JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ENVIRONMENTAL DETOXIFICATION POTENTIAL OF AXENIC AND MIXED CULTURES OF *BACILLUS* SPECIES ON PESTICIDES USING AN *IN VITRO* BIODEGRADATION ASSAY

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https://doi.org/10.55251/jmbfs.9272

ARTICLE INFO

Received 30. 7. 2022
Revised 22. 12. 2022
Accepted 10. 1. 2023
Published 1. 4. 2023

Regular article

OPEN ACCESS

ABSTRACT

Pesticide use is an integral part in global agricultural practice, however, these chemicals have recently been identified as emerging pollutants that impact on ecosystem health and sometimes require remediative intervention. This study assessed the biodegradative ability of species of *Bacillus* on the organochlorine herbicide, paraquat and the organophosphorus insecticide, dichlorvos. The *Bacillus* isolates were identified based on their genomic, biochemical and morphological characteristics while the ability of the isolates to degrade the pesticides was determined by monitoring changes in optical density, pH, total viable count and pesticide concentration in *in vitro* systems over an 18-day period using pesticide-modified mineral salts broth. The two Bacilli isolates were identified as *Bacillus crassostreae* and *Bacillus niabensis.* The organochlorine herbicide was more readily utilised by the axenic *Bacillus* isolates and their mixed culture than the organophosphorus insecticide as evidenced by variations in turbidity and counts. Biodegradation was more effective with the mixed culture than with the axenic cultures. Pesticide degradation levels of 64.26 % – 93.70 % and 70.43 % – 98.20 % were observed for the insecticide and herbicide respectively for both the bacilli and their mixed culture as revealed by gas chromatography analysis. The degradation efficiencies of the two bacilli for both dichlorvos and paraquat showed significant differences from each other and from the mixed culture at 95% confidence interval. The study established the potential of *Bacillus crassostreae*, *Bacillus niabensis* and their mixed culture to be employed as inocula in the detoxification of pesticide-contaminated environmental media.

Keywords: *Bacillus*; Biodegradation; Dichlorvos; Emerging pollutants; Paraquat; Pesticide

INTRODUCTION

Contemporary agricultural practice goes hand-in-hand with pesticide use. Modern pesticides are largely semi-volatile synthetic compounds characterised by high environmental mobility, poor biodegradability and eco toxicity **(Doolotkeldieva *et al*., 2018; Osadebe *et al*., 2018a)**. This group of xenobiotics become pollutants when they persist in environmental media occurring in quantities higher than permissible rates. They are, thus, categorised as persistent organic pollutants (POPs) impacting both terrestrial ecosystems and the aquifer through leaching **(Megson *et al*., 2016)**. Furthermore, surface run-offs quite often introduce these persistent pollutants to the surrounding water bodies. Evaporation from soils is also a concern as it could contribute to air pollution. Pesticides, as pollutants, are therefore, a threat to both aquatic, atmospheric and edaphic environments **(Parte *et al*., 2017; Lehmann *et al*., 2018)**. The ideal pesticide should terminate target organisms without any impact on non-target groups; this is, unfortunately, rarely the case as findings reveal that only under 5 % of applied pesticides reach the target organisms **(Javaid *et al*., 2016)**. Pesticides are considered emerging pollutants because they have only recently been identified as environmental pollutants. This is owing to their capacity for persistence in environmental media and their ecological and human health impacts **(Ali *et al*., 2022)**.

These xenobiotic hydrocarbon compounds are often classed based on their target organism or their chemical makeup. Pesticides used for the management of unwanted plants, insect life, rodents and fungi are referred to as herbicides, insecticides, rodenticides and fungicides respectively. Based on their chemical structures and constituent functional groups, they are described as organochlorines, organophosphates, organometallic compounds, pyrethroids, and carbamates amongst others **(Arora *et al*., 2020)**. Global use of pesticides is on a steady rise; the **USEPA (2011)** estimate that roughly 2.5 billion kilogrammes of pesticides are used annually across the globe with about 17.9 % of that utilised in the United States alone. Other reports highlight that, worldwide, insecticides and herbicides are the most habitually employed pesticide groups making up about 29.5 % – 79.0 % and 47.0 % – 47.5 % of global pesticide use respectively (**Zhang *et al*., 2011; De *et al*., 2014)**. Paraquat dichloride is a widely used organochlorine herbicide whose use is strongly regulated in the United States due to toxicity though it is the herbicide of choice in several developing countries **(Nesheim *et al*., 2005)**. Similarly, the organophosphorus insecticide, dichlorvos, is equally highly toxic to humans and other non-target species, but is used extensively in developing countries for the management of insects that destroy crops and plague livestock **(Binukumar and Gill, 2010)**. This insecticide is currently banned in Europe **(EC, 2011)** and is in the class 1B category of "highly hazardous" chemicals **(WHO, 2012)** and group 2B for possible carcinogens **(Cancer IAFR, 2021)**.

Microorganisms, particularly bacteria, are the main detoxification and degradation agents within a thriving ecosystem. They are, therefore, important players in the management of environmental media tainted by pesticides. The key bacterial degraders associated with pesticide degradation belong to the Proteobacteria. Several studies have implicated bacterial genera such as *Arthrobacter, Pseudomonas, Actinobacter, Vibrio, Bacillus, Sphingomonas, Novosphingobium, Flavobacterium, Burkholderia* and *Cupriavidus*, amongst others, in pesticide degradation in both soil and water systems **(Bano and Musarrat, 2004; Yan *et al*., 2007; Rani and Dhania, 2014; Javaid *et al*., 2016; Osadebe *et al*., 2018a; Gupta *et al*., 2019; Duc, 2022)**. Bacterial detoxification in contaminated land management systems are fundamental to ecosystem restoration if further impact on the environment is to be mitigated. The genus *Bacillus* of the order Bacillales are Gram positive, endospore forming rods with a remarkable capacity to proliferate in high densities even under somewhat adverse environmental conditions. They are known to synthesise an array of metabolites like antibiotics, amino acids, pheromones and biosurfactants that have extensive applications in industry, environment, health and agronomy. Bacilli demonstrate a competence for degradation of an array of compounds whether natural and synthetic **(Caulier *et al*., 2019; Patel and Gupta, 2020)**. They have been linked to the utilisation and breakdown of antibiotics, detergents, petroleum hydrocarbons, lubricating oils, explosives and dyes in several studies **(Singh and Singh, 2016; Osadebe *et al*., 2018b; Osadebe *et al*., 2018c; Sultana *et al*., 2021; Fareed *et al*., 2022; Monga *et al*., 2022; Zhang *et al*., 2022)**. These reports highlight the importance of bacilli in environmental detoxification and bioremediation.

The current study sought to establish the capacity of axenic and mixed cultures of *Bacillus* to utilise selected pesticides as their sole carbon source under aerobic conditions and eliminate them from pesticide-tainted media. The pesticides tested were the organophosphate insecticide, dichlorvos (with chemical name 2,2-dichlorovinyl dimethylphosphate, $C_4H_7Cl_2O_4P$) and the organochlorine herbicide, 1,1-dimethyl-4,4-bipyridinium dichloride, $C_{12}H_{14}Cl_2N_2$ (regularly referred to as paraquat or paraquat dichloride). The pesticides were tested at levels akin to their recommended field application rates.

MATERIAL AND METHODS

Sample Collection

The pesticides used was obtained from the local town market in Port Harcourt metropolis, Rivers State, Nigeria while the *Bacillus* species used in the study were isolated from crude oil agricultural soil in Bomu area of Ogoniland, Nigeria using the composite sampling approach. Samples were collected from the soil surface up to around 20 cm using an auger. The soil samples were conveyed to the laboratory in sterile black sample bags.

Isolation of *Bacillus* species

Isolation was done using nutrient agar (Merck, Germany). A portion of the soil composite was suspended in sterile normal saline (1:10 w/v) and shaken vigorously. The settled suspension was then serially diluted and aliquots from selected dilutions were inoculated unto sterile medium with incubation for 24 h – 48 h at approximately 30 ± 2 °C. Discrete colonies were purified by streaking technique on fresh media. The pure isolates obtained were stored on media slants until required for further analysis **(Cheesbrough, 2006)**.

Preliminary Characterisation of the Isolates

The isolated bacteria were characterized based on their morphological, microscopic, and biochemical properties as proposed **by Cheesbrough (2006)** and **Holt *et al*. (1994)** in order to putatively identify the *Bacillus* spp.

Confirmatory Identification of *Bacillus* Isolates via Sanger Sequencing

The identities of the two isolates ascertained as *Bacillus* spp. were confirmed via genomic analysis. The cells of pure isolates cultivated in Luria-Bertani medium were harvested by centrifugation and the bacterial DNA extracted using ZR fungal/bacterial DNA Miniprep (Zymo research, USA) as outlined by the manufacturer. Following DNA extraction, the concentration and purity of the DNA obtained was established using the Nanodrop 2000 spectrophotometer while the integrity of the DNA sample was established by quantifying and visualizing the DNA using a UV transilluminator on 1% w/v agarose gel. The 16S region of the rRNA genes of the isolates were amplified using the 27F and 1492R forward and reverse universal primers on GeneAmp® PCR System 9700 (Applied Biosystems, USA) at a final volume of 50 µl. The PCR parameters were: initial denaturation at 94 °C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 s, annealing at 56 °C for 30 s, elongation at 72 °C for 45 s and then final elongation at 72°C for 7 minutes. Hold temperature was 10 °C. The amplified PCR products were resolved on a 1.5 % agarose gel at 120V for 15 minutes then visualized using a UV transilluminator. Sequencing was done using the BigDye® Terminator 117 v3.1 kit on 3510 ABI sequencer (Inqaba Biotechnological, South Africa) **(Nasser *et al.*, 2017)**.
Sequence identification entailed checking the 16S rRNA sequences obtained for each test isolate against the National Centre for Biotechnology Information (NCBI) database using the basic local alignment search tool (BLAST) analysis. Blast hits with e-values closest to 0.0 were concluded to be closest to the isolate and were used for alignment and assembly of the phylogenetic tree.

Biodegradation Assay - *In Vitro* Pesticide Degradation in Mineral Salts Broth (MSB)

The two pure isolates of *Bacillus* spp. obtained and their mixed culture were subjected to a biodegradation assay to assess their capacities for pesticide utilisation when it is available as the sole carbon source. Bacterial standard enrichment cultures consisted of the axenic isolates introduced into 100 ml mineral salts broth containing the specific test pesticide as the sole carbon source. The inoculated broths were incubated at room temperature for 24 h.
The technique outlined by **Okpokwasili and Okorie (1988)** was adapted for the biodegradation assay using pesticide supplemented MSB in replicated 250 ml Erlenmeyer flasks at a concentration of 20 µg ml^{-1} which represents roughly 2.0 kg/ha. This is over the recommended field application rate of 0.25 – 1.5 kg/ha for both pesticides. The flasks were then inoculated with about 1 ml of 24 h bacterial culture (optical density of 0.8). Incubation was at 30 °C on a rotary shaker for 18 days with monitoring for pesticide concentration, viable bacterial counts and turbidity of the medium at regular intervals. The initial pH of the set-up was 7.0. Control studies consisted of uninoculated pesticide-laden MSB flasks.

Determination of Growth by Turbidimetry

After a 24 h acclimatisation period, known portions of the MSB from the biodegradation assay set-ups were extracted at regular intervals and the optical density determined at 540 nm using a UV Visible Spectrophotometer. The uninoculated MSB served as the standard.

Enumeration of *Bacillus* spp. in the Assay Set-ups

Roughly 0.1 ml aliquots were collected from the set-ups and plated out unto sterile nutrient agar with incubation at 30 °C for 24 h – 48 h. Only plates with counts of 30 – 300 colonies were selected for determination of counts. Plate counts were done at the end of the incubation period using an automated digital colony counter (Balance Instrument Co., China). Plates with counts in excess of 300 colonies were discarded.

Determination of Pesticide Content Using Gas Chromatography-Mass Spectrometry (GC-MS)

The residual pesticide concentrations during the study were monitored to establish biodegradation. About 5 ml of MSB from the set-ups containing the pesticides and the bacterial inocula was centrifuged and the supernatant obtained analysed using GC-MS (Agilent 6890/ 5973N, USA). The sample was first dehydrated using anhydrous sodium sulphate then the pesticide residue was extracted using 30 ml dichloromethane via mechanical agitation. The organic layer was concentrated in an evaporator and mixed with cyclohexane. The extract was eluted using pentane in a capillary column system. The eluted sample was allowed to stand overnight at room temperature in a fume cupboard for evaporation to take place. An injection volume of 3 µl was used. Operating conditions were as recommended by the manufacturer. The pesticide concentrations were established at the onset and at the end of the 18-day study. The pesticide removal efficiency for each isolate and the mixed culture was determined as outlined in Equation 1:

$$\text{Removal Efficiency (\%)} = \frac{Ci - Cf}{Ci} \times 100 \qquad \text{Eq. (1)}$$

Where: Cf – Residual pesticide concentration; Ci – Initial pesticide concentration

Data Analysis

The data obtained in the study was analysed statistically using Microsoft Excel® 2016 and SPSS® version 23. Basic statistical distribution analysis was carried out on the data. One and two factor ANOVA evaluated the relationships between the pesticide removal efficiencies amongst the two isolates and the mixed culture as well as within groups comparing removal efficiencies for each isolate and the mixed culture on the insecticide and herbicide. Comparisons were done at 95% confidence interval.

RESULTS AND DISCUSSION

Identity of isolates

The two isolates were recognised as *Bacillus* spp. from their biochemical and morphological properties (Table S1 in supplementary files). Their identities were established as *Bacillus niabensis* (isolate code: A4) and *Bacillus crassostreae* (isolate code: A7) based on results from DNA sequencing and phylogenetic analysis (Figure 1). The nucleotide sequences obtained for the isolates were registered in the NCBI GenBank® under accession numbers MF547449 and MF547451 for *B. niabensis* and *B. crassostreae* respectively.

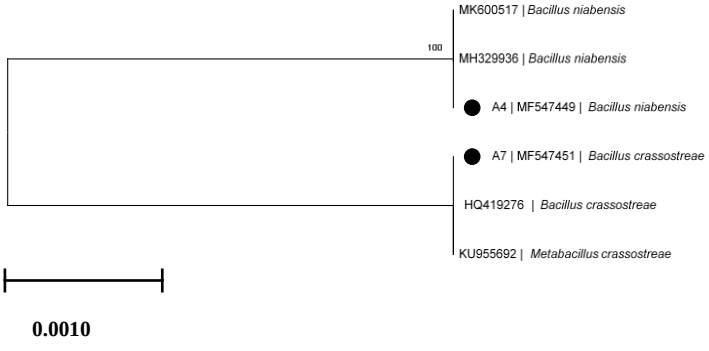
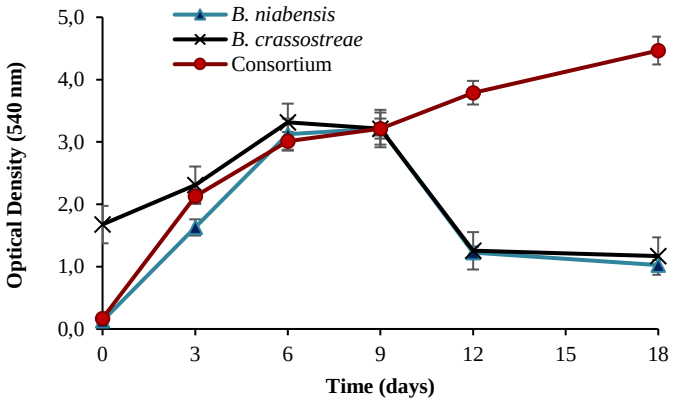


Figure 1 Phylogenetic tree based on 16S rRNA gene sequences showing the evolutionary relationships between the two isolates of *Bacillus* sp. The black circles indicate the isolates from the current study

Growth profile, pH and removal efficiency of the isolates and their mixed culture in pesticide-tainted medium

The growth curve studies using turbidimetry and plate counts (Figures 2 and 3) revealed that while both species and the mixed culture were able to utilize the pesticides as a carbon source and grow extensively in the tainted broths, *B. crassostreae* showed more proficiency in both the herbicide and the insecticide. The mixed culture consisting of the two bacilli exhibited a steady rise in optical density (representing cell density) till the conclusion of the investigation while both

axenic cultures peaked between days 6 and 9 and then entered a steady decline. A similar trend was noted for the bacterial growth curves. No distinct lag phase was observed in the growth profiles.



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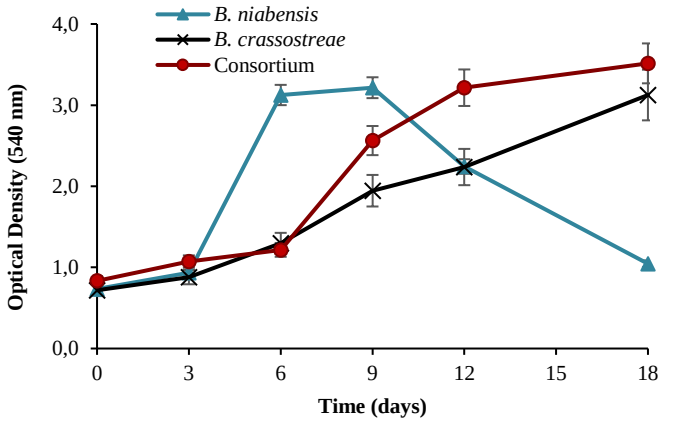
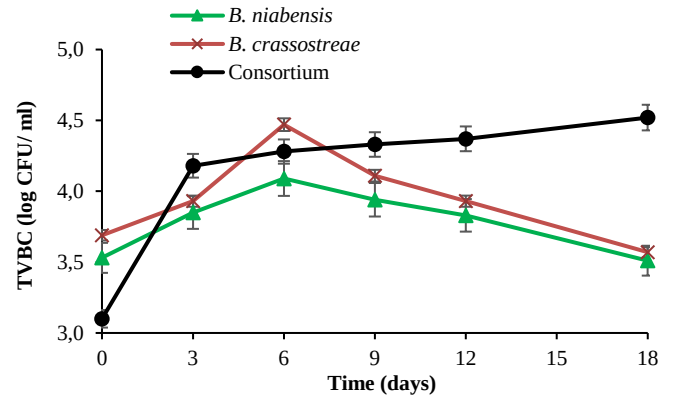


B

Figure 2 Changes in optical density (O.D.) of the mineral salts broth inoculated with the species of *Bacillus* and their mixed culture for (a) the organophosphate insecticide, dichlorvos and (b) the organochlorine herbicide, paraquat during the biodegradation assay. Values are means of triplicates. Bars represent the standard error.

While the mixed culture seemed to remain in the exponential phase of growth on both the insecticide and the herbicide till the end of the study, active growth for the axenic cultures of *B. niabensis* and *B. crassostreae* peaked on day 6 for the insecticide (dichlorvos) and then displayed a steady decline signifying the onset of the death phase of the growth cycle. With the herbicide, *B. crassostreae* continued to show increased viable cell count as contact time increased, up till the end of the study while counts were observed to decline from day 9 with *B. niabensis*. By the end of the study, counts of 3.51 log CFU/ml, 3.57 log CFU/ml and 4.52 log CFU/ml were obtained for *B. niabensis*, *B. crassostreae* and the mixed culture respectively for dichlorvos. For paraquat, *B. niabensis*, *B. crassostreae* and the mixed culture had terminal counts of 4.23 log CFU/ml, 4.27 log CFU/ml and 4.92 log CFU/ml respectively. These results indicated that *B. crassotreae* was a more efficient degrader than *B. niabensis*.



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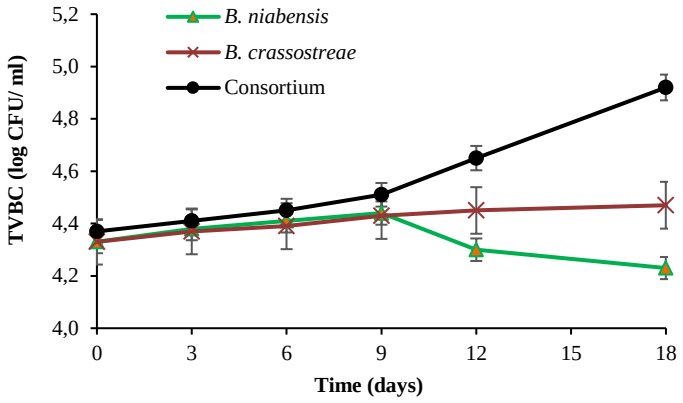
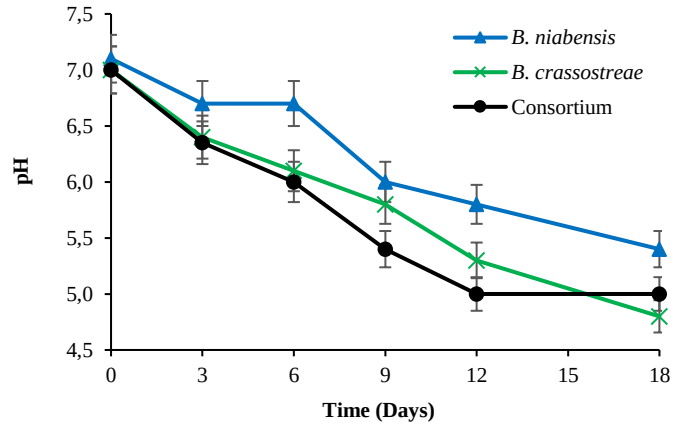


B

Figure 3 Changes in total viable bacterial counts (TVBC) of the mineral salts broth inoculated with the species of *Bacillus* and their mixed culture for (a) the organophosphate insecticide, dichlorvos and (b) the organochlorine herbicide, paraquat during the biodegradation assay. Values are means of triplicates. Bars represent the standard error.

All the set-ups demonstrated comparable trends in pH profile. During biodegradation of dichlorvos, the pH of the system for the bacilli and their consortium dropped from neutral to somewhat acidic conditions while for paraquat, the pH remained relatively neutral till day 6 then showed slight tendencies towards alkalinity rising to about 8.5 – 8.6 on average by the end of the study (Figure 4). The organophosphorus insecticide proved to be more recalcitrant as depicted in Figure 5. Pesticide degradation levels of 64.26 % – 93.70 % and 70.43 % – 98.20% were observed for the insecticide and herbicide respectively for both the bacilli and their mixed culture with the consortium being the most efficient degrader. The bacterial consortium almost completely eliminated the pesticides in the in vitro systems. The consortium was most efficient displaying mean removal efficiencies of up to 93.7 % for dichlorvos (organophosphorus insecticide) and 98.2 % for paraquat (organochlorine herbicide).



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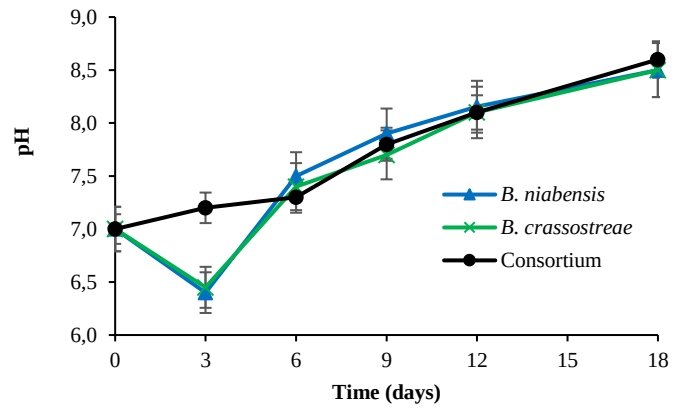

B

Figure 4 Changes in pH of the mineral salts broth inoculated with the species of *Bacillus* and their mixed culture for (a) the organophosphate insecticide, dichlorvos

and (b) the organochlorine herbicide, paraquat during the biodegradation assay. Values are means of triplicates. Bars represent the standard error.

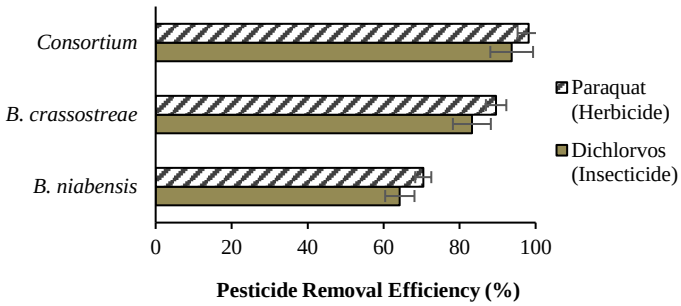

Figure 5 Removal efficiency of the organophosphorus insecticide and organochlorine herbicide by the *Bacillus* isolates and their mixed culture during the study. The bars represent the standard error.

Statistical comparisons

Output from Levene's test indicated homogeneity of variance across board. With p values less than 0.05, the removal efficiencies of *B. niabensis* and *B. crassostreae* for both dichlorvos and paraquat showed significant differences from each other and from the mixed culture at 95 % confidence interval. For both isolates as well, there were statistically significant differences when comparing their removal efficiencies for dichlorvos and paraquat; however, for the mixed culture, the removal efficiencies for dichlorvos and paraquat did not differ significantly from each other.

DISCUSSION

Only a diminutive fraction of soil bacteria have capacity for pesticide degradation. For the insecticide vetox-85, a previous study showed that only 0.32 % of soil microorganisms were utilisers **(Osadebe *et al*., 2018a)**. **Doolotkeldieva *et al.* (2018)**, in contrast, found pesticide-degrading bacteria to make up 32% – 47 % of autochthonous organisms in a soil ecosystem in Kyrgyzstan. In spite of this, several species of *Bacillus* have been associated with pesticide degradation in different ecosystems. Research has demonstrated that pesticides like diazinon, chlorpyrifos, endrin; parathion and dichlorodiphenyltrichloroethane (DDT) can be readily utilised by *Bacillus* spp. (**Verma *et al*., 2014; Upadhyay and Dutt, 2017; Huang *et al*., 2018; Osadebe *et al*., 2018a)**. *Bacillus* was noted as one of several resilient bacterial isolates from dichlorvos- and paraquat-treated soils **(Ataikiru *et al*., 2020; Adigun *et al*., 2022)** even though **Adigun *et al*. (2022)** confirmed a drop in soil microbial populations following the application of dichlorvos. Strains of *B. aryabhattai* were isolated from soil impacted by paraquat in investigations in Thailand **(Inthama *et al*., 2021)**. **Lamoreaux and Newland (1978)** as cited in **Okoroiwu and Iwara (2018)** linked the removal of dichlorvos in soil to the presence of *B. cereus* with removal levels of 50 % in 3.9 days in soil and 49 % in 4 days when the herbicide was included as the sole carbon source in MSM. Marine-derived *B. niabensis* has been associated with the degradation of the surfactant, benzyldimethyl hexadecylammonium by about 90 % in 7 days when it was present at relatively low concentrations **(Bassey and Grigson, 2011)**.

The greater proficiency of the isolates and the mixed culture in the utilisation of the organochlorine herbicide, paraquat, is fairly unusual as chlorinated pesticides are well known to inhibit bacterial cell viability and impede cell metabolism even under structured laboratory conditions **(El-Bestawy *et al*., 2014)**. The microbial growth cycle predicts the biodegradation rates of the substrate such that optimal biodegradation levels typically occur when microbial counts associated with the substrate are greatest **(Ojo-Omoniyi, 2013)**. Based on the proficiency of growth and cell density (optical density) obtained in the current study, the mixed culture was the most effective degrader followed closely by *B. crassostreae*. The high rate of pesticide removal obtained in the current study is supported by similar studies on pesticide elimination by species of *Bacillus*. The *B. niabensis* and *B. crassostreae* isolates from the present study have proved to be equally as effective as those in comparable studies. As seen with the mixed culture in the current study, immobilised cells of *B. subtilis* alongside *P. aeruginosa* removed up to 98 % of paraquat in *in vitro* investigations **(Jindakaraked *et al*., 2021)**. **Duc (2022)** established a 97.5 % degradation of the insecticide, cabofuran, by a strain of *Bacillus* immobilised on rice straw. *B. cereus* proved able to utilise cypermethrin, imidacloprid, fipronil and sulfosulfuron as its sole carbon source in MSB with biodegradation levels of 94 %, 91 %, 89 % and 86 % respectively obtained during a 15-day period **(Gangola *et al*., 2021)**. Likewise, degradation of cypermethrin in another 15-day study by *Bacillus* sp. resulted in elimination of around 85 % of the pesticide **(Bhatt *et al*., 2019)** while *B. licheniformis* removed up to 64 % of cypermethrin after 5 days in field-based investigations **(Dang *et al*., 2015)**.

Akin to the present study, **El-Bestawy *et al.* (2014)** also confirmed improved pesticide biodegradation with increasing contact time. Contact time is considered an important function of pesticide degradation by microorganisms alongside concentration. The presence of microbial groups with the metabolic capacity for degradation of hydrocarbon compounds is fundamental to the environmental biodetoxification process. Previous exposure and adaptation could play a role in the enhanced pesticide removal obtained in the present study. The isolates used in the current study were obtained from crude oil impacted soil and, thus, have a history of exposure to various groups of hydrocarbons. Microorganisms will typically adapt to measured and regular contact to different groups of substances. Adaptation such that subsequent exposure to similar categories of those compounds would result in more rapid and efficient degradation as the organisms already possess the requisite catabolic enzymes for mineralisation. The more regular the exposure, the more superior its degradation competence and skill at utilising present and imminent hydrocarbon compounds upon contact **(Bakar *et al*., 2022)**. Microbial species breakdown xenobiotics to which they have been previously exposed more readily than they would "new" compounds **(Biello, 2015)**.

The results of several similar studies confirm this assertion that previous contact with a specific group of compounds enhances the capacity of degrading microorganisms to utilise similar compounds upon future exposure. One comparable study found that species from sediments with chronic petroleum contamination showed degradation rates exceeding 10 – 400 times those seen with microorganisms from pristine sediment. The experienced hydrocarbon degrading bacteria has been known to transfer this advanced ability for hydrocarbon degradation to subsequent generations via genetic adaptation. Certain species of *Vibrio* have been extensively studied for exhibition of this trait **(Chaillan *et al*., 2006; Biello, 2015)**. In another study, 55 % pyrene degradation was recorded when using a suspension of bacterial cells previously cultivated in pyrene-laden media compared to 1 % obtained with the autochthonous previously unexposed species **(Laleh *et al*., 2006)**. Similarly, greater metabolism was noted when the inoculum was enriched in media containing crude oil as carbon source prior to biodegradation testing in contrast to those species cultivated using plain unmodified nutrient broth **(Chaîneau *et al*., 2005)**.

The enhanced biodegradation of the mixed culture in the present study is somewhat anticipated. A consortium of organisms will typically give better substrate utilisation results than pure cultures. Complete biodegradation requires a community of species to achieve. Each individual species has a niche range of organic compounds it can utilise for energy. A mixed population would, therefore, provide individuals with metabolic capacity for different groups of compounds working together to ensure that the biodegradation proceeds at maximum levels. Each species plays a unique role in hydrocarbon transformation process. Several studies conclude that each individual in a mixed culture involved in biodegradation, depends on the presence of the other individuals to survive and effectively function in their niche. An eight-strain consortium with six genera effectively eliminated hydrocarbon compounds from environmental media, however, only five members of the consortium were culturable as axenic forms on the different hydrocarbon compounds and the efficiency of biodegradation dropped drastically with the exclusion of three selected organisms from the association **(Pizarro-Tobias *et al*., 2014)**. The interaction within a microbial community or consortium during biodegradation is relatively unclear, it has been opined that the different microorganisms function in synergy with one member eliminating the toxic metabolites produced by its counterpart. Often times, one species has the capacity to fully metabolise the by-products resulting from the partial degradative activities of another member **(Cerqueira *et al*., 2012; McGenity *et al*., 2012)**.

The observed variation in the pH of the *in vitro* systems may be due to the introduction of degradation by-products whether acidic in the case of the insecticide, dichlorvos, or alkaline as seen in the set-ups containing the herbicide, paraquat, in the present study. **Zhang *et al*. (2021)** have described dichloroacetic acid and ethanol as products of aerobic bacterial degradation of dichlorvos; these could result in a drop in pH in a closed system. The drop in pH, in turn, impacts negatively on further bacterial growth and the degradative activities of the *Bacillus* isolates. This would explain the lower degradation efficiency seen with the organophosphorus insecticide. Changes in pH from optimum levels could impede further degradative activities on the part of the bacteria. The optimal pH for paraquat degradation and the growth of the degrader, *B. aryabhattai* was 7.0 at 30 °C **(Inthama *et al*., 2021)**. The pH levels impact on both microbial activity and the availability of ions in the water. **Dwivedi (2012)** described pH as the major factor influencing the adsorption of contaminant molecules and further maintained that the particle surface charge and surface accessibility also hinged on the pH. Furthermore, as pH rises, the availability of micronutrients such as phosphorus and nitrogen derivatives, exchangeable sodium and potassium, magnesium and calcium declines, in contrast, as pH decreases so does nitrate and chloride availability **(Dhankhar and Guriyan, 2011)**. Biodegradation rates are often strongest at neutral pH levels; the exception being the extremophiles **(Hazen *et al*., 2016)**. Most bacteria are incapacitated under acidic conditions hence fungi constitute the chief players during biodegradation in acidic soils owing to their tolerance of lower pH levels **(Obahiagbon *et al*., 2014)**. The acidic conditions observed during the biodegradation of the insecticide in the present study could have played a role in the reduced elimination of the insecticide from media compared to the herbicide.

Another possibility for the diminished removal efficiency seen with the insecticide compared to the herbicide could be that dichlorvos was toxic to the test bacteria perhaps owing to the mode of action of these chemicals. Insecticides will often function as enzyme and cellular pathway inhibitors. For instance, they are known to interrupt acetylcholinesterase, voltage-gated chloride channel and the acetylcholine receptor systems; similar systems are also present in bacteria. Herbicides in contrast, block more plant-specific pathways like photosynthesis and synthesis of carotenoid, 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase and aromatic and branched chain amino acids essential to only plants **(Casida, 2009; Combarnous, 2017)**. Paraquat, in particular, functions by inhibiting photosynthesis within the chloroplast **(Qian *et al*., 2009)** while dichlorvos like most insecticides blocks acetylcholinesterase function **(Binukumar and Gill, 2010)**.

CONCLUSION

The pesticides were shown to be relatively biodegradable. *Bacillus niabensis* and *Bacillus crassostreae* and their mixed culture were able to remove the organophosphate pesticide, dichlorvos and the organochlorine herbicide, paraquat from mineral salts broth in 18-day *in vitro* investigations. The mixed culture displayed the greatest removal efficiency of 93.7 % and 98.2 % for dichlorvos and paraquat respectively. Of the two bacilli isolates, *B. crassostreae* proved to be the stronger degrader for both pesticides studied having removal efficiencies approximately 19 % greater than those of *B. niabensis* for both dichlorvos and paraquat. The study established that *B niabensis*, *B. crassostreae* and the mixed culture have capacity for detoxification of pesticide-tainted environmental media.

Funding: This research received no external funding.

Conflicts of Interest Statement: The authors declare no conflicts of interest.

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