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# Biosurfactant Production Potential of Atrazine-tolerant Pseudomonas aeruginosa and Providencia species Isolated from Oke Osun Farm Settlement, Osogbo

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# Biosurfactant Production Potential of Atrazine-tolerant *Pseudomonas aeruginosa* and *Providencia species* Isolated from Oke Osun Farm Settlement, Osogbo

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**Abstract**

**Background**: The ever-growing global population and increasing demand for food to feed the population have led to the increasing use of herbicides in controlling weeds. The widespread and long-term use of atrazine results in large residual levels of atrazine in soil, which further causes water contamination and soil pollution. This research aimed to isolate and characterize microorganisms capable of tolerating atrazine as a sole carbon source.

**Methods:** Soil and water samples were collected from the Oke Osun farm settlement. Mineral salt medium was utilized to isolate the bacteria via the spread plate technique. Various concentrations of atrazine were utilized to carry out the atrazine tolerance assay. The isolates were identified morphologically, biochemically and 16S rRNA characterization. The influence of pH, temperature, inoculum size and agitation rate on the breakdown of atrazine was examined in mineral salt medium supplemented with atrazine as the sole carbon source.

**Results**: Compared with the water samples, the soil samples presented more atrazine-tolerant bacteria, and five isolates passed the atrazine tolerance assay. The five isolates were identified as *Proteus mirabilis, Pseudomonas aeruginosa*, *Providencia manganoxydans*, *Providencia rettgeri* and *Bacillus atrophaeus*. The growth of bacteria and breakdown of atrazine were greater at pH 9, temperatures ranging from 35–40°C, inoculum sizes ranging from 6% and agitation rates ranging from 150–200 rpm. The isolate with a high rate of efficient atrazine utilization was found to be

*Providencia manganoxydans* on the basis 16S rRNA sequencing and molecular phylogenetic analysis.

**Conclusion**: Atrazine-tolerant isolates, especially *Providencia manganoxydans* could be promising candidates for the bioremediation of atrazine-polluted soils because of their ability to tolerate atrazine and potential for biosurfactant production.

**Keywords**: Atrazine–tolerant, biosurfactant, potential, *Pseudomonas aeruginosa*, *Providencia* sp.

## Background

Herbicides, also popularly known as weed killers, are chemicals used to kill undesired plants. Although specific herbicides destroy specific targets, the target crop is relatively unscathed. Some of these act by interfering with the growth of the weed and are often synthetic mimics of natural plant hormone inhibitors. Some herbicides are not selective and kill all plant material with which they come into contact. Smaller quantities are employed in forestry, pasture systems, and the management of areas designated as wildlife habitats (Paul *et al* 2024). Herbicides are employed in agricultural, aquatic, forest, and wild-land ecosystems to lower the density of undesired plants (weeds) and to facilitate the establishment of desirable species. Over time, the use of herbicides in agroecosystems reduces weed density and may also lead to the selection of species that are adapted to chemicals, hence diminishing weed species diversity (von Redwitz, *et al*, 2025).

The triazine family of herbicides, which includes atrazine, was introduced in the 1950s (Merotto, *et al* 2022); these herbicides are currently the herbicide family of greatest concern regarding groundwater contamination. Atrazine does not break down readily (within a few weeks) after being applied to soils above neutral pH. Under alkaline soil conditions, atrazine may be transported into the soil profile as far as the water table by soil water following rainfall, causing pollution. Atrazine is thus termed "carryover", a typically unfavourable quality for herbicides.

Many current chemical herbicides for agriculture are intended to disintegrate within a short period after application. This is advantageous, as it allows crops that may be damaged by the herbicide to be grown on the land in future seasons. However, herbicides with poor residual action (i.e., those that break down quickly) frequently do not provide season-long weed control.

The ever-growing global population and increasing demand for food to feed the population have led to the increasing use of herbicides in controlling weeds. The advancement of agriculture with improved agricultural technology to meet this need and the productive quality of soil play a major role in most of today's productivity. Concern about herbicide pollution in the environment in the current setting of herbicide use has gained enormous importance (Kinyua *et al*., 2022).

The ability of herbicides in the soil environment to affect weed control efficacy, nontarget organism exposure and off-site mobility has become a source of environmental concern (Elgaber *et al* 2025). The harmful effects of pesticide compounds on soil microorganisms may impact soil fertility (Meena *et al* 2020). Ideally, the applied pesticides should only be poisonous to the target organisms and should be biodegradable and eco-friendly to some degree (Ahmad *et al* 2024).

Atrazine is considered highly mobile in soil and is regularly discovered in surface water and groundwater because of its very poor adsorption capacity, moderate water solubility, and persistence (Chowdhury *et al* 2021). Major degradation routes of atrazine include either biotic degradation governed by microbes or abiotic degradation such as chemical and photochemical reactions (Chowdhury *et al.,* 2021). Environmental factors such as temperature, soil moisture content, pH, the oxygen concentration of the surrounding matrix, and soil type (Chowdhury *et al.*, 2020) affect the persistence of atrazine in soil and natural environments.
Chowdhury *et al.* (2021) reported that the persistence of atrazine and trifluralin in clay loam soil is strongly influenced by temperature, with both herbicides lasting for several years at temperatures lower than 20 °C. Higher temperatures and specific moisture levels accelerated degradation, whereas significantly extended their half-lives.

According to the Environmental Protection Agency (EPA), 60% of herbicides, 90% of fungicides and 30% of insecticides are known to be carcinogenic. The possible risks to all living organisms stemming from the indiscriminate application of these agricultural pesticides have now emerged as one of the most potent environmental or health problems, particularly in underdeveloped countries (Modu *et al* 2025).

The toxicity of herbicides varies wildly. In addition to acute toxicity from high exposure levels, there is concern probable carcinogenicity (Esqueda-Esquivel *et al*., 2025), as well as various long-term difficulties, such as contributing to Parkinson's disease (Atterling *et al*, 2025). Some herbicides induce a range of health problems, ranging from skin rashes to death. The channel of attack can emerge from intentional or unintentional direct intake, inappropriate application that results in herbicide coming into direct contact with people or wildlife, inhalation of aerial sprays, or consumption of food before the specified preharvest interval. Under specific situations, some herbicides can be transported by leaching or surface runoff to contaminate groundwater or distant surface water sources.

Generally, the factors that enhance herbicide movement include violent storm occurrence (especially quickly after herbicide application) and the ability of soils with limited capacity to absorb or store herbicides. Herbicide qualities that improve the chance of transport include persistence (resistance to degradation) and high water solubility (Kinyua *et al*., 2022). Triazine exposure is potentially related to an increased risk of breast cancer, but the causal relationship between triazine and breast cancer remains uncertain (Werder *et al*., 2020).

Currently, there are numerous alternative procedures for the removal of herbicides from soil, such as chemical treatment, volatilization and cremation. Chemical treatment and volatilization, however, are difficult as large amounts of acids and alkalis are created and thereafter must be disposed of (Mahendra, 2024). Incineration, which is a very dependable physical–chemical process for the destruction of these substances, has received severe public criticism because of its potentially harmful emissions and elevated economic expenses (Kosimov *et al*, 2025). Overall, most of these physical–chemical cleaning procedures are expensive and inefficient (Moteguo, 2024) since the contaminated soil must be excavated at a site and carried to a storage facility where it can be processed. Owing to environmental concerns related to the accumulation of herbicides in food products and water supplies, there is a considerable need to find safe, convenient and economically feasible technologies for herbicide cleanup (Sami *et al*, 2025). For this reason, numerous biological approaches involving the biodegradation of chemical substances by microorganisms have been developed (Shah *et al*., 2024).

The use of microorganisms (fungi and bacteria), either naturally existing or introduced, to reduce contaminants is called bioremediation (Ayilara and Babalola, 2023). Microbial metabolism is arguably the most important herbicide degradative mechanism in soils (Kumar *et al.*, 2021) and is the basis for bioremediation, as degrading microorganisms receive C, N or energy from herbicide molecules (Hassaan and El Nemr, 2020). The goal of bioremediation is to at least lower pollutant levels to undetectable, nontoxic or acceptable levels, i.e., within the limitations specified by regulatory bodies (Mukherjee *et al.*, 2021), or ideally to totally mineralize organo-pollutants to carbon dioxide. From an environmental point of view, this entire mineralization process is beneficial since it signifies complete detoxification (Mukherjee *et al.*, 2021).

The use of bioremediation to remove contaminants is often less expensive than the equivalent physical–chemical approaches. This method offers the possibility to treat contaminated soil and groundwater onsite without the need for excavation (Alori *et al*., 2022), and it requires little energy input and retains the soil structure (Sharma, 2021). Perhaps the most attractive element of bioremediation is the decreased influence on natural ecosystems, which should be more acceptable to the public (Darshan and Yogesh 2021). The intricacy of microbial mechanisms for the breakdown of organo-pollutants, as well as the period before microbial degradation occurs, which requires weeks to months, has led to the slow emergence of this technology as a viable technique for remediation (Sharma, 2021). More extensive research on the principles of biodegradation and the development of efficient decontamination technologies are needed to overcome the hazardous waste problem (Elshafei and Mansour 2024).

Atrazine is one of the most extensively used herbicides in agriculture because of its efficiency in controlling broadleaf weeds. However, it is a persistent pesticide typically found in soil and water, which has caused major environmental and health issues. Continuous application in farm villages such as Oke–Osun leads to the accumulation of atrazine residues, which can disturb microbial diversity, contaminate groundwater and pose dangers to non–target creatures, including humans. Few investigations have been conducted on the atrazine contamination status of the Oke–Osun farm settlement and have focused on finding indigenous microorganisms capable of tolerating and

perhaps decomposing atrazine. The absence of such data hampers the development of bioremediation solutions customized for the local environment.

Atrazine contamination harms soil fertility, groundwater quality and biodiversity in agricultural lands. The use of chemical surfactants could lead to the eradication of important microbial flora that contribute to soil fertility. Identifying atrazine–tolerant and biosurfactant–producing bacteria could provide eco-friendly solutions to the problem of soil contamination by atrazine through detoxification of polluted soils.

The purpose of this investigation was to isolate and characterize atrazine–tolerant and biosurfactant–producing bacteria from the Oke–Osun Farm Settlement, to evaluate their potential significance in the bioremediation of atrazine–contaminated soils.

## Methods

### Isolation of Atrazine–tolerant and Biosurfactant–producing Bacteria

The isolation and detection of atrazine–resistant and biosurfactant–producing bacteria were performed via an enrichment approach from soil samples obtained from the Oke–Osun farm settlement area in Osogbo, Osun State. The farm hamlet has a long history of atrazine usage. Soil profiles 5–30 cm below the surface were sampled via a sterile trowel following removal of the top layer of the soil up to 1–5 cm. Three soil samples were taken from three locations of the Oke-Osun farm settlement. Three water samples were also collected from the stream running through the farm. All samples were taken to the Microbiology Laboratory of Fountain University, Oke-Osun, Osogbo, and stored in a refrigerator until needed.

### Cultivation of Atrazine–tolerant Bacteria in Broth Medium.

The enrichment process with a mineral salt medium (MSM) was utilized. The medium was made with 2.0 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $CaCl_2.2H_2O$, 0.001 g of $FeSO_4 .7H_2O$, 1.5 g of $Na_2HPO_4.12H_2O$, and 1.5 g of $KH_2PO_4$ per litre of deionised water. The final pH was adjusted to 7.2. After autoclaving at 121 °C for 20 minutes, the medium was supplemented with various concentrations (20 mg/L, 30 mg/L, and 40 mg/L) of atrazine as the sole carbon source. A

total of 10 g of each soil sample was inoculated and homogenized in 250 mL Erlenmeyer flasks. The flasks were then incubated under orbital agitation (100,150 and 200 rpm) at 37 °C for 7 days. After 7 days, a 10 mL aliquot of each culture was transferred to new MSM broth (100 mL) with a similar atrazine volume. Each culture was subsequently serially diluted from $10^{-1}$ to $10^{-5}$ in sterile distilled water, and dilutions of $10^{-1}$, $10^{-3}$ and $10^{-5}$ were subsequently plated on MSM agar, supplemented with 0.1 mL of atrazine. The Petri dishes were then incubated at 37 °C for 48 hours. On the basis of morphological features, individual bacterial colonies were selected and subcultured to generate pure cultures.

**Identification of isolates**

The identification of selected bacteria was done on the basis of Gram's staining, microscopy and sugar utilization was performed. In addition, the biochemical characteristics of the isolates and substrate usage patterns were evaluated.

**Subculturing**

Before the isolates were subcultured, the total viable count was determined. Distinct viable colonies were streaked on freshly prepared nutrient agar plates to obtain a pure culture.

**Atrazine–tolerance assay**

Pure cultures from each subcultured plate were selected using an inoculation loop and streaked onto freshly prepared nutrient agar plates containing various concentrations of atrazine (20 mg/L, 30 mg/L, and 40 mg/L). The plates were then incubated at 37 °C for 48 hours.

**Stock Culture**

The cultures that passed the assay and thrived best were subcultured in a stock bottle. MacCartney bottles were filled with 10 mL of prepared nutrient agar and sterilized in an autoclave. The bottles were kept slanted and allowed to harden. The atrazine-tolerant isolates were subcultured into each bottle and incubated at 37 °C for 24 hours.

**Optimal Conditions for Atrazine–tolerant Bacteria Using One Factor at a Time.**

The effects of temperature (20 °C, 30°C and 40 °C), inoculum size (2%, 4% and 6%) of 0.5 McFarland, pH (5, 7 and 9) and agitation rate (100 rpm, 150 rpm, and 200 rpm) were varied. A volume of 100 ml of mineral salt medium was added to a conical flask, the pH was tested adjusted to the required pH (5, 7, 9), and the mixture was then sterilized at 121 °C for 15 minutes. Atrazine (0.004g, 40mg/L) was added to each flask, and the mixture was allowed to cool. An initial inoculum size of 2 mL (2%) was added, and the mixture was cultured in a shaking incubator at an initial temperature of 30 °C and an agitation rate of 100rpm for 24 hours. A bacterial aliquot of 5 mL was obtained from each conical flask after 24 hours to determine the rate of absorbance at a wavelength of 600 (OD 600).

**Carbon and Energy Source**

The ideal temperature, inoculum size, pH and agitation rate were changed for this test. Glucose and sucrose were introduced as sources of carbon and energy. Each sugar (0.02g) was added to a 10 mL test tube apiece. A total of 100 mL of MSM was added to a conical flask with the optimal pH; the test tubes containing the sugars and the MSM flasks were sterilized in an autoclave. Atrazine (0.004 g) of was added to the MSM, and the test tubes were poured into each flask. The appropriate inoculum size was used, and the flasks were incubated in a shaking incubator at the optimal temperature and agitation rate for 48 hours. A bacterial aliquot of 5 ml was obtained from each conical flask after 48 hours to determine the rate of absorbance at a wavelength of 600 (OD 600).

**Nitrogen Source**

The ideal temperature, inoculum size, pH and agitation rate were unchanged for this test. Sodium nitrite ($NaNO_2$) and sodium nitrate ($NaNO_3$) were used as nitrogen sources. Each salt (0.2 g) was added to 100ml of MSM produced in conical flasks at the optimal pH. The conical flasks were sterilized in an autoclave. Atrazine (0.004 g) of atrazine was added, and the mixture was allowed to cool. The appropriate inoculum size was employed, and the flasks were incubated in a shaking incubator utilizing the optimal temperature and agitation rate for 24 hours. A bacterial aliquot of 5ml was obtained from each conical flask after 48 hours to determine the rate of absorbance using a wavelength of 600 (OD 600).

**Preliminary screening for biosurfactant production**

For the preliminary screening, a mineral salt medium with the following composition (g/L) was used: $Na_2HPO_4$ (1.5), $KH_2PO_4$ (1.5), $MgSO_4$. $7H_2O$ (0.2), $NH_4SO_4$ (2.0), $FeSO_4.7H_2O$ (0.01), NaCl (1.1), KCl (1.1), yeast extract (0.02). The medium was supplied with 2ml of the following trace element mixture (g/L): $ZnSO_4.7H_2O$ (0.29), $MnSO_4.4H_2O$ (1.78), $CuSO_4.5H_2O$ (1.0), $NaNO_3$(1.5), $CaCl_2.4H_2O$ (0.24). pH= 7.0 ± 0.2

After the mineral salt medium was prepared, it was sterilized at 121 °C for 15 minutes. Then, 0.5 mL of sterile spent vegetable oil was added to 50 mL of mineral salt medium as a carbon source, inoculated separately with 1 mL of 0.5 McFarland standardized isolates of different atrazine–tolerant bacteria and incubated at 37 °C in an incubator shaker at 150 rpm for 3 days.

After the 3rd day, the fermentation medium was centrifuged at 5000 rpm for 20 minutes to obtain the crude supernatant required for preliminary screening of the biosurfactant produced by the isolates via the following techniques: drop collapse test, oil spreading technique, thermostability, blood hemolysis test and emulsification Index.

**Molecular characterization of isolates and phylogenetic study**

Molecular characterization was carried out on the isolate with the best growth after the optimization experiments were carried out. The molecular characterization was carried out at Inqaba Biotech Company via polymerase chain reaction (PCR) and gene sequencing. Genomic DNA was extracted from the isolates via the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, catalogue No. D6005).

Universal primers for amplification of the bacterial 16S rRNA gene were used. The forward primer was FT1 (5'-AGAGTTTGATCATGGCTCAG -3'), and the reverse primer was RT5 (5'-ACGGTTACCTTGTTACGACTT-3'). The PCRs were performed as follows: 95 °C for 5 min; 30 cycles of 95 °C for 20 s, 50 °C for 20 s and 72 °C for 3 min; and a final extension at 72 °C for 10 min. The PCR products were stored at 4 °C. The PCR products were sequenced at automated sequencing facilities. A homology comparison of the partial 16S rRNA gene sequence was performed via the NCBI BLAST database (http://www. ncbi.nlm.nih.gov/). Sequence alignment

was performed via the MEGA 12 software package. The phylogenetic tree was constructed via the neighbour–joining (NJ) method.

The phylogenetic tree was constructed using neighbour-joining sequences of different species extracted from the NCBI database. The downloaded sequences were aligned using the ClustalW tool in MEGA version 12 software via a bootstrapped neighbour-joining approach.

## Results and discussion

### Total Viable Count

The total viable count was performed on each plate incubated for 48 hours, and the results are shown in Table 1. The highest soil microbial counts were found in Maize Farms 1 and 2, implying more abundant or vigorous microbial activity, which might be associated with organic matter, fertilizer application, or moisture availability. Maize Farm 3 has noticeably lower counts than Farms 1 and 2 do, which could indicate poorer soil health, different farming practices, or drier conditions. Stream Point 1 has significantly higher counts than Stream Points 2 and 3 do, possibly indicating organic pollution, runoff from nearby farms, or proximity to animal activity. Stream Points 2 and 3 have very low counts, suggesting relatively little microbiological activity.

**Table 1: Total viable count of atrazine-tolerant bacteria found in the soil and water samples.**

| Location | Type of sample | Dilution | 24 hours | 48 hours |
|---|---|---|---|---|
| Maize farm 1 | Soil | $10^2$ | 33 | 40 |
| | | $10^5$ | 29 | 40 |
| Maize farm 2 | Soil | $10^2$ | 40 | 46 |
| | | $10^5$ | 37 | 45 |
| Maize farm 3 | Soil | $10^2$ | 19 | 23 |
| | | $10^5$ | 8 | 10 |
| Stream point 1 | Water | $10^2$ | 38 | 44 |
| | | $10^5$ | 35 | 41 |
| Stream point 2 | Water | $10^2$ | 3 | 3 |
| | | $10^5$ | 2 | 4 |
| Stream point 3 | Water | $10^2$ | 1 | 2 |
| | | $10^5$ | 0 | 1 |

**Table 2: Average number of viable bacteria (CFU/g) in the soil and water samples.**

| Location | Type of sample | Dilution factor | 24 hours | 48 hours |
|---|---|---|---|---|
| Maize farm 1 | Soil | $10^2$ | $3.3 \times 10^3$ | $4 \times 10^3$ |
| | | $10^5$ | $2.9 \times 10^6$ | $4 \times 10^6$ |
| Maize farm 2 | | $10^2$ | $4 \times 10^3$ | $4.6 \times 10^3$ |
| | | $10^5$ | $3.7 \times 10^6$ | $4.5 \times 10^6$ |
| Maize farm 3 | Soil | $10^2$ | $1.9 \times 10^3$ | $2.3 \times 10^3$ |
| | | $10^5$ | $8 \times 10^5$ | $1 \times 10^6$ |
| Point 1 of Stream | Water | $10^2$ | $3.8 \times 10^3$ | $4.4 \times 10^3$ |
| | | $10^5$ | $3.5 \times 10^6$ | $4.1 \times 10^6$ |
| Point 2 of Stream | Water | $10^2$ | $3 \times 10^2$ | $3 \times 10^2$ |
| | | $10^5$ | $4 \times 10^5$ | $4 \times 10^5$ |
| Point 3 of Stream | | $10^2$ | $1 \times 10^2$ | $2 \times 10^2$ |
| | | $10^5$ | $1 \times 10^0$ | $1 \times 10^0$ |

**Colony-forming units (CFU/g)**

Tables 1 and 2 show the total viable and average bacterial counts obtained from the water and soil samples at different locations. The soil from Maize Farm 1 had one of the highest bacterial loads among the farm soils, indicating that the microbiologically active and healthy soil had good organic matter or fertilizer input. The soil in Maize Farm 2 has slightly greater activity than Farm 1, making it the most microbially active soil in this study. This may reflect more organic input, better moisture retention, or more fertile soil conditions, while Maize Farm 3's average microbial counts are notably lower than those of Farms 1 and 2. This could suggest poorer soil quality, less organic matter, different land management practices, or environmental stress.

**Atrazine tolerance assay**

Among the 36 distinct viable colonies used for the atrazine tolerance assay, 5 isolates that were able to tolerate all concentrations of atrazine were used for the main study.

**Gram Staining and Biochemical characterization**

The results of Gram's staining and biochemical characterization are shown in Table 3.

**Table 3: Gram's Staining and Biochemical Characteristics of Isolates**

| Morphology/ biochemical test | Isolate 2 | Isolate 22 | Isolate 31 | Isolate 33 | Isolate 35 |
|---|---|---|---|---|---|
| Spore staining | - | - | - | - | + |
| Gram reaction | - | - | - | - | + |
| Shape | Rod | Rod | Rod | Rod | Rod |
| Motility | - | - | - | - | - |
| Catalase | + | + | + | + | + |
| Oxidase | - | - | + | - | - |
| Citrate utilization | + | + | + | + | + |
| Indole | + | + | - | + | + |
| Urease | + | + | - | + | + |
| Starch hydrolysis | - | - | + | - | - |
| Sugar fermentation | | | | | |
| Lactose | - | - | - | - | - |
| Glucose | + | + | - | + | + |
| Fructose | + | + | - | + | + |
| Organism Identity | *Proteus mirabilis* | *Providencia rettgeri* | *Pseudomonas aeruginosa* | *Providencia manganoxydans* | *Bacillus atrophaeus* |

**Key**: + is positive
- is negative

## Optimal Conditions for the Growth of Atrazine-Tolerant Bacteria

The effects of temperature, pH, inoculum size and agitation rate on biomass yield during atrazine biodegradation are shown in Figures 1 to 4.

### Effects of Carbon and Nitrogen Sources

Figures 5 and 6 show the effects of carbon and nitrogen sources on biomass yield during atrazine biodegradation.

### Optimal conditions for atrazine tolerance

The results revealed that a temperature of 40 °C, a pH of 9, an inoculum size of 6% and an agitation rate of 150 rpm were the best and optimal conditions for maximum atrazine degradation. These results are similar to those of other authors, who used species such as *Bacillus safensis* (60%), *Pseudomonas aeruginosa* (80%), *Bacillus badius* (73.5%), *Serratia marcescens, Klebsiella species,* and *Comamonas species* (83.3%) (Muhammad *et al.,* 2021).

Temperature is a significant factor that affects the growth and activity of microorganisms involved in atrazine breakdown. All the biological activities leading to growth are catalyzed by the enzymes present in the cell. The activity of enzymes is controlled by temperature, and consequently, temperature is able to influence growth (McLeod *et al*., 2024). The effects of various temperatures on the biomass yield of atrazine-tolerant isolates are displayed in Figure 1. The results of this study revealed that the biomass output during atrazine breakdown reached a maximum at an ideal temperature of 40 °C. This finding is consistent with certain earlier investigations that demonstrated higher atrazine degradation rates at elevated temperatures. Maijama'a *et al*. (2024) demonstrated that a mesophilic temperature range of 30 °C to 40 °C promoted atrazine breakdown. Similarly, Liang *et al*. (2022) reported increased microbial activity and atrazine breakdown efficiency at relatively high temperatures.

However, it is crucial to remember that certain  shown differing trends. Khatoon and Rai (2020) reported that extreme temperatures exceeding 40 °C led to a decrease in microbial biomass and a decrease in atrazine degradation rates due to thermal stress on the microbial population. Muhammad *et al*. (2021) also reported that growth and degradation capacities were both inhibited when the temperature was greater than 40 °C. Therefore, while a temperature of 35–40 °C appears

to be optimal for atrazine decomposition in this study, the greater range of temperatures explored in other studies may alter the applicability of this specific condition.
pH is among the most critical parameters regulating the efficiency of various chemical and biological activities (Muhammad *et al*., 2021). Changes in pH impact both the structure and activity of proteins; this also affects the activity of cellular enzymes. A severe change in pH also affects the cell membrane and membrane proteins, which in turn debilitate the permeability barrier of the cell. The pH of the surroundings of an organism is frequently affected by the metabolic activity of the organism itself or by other activities in nature. This is inferred when culture media are combined with buffers that can resist a shift in pH (Subberwal *et al*., 2025).

The effects of various pH values on the growth of atrazine-degrading isolates are presented in Figure 2. An optimum pH of 7–9 had a significant effect on the biomass yield during atrazine bioconversion and biomass growth.
In contrast, previous research by Liang *et al*. (2022) revealed that an acidic to slightly alkaline pH ranging from 6.5--7.5 was optimal for atrazine degradation. Therefore, the pH preferences of atrazine-degrading bacteria may vary depending on their ecological niche.

Agitation has a critical function in maintaining consistent conditions and preventing substrate stratification in liquid cultures. The deterioration rate decreases as the agitation speed increases. (Buaroy *et al*., 2025). In this study, as the agitation rate increased, the atrazine tolerance likewise increased but then decreased (Figure 4). This was due to the contact between the microorganisms and atrazine, which increased the degradation of atrazine. A supplementary increase in agitation speed led to a decrease in atrazine decomposition. This occurred because, at high speeds, disruption of cells occurs, which leads to poor degradation (Khatoon *et al*., 2020).

The inoculum size refers to the amount of microbial culture added to the growth medium. In our study, an inoculum size of 6% resulted in the highest biomass yield during atrazine degradation (Figure 3). This finding is in line with several studies that reported positive correlations between inoculum size and atrazine degradation efficiency. A larger inoculum size introduces a greater number of degrader cells, leading to more effective atrazine degradation. However, increasing the inoculum size beyond an optimal point might not always lead to enhanced degradation. Some

studies, such as the work of Smith *et al.* in 2021, have shown that excessively high inoculum sizes can lead to competition among microbial species, leading to a decreased biomass yield and less efficient atrazine degradation. Therefore, the optimal inoculum size should be carefully determined on the basis of the specific microbial community and environmental conditions.

**Effects of carbon and nitrogen sources**

The influence of glucose and sucrose as carbon sources was studied, as shown in Figure 5. On the basis of these results, some isolates easily assimilate sucrose better, whereas others assimilate glucose better. A study by Reyad *et al*. in 2017 investigated the use of glucose and sucrose as carbon sources in microbial cultures for atrazine degradation. They reported that the microbial biomass was generally greater when glucose was used than when sucrose was used. With respect to the influence of sodium nitrate and sodium nitrite as nitrogen sources, sodium nitrate was the preferred nitrogen source for the atrazine-tolerant isolates (Figure 6).

Culture conditions play a vital role in determining the biomass yield during atrazine degradation. Optimizing the temperature, pH, inoculum size, agitation rate, carbon, and nitrogen source is essential for increasing atrazine biodegradation efficiency. These findings provide valuable insights for designing effective bioremediation strategies to combat atrazine contamination in the environment.

The results of the optimization revealed that *Providencia manganoxydans* presented the best biomass yield during atrazine tolerance. The biomass yield and degradation of atrazine in *Providencia manganoxydans* were optimal at 40 °C, a pH of 9, an inoculum size of 6% and an agitation rate of 150 rpm. In addition, a greater biomass yield was produced when sucrose and sodium nitrate were supplemented as carbon and nitrogen sources, respectively. In a study by Mirzavand *et al.* (2023), *Pseudomonas species* were identified as prominent atrazine-degrading bacteria. While *Providencia manganoxydans* was a better isolate, *Pseudomonas species* presented comparable atrazine degradation abilities under certain conditions.

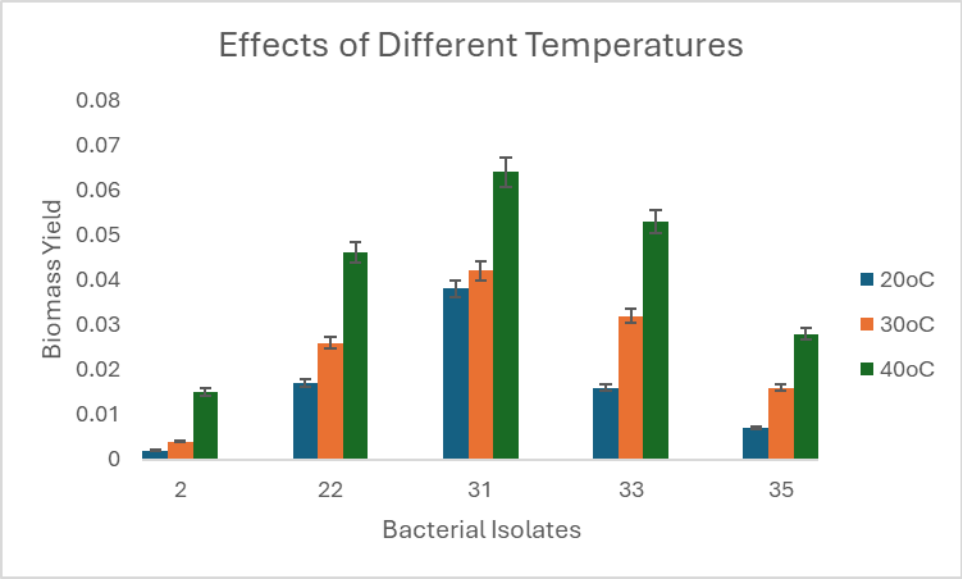



**Figure 1: Effects of varying temperatures on the yield of biomass**

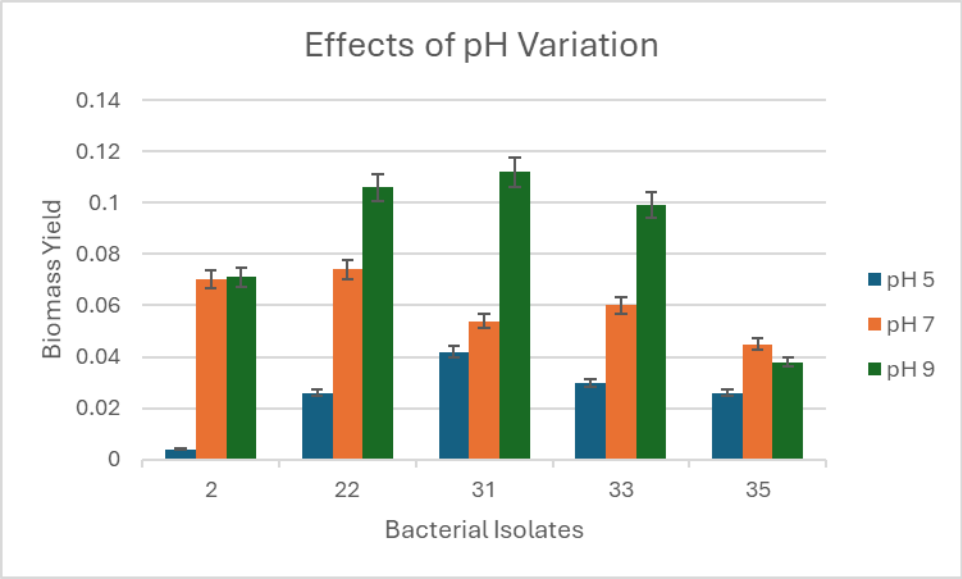



**Figure 2: Effects of varying pH on biomass yield.**

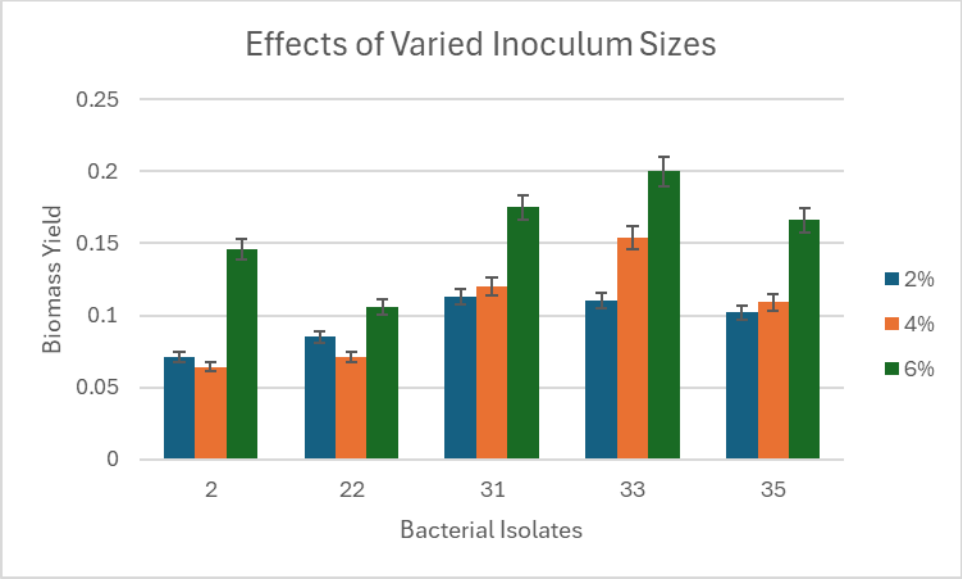



**Figure 3: Effects of varying inoculum sizes on biomass yield**

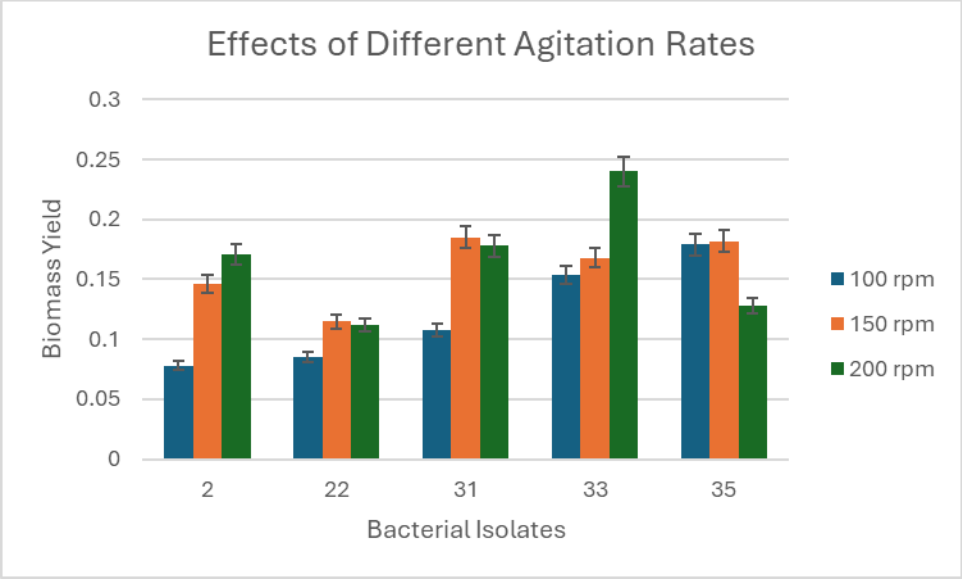



**Figure 4: Effects of various agitation rates on the yield of biomass**

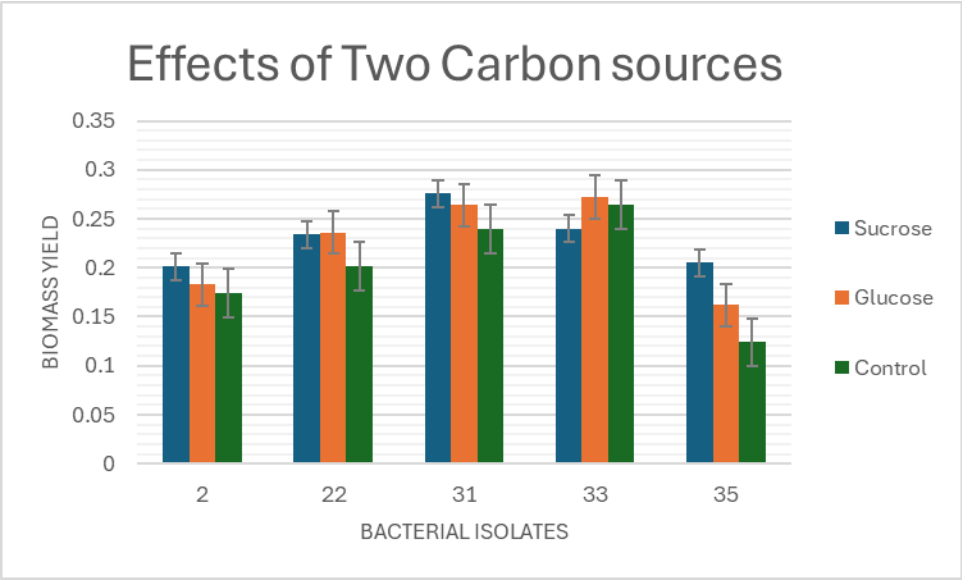



**Figure 5: Effects of carbon sources on the yield of biomass**

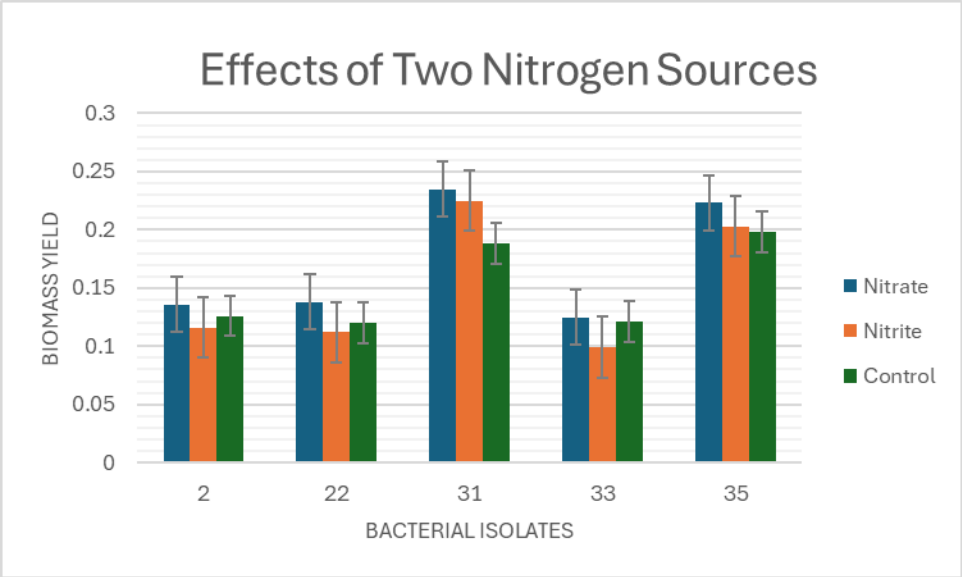



**Figure 6: Effects of nitrogen sources on the yield of biomass**

**Biosurfactant production potentials of the isolates**

Some of the isolates also presented significant biosurfactant production abilities, as shown in Table 4 and Figure 7. All the isolates were positive for the drop collapse test, hemolytic activity and emulsification index, ranging from 50–57%[i] (Table 4). This makes them good candidates for bioremediation studies involving the use of biosurfactants to restore polluted soils.

All five isolates tested positive for the primary screening methods, such as oil spreading and drop collapse, with isolate 22 showing negative hemolytic activity. This indicates their potential to produce surfactants. The emulsification index values in this study are comparable to those reported by Abdel-Mawgoud in 2009 for other biosurfactant producers, such as *Pseudomonas aeruginosa* isolate Bs20, which has been reported to have an emulsification index of 59–66.0%.

The FTIR spectra of the biosurfactants from the three species (Figure 8) are shown in Figures 10, 11 and 12. The spectra displayed a prominent broad band at approximately 3300–3400 $cm^{-1}$, which is characteristic of O–H and N–H stretching vibrations, indicating the presence of hydroxyl or amine groups. Peaks in the 2850–2950 $cm^{-1}$ region correspond to the symmetric and asymmetric stretching of C–H bonds in aliphatic chains. A strong absorption peak near 1640–1650 is indicative of C = O stretching in amide groups, a feature common in lipopeptide biosurfactants. Additionally, peaks in the 1000–1100 $cm^{-1}$ range are associated with C–O–C and C–O stretching vibrations, suggesting the presence of carbohydrate moieties or ester linkages. The FTIR spectra are consistent with those of known biosurfactants, especially biosurfactants produced by *Pseudomonas aeruginosa*. These functional groups are also typical of rhamnolipids and lipopeptides. The FTIR spectrum of *Providencia manganoxydans* (Figure 11) shows peaks at 3390.86 cm-1, 2927.77 cm-1, 1643.35 cm-1 and 1014.56 cm-1, which are characteristic functional groups of glycolipid biosurfactants.

To identify the specific chemical compounds present in the biosurfactants, GC–MS analysis was performed (Figures 5, 6 and 7). The major compounds present in biosurfactants include fatty acids, hydrocarbons and esters. There are methyl esters of fatty acids, such as methyl stearate ($C_{19}H_{38}O_2$), hexadecenoic acid methyl ester ($C_{17}H_{34}O_2$), and cis–11–eicosenoic acid methyl ester ($C_{21}H_{40}O_2$), present in all three *Providencia* and *Pseudomonas* biosurfactants. The identification of 9,12–

octadecadienoic acid and oleic acid also revealed the presence of fatty acids, which are common components of biosurfactants.

**Table 4: Biosurfactant-producing activity of bacterial isolates.**

| Bacteria isolates | Oil spreading (cm) | Drop collapse | Haemolytic activity | Thermostability | Emulsification index (%) |
|---|---|---|---|---|---|
| Isolate 2 | 1.7 | + | + (β) | 1.8 | 52.00 |
| Isolate 22 | 1.4 | + | – (γ) | 2.3 | 55.20 |
| Isolate 31 | 2.0 | + | + (β) | 3.1 | 50.00 |
| Isolate 33 | 1.7 | + | + (α) | 2.8 | 52.40 |
| Isolate 35 | 1.5 | + | + (β) | 2.4 | 57.00 |

Key: – = negative reaction.
+ = positive reaction.

Table 5. FTIR SPECTRA OF THREE BIOSURFACTANTS

| *Providencia rettgeri* (22 | | *Pseudomonas aeruginosa* (31) | | *Providencia manganoxydans* (33) | |
|---|---|---|---|---|---|
| Peak | Intensity | Peak | Intensity | Peak | Intensity |
| 3456.64 | 0 | 3429.43 | 0 | 3390.86 | 0 |
| 2848.86 | 38 | 2848.86 | 38 | 2848.86 | 37 |
| 2366.66 | 42 | 2368.59 | 42 | 2366.66 | 45 |
| 2090.84 | 36 | 2092.77 | 36 | 2092.77 | 31 |
| 1645.28 | 06 | 1643.35 | 06 | 1643.35 | 02 |
| 1458.18 | 27 | 1261.45 | 27 | 1421.54 | 25 |
| 1107.14 | 30 | 1105.21 | 30 | 1107.14 | 23 |
| 1014.56 | 24 | 1014.56 | 24 | 1014.56 | 15 |
| - | 06 | 748.38 | 06 | - | - |
| 592.15 | 02 | 435.91 | 02 | 532.35 | 0 |



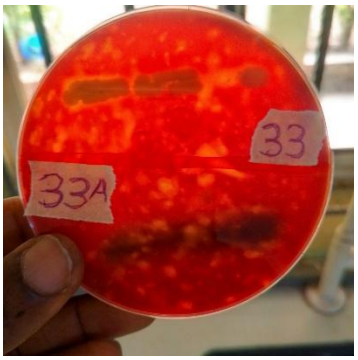

*Proteus mirabilis*




*Providencia rettgeri*




*Pseudomonas aeruginosa*




*Providencia manganoxydans*




*Bacillus atrophaeus*

Figure 7: Blood hemolysis screening for biosurfactant potential of the isolates

KEY:
02 – *Proteus mirabilis* (wild-type) β – hemolysis
02A – *Proteus* (Mutant) β – hemolysis
22 – *Providencia rettgeri* (wild-type) γ – hemolysis
22A – *Providencia rettgeri* (Mutant) γ – hemolysis
31 – *Pseudomonas aeruginosa* (wild-type) β – hemolysis
31A – *Pseudomonas aeruginosa* (Mutant) β – hemolysis
33 – *Providencia manganoxydans* (wild-type) α – hemolysis
33A – *Providencia manganoxydans* (Mutant) α – hemolysis
35 – *Bacillus atrophaeus* (wild-type) β – hemolysis
35A – *Bacillus* (Mutant) β – hemolysis

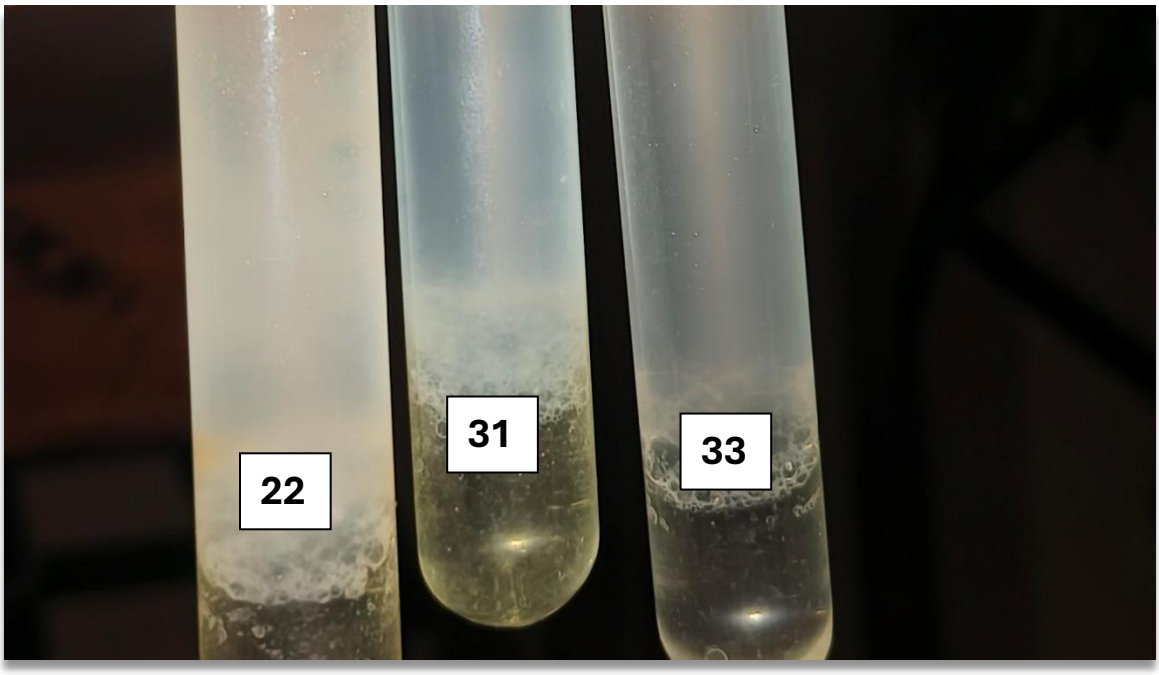



**Figure 8: Biosurfactants produced by three of the isolates via spent vegetable oil**

Table 6: GCMS peak reports of rhamnolipids from *Providencia rettgeri*

| S/N | Retention time | Molecular Formula | Library/ID | molecular weight(mW) |
|---|---|---|---|---|
| **1** | 11.215 | $C_{11}H_{24}$ | Undecane | 156 |
| **2** | 15.709 | $C_{12}H_{24}O$ | Dodecane | 184 |
| **3** | 16.879 | $C_{14}H_{22}O$ | Phenol, 3,5-bis(1,1-dimethylethyl)- | 206 |
| **4** | 17.739 | $C_{29}H_{62}O_4Si_2$ | Eicosenoic acid, 2,3-bis(trimethyl ester | 530 |
| **5** | 18.261 | $C_{18}H_{32}O_2$ | 9,12-Octadecadienoic acid (Z,Z)- | 280 |
| **6** | 18.495 | $C_{35}H_{70}$ | 17-Pentatriacontene | 490 |
| **7** | 19.007 | $C_{15}H_{30}O_2$ | Tridecanoic acid, 12-methyl, methyl ester | 242 |
| **8** | 20.085 | $C_{24}H_{38}O_4$ | Diisooctyl phthalate | 390 |
| **9** | 20.996 | $C_{11}H_{20}O_2$ | 2-Decenoic acid, methyl ester | 184 |
| **10** | 21.143 | $C_{17}H_{34}O_2$ | Hexadecanoic acid, methyl ester | 270 |
| **11** | 21.633 | $C_{17}H_{24}O_3$ | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien | 276 |
| **12** | 23.207 | $C_{18}H_{31}ClO$ | 9,12-octadecadienoyl chloride | 298 |
| **13** | 23.438 | $C_{19}H_{38}O_2$ | Methyl stearate | 298 |
| **14** | 25.986 | $C_{21}H_{40}O_2$ | cis-11-Eicosenoic acid, methyl ester | 324 |
| **15** | 26.099 | $C_{37}H_{74}NO_8P$ | Hexadecanoic acid, 1-(2-amino ethoxy) hy | 691 |

Table 7: GCMS peak report of rhamnolipids from *Pseudomonas aeruginosa*

| **Retention time** | **Molecular Formular** | **Compound name** | **molecular weight(mW)** |
|---|---|---|---|
| **11.245** | $C_{11}H_{24}$ | Undecane | 156 |
| **13.992** | $C_9H_{10}O$ | Benzaldehyde,2,4-dimethyl- | 134 |
| **15.695** | $C_{12}H_{24}O$ | Dodecane | 184 |
| **16.877** | $C_{14}H_{22}O$ | Phenol, 3,5-bis(1,1-dimethylethyl)- | 206 |
| **17.999** | $C_{13}H_{26}O$ | Tridecanal | 198 |
| **18.246** | $C_{18}H_{34}O_2$ | Oleic Acid | 530 |
| **19.001** | $C_{15}H_{30}O_2$ | Methyl tetradecanoate | 280 |
| **20.076** | $C_{24}H3_8O_4$ | Di-n-octyl phthalate | 490 |
| **20.987** | $C_{17}H_{33}NO_3$ | Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexane | 242 |
| **21.137** | $C_{17}H_{34}O_2$ | Hexadecanoic acid, methyl ester | 390 |
| **21.628** | $C_{17}H_{24}O_3$ | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien | 184 |
| **21.976** | $C_{16}H_{22}O_4$ | Dibutyl phthalate | 270 |
| **23.201** | $C_{19}H_{36}O_2$ | 9-Octadecadienoic acid (Z)-, methyl ester | 276 |
| **23.430** | $C_{19}H_{38}O_2$ | Methyl stearate | 298 |
| **26.086** | $C_{37}H_{74}NO_8P$ | Hexadecanoic acid, 1-(2-amino ethoxy) hy | 298 |

Table 8: GCMS peak reporting of rhamnolipid from *Providencia manganoxydans*

| **Retention time** | **Molecular Formular** | **Compound name** | **molecular weight(mW)** |
|---|---|---|---|
| **11.226** | $C_{11}H_{24}$ | Undecane | 156 |
| **15.609** | $C_{12}H_{24}O$ | Dodecane | 184 |
| **16.872** | $C_{14}H_{22}O$ | Phenol, 3,5-bis(1,1-dimethylethyl)- | 206 |
| **17.734** | $C_{13}H_{26}O$ | Tridecanal | 198 |
| **18.224** | $C_{18}H_{32}O_2$ | 9,12-Octadecadienoic acid (Z,Z)- | 280 |
| **18.648** | $C_{35}H_{70}$ | 17-Pentatriacontene | 490 |
| **19.156** | $C_{15}H_{30}O_2$ | Tridecanoic acid, 12-methyl, methyl ester | 242 |
| **20.108** | $C_{24}H_{38}O_4$ | Diisooctyl phthalate | 390 |
| **20.864** | $C_{11}H_{20}O_2$ | 2-Decenoic acid, methyl ester | 184 |
| **21.164** | $C_{17}H_{34}O_2$ | Hexadecanoic acid, methyl ester | 270 |
| **21.606** | $C_{17}H_{24}O_3$ | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien | 276 |
| **23.232** | $C_{18}H_{31}ClO$ | 9- Octadecenoic acid (Z)-,methyl ester | 298 |
| **23.432** | $C_{19}H_{38}O_2$ | Methyl stearate | 298 |
| **25.848** | $C_{21}H_{40}O_2$ | cis-11-Eicosenoic acid, methyl ester | 324 |
| **26.092** | $C_{37}H_{74}NO_8P$ | Hexadecanoic acid, 1-(2-amino ethoxy) hydro | 691 |

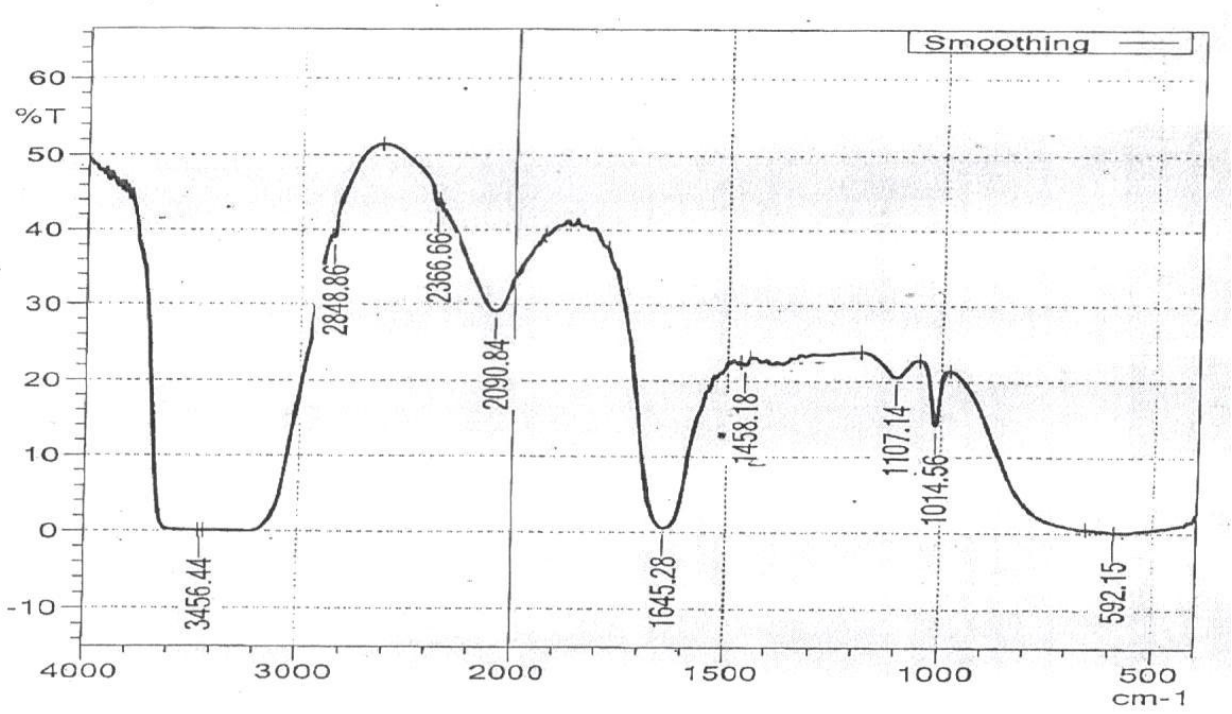



**Figure 9: FTIR spectra of Biosurfactants produced by *Providencia rettgeri***

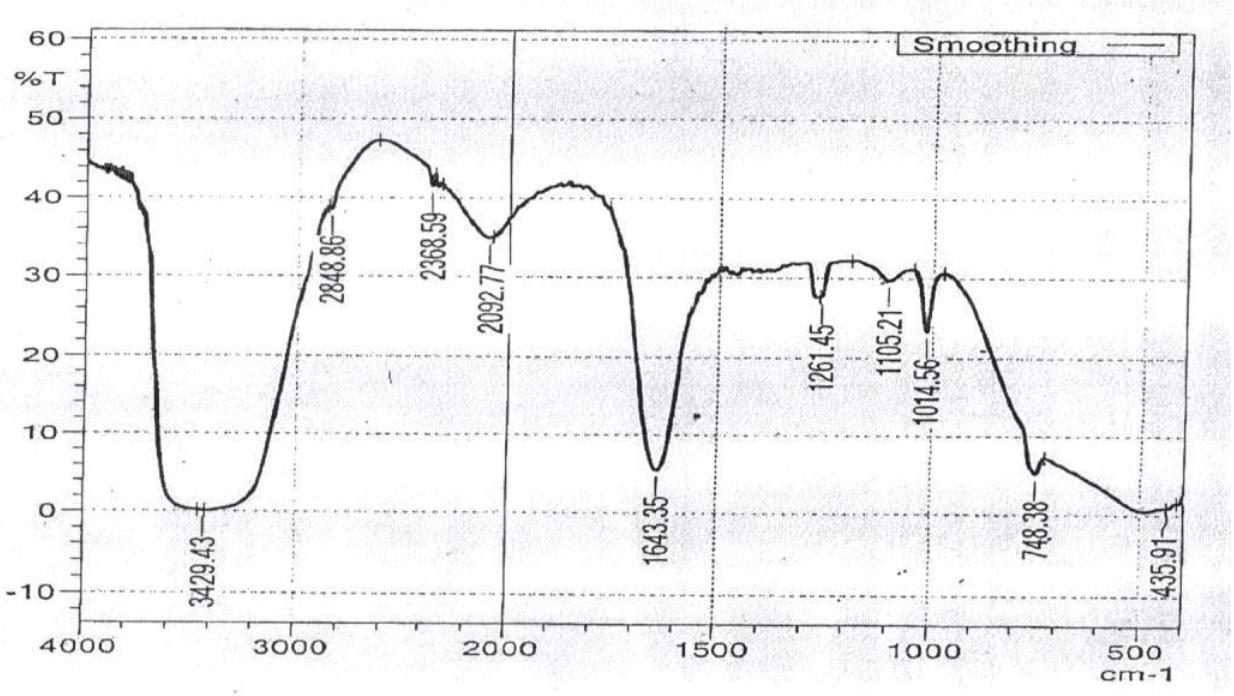



**Figure 10: FTIR spectra of biosurfactants produced by *Pseudomonas aeruginos***

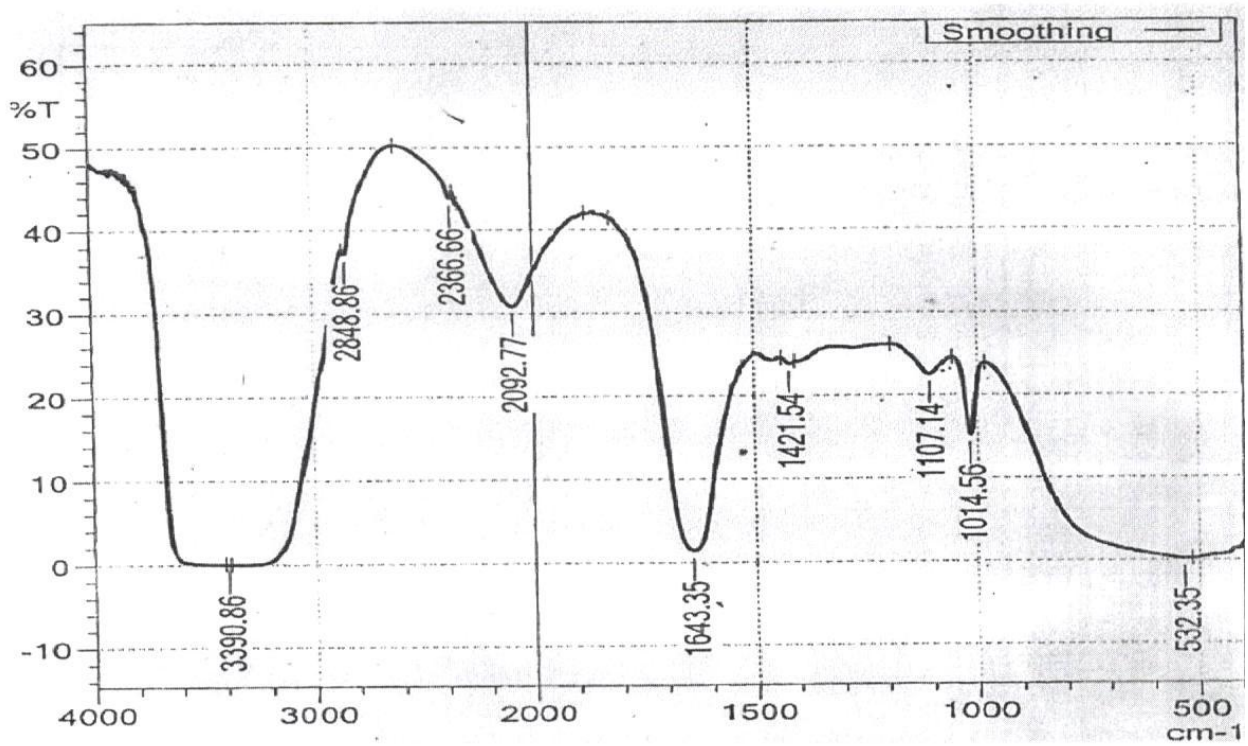



**Figure 11: FTIR spectra of Biosurfactants produced by *Providencia manganoxydans***

**Molecular identification and phylogenetic analysis of isolates**

There is a great diversity of bacteria capable of efficiently degrading atrazine (e.g., *Pseudomonas, Bacillus, Providencia, Enterobacter, Ochrobactrum, and Actinomycetes*) (Guerrero Ramírez *et al.* 2023).

The total viable count of atrazine-degrading bacteria is essential for assessing the potential for bioremediation of atrazine-contaminated sites. Compared with the water samples, the Oke Osun farm settlement samples presented a greater count of atrazine-degrading bacteria. (Table 1). The higher count in the soil samples could be a result of the availability of diverse niches, favourable soil characteristics, and reduced dilution effects. This finding agrees with previous research in the field, emphasizing the importance of soil environments for supporting the growth and metabolic activities of atrazine-degrading bacteria. Knowledge of the diversity and abundance of these organisms in polluted ecosystems is vital for developing effective bioremediation strategies to mitigate atrazine contamination. (Urseler, *et al*, 2022).

Five bacterial isolates with varying abilities to tolerate atrazine were identified: *Proteus mirabilis* (OKOS 4), *Pseudomonas aeruginosa* (OKOS 5), *Providencia manganoxydans* (OKOS 3),

*Providencia rettgeri* (FUO OS 04) and *Bacillus atrophaeus* (OSFUO 02) (Table 5). Among them, two isolates belong to the genus *Providencia*, and one belongs to the genera *Proteus*, *Pseudomonas* and *Bacillus*. The phylogenetic trees for *Providencia, Proteus, Pseudomonas* and *Bacillus* are shown in Figures 12, 13, 14, 15 and 16.

The 16S RNA sequences of the isolates have been deposited in the NCBI database, with accession numbers assigned as shown in Table 9.

**Table 9: BLAST PREDICTION**

| Isolate code | Predicted organism | Percentage ID | GenBank Accession number | Strain |
|---|---|---|---|---|
| 2 | *Proteus mirabilis* | 83.94% | PZ522006.1 | OKOS 04 |
| 22 | *Providencia rettgeri* | 99.80% | PX768974.1 | FUO OS 04 |
| 31 | *Pseudomonas aeruginosa* | 99.19% | PQ314333.1 | OKOS 5 |
| 33 | *Providencia manganoxydans* | 99.21% | PQ328881.1 | OKOS 3 |
| 35 | *Bacillus atrophaeus* | 93.79% | PZ517645.1 | OSFUO 02 |

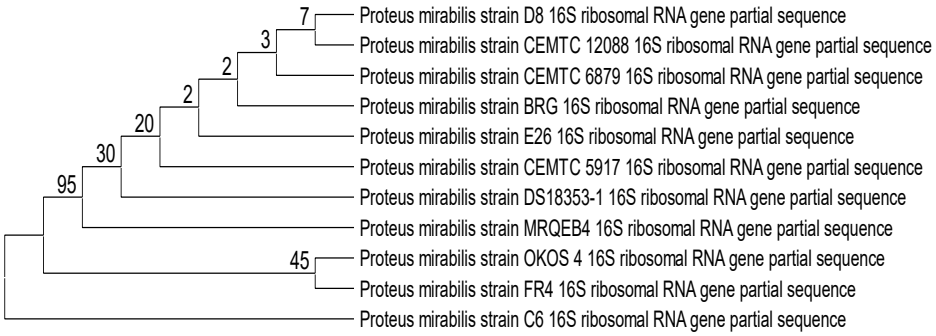



**Figure 12: Neighbour-joining phylogenetic tree of the *Proteus mirabilis* (02) species used in this study in relation to other *Proteus* species**

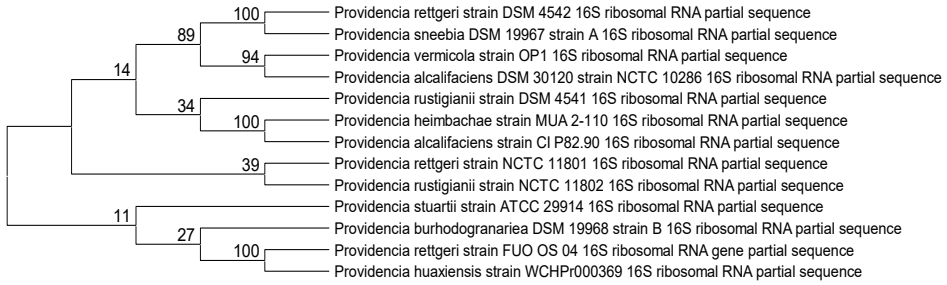



**Figure 13: Neighbour-joining phylogenetic tree of *Providencia rettgeri* (22) species used in this study in relation to other *Providencia* species**

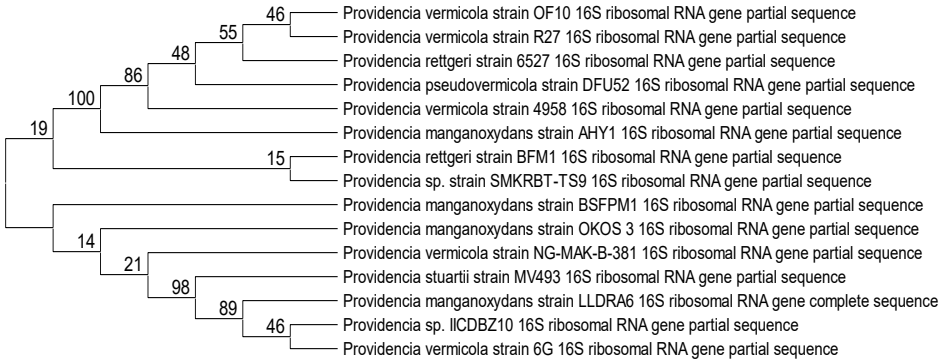



**Figure 14: Neighbour-joining phylogenetic tree of *Providencia manganoxydans* (33) species used in this study in relation to other *Providencia* species**

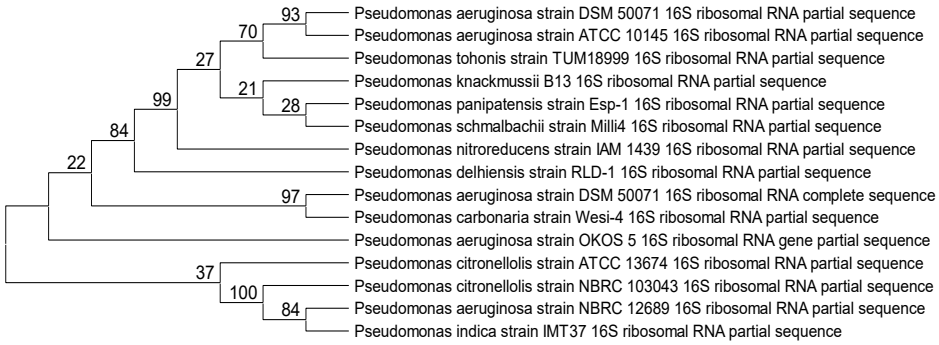



**Figure 15: Neighbour-joining phylogenetic tree of *Pseudomonas aeruginosa* (31) OKO 5 in relation to other *Pseudomonas* species**

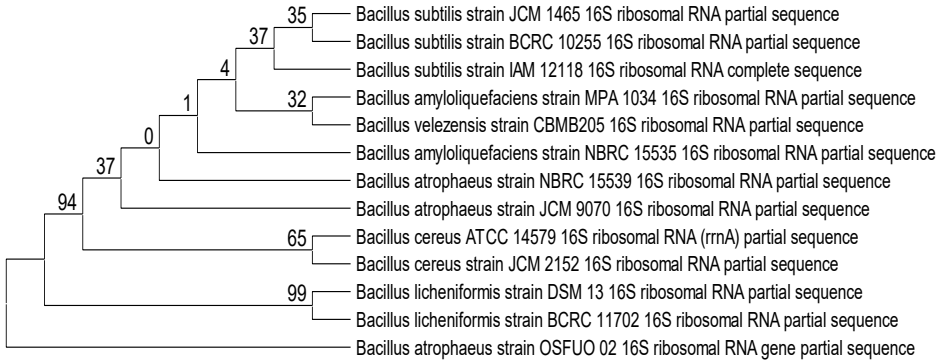



**Figure 16: Neighbour-joining phylogenetic tree of *Bacillus atrophaeus* (35) species used in this study in relation to other *Bacillus* species**

**Conclusion**

In this study, atrazine-tolerant bacterial strains were isolated from representative soil and water samples from the Oke Osun Farm Settlement. The soil samples had more atrazine-tolerant bacteria than the water samples did, even though the water samples should have had a higher level of atrazine pollution from runoff into aquatic systems. This is because the physicochemical properties of soil environments make them more likely to support microbial growth. Five different types of bacteria were identified and characterized. Two of them were from the genus *Providencia* (*Providencia manganoxydans* and *Providencia rettgeri*), and the others were identified as *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Bacillus atrophaeus*. These strains all had different levels of tolerance to atrazine and some potential for biosurfactant production.

Optimization of the culture conditions indicated that 35–40 °C, pH 7–9, an inoculum size of 6% and an agitation rate of 150–200 rpm are the optimum conditions for attaining maximum atrazine degradation. When sucrose and sodium nitrate were used as sources of carbon and nitrogen, respectively, *Providencia manganoxydans* had the highest biomass yield and degradation potential of all the isolates. These results show how important nutrition and the environment are for microorganisms that can breakdown atrazine.

Additionally, the importance of these isolates resides not only in their degradation abilities but also in their capacity to produce biosurfactants, as evidenced by positive drop collapse tests, hemolytic activity, and satisfactory emulsification indices (52–57%). These findings indicate that their biotechnological potential extends beyond simple degradation, positioning them as viable candidates for biosurfactant-assisted bioremediation strategies in atrazine-contaminated environments. Consequently, the results of this study establish a technological basis for the bioremediation of atrazine pollution and underscore the need for additional investigations into the molecular mechanisms required to attain this objective via the identified isolates.

Owing to its ability to tolerate atrazine, *Providencia manganoxydans* has significant potential for bioremediation applications. Its efficient atrazine tolerance capabilities, especially under optimized conditions, make it a promising candidate for remediating atrazine-contaminated environments. By harnessing the intrinsic abilities of *Providencia manganoxydans,* it is possible

to reduce atrazine levels and mitigate the environmental risks associated with this persistent herbicide, which aligns with the Sustainable Development Goals (SDG 15) of environmental protection, restoration, reversal of land degradation and sustainable soil management.

REFERENCES


Abdel-Mawgoud, A. M., Aboulwafa, M. M. and Hassouna, N. A. H. (2009). Characterization of rhamnolipid produced by *Pseudomonas aeruginosa* isolate Bs20. *Applied biochemistry and biotechnology*, *157*(2), 329-345.

Ahmad, M. F., Ahmad, F. A., Alsayegh, A. A., Zeyaullah, M., AlShahrani, A. M., Muzammil, K., Saati, A. A., Wahab, S., Elbendary, E. Y., Kambal, N., Abdelrahman, M. H. and Hussain S. (2024). Pesticides' impacts on human health and the environment, with their mechanisms of action and possible countermeasures. *Heliyon.* **4**;10(7)

Alori E. T, Gabasawa A. I, Elenwo C. E and Agbeyegbe O. O (2022). Bioremediation techniques as affected by limiting factors in soil environment. *Front. Soil Sci*. 2:937186. doi:10.3389/fsoil.2022.937186

Atterling Brolin, K., Schaeffer, E., Kuri, A., Rumrich, I.K., Schumacher Schuh, A.F., Darweesh, S.K.L., Kaasinen, V., Tolppanen, A.-M., Chahine, L.M. and Noyce, A.J. (2025), Environmental Risk Factors for Parkinson's Disease: A Critical Review and Policy Implications. Mov Disord, 40: 204-221. https://doi.org/10.1002/mds.30067

Ayilara M. S and Babalola O. O (2023). Bioremediation of environmental wastes: the role of microorganisms. *Front. Agron*. **5**:1183691. doi: 10.3389/fagro.2023.1183691

Buaroy, C., Detchomphoo, K., Pasriwong, N., Thanee, L., Kongruang, S. (2025). Effect of agitation rate for enhanced growth, metabolite production and kinetic modelling of *Bifidobacterium bifidum* fermentation. *International Journal of Applied Science and Engineering,* 22, 2025162. https://doi.org/10.6703/IJASE.202512_22(4).001

Chowdhury, I. F., Doran, G. S., Stodart, B. J., Chen, C., & Wu, H. (2020). Trifluralin and Atrazine Sensitivity to Selected Cereal and Legume Crops. *Agronomy*, **10**(4), 587. https://doi.org/10.3390/agronomy10040587

Chowdhury, I. F., Rohan, M., Stodart, B. J., Chen, C., Wu, H. and Doran, G. S. (2021). Persistence of atrazine and trifluralin in a clay loam soil undergoing different temperature and moisture conditions. *Environ Pollut.* **1**;276:116687. doi: 10.1016/j.envpol.2021.116687.

Darshan, R. and Yogesh, P. (2021). Bioremediation of Metals, Metalloids, and Nonmetals. (chapter 2). In "Microbial Rejuvenation of Polluted Environment, Microorganisms for Sustainability 26", D. G. Panpatte, Y. K. Jhala (eds.), Springer Nature Singapore Pte Ltd. https://doi.org/10.1007/978-981-15-7455-9_2

Elgaber, A.K.A.; Yasin, E.H.E.; Mustafa, M.; Zubairu, A.M.; Czimber, K.; Osman, A.G.; Elsheikh, E.A.E. (2025). Impact of NPK, Plant Residue, Soil Type, and Temperature on the Half-Life of Atrazine Herbicide. *Nitrogen*, **6**, (3) 79. https://doi.org/10.3390/nitrogen6030079

Elshafei, A.M. and Mansour, R. (2024). Microbial bioremediation of soils contaminated with petroleum hydrocarbons. *Discov. Soil* **1**, 9. https://doi.org/10.1007/s44378-024-00004-5 *Environment and Social Science* **11**(1):238-255 doi.org/10.56160/jaeess/2025/11/1/020

Esqueda-Esquivel, A. V., Uribe-Gómez, S., Zetina-Lezama, R., Reynolds-Chávez, M. A. and Peralta-Antonio, N. (2025). Comparison of natural and chemical herbicides for weed management in Veracruz, Mexico. *Tropical and Subtropical Agroecosystems*. 28. 10.56369/tsaes.6447.

Guerrero Ramírez, J. R., Ibarra Muñoz, L. A., Balagurusamy, N., Frías Ramírez, J. E., Alfaro Hernández, L., & Carrillo Campos, J. (2023). Microbiology and Biochemistry of Pesticides Biodegradation. *International journal of molecular sciences*, *24*(21), 15969. https://doi.org/10.3390/ijms242115969

Hassaan, A. M. and El Nemr, A. (2020). Pesticides pollution: Classifications, human health impact, extraction and treatment techniques. *Egyptian Journal of Aquatic Research*. **46.** (3) 207-220. https://doi.org/10.1016/j.ejar.2020.08.007

Khatoon, H. and Rai J. P. N. (2020). Optimization studies on biodegradation of atrazine by *Bacillus badius* ABP6 strain using response surface methodology. *Biotechnol Rep* (Amst). 26:e00459. doi: 10.1016/j.btre.2020.e00459.

Kinyua, A., Mbugua, J. K., Waswa, A. G., Mbui, D. N., Kithure, J., Michira, I. and Wandiga, S. O. (2022). Bio-Remediation of Lambda Cyhalothrin, Malathion and Chlorpyrifos Using Anaerobic Digestion Bio-Slurry Microbes. *Medicon Agriculture & Environmental Sciences* 2(5) 03-12.

Kosimov, D., Ergashev, R., Mavjudova, A. and Kuziev, S. (2025). Organophosphorus Pesticide Degradation by Microorganisms: A Review. *Front Biosci* (Elite Ed). 26;17(3):38805. doi: 10.31083/FBE38805. PMID: 41074463.

Kumar, M., Yadav, A. N., Saxena, R., Paul, D. and Tomar, R. S. (2021). Biodiversity of pesticides degrading microbial communities and their environmental impact. *Biocatalysis and Agricultural Biotechnology*, **31**,101883, doi.org/10.1016/j.bcab.2020.101883.

Liang, Y., Ding, L., Song, Q., Zhao, B., Wang, S., and Liu, S. (2022). Biodegradation of atrazine by three strains: identification, enzymes activities, and biodegradation mechanism. *Environmental Pollutants and Bioavailability*, **34**(1), 549–563. https://doi.org/10.1080/26395940.2022.2151515

Maijama'a, S., Yuguda, A. U., Tafida, U. I., Ibrahim, A. S., Yahaya, A., Adamu, H. and Sabo, A. (2024) ). Influence Of Some Selected Environmental Factors on the Natural Attenuation of Atrazine In Tropical Agricultural Soils. *Open Journal of Environmental Research* (ISSN 2734-2085). **5**. 77-88. 10.52417/ojer.v5i1.617.

Maiva, I. M. (2025). Examining The Pollution Risks of Agrochemicals and Paths To Environmental Sustainability: A Systematic Review. *Journal of Agricultural Economics,*

McLeod, M., Barwell, S., Holyoak, T. and Thorne, R. (2024). A structural perspective on the temperature-dependent activity of enzymes. bioRxiv : the preprint server for biology. 10.1101/2024.08.23.609221.

Meena, R. S., Kumar, S., Datta, R., Lal, R., Vijayakumar, V., Brtnicky, M., Sharma, M. P., Yadav, G. S., Jhariya, M. K., Jangir, C. K., Pathan, S. I., Dokulilova, T., Pecina, V. and Marfo, T. D. (2020) Impact of Agrochemicals on Soil Microbiota and Management: A Review. *Land* **9**, 34; doi:10.3390/land9020034 www.mdpi.com/journal/land

Merotto, A., Gazziero, D., Oliveira, M., Scursoni, J., Garcia, M., Figueroa, R. and Turra, G. (2022). Herbicide use history and perspective in South America. *Advances in Weed Science*. **40**. 10.51694/AdvWeedSci/2022;40:seventy-five010.

Mirzavand, S., Aeini, M., Mirzaei Najafgholi, H., Moazen-Rezemehale, H. (2023). Identification and Characterization of Bacterial Strains Capable of Degrading Atrazine and Metribuzin Herbicides in Sugarcane Fields. *Sugar Tech*. **26**. 10.1007/s12355-023-01323-7.

Modu, M. A., Kodiya, M. A., Babangida, M. U., Kabara, A. D., Ishaq, K., Ngoshe, H. S. and

Maiva, I. M. (2025). Examining The Pollution Risks of Agrochemicals and Paths To Environmental Sustainability: A Systematic Review. *Journal of Agricultural Economics, Environment and Social Science* **11**(1):238-255 doi.org/10.56160/jaeess/2025/11/1/020

Muhammad, F., Yusuf, F., Ahmad, F. A., Shehu, U., Yakasai, H. M. (2021). Optimizing the Effect of pH and Temperature on Atrazine Degradation by *Bacillus safensis* strain BUK_BCH_BTE6 an Efficient Atrazine Tolerating Bacteria from an Agricultural Soil in Kura Local Government Area of Kano State, *Nigeria Nig. J. Biotech*. 38 (2) :92-100 DOI: https://dx.doi.org/10.4314/njb.v38i2.10

Mukherjee, S., Narula, R., Bhattacharjee, S., Dutta, D., Bose, I., Mahakud, J., Paul, S., Bhattacharjee, S. and Paul, S. (2021). Bioremediation: the eco-friendly solution to the hazardous problem of environmental pollution. *Journal of Environmental Engineering and Landscape Management*, *29*(4), 477–483. https://doi.org/10.3846/jeelm.2021.14439

Paul, S. K., Mazumder, S. and Naidu, R. (2024). Herbicidal weed management practices: History and future prospects of nanotechnology in an eco-friendly crop production system. *Heliyon.*;10(5):e26527. doi: 10.1016/j.heliyon.2024.e26527. PMID: 38444464; PMCID: PMC10912261.

Reyad, A., Radwan, T., Ibrahim, W. and Essa, A. (2017). Occurrence of Atrazine Biodegrading Bacterium *"Ochrobactrum oryzae"* In Agricultural Wastewater. *Egyptian Journal of Botany*, *57*(2), 303-316. doi: 10.21608/ejbo.2017.647.1032

Sami, S. A., Akter, B., Nirob, N. H., Jahan, I., Lokman, M., Khatun, M. H., Hossain, M. K. and Barmon, J. (2025). A comprehensive review on environmental dynamics of chlorpyrifos: Fate, toxicity, remediation, and regulatory aspects. *Ecotoxicology and Environmental Safety*, 308, https://doi.org/10.1016/j.ecoenv.2025.119484.

Shah, B. A., Malhotra, H., Papade, S. E., Dhamale, T., Ingale, O. P., Kasarlawar, S. T. and Phale, P. S. (2024). Microbial degradation of contaminants of emerging concern: metabolic, genetic and omics insights for enhanced bioremediation. *Front Bioeng Biotechnol*. 12:1470522. doi: 10.3389/fbioe.2024.1470522. PMID: 39364263; PMCID: PMC11446756.

Sharma, I. (2021). "Bioremediation Techniques for Polluted Environment: Concept, Advantages, Limitations, and Prospects," Chapters, in: Mario Alfonso Murillo-Tovar & Hugo Albeiro

Saldarriaga Norena & Agnieszka Saeid (ed.), Trace Metals in the Environment - New Approaches and Recent Advances, IntechOpen.

Subberwal, M., Giles, M., Neilson, R., Roberts, D., Caul, S., Susan Mitchell and Ijaz U. Z. (2025). From farm to microbe: organic amendments and soil texture as drivers of soil Microbiome composition. *Environmental Microbiome* (2025) 20:158 https://doi.org/10.1186/s40793-025-00815-1

Urseler, N., Bachetti, R., Carolina, M. and Agostini, E. (2022). Bioremediation Strategies to Mitigate the Impact of Atrazine on the Environment: Recent Advances and Prospects. 10.1007/978-981-16-9310-6_21.

von Redwitz, C., Andert, S., Bensch, J., Forster, R., Schatke, M., Strehlow, B. and Ulber, L. (2025). Enhancing Arable Weed Diversity by Reduced Herbicide Use?. *Journal of Crop Health.* **77**. 10.1007/s10343-025-01127-7.

Werder, E. J., Engel, L. S., Satagopan, J., Blair, A., Koutros, S., Lerro, C. C., Alavanja, M. C., Sandler, D. P., & Beane Freeman, L. E. (2020). Herbicide, fumigant, and fungicide use and breast cancer risk among farmers' wives. *Environmental epidemiology (Philadelphia, Pa.)*, *4*(3), e097. https://doi.org/10.1097/EE9.0000000000000097

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