Biodegradation of Paraquat. J Biopestic Environ. 2015;1:80-85.

63. Devi KY, Iyer P. Fungal biodegradation of Carbofuran pesticide. IJRSB. 2016;4(12): 54-57.

64. Ortiz-Hernandez ML, Sanchez-Salinas E, Olvera-Velona A, Folch-Mallol JL. Pesticides in the environment: Impacts and its biodegradation as a strategy for residues treatment. In: Stoytcheva M, (Ed.). Pesticides-formulations, effects, fate. InTech, Croatia. 2011;551-574.

65. Okerentugba PO, Ezeryone OU. Petroleum degrading potentials of single and mixed microbial cultures isolated from Rivers and Refinery Effluent in Nigeria. Afr J Biotechnol. 2003;2:288-292.

66. Ortiz-Hernandez LM, Sanchez-Salinas E, Castrejon-Godinez ML, Dantan-Gonzalez E, Popoca - Ursino EC. Mechanisms and strategies for pesticide biodegradation: Opportunity for waste, soils and water cleaning. Rev Int. 2013;29:85-104.

© 2020 Ataikiru et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.