IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 5 issue 1 Jan, 2026 Research Article

# Hidden Dangers beneath Our Feet: How Organophosphorus and Alkylphenol Pollutants Threaten Soil Nitrifying Bacteria Balance and Earthworm Health

**Oshilonyah, Lucy Unoma [1]*., Jaboro, Grandy Austin[2], and Igborgbor, Jude Chukwuemeke[3]**
[1,2&3]Department of Biological Sciences, Faculty of Science,
University of Delta, Agbor, Delta State,
***Corresponding Author:** *lucy.oshilonyah@unidel.edu.ng*

## ABSTRACT

Pesticides are widely utilized in agriculture to control plant diseases and enhance crop yield. However, their persistence as metabolite residues following degradation and their adverse effects on ecological communities are a global concern. This study evaluated the bioavailability and ecotoxicological impacts of organophosphate and alkyl-phenol pesticides on earthworms and nitrifying bacteria isolated from legume plant soil using standard methods. Bacterial isolates were cultured on Winogradsky medium and identified based on cultural, morphological, and biochemical characteristics. Molecular identification and PCR detection confirmed the nitrifying bacteria as *Nitrosomonas europaea* strain AL 954747.1 and *Nitrobacter winogradskyi* strain NR 074324.1, with percentage identities of 98.0 and 99.0, respectively. Experimental concentrations (100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l, 500 mg/l, 1000 mg/l, and 2000 mg/l) were derived from pesticide stock solutions prepared according to manufacturer's instructions (100 mg in 1000 ml distilled water) and applied to *Nitrobacter* sp., *Nitrosomonas* sp., and to earthworms for 96 hours. Toxicity tests results indicated that at 100 mg/l organophosphate pesticide, *Nitrosomonas* sp. growth ranged from 75.67±6.13 to 40.32±3.20, and *Nitrobacter* sp. from 78.37±6.26 to 43.34±3.25 after 96 hours compared to controls of 100.54±7.34 and 105.29±7.68, respectively. While for alkyl-phenol pesticides at 100 mg/l, *Nitrosomonas* sp. growth ranged from 94.31±6.45 to 63.48±3.40, and *Nitrobacter* sp. from 92.56±6.61 to 62.59±3.50. Generally, the order of growth of both nitrifying bacteria in various concentrations of both pesticides was Control > 100mg/l > 200mg/l > 300mg/l > 400mg/l > 500mg/l > 1000mg/l > 2000mg/l. The Organo-phosphorus and Alkyl-phenol pesticide toxicity on earthworms showed similar reductions. At 100 mg/l, numbers declined from 10 (24 hours) to 5 (120 hours); at 500 mg/l, from 6 (24 hours) to 1 (120 hours); and at 2000 mg/l, from 2 (24 hours) to 0 (72 hours), while controls remained at 10 throughout. These findings suggest that autotrophic transformation by nitrifying bacteria and earthworms, which supports soil fertility, may be impaired in ecosystems contaminated with these pesticides, potentially reducing or halting nitrification processes. It is therefore recommended that pesticides be applied strictly according to manufacturer instructions to prevent ecosystem disruption.

**Keywords**: Pesticides, Organophosphorus, Alkylphenol, Pollutants, Nutrifying Bacteria, Earthworm.

## Introduction

Most of the seriously polluted soils in the world originate from practices between 1960 and 1980, including dumping of chemical wastes and disposal of dredge materials from harbors (Alves *et al.,* 2013). Risk assessment for these contaminated sites is seriously hampered by a lack of quantitative knowledge about bioavailability of the pollutants. It is generally accepted that the total concentration is a poor measure for predicting accumulation and toxic effects and that bioavailability tends to decrease with increasing contact time between chemical and soil (sequestration) (Hasenbein *et al.,* 2015). Nitrifying bacterial and Earthworms are appropriate model organisms for bioavailability as they live in close contact with the soil, have a thin and permeable cuticle, and also consume large amounts of soil nutrients.

However, bioassays with earthworms and nitrifying bacterial have several limitations in providing a general measure of bioavailability (Bandeira *et al.,* 2020).



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

First, bioavailability may depend on the behavior of the organism and may thus differ between species; second, assays are generally performed with homogenized and sieved soil samples whereas exposure in the field is more heterogeneous.

Bioavailability is a function of soil properties, time, environmental conditions and plant and microbial characteristics (Naidu, 2011). Ecotoxicology can be defined as the study of impacts of pollutants on the structure and function of ecosystems (Zhou *et al.,* 2020). It can be by manmade poisonous chemicals and their effects on the environment, it does not include the study of naturally occurring toxins. It can also be referred to as a scientific discipline combining the methods of ecology and toxicology in studying the effects of toxic substances and especially pollutants on the environment and living things.

Ecotoxicology looks at the impacts of contaminants including populations, pesticides on individuals, natural communities and ecosystems (Yang *et al.,* 2018). Communities of living things and the environments they live in form ecosystems. Ecosystems include rivers, ponds, deserts, grasslands and forests and they too can be affected by pesticides.

Organophosphates (OP) are chemical substances produced by the process of esterification between phosphoric acid and alcohol (Xu *et al.,* 2020). Organophosphates can undergo hydrolysis with the liberation of alcohol from the ester bond. These chemicals are the main components of herbicides, and pesticides. For example, there are indications that uptake of dieldrin, alkyltin was limited by chemical transport in the soil and that the bioavailable phase for PAHs can be depleted.

Furthermore, the rate constant in the elimination stage in uncontaminated soil will be smaller than the apparent rate constant from the accumulation stages (Brami *et al.,* 2013). When desorption is slow and rate limiting, we should observe a first rapid increase in body residues, followed by a slower rate of increase (governed by desorption). Several authors have reported peak-shaped accumulation curves, most often for PAHs. The exact cause of this pattern is unknown, but suggested explanations include the induction of active excretion by the worm, an increase of sorption in soil, or biodegradation coupled to slow desorption from organic matter.

When there is rapid degradation or sequestration, the bioavailable concentration will decrease during the experiment, leading to peak-shaped uptake curves (Castillo-Diaz *et al.,* 2016). When this shape is caused by induced biotransformation in the organism, one can expect that the second accumulation stage will be identical to the first stage (as bioavailability is unaffected) (Atuanya and Nwadibie, 2018).

Uneze *et al*. (2024) reported a reduction in microbial populations in farm soils applied with pesticides. Assessing the bioavailability and ecotoxicological effects of organophosphates and alkyphenol pesticides on nitrifying bacteria and earthworm is important because Xenobiotics pesticides are a major cause for concern all over the world, given their persistence to their metabolite residues after degradation by artificial or natural means and adverse effects on the eco-biota (Atuanya and Nwadibie, 2018).

The aim of this study is to assess the bioavailability and ecotoxicological effects of organophosphates and alkyphenol pesticides on earthworm and nitrifying bacteria, because Xenobiotics pesticides are a major cause for concern all over the world

## Materials and Methods

### Sample Collection

Soil sample (1kg) at 10cm depth was collected with a standard soil auger from uncontaminated leguminous farm (green manure legumes) land in Faculty of Agriculture in University of Benin, Benin City. Organo-phosphate (Dichlorvos) and Alky-phenol (m-cresol) chemical was also purchased from Rovet Scientific (NIG) Limited at Benin City, Edo State Nigeria.

### Preparation of Samples

About 500g of soil was passed through a mesh sieve (2 mm pore size) to remove large particles and was thoroughly mixed. Thereafter, 5g of the soil sample was suspended in 45 ml of sterile phosphate buffer containing 139 mg of $K_2HPO_4$ and 27 mg of $KH_2PO_4$ per litre. The pH was adjusted to 7.0 and the mixture was shaken in a rotary shaker at 100 rpm at room temperature (28±2°C) for 2 hours in order to liberate the organisms into the liquid medium.



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

**Collection of Earthworms**

The earthworms were collected from uncultivated farm land in University of Benin, Ugbowo campus Benin City, Edo State, Nigeria. It lies within Latitude $6.308^0$N and Longitude $5.6186^0$E. The upper layer of the soil was dogged and earthworms found were hand sorted from liters of plant materials.

**Preparation of Media**

**Preparation of Winogradsky broth**

Winogradsky broth medium (phase 1) was prepared with the following composition (g/l) in sterile distilled water: $(NH_4)_2SO_4$, 2.0 g; $K_2HPO_4$, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; NaCl, 2.0 g; $FeSO_4.7H_2O$, 0.4 g; and $CaCO_3$, 0.01 g.

Ten test tubes were each filled with 9 ml of the Winogradsky broth medium, autoclaved at 121°C for 15 minutes at 15 psi and allowed to cool (Atuanya *et al.,* 2016). The test tubes were used to carry out ten - fold serial dilutions of the soil suspension.

The Winogradsky broth medium (phase 1) was used for the isolation of nitrifying bacteria responsible for nitrification phase 1. Another Winogradsky broth medium (phase 11) used for the isolation of nitrifying bacteria responsible for nitrification phase II was also prepared.

The Winogradsky broth medium (phase 11) was prepared with the following composition (g/l): $KNO^2$, 0.1 g; $Na_2CO_3$, 1.0 g; NaCl, 0.5 g; and $FeSO_4.7H_2O$, 0.4 g. Another set of ten test tubes were each filled with 9 ml of the Winogradsky broth medium phase I1 and sterilized at 121°C for 15 minutes at 15 psi. The contents of the test tubes were allowed to cool. The test tubes were used to carry out another ten - fold serial dilutions of the soil sample suspension.

**Preparation of Winogradsky agar media**

Winogradsky agar media for nitrification phases 1 and 11 were prepared by adding 15.0 g agar to 1000 ml of the fresh broth and sterilized at 121 °C for 15 minutes at 15 psi. The media were allowed to cool to about 45 °C before they were dispensed into sterile Petri dishes and allowed to stay overnight in order to test for sterility.

**Isolation and Enumeration of Nitrifying Bacteria**

Winogradsky agar plates were aseptically inoculated with 0.1ml aliquot of the appropriate dilution of ($10^{-3}$) of the soil suspension using spread plate technique. The inoculum was spread over the entire surface of the solid agar with a sterile glass rod. All the inoculated Petri dishes were incubated aerobically at room temperature (28±2 °C) for 3 weeks. The colonies which developed were counted and expressed as colony forming unit (CFU).

**Purification of Isolates**

Different discrete colonies that developed on the Winogradsky agar media for nitrification phases I and II after 3 weeks of incubation was aseptically sub-cultured repeatedly on freshly prepared Winogradsky agar media, phases 1 and II, respectively. All the plates were incubated aerobically at room temperature (28±2 °C) for another 5 days. The pure isolates were transferred to Winogradsky agar slants and stored in the refrigerator till further use (Udochukwu *et al.,* 2018).

**Identification of Isolates**

Pure isolates from the corresponding agar slants was characterized and identified using morphological (cell and colonial morphology, shape, motility, and gram reaction), biochemical and physiological attributes characterized included.

**Physiological Characterization**

**Ammonia oxidation test**

Five millilitres of aqueous solution of ammonium sulphate (0.2% w/v) was added into two different test tubes containing 5 ml of Winogradsky mineral basal medium. The tubes were sterilized by autoclaving at 121°C for 15 minute at 15 psi and then allowed to cool. One loopful of each isolate was added into each tube and incubated aerobically for 5 days at room temperature. At the end of the incubation period, the presence of nitrite was tested using GriessIlosvay reagent. This was done by drop plate method. Four drops of the liquid culture from the test tube were removed with a sterile Pasteur pipette and to it , was added, one drop of the reagent and then observed for the development of purplish red colouration within 5 minutes.



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

**Biochemical Characterization**

The tests for the biochemical characterization included Catalase test, Urease test, Oxidase test, Indole test, Citrate utilization, Methyl red test, and Voges – Proskauer test.

**Sugar fermentation test**

The sugar fermentation test was carried out to determine the ability of the isolates to ferment various sugars which is indicated by the production of acids/gas. The following sugars were used: mannitol, maltose, glucose and lactose. From each sugar, 0.5g were dissolved in 50 ml of peptone water and sterilized by membrane filtration. A pinch of phenol red were added as indicator and 5 ml aliquots were aseptically dispensed into sterile bijou bottles containing sterile Durham tubes which were inverted in the sterile broth. The broth were inoculated with the isolates using sterile wire loop and incubated at 30 $^{o}$C for 48 hours. The content was observed for change in colour and/or the production of gas.

**Preparation and Standardization of Inoculum**

Viable cell counts were carried out by serially diluting the cultures of the isolates and the dilutions plated in sterile Winogradsky agar medium using pour plate method and incubated aerobically at room temperature (28±2 $^{o}$C) for 5 days. The concentrations of viable cells in the original cultures of the isolates (expressed as colony forming unit per ml or CFU/ml) were 38 calculated from the plate counts on the pour plate to produce standard inoculum size of each isolate that were used during the experiment.

**Toxicity Test of Organophosphate and Alkyphenol on Nitrifying Bacteria**

Changes in population of the two nitrifying bacteria isolated from soil samples (*Nitrosomonas* and *Nitrobacter*) were monitored following their exposure to different concentrations of organophosphate and alkyphenol pesticides for 96 hours.

The effects of organophosphate and alkyphenol pesticides on the two nitrifying bacteria were performed using mineral salts medium of the following composition (g/l): $(NH_4)_2SO_4$, 1.0 g; $KH_2PO_4$, 1.0 g; $K_2HPO_4$, 1.0 g; $MgSO_4$,0.2 g; $CaCl_2$, 0.02 g and $FeCl_3.6H_2O$, 0.004 g.

Thereafter, 100 ml , 99.5 ml, 99 ml, 98 ml, 95 ml and 90 ml of the mineral basal medium (MBM) were dispersed into each of 250 ml capacity Erlenmeyer flasks, respectively, and sterilized at 121 $^{o}$C at 15 psi for 15 minutes. Seven different concentrations of organophosphate (dichlorvos) and alkyphenol (m-cresol) (100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l, 500 mg/l, 1000 mg/l, and 2000 mg/l) were, respectively, used. A set up without the pesticide (toxicant) served as the control (Atuanya *et al*., 2016). About 2 ml of the inoculum were carefully transferred into each of the flasks containing the mineral salts medium. They were shaken thoroughly to mix and were all properly labeled and incubated aerobically at room temperature for 96 hours. All the flasks containing sterile MBM supplemented with different concentrations of organophosphate and alkyphenol and 2 ml of the inoculum were agitated at 180 rpm at room temperature for 96 hours on a rotary shaker. The growths were measured by withdrawing samples from the medium every 24 hours and the turbidity measured at 600 nm using spectrophotometer. Thus, during incubation, representative samples from all the flasks were withdrawn at intervals of 24 h, 48 h, 72 h and 96 h exposure periods and the absorbance of the turbidity measured at 600 nm using spectrophotometer (Atuanya *et al*., 2016).

**Toxicity Test of Organophosphate and Alkyphenol pesticides on Earthworm**

Earthworms were collected by digging and hand sorting from uncontaminated soil and taken to the laboratory for experimentation. They were first washed free of adhering particles using distilled water and selected for experiment based on their maturity (presence of clitellum). Seven concentrations of organophosphate and alkyphenol; 100mg/l, 200mg/l, 300mg/l, 400mg/l, 500mg/l, 1000mg/l and 2000mg/l were prepared previously in glass beaker then 10 selected earthworms were introduced and were observed for a period of 120 hrs (24, 48, 72, 96 and 120). Dead earthworms were removed and the ones alive were recorded (Bonnard *et al*., 2009).

**Bacteria genomic DNA Extraction protocol**

Single colonies grown on medium was transferred to 1.5 ml of liquid medium and cultures was grown on a shaker for 48 h at 28$^{o}$C. After this period, cultures were centrifuged at 4600xg for 5 min.



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

The resulting pellets were resuspended in 520µl of TE buffer (10mM Tris-HCL, 1mM EDTA, pH 8.0). Fifteen micro liters of 20% SDS and 3µl of Proteinase K (20mg/ml) was added. The mixture was incubated for 1 h at 37°C, then 100µl of 5M Nacl and 80µl of a 10% CTAB solution in 0.7M Nacl was added and mixed. The suspension was incubated for 10 min. at 65°C and kept on ice for 15 min. an equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min. and centrifuged at 7200xg for 20 min. the aqueous phase was transferred into a new tube, isopropanol (1:0:6) was added and DNA will be precipitated at -20°C for 16 h. DNA was collected by centrifugation at 7200xg for 10 min, and was washed with 500µl of 70% ethanol, air dried at room temperature for approximately 3 h and finally dissolved in 50µl of TE buffer (Brosius *et al*., 1981).

**Polymerase Chain Reaction procedure**

The PCR consist of final volume of 50µl which included 8µl DNA and 42µl reaction cocktail consisting of 5x GoTaq green reaction, 10 Mm of each dNTPs, 10 pmol each 27F:5′-AGAGTTTGATCM TGGCTCAG-3′ and 1525 R: 5′-AAGGAGGTGWT CCARCC-3′ specific for 800bp conserved domain of the 16S rRNA polymerase. PCR was carried out using the following thermal cycles regime; an initial denaturation at 94°C for 1 min, this was followed by 29 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 1 min and an extension at 72°C for 1.5 min, a final extension at 72°C for 5 min ended the PCR experiment (Brosius *et al*., 1981).

**Agarose Gel Electrophoresis**

Agarose powder of 1.5g was weighed. 1.5ml of 0.5x TAE buffer was added and dissolved by boiling using microwave oven. The mixture was allowed to cool to about 60$^0$C. Ten (10ml) of ethidium bromide was added and mixed by swirling gently and then poured into electrophoresis tank with the comb in place to obtain a gel thickness of about 4-5mm while trying as much as possible to avoid bubbles. One (1x) TAE buffer was poured into the tank with the buffer covering the gel. The comb was then removed. Ten (10µl) of sample was mixed with 1µl of the 10x loading dye. The samples were carefully loaded into the wells created by the combs.

The electrodes were connected to the power pack in such a way that the negative terminal was at the end where the sample has been loaded about three-quarter of the electrodes. Electrodes are turned off and disconnected after the process and the gel is observed on UV-trans illuminator (Opere and Ojo, 2013).

**Sequencing of the 16S rDNA gene**

The purified DNA samples was sequenced at the Bioscience Laboratory International institute for tropical Agriculture (I.I.T.A), Ibadan, and Oyo State with an automated DNA sequencing analyzer (ABI 3730 x) using 27F and 1492R primers. Sequence assembly and alignment was carried out using CLC bio software, followed by searching the homology in the Gene Bank DNA data base using Basic Local Alignment Search Tool (BLAST) program of CLC bio software (Marino *et al*., 1992).

**Statistical Analysis**

Analysis of variance (ANOVA) and Dunnet's method were employed for data evaluation; $p<0.05$ was taken as statistically significant. The software package, Graph pad prism 5 was used for data analysis.

## Results

Table 1 shows the cultural, morphological and biochemical characteristics of the Nitrifying bacteria isolated from the green legume farm soil samples. The cultural characteristics used to identify the bacterial isolate were shape, elevation, margin, size, wetness/dryness, transparency and colour.

The morphological characteristics used were gram staining, cell type, cell arrangement and motility. While the biochemical characteristics include catalase, oxidase, coagulase, indole, urease, citrate, nitrate reduction, ammonium reduction, sugar fermentation: glucose and lactose. The probable bacterial isolates were *Nitrosomonas* sp. and *Nitrobacter* sp.

Plate 1 shows the gel electrophoresis result of the PCR detection of Nitrifying bacteria species. Lane M is the Molecular marker, Lane 1 is the *Nitrobacter winogradskyi's*. (700 bp), and Lane 2 is *Nitrosomonas europae* (650 bp).



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

**Table 1: Cultural, morphological and biochemical characteristics of the Nitrifying bacteria isolated from the green legume farm soil samples**

| Cultural characteristics | A | B |
|---|---|---|
| Shape | Round | Round |
| Elevation | Raised | Raised |
| Margin | Entire | Entire |
| Size | Small | Small |
| Wetness/dryness | Wet | Wet |
| Transparency | Opaque | Opaque |
| Colour | Brownish | Brownish |
| **Morphological** | | |
| Gram Staining | Negative | Negative |
| Cell type | Rod | Rod |
| Cell Arrangement | Single | Single |
| Motility | - | - |
| **Biochemical** | | |
| Catalase | + | + |
| Oxidase | - | - |
| Coagulase | - | - |
| Indole | - | - |
| Urease | - | - |
| Citrate | - | + |
| Nitrate reduction | + | - |
| Ammonium reduction | - | + |
| **Sugar fermentation** | | |
| Glucose | + | + |
| Lactose | - | - |
| **Possible Organism** | *Nitrobacter* sp. | *Nitrosomonas* sp. |

**Key:** + positive, - negative

Figure 1shows the Phylogenetic association between *sequences* of *Nitrosomonas europae* strain AL954747.1 with other *Nitrosomonas* strains ranged between 53% to 81% respectively.

Figure 2 presents the Phylogenetic association between *sequences* of *Nitrobacter winogradskyi* strain NR_074324.1 with other *Nitrobacter* strains ranged between 47% to 80% respectively.

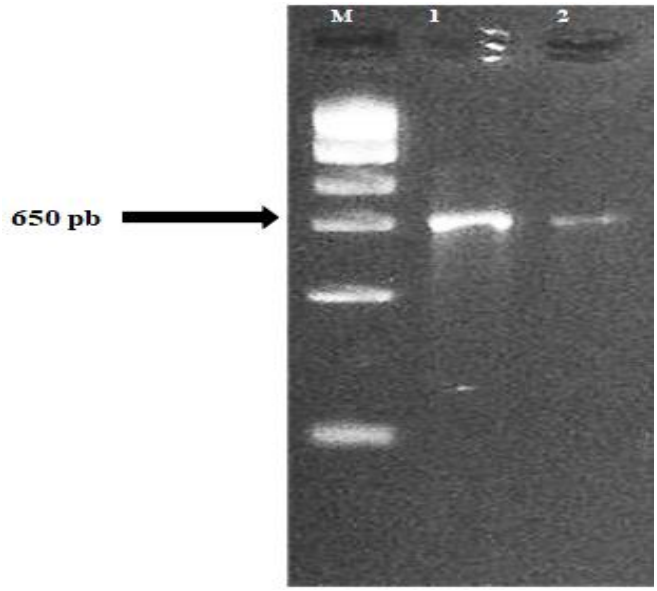


**Plate 1: PCR detection of Nitrifying bacteria species using gel electrophoresis**

Lane M: Molecular marker, Lane: *Nitrobacter winogradskyi's*. (700 bp), Lane 2: *Nitrosomonas europae* (650 bp)

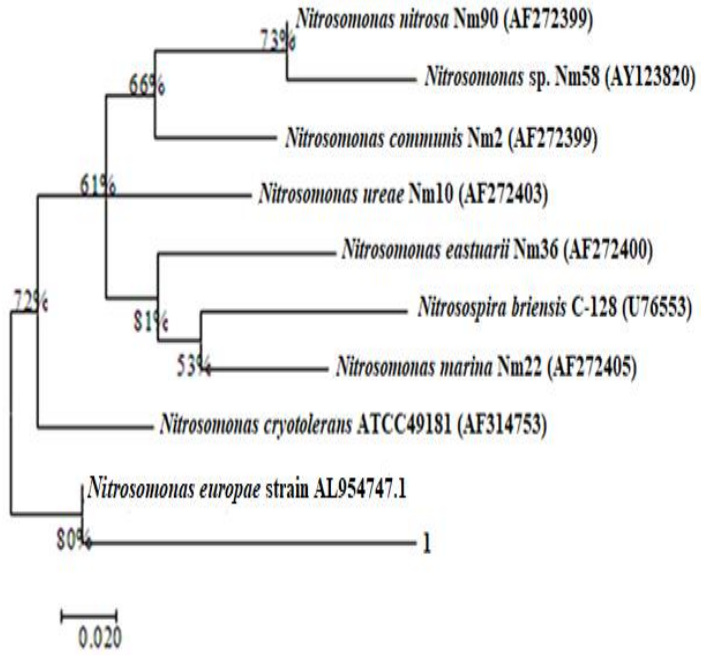


**Figure 1: Phylogenetic association between *sequences* of *Nitrosomonas europae* strain AL954747.1 with other *Nitrosomonas* strains ranged between 53% to 81% respectively.**



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

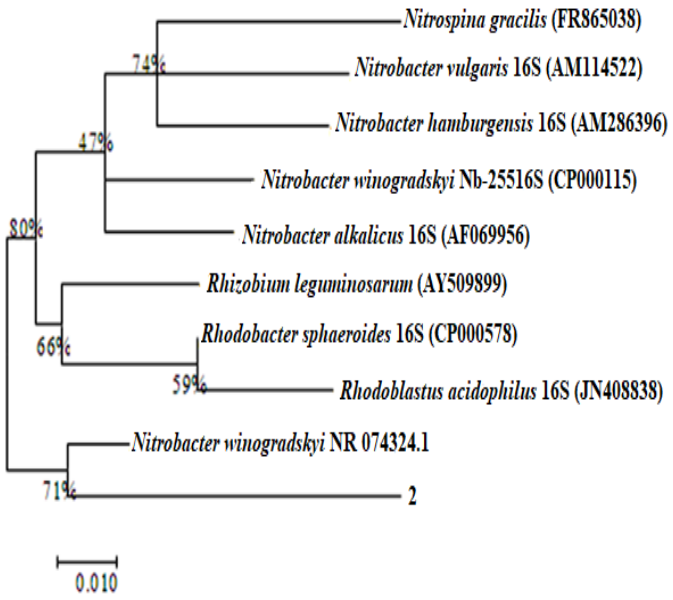


**Figure 2: Phylogenetic association between *sequences* of *Nitrobacter winogradskyi* strain NR_074324.1 with other *Nitrobacter* strains ranged between 47% to 80% respectively.**

Table 2 presents the 16SrRNA analysis of Nitrifying bacteria, using PCR methods confirmed the identity of the Nitrifying bacteria isolates to be *Nitrosomonas europae* and *Nitrobacter winogradskyi*, with a percentage identity of 98% and 99% respectively. The PCR detection of Nitrifying bacteria showed band size of 650bp when compaired to a molecular ladder. The size of the amplicon was about 1500 bp, and the DNA ladder used is a 1kbp ladder from NEB.

Table 3 and Table 4 show the growth response of *Nitrosomonas* sp. and *Nitrobacter* sp. to various concentrations of organo-phosphorus pesticides respectively. At 24 hr the growth response of *Nitrosomonas* sp. at 100 mg/l concentrations was 75.67±6.13, while *Nitrobacter* sp. was 78.37±6.26. Also at 96 hrs the growth response further decreased to 40.32±3.20 for *Nitrosomonas* sp. and 43.34±3.25 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 100 mg/l concentrations organo-phosphorus pesticides have significant effect on the growth of *Nitrosomonas* sp and *Nitrobacter* sp population after 96 hrs exposure.

**Table 2: Molecular identification of *Nitrosomonas* and *Nitrobacter* isolates**

| Bacterial identity | Max. Score | Query cover (%) | Percent identity | Accession No. |
|---|---|---|---|---|
| *Nitrosomonas europae* | 12538 | 100 | 98.00 | AL 954747.1 |
| *Nitrobacter winogradskyi* | 2752 | 1000 | 99.00 | NR_074324.1 |

**Table 3: Growth response of *Nitrosomonas europaea* strain AL954747.1. in organo-phosphorus pesticides**

| Organo-phosphorus Pesticides Concentrations (mg/l) | Growth response of *Nitrosomonas* sp (CFU/ml x$10^3$) to various concentrations of Organo-phosphorus Pesticides with Time (hr) | | | | |
|---|---|---|---|---|---|
| | Control | 24 | 48 | 72 | 96 |
| **100** | 100.54±7.34 | 75.67±6.13 | 62.34±5.39 | 53.10±4.51 | 40.32±3.20 |
| **200** | 100.54±7.34 | 63.93±5.42 | 54.51±4.44 | 41.28±3.36 | 30.33±2.10 |
| **300** | 100.54±7.34 | 50.63±4.56 | 44.42±3.53 | 31.19±2.59 | 21.25±2.10 |
| **400** | 100.54±7.34 | 41.15±3.37 | 32.23±2.72 | 19.40±1.96 | 09.51±1.83 |
| **500** | 100.54±7.34 | 30.38±2.64 | 21.03±2.32 | 10.53±1.41 | 5.62±1.22 |
| **1000** | 100.54±7.34 | 15.48±1.33 | 7.39±1.15 | 3.47±0.82 | 1.81±0.41 |
| **2000** | 100.54±7.34 | 6.98±1.26 | 3.48±0.75 | 1.25±0.17 | 0.00±0.00 |

Values are expressed as mean±SEM, n=3.



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

**Table 4: Growth response of *Nitrobacter winogradskyi* strain NR 074324.1 in organo-phosphorus pesticides**

| Organo-phosphorus Pesticides Concentrations (mg/l) | Growth response of *Nitrobacter* sp (CFU/ml x$10^3$) to various concentrations of Organo-phosphorus Pesticides with Time (hr) | | | | |
|---|---|---|---|---|---|
| | Control | 24 | 48 | 72 | 96 |
| **100** | 105.29±7.68 | 78.37±6.26 | 66.45±5.27 | 57.28±4.43 | 43.34±3.25 |
| **200** | 105.29±7.68 | 67.36±5.36 | 56.63±4.45 | 45.42±3.33 | 38.30±2.12 |
| **300** | 105.29±7.68 | 51.37±4.48 | 51.37±4.48 | 46.46±3.62 | 35.38±2.35 |
| **400** | 105.29±7.68 | 40.84±3.50 | 31.18±2.41 | 22.21±2.19 | 11.36±1.61 |
| **500** | 105.29±7.68 | 31.19±2.59 | 20.52±2.11 | 11.33±1.39 | 6.27±1.18 |
| **1000** | 105.29±7.68 | 16.72±1.36 | 8.35±1.28 | 4.11±0.56 | 2.39±0.54 |
| **2000** | 105.29±7.68 | 7.58±1.38 | 4.00±0.51 | 2.10±0.24 | 1.15±0.31 |

Values are expressed as mean±SEM, n=3.

At 24 hr the growth response of *Nitrosomonas* sp. at 200 mg/l concentrations was 63.93±5.42, while *Nitrobacter* sp. was 67.36±5.36. Also at 96 hrs the growth response further decreased to 30.33±2.10 for *Nitrosomonas* sp. and 38.30±2.12 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 200 mg/l concentrations organo-phosphorus pesticides have significant effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure. At 24 hr the growth response of *Nitrosomonas* sp. at 300 mg/l concentrations was 50.63±4.56, while *Nitrobacter* sp. was 51.37±4.48. Also at 96 hrs the growth response further decreased to 21.25±2.10 for *Nitrosomonas* sp. and 24.11±2.19 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 300 mg/l concentrations organo-phosphorus pesticides have significant effect on the growth of *Nitrosomonas* sp and *Nitrobacter* sp population after 96 hrs exposure.

At 24 hr the growth response of *Nitrosomonas* sp. at 400 mg/l concentrations was 41.15±3.37, while *Nitrobacter* sp. was 40.84±3.50. Also at 96 hrs the growth response further decreased to 09.51±1.83 for *Nitrosomonas* sp. and 11.36±1.61 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 400 mg/l concentrations organo-phosphorus pesticides have significant effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

At 24 hr the growth response of *Nitrosomonas* sp. at 500 mg/l concentrations was 30.38±2.64, while *Nitrobacter* sp. was 31.19±2.59. Also at 96 hrs the growth response further decreased to 5.62±1.22 for *Nitrosomonas* sp. and 6.27±1.18 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 500 mg/l concentrations organo-phosphorus pesticides have significant effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

At 24 hr the growth response of *Nitrosomonas* sp. at 1000 mg/l concentrations was 15.48±1.33, while *Nitrobacter* sp. was 16.72±1.36. Also at 96 hrs the growth response further decreased to 1.81±0.41 for *Nitrosomonas* sp. and 2.39±0.54 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 1000 mg/l concentrations organo-phosphorus pesticides have drastic effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

At 24 hr the growth response of *Nitrosomonas* sp. at 2000 mg/l concentrations was 6.98±1.26, while *Nitrobacter* sp. was 7.58±1.38. Also at 96 hrs the growth response further decreased to 0.00±0.00 for *Nitrosomonas* sp. and 1.15±0.31 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 2000 mg/l concentrations organo-phosphorus pesticides have drastic effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

Table 5 and Table 6 show the growth response of *Nitrosomonas* sp. and *Nitrobacter* sp. to various concentrations of alkyl-phenol pesticides respectively.



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

**Table 5: Growth response of *Nitrosomonas europaea* strain AL954747.1. in alkyl-phenol pesticides**

| Alkyl-phenol Pesticides Concentrations (mg/l) | Growth response of *Nitrosomonas* sp (CFU/ml x$10^3$) to various concentrations of Alkyl-phenol Pesticides with Time (hr) | | | | |
|---|---|---|---|---|---|
| | Control | 24 | 48 | 72 | 96 |
| **100** | 100.54±7.34 | 94.31±6.45 | 83.63±5.48 | 72.81±4.93 | 63.48±3.40 |
| **200** | 100.54±7.34 | 84.45±5.43 | 75.10±4.81 | 63.34±3.51 | 51.48±2.73 |
| **300** | 100.54±7.34 | 75.64±4.60 | 64.95±3.25 | 53.19±2.48 | 42.40±2.36 |
| **400** | 100.54±7.34 | 60.57±3.34 | 49.85±2.56 | 38.59±2.10 | 29.73±1.80 |
| **500** | 100.54±7.34 | 50.21±2.43 | 39.43±2.68 | 28.67±1.75 | 19.49±1.47 |
| **1000** | 100.54±7.34 | 19.58±1.61 | 9.15±0.85 | 4.72±0.44 | 2.63±0.19 |
| **2000** | 100.54±7.34 | 4.13±0.32 | 1.36±0.15 | 0.00±0.00 | 0.00±0.00 |

Values are expressed as mean±SEM, n=3.

**Table 6: Growth response of *Nitrobacter winogradskyi* strain NR 074324.1in alkyl-phenol pesticides**

| Alkyl-phenol Pesticides Concentrations (mg/l) | Growth response of *Nitrobacter* sp (CFU/ml x$10^3$) to various concentrations of Alkyl-phenol Pesticides with Time (hr) | | | | |
|---|---|---|---|---|---|
| | Control | 24 | 48 | 72 | 96 |
| **100** | 105.29±7.68 | 92.56±6.61 | 81.47±5.59 | 70.21±4.65 | 62.59±3.50 |
| **200** | 105.29±7.68 | 82.34±5.62 | 70.75±4.76 | 61.63±3.44 | 50.55±2.67 |
| **300** | 105.29±7.68 | 72.40±4.53 | 61.73±3.38 | 50.50±2.41 | 40.68±2.33 |
| **400** | 105.29±7.68 | 60.20±3.30 | 49.62±2.66 | 37.65±2.25 | 26.56±1.63 |
| **500** | 105.29±7.68 | 49.60±2.41 | 38.62±2.54 | 27.53±1.69 | 18.19±1.40 |
| **1000** | 105.29±7.68 | 17.84±1.48 | 7.61±0.61 | 3.96±0.50 | 1.82±0.11 |
| **2000** | 105.29±7.68 | 3.39±0.22 | 1.10±0.05 | 0.00±0.00 | 0.00±0.00 |

Values are expressed as mean±SEM, n=3.

At 24 hr the growth response of *Nitrosomonas* sp. at 100 mg/l concentrations was 94.31±6.45, while *Nitrobacter* sp. was 92.56±6.61. Also at 96 hrs the growth response further decreased to 63.48±3.40 for *Nitrosomonas* sp. and 62.59±3.50 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 100 mg/l concentrations alkyl-phenol pesticides have significant effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

At 24 hr the growth response of *Nitrosomonas* sp. at 200 mg/l concentrations was 84.45±5.43, while *Nitrobacter* sp. was 82.34±5.62. Also at 96 hrs the growth response further decreased to 51.48±2.73 for *Nitrosomonas* sp. and 50.55±2.67 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 200 mg/l concentrations alkyl-phenol pesticides have significant effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

At 24 hr the growth response of *Nitrosomonas* sp. at 300 mg/l concentrations was 75.64±4.60, while *Nitrobacter* sp. was 72.40±4.53. Also at 96 hrs the growth response further decreased to 42.40±2.36 for *Nitrosomonas* sp. and 40.68±2.33 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 300 mg/l concentrations alkyl-phenol pesticides have significant effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

At 24 hr the growth response of *Nitrosomonas* sp. at 400 mg/l concentrations was 60.57±3.34, while *Nitrobacter* sp. was 60.20±3.30. Also at 96 hrs the growth response further decreased to 29.73±1.80 for *Nitrosomonas* sp. and 26.56±1.63 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 400 mg/l concentrations alkyl-phenol pesticides have significant effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

At 24 hr the growth response of *Nitrosomonas* sp. at 500 mg/l concentrations was 50.21±2.43, while *Nitrobacter* sp. was 49.60±2.41. Also at 96 hrs the growth response further decreased to 19.49±1.47 for *Nitrosomonas* sp. and 18.19±1.40 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 500 mg/l concentrations alkyl-phenol pesticides have significant effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

At 24 hr the growth response of *Nitrosomonas* sp. at 1000 mg/l concentrations was 19.58±1.61, while *Nitrobacter* sp. was 17.84±1.48. Also at 96 hrs the growth response further decreased to 2.63±0.19 for *Nitrosomonas* sp. and 1.82±0.11 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 1000 mg/l concentrations alkyl-phenol pesticides have drastic effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

At 24 hr the growth response of *Nitrosomonas* sp. at 2000 mg/l concentrations was 4.13±0.32, while *Nitrobacter* sp. was 3.39±0.22.

Also at 96 hrs the growth response further decreased to 0.00±0.00 for *Nitrosomonas* sp. and 0.00±0.00 for *Nitrobacter* sp. Compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. This result further reveals that 2000 mg/l concentrations alkyl-phenol pesticides have drastic effect on the growth of *Nitrosomonas* sp. and *Nitrobacter* sp. population after 96 hrs exposure.

Table 5 shows the toxicity effect of alkyl-phenol pesticides on earthworm at different concentrations level. At 100 mg/l concentration the number of earthworm reduced from 10 (24hr) to 6 (120hrs). At 200 mg/l concentration the number of earthworm reduced from 9 (24hr) to 5 (120hrs). At 300 mg/l concentration the number of earthworm reduced from 8 (24hr) to 4 (120hrs). At 400 mg/l concentration the number of earthworm reduced from 7 (24hr) to 3 (120hrs). At 500 mg/l concentration the number of earthworm reduced from 6 (24hr) to 2 (120hrs). At 1000 mg/l concentration the number of earthworm reduced from 4 (24hr) to 0 (120hrs). While at 2000 mg/l concentration the number of earthworms reduced from 2 (24hr) to 0 (72hrs). Compared to the control which was 10 from 24 hr to 120 hrs. The result further reveals that effect of alkyl-phenol pesticides on earthworm was dose dependent.

**Table 5: Toxicity effect of alkyl-phenol pesticides on earthworm at different concentrations level**

| Time (hr) | Alkyl-phenol Pesticides Concentrations (mg/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 100 | 200 | 300 | 400 | 500 | 1000 | 2000 |
| 24 | 10 | 10 | 9 | 8 | 7 | 6 | 4 | 2 |
| 48 | 10 | 9 | 8 | 7 | 6 | 5 | 3 | 1 |
| 72 | 10 | 8 | 7 | 6 | 5 | 4 | 2 | 0 |
| 96 | 10 | 7 | 6 | 5 | 4 | 3 | 1 | 0 |
| 120 | 10 | 6 | 5 | 4 | 3 | 2 | 0 | 0 |

Table 6 shows the toxicity effect of organo-phosphorus pesticides on earthworm at different concentrations level. At 100 mg/l concentration the number of earthworm reduced from 10 (24hr) to 5 (120hrs). At 200 mg/l concentration the number of earthworm reduced from 9 (24hr) to 4 (120hrs).

At 300 mg/l concentration the number of earthworm reduced from 8 (24hr) to 3 (120hrs). At 400 mg/l concentration the number of earthworm reduced from 7 (24hr) to 2 (120hrs).

At 500 mg/l concentration the number of earthworm reduced from 6 (24hr) to 1 (120hrs). At 1000 mg/l concentration the number of earthworm reduced from 4 (24hr) to 0 (96hrs).

On the other hand, at 2000 mg/l concentration, the number of earthworms reduced from 2 (24hr) to 0 (72hrs) when compared to the control which was 10 from 24 hr to 120 hrs. The result further reveals that effect of alkyl-phenol pesticides on earthworm was dose dependent.



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

**Table 6: Toxicity effect of organo-phosphorus pesticides on earthworm at different concentrations level**

| | Organo-phosphorus Pesticides Concentrations (mg/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Time (hr)** | **Control** | **100** | **200** | **300** | **400** | **500** | **1000** | **2000** |
| 24 | 10 | 10 | 9 | 8 | 7 | 6 | 4 | 2 |
| 48 | 10 | 9 | 8 | 7 | 6 | 5 | 3 | 1 |
| 72 | 10 | 8 | 7 | 6 | 5 | 4 | 2 | 0 |
| 96 | 10 | 7 | 6 | 5 | 4 | 3 | 0 | 0 |
| 120 | 10 | 5 | 4 | 3 | 2 | 1 | 0 | 0 |

## Discussion

This study was carried out to assess the bioavailability and ecotoxicological effects of organophosphates and alkyphenol pesticides on earthworm and nitrifying bacteria, because Xenobiotics pesticides are a major cause for concern all over the world, given their persistence to their metabolite residues after degradation by artificial or natural means and adverse effects on the eco-biota (Atuanya and Nwadibie, 2018). Uneze *et al*. (2024) reported a reduction in microbial populations in farm soils applied with pesticides.

In this present study, the probable bacterial isolates from the green legume farm soil samples, cultured on Winogradsky medium and identified based on cultural, morphological, and biochemical characteristics were *Nitrosomonas* sp. and *Nitrobacter* sp. Molecular identification and PCR detection confirmed the nitrifying bacteria as *Nitrosomonas europaea* strain AL 954747.1 and *Nitrobacter winogradskyi* strain NR 074324.1, with percentage identities of 98.0 and 99.0, respectively.

The results of this present study shows that at 100 mg/l concentrations of organo-phosphorus pesticides, the growth response of *Nitrosomonas* sp. ranged from 75.67±6.13 x $10^3$CFU/ml to 40.32±3.20 x $10^3$CFU/ml, while *Nitrobacter* sp. ranged from 78.37±6.26 x $10^3$CFU/ml to 43.34±3.25 x $10^3$CFU/ml within 96hrs exposure. Also, at 500 mg/l concentrations of organo-phosphorus pesticides, the growth response of *Nitrosomonas* sp. ranged from 30.38±2.64 x $10^3$CFU/ml to 5.62±1.22 x $10^3$CFU/ml, while *Nitrobacter* sp. ranged from 31.19±2.59 x $10^3$CFU/ml to 6.27±1.18 x $10^3$CFU/ml within 96hrs exposure.

While at 2000 mg/l concentrations of organo-phosphorus pesticides, the growth response of *Nitrosomonas* sp. ranged from 6.98±1.26 x $10^3$ to 0.00±0.00 x $10^3$CFU/ml, and *Nitrobacter* sp. ranged from 7.58±1.38 x $10^3$CFU/ml to 1.15±0.31 x $10^3$CFU/ml within 96 hrs exposures when compared to the control which was 100.54±7.34 x $10^3$CFU/ml for *Nitrosomonas* sp. and 105.29±7.68 x $10^3$CFU/ml for *Nitrobacter* sp. This is in line with the study of Atuanya *et al.* (2018) who stated that *Nitrosomonas* sp. count was 13.02 - 1.78 x$10^4$ CFU/ml, while *Nitrobacter* sp. count was 15.17 - 2.43 x$10^4$ CFU/ml at the first to fourth exposure to Aldrin and Lindane pesticides. According to Daims *et al.* (2015) the toxicity level of a pesticide depends on the deadlines of the chemical, the dose, the length of exposure, and the route of entry or absorption by the body. Pesticide degradation in soil genetically results in a reduction in toxicity, however some pesticides have breakdown products (metabolites) that are more toxic than the parent compound (Rizzati *et al.,* 2016). According to Singh *et al.* (2015) organo-phosphorus pesticides use with in North and South America has increased dramatically with use of transgenic herbicide-resistant crops, thus effects on soil ecological processes are of concern. Utami *et al.* (2020) observed toxic effect of organo-phosphorus on soil microbial biomass 3 days after application. However, Wang *et al.* (2016) demonstrated that field application rates of the organo-phosphorus decreased microbial biomass carbon by 17% and microbial biomass nitrogen by 76% in nine soils at 14 days after treatment. Xu *et al.* (2020) showed that organo-phosphorus decreased microbial biomass carbon by 16%, 56 days after field application. However, under laboratory conditions, doses 10 and 100x field application rates elicited reduced soil biomass (Atuanya and Tudararo-Aherobo, 2014). Soil microbial biomass measurements has been reported to give an early indication of long-term changes in soil organic matter content, long before such changes could be measured by conventional techniques (Belden and Brain, 2017).



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

Microorganisms form a vital part of the soil food web, therefore microbial biomass is considered to be a measure of potential microbiological and ecosystem functioning (Castillo-Diaz *et al.,* 2016). However for proper understanding of ecosystem functioning and determining soil disturbances because of various agricultural management practices, microbial activities must also be determined along with microbial activity in soil has been reported as a criteria for evaluating pesticide toxicity (Zhou *et al.,* 2020).

The results obtained from this study also showed that at 100 mg/l concentrations of alkyl-phenol pesticides, the growth response of *Nitrosomonas* sp. ranged from 94.31±6.45 to 63.48±3.40, while *Nitrobacter* sp. ranged from 92.56±6.61 to 62.59±3.50 within 96hrs exposure. Also, at 500 mg/l concentrations of alkyl-phenol pesticides, the growth response of *Nitrosomonas* sp. ranged from 50.21±2.43 to 19.49±1.47, while *Nitrobacter* sp. ranged from 49.60±2.41 to 18.19±1.40 within 96hrs exposure. While at 2000 mg/l concentrations of alkyl-phenol pesticides, the growth response of *Nitrosomonas* sp. ranged from 4.13±0.32 to 0.00±0.00, while *Nitrobacter* sp. ranged from 3.39±0.22 to 0.00±0.00 within 72 hrs exposure as compared to the control which was 100.54±7.34 for *Nitrosomonas* sp. and 105.29±7.68 for *Nitrobacter* sp. The study of Fang *et al.* (2019) reported that both organo-phosphorus and alkyl-phenol pesticides significantly decreased the numbers of nitrogen fixating bacteria, although total bacterial biomass was little affected. The study indicated that organo-phosphorus and alkyl-phenol pesticides application may negatively affect non-target soil microorganisms and their activities. Hasenbein *et al.* (2015) reported that in alkyl-phenol pesticides treated soil, a drastic decrease in microbial biomass was observed as compared to the control. However, MBC content increased with time in alkyl-phenol treated soil. The results of toxicity studies showed that the toxicity of the organo-phosphorus and alkyl-phenol formulations on *Nitrobacter* sp. and *Nitrosomonas* sp. depended on the contact time and pesticide concentrations (John and Shaike, 2015). The count for *Nitrobacter* sp. and *Nitrosomonas* sp. decreased with increase in exposure time for all the test pesticides at different concentrations. The decrease in value with time may be attributed to increased water solubility with time or interference with microbial enzyme activity by the two pesticides.

Li *et al.* (2020) observed that alkyl-phenol at 1000 mg/l and 2000 mg/l inhibited the enzyme responsible for the metabolism of glucose in acetic acid bacteria. Generally, the order of growth of both nitrifying bacteria in various concentrations of both pesticides was Control > 100mg/l) > 200mg/l > 300mg/l > 400mg/l > 500mg/l > 1000mg/l > 2000mg/l.

The toxicity effect of organo-phosphorus pesticides on earthworm at different concentrations level revealed that at 100 mg/l concentration the number of earthworm reduced from 10 (24hr) to 5 (120hrs). At 500 mg/l concentration the number of earthworm reduced from 6 (24hr) to 1 (120hrs). While at 2000 mg/l concentration the number of earthworm reduced from 2 (24hr) to 0 (72hrs). Compared to the control which was 10 from 24 hr to 120 hrs. Also alkyl phenol pesticides toxicity effect on earthworm at different concentrations level revealed that at 100 mg/l concentration the number of earthworm reduced from 10 (24hr) to 5 (120hrs). At 500 mg/l concentration the number of earthworm reduced from 6 (24hr) to 1 (120hrs). While at 2000 mg/l concentration the number of earthworm reduced from 2 (24hr) to 0 (72hrs). Compared to the control which was 10 from 24 hr to 120 hrs. Generally, the order of growth of earthworms in various concentrations of both pesticides was Control > 100mg/l > 500mg/l > 2000mg/l. According to Alves *et al.* (2013) soil fauna (earthworms, nematodes, microarthropods, protozoans) are important in organic matter (OM) transformations and soil structure formation, and are useful bio-indicators to study Xenobiotic ecotoxicity in soil. The study of Brami *et al.* (2017) showed that organo-phosphorus had no significant effect on herbivores, but significantly reduced certain fungal feeders (Tylenchidae) by 13%, predatory nematodes (Dorylamidae) by 33% and earthworms (Annelida) by 55%. Soil nematodes, earthworms and protozoa were also affected by field application rates of the test pesticides: Aldrin and Lindane (Atuanya and Nwadibie, 2018). Earthworms were also affected in general by application of pesticides (Uwizeyimana *et al.,* 2017).Wang *et al.* (2015) however reported no effect on earthworms in top 10 cm soil layer by low concentrations of pesticides. Stanley *et al.* (2015) found out that earthworms were extremely sensitive to organophosphate and carbamates and less sensitive to organochlorines. Copper oxychloride as fungicide is very toxic for earthworms (Olawoyin *et al.,* 2012).



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

## Conclusion and Recommendation

The findings from this study has shown that, pesticides in the soil affect the non-target and beneficial microorganisms and their activities which are essential for maintaining soil fertility. The result of this study suggest that the activities of nitrifying bacteria and earthworm which enhances soil fertility may be hindered in an ecosystem polluted with these pesticide formulations, as nitrification processes will be reduced or totally stopped. Therefore, it is recommended that pesticides should be applied according to manufacturer prescription and not misapplied.

## References


Alves, P.R.L., Cardoso, E.J.B.N., Martines, A.M., Sousa, J.P. & Pasini, A. (2013). Earthworm ecotoxicological assessments of pesticides used to treat seeds under tropical conditions. *Chemosphere*, 90, 2674– 2682.

Atuanya, E. I. & Nwadibie, D. (2018). Effects of Dumpsite Leachates on Soil Biological Sentinels in Benin City, Nigeria. *Journal of Applied Science and Environmental Management*, 22(11), 1757–1760.

Atuanya, E. I., Dave-Omoregie, A. O., Udochukwu, U. & Inetianbor, J. (2016). Toxicological effects of plastic composted soil on nitrifying bacteria. *British Biotechnology Journal,* 13(4),1-7.

Atuanya, E.I. & Tudararo-Aherobo, L (2014). Ecotoxicological Effects of Discharge of Nigerian Petroleum Refinery Oily Sludge on the Biological sentinels. Afr. *Journal of Environmental Science and Technology* 9(2), 95-103.

Bandeira, F.O., Alves, P.R.L., Hennig, T.B., Schiehl, A., Cardoso, E.J.B.N. & Baretta, D. (2020). Toxicity of imidacloprid to the earthworm Eisenia andrei and collembolan Folsomia candida in three contrasting tropical soils. *Journal of Soils Sediments,* 20, 1997–2007.

Belden, J.B. & Brain, R.A. (2017). Incorporating the joint toxicity of co-applied pesticides into the ecological risk assessment process. *Integrated Environmental Assessment and Management,* 27, 273-294.

Bishop, C.A., Woundneh, M.B., Maisonneuve, F., Common, J., Elliott, J.E. & Moran, A.J. (2020). Determination of neonicotinoids and butenolide residues in avian and insect pollinators and their ambient environment in Western Canada. *Science of the Total Environment,* 773, 197-203.

Bonnard, M., Eon, I.C., Morel, J.L.& Vasseur, P. (2009). Genotoxic and reproductive effect of an industrially contaminated soil on the earthworm *Eisenia fetida*. Environmental and Molecular Mutagenesis, 50 (1), 60-67.

Brami, C., Glover, AR, Butt, K.R, & Lowe C.N (2017) Avoidance, biomass and survival response of soil dwelling (endogeic) earthworms to OECD arti¦cial soil: potential implications for earthworm ecotoxicology. *Science,* 284, 483-501.

Brosuis, J., Dull, T.L., Sleeter, D.D., & Noller, H.F. (1981) . Gene Organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *Journal of Molecular Biology,* 148, 107-127

Caranto, J. D. & Lancaster, K. M. (2017). Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. *Proceedings of the National Academy of Sciences,* 114(31), 8217–8222.

Castillo-Diaz, J.M., Martin-Laurent, F., Beguet, J., Nogales, R. & Romero, E. (2016). Fate and effect of imidacloprid on vermicompost-amended soils under dissimilar conditions: Risk for soil functions, structure, and bacterial abundance. *Science of the Total Environment*, 579, 1111–1119.

Cimino, A.M., Boyles, A.L., Thayer, K.A. & Perry, M.J. (2017). Effects of Neonicotinoid Pesticide Exposure on Human Health: A Systematic Review. *Environmental Health Perspective,* 125, 155–162.

Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., & Kirkegaard, R. H. (2015). Complete nitrification by Nitrospira bacteria. *Nature,* 528(7583), 504–509.




**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

Dong-Mei, X.U., Yan-Hua, W., Nan, W. & Gui-Wei, R. (2015). Effects of Single and Co-Exposure of Cu and Chlorpyrifos on the Toxicity of Earthworm. *Environmental Science,* 36, 280–285.

Fang, W., Peng, Y., Muir, D., Lin, J. & Zhang, X. (2019). A critical review of synthetic chemicals in surface waters of the US, the EU and China. *Environment international,* 131,104-109.

Hallmann, C.A., Foppen, R.P.B., Turnhout, C.A.M.V., Kroon, H.D. & Jongejans, E. (2014). Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature* 511, 341–343.

Hasenbein, S., Lawler, S.P., Geist, J. and Connon, R.E. (2015). The use of growth and behavioral endpoints to assess the effects of pesticide mixtures upon aquatic organisms. *Ecotoxicology,* 10, 261-269.

Ikarashi, Y., Yarimizu, J., Yokoyama, K., Kobayashi, T. & Nakazawa, H. (2016). Characterization of two endoglucanases for the classi¦cation of the earthworm, *Eisenia fetida* Waki. *Bioscience Biotechnology and Biochemistry,* 11, 213-220.

John, E.M. & Shaike, J.M. (2015). Chlorpyrifos: pollution and remediation. *Environmental Chemistry Letters* 13, 269–291.

Kits, K., Dimitri, S., Christopher, J.; Lebedeva, E. V., Han, P., Bulaev, A., Pjevac, P., Daebeler, A., Romano, S., Albertsen, M., Stein, L. Y. & Daims, H. (2017). Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. *Nature* 549(7671), 269–272.

Li, A.J., Banjabi, A.A., Takazawa, M., Kumosani, T.A., Yousef, J.M. and Kannan, K. (2020). Serum concentrations of pesticides including organophosphates, pyrethroids and neonicotinoids in a population with osteoarthritis in Saudi Arabia. *Science of the Total Environment,* 737,139-146.

Marino, S., Ignacimuthub, S., Duraipandiyanb, V. & Egedigwe, C.A. (1992). Effect of dietary incorporation of *Vernonia amygdalina* and *Vernonia colorata* on blood lipid profile and relative organ weight in albino rats (Thesis). Department of Biochemistry, MOUAU, Nigeria.

Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.A. & Cedervall, T. (2017). Brain damage and behavioural disorders induced by plastic nanoparticles delivered through the food chain. *Science Reports,* **7**, 114-122.

Okpokwasili. G. C. & Odokuma, L. O. (1996). Response of *Nitrobacter* to toxicity of drilling chemicals. *Journal of Petroleum Science and Engineering*, 16, 81-87.

Olawoyin, R., Oyewole, S. A. & Grayson, R L. (2012). Potential risk effect from elevated levels of soil heavy metals on human health in the Niger delta. *Ecotoxicology and Environmental Safety,* 85, 120–130.

Opere, B.O., Ojo, J.O., Omonigbehin, E. & Bamidele, M. ( 2013). Antibiotics susceptibility and plasmid profile analysis of pathogenic bacteria isolated from environmental surfaces in public toilets. Transitional Journal of Science and Technology, 3(2), 22-30.

Rizzati, V., Briand, O., Guillou, H. & Gamet-Payrastre, L. (2016). Effects of pesticide mixtures in human and animal models: An update of the recent literature. *Chemical and Biology Interaction,* 254: 231–246.

Rundlof, M., Andersson, G.K.S., Bommarco, R., Fries, I., Hederstrom, V., Herbertsson, L., Jonsson, O., Klatt, B.K., Pedersen, T.R., Yourstone, J. and Smith, H.G. (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature,* **521**:77–162.

Silva, V., Silva, C., Soares, P., Garrido, E., Manuela, B., & Fernanda, G. J. (2020). Isothiazolinone Biocides: Chemistry, Biological and Toxicity Profiles. *Molecules,* 25(4), 991-100.

Singh, S., Gupta, R. & Sharma, S. (2015). Effects of chemical and biological pesticides on plant growth parameters and rhizospheric bacterial community structure in *Vigna radiata*. *Journal of Hazard Mater,* 291,102–110.



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.

Stanley, D.A., Garratt, M.P.D., Wickens, J.B., Wickens, V.J., Potts, S.G. & Raine, N.E. (2015). Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. *Nature,* 528, 548–548.

Tenorio-Nunes, M.E. & Gaeta-Espindola, E.L. (2012). Sensitivity of *Eisenia andrei* (Annelida, Oligochaeta) to a commercial formulation of abamectin in avoidance tests with arti¦cial substrate and natural soil under tropical conditions. *Ecotoxicology,* 21,1063–1071.

Uneze, D. P., Kugbenu, G. J. & Obire, O. (2024). Influence of Pesticides Contamination on Microbial Population of Selected Farmlands. *British Journal of Environmental Sciences*, 12 (5), 14-56.

Utami, R.R., Geerling, G.W., Salami, I.R.S., Notodarmojo, S. & Ragas, A.M.J. (2020). Environmental prioritization of pesticide in the Upper Citarum River Basin, Indonesia, using predicted and measured concentrations. *Science of the Total Environment,* 787, 1401–14010.

Uwizeyimana, H., Wang, M., Chen, W. Khan, K. (2017). The eco-toxic effects of pesticide and heavy metal mixtures towards earthworms in soil. *Environmental Toxicology and Pharmacology,* 55, 20–29.

Wang, K., Pang, S., Mu, X., Qi, S., Li, D., Cui, F. & Wang, C. (2015). Biological response of earthworm, *Eisenia fetida*, to neonicotinoid pesticides. *Chemosphere,* 132, 120–126

Wang, Y., Chen, C., Qian, Y., Zhao, X. & Wang, Q. (2016). Ternary toxicological interactions of pesticides, herbicides, and a heavy metal on the earthworm *Eisenia fetida*. *Journal of Hazard Mater,* 284, 233–240.

Xu, B., Xue, R., Zhou, J., Wen, X., Shi, Z., Chen, M., Xin, F., Zhang, W., Dong, W. & Jiang, M. (2020). Characterization of Acetamiprid Biodegradation by the Microbial Consortium ACE-3 Enriched From Contaminated Soil. *Frontiers in Microbiology,* 11, 217-223.

Yang, G., Chen, Yu., Yijun, Zhao, H., Wen, W. & Yanhua, D. (2018). Combined effects of four pesticides and heavy metal chromium (VI) on the earthworm using avoidance behavior as an endpoint. *Ecotoxicology and Environmental Safety,* 12, 157-166.

Zhou, Y., Guo, J., Wang, Z., Zhang, B., Sun, Z., Yun, X. & Zhang, J. (2020). Levels and inhalation health risk of neonicotinoid pesticides in ¦ne particulate matter (PM2.5) in urban and rural areas of China. *Environment International Journal,* 142, 284-293.



**Citation:** Oshilonyah *et al.* (2026). Hidden dangers beneath our feet: how organophosphorus and alkylphenol pollutants threaten soil nitrifying bacteria balance and earthworm health. *International Journal of Microbiology and Applied Sciences*, *5(1)*, 96 – 110.