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Isolation and Characterization of Bacteria Responsible for the Degradation of Tributyltin (TBT) from Freshwater Sediment

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Abstract:
Bacteria in freshwater sediments usually break down Tributyltin compound (TBT) that has been discharged into the aquatic environment. The current study was conducted to isolate and characterize bacteria responsible for the degradation of TBT from freshwater sediments. Five distinct locations in River Benue, Makurdi, Benue state, were selected to collect samples of sediments using an Eckmangrab (Wildlife Supplies Co., Nig.), from a depth of 10 meters below the surface, placed into sterile polythene bags, and sent immediately to the laboratory for examination. Following homogenization, the sediment samples were divided into three treatments, denoted A, B, and C. Treatment A underwent heat treatment, treatment B was taken as control and TBT was added to treatment C. The bacterial identification was done using morphological and biochemical characters and the degradation experiment was done using the pour plate method. The total viable counts of freshwater sediments show that sediment A (heat treated) days 0, 14th, and 28th has the lowest total viable count of (0.00Cfu/g) while water sediment B (control) day 14th has the highest total viable count of ($6.1 \times 10^1 \pm 3.5 \times 10^1$CFU/g). Bacteria isolates include *Bacillus spp., Enterobacter, Shigella* spp.*, Staphylococcus species*, *Pseudomonas* spp., and *Escherichia coli*. Both *Bacillus* and *Pseudomonas* species have the highest percentage prevalence of 30.77% each and *Shigella* spp., has the lowest of 3.85% across samples. The observed TBT degradation efficiency and resistivity under the studied conditions suggested that *Bacillus* and *Pseudomonas* were more efficient probably due to their ability to withstand till 42 days of the experiment. There is still considerable interest in using these organisms to remove heavy metals and harmful organic contaminants. Therefore, further studies should be performed on this, in order to use bacteria in controlling organotins (TBT) environmental pollution.

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INTRODUCTION

Tributyltin is one of the most hazardous compounds frequently used as a wood preservative and antifouling paint for boats, ships, marine cages, fishing nets, and docks owing to its biocidal properties (Bangkedphol *et al.*, 2009; Omae, 2003). Its distribution in the aquatic environment has seriously harmed the coastal ecology, particularly the living biota (Choi *et al.*, 2023; Ebah *et al.*, 2016; Parmentier *et al.*, 2019).

Tributyltin compounds build up in harbor waters, higher organisms, and sediments due to increased maritime activity, erosion, and transport (Kline *et al.*, 1989; Zhakovskaya *et al.*, 2022). Freshwater and marine harbor settings, which are mostly connected to boating activities, have been found to contain higher levels of TBT (Bandara *et al.*, 2021).

The use of TBT and its derivatives has raised concerns about possible harm to the aquatic environment, as TBT is harmful at low concentrations to organisms that are not its intended targets. Moreover, it interacts with the endocrine system in humans and other organisms (Ebah *et al.*, 2016; Metcalfe *et al.*, 2022). It damages the body, has an impact on reproduction, immune system, and induces imposex, but its carcinogenic potential is still unknown (da Silva *et al.*, 2023; Wu *et al.*, 2020).

Despite being categorized as a persistent organic pollutant (POP), a significant quantity of TBT has already been released into the terrestrial and aquatic environments (Ebah *et al.*, 2016). TBT buildup in ambient media and organism tissue has become a significant problem, especially as it may be biomagnified in the food chain and has a major negative influence on humans, the top predator (Sham *et al.*, 2020).

Biodegradation is an efficient method by which microorganisms become acclimated to appropriate conditions leading to sustainable remediation. The amount of TBT can be reduced via biodegradation to a level where it has no effect on living things. Numerous environmental contaminants are known to be effectively degraded by marine microbes (Ebah *et al.*, 2016). The study aims to isolate and identify bacteria responsible for the degradation of tributyltin from freshwater sediments.

MATERIAL AND METHODS

Sample collection

Five separate locations in River Benue, Makurdi, Benue state, provided samples of sediments. The sediments were extracted using an Eckman grab (Wildlife Supplies Co., Nig.) at a depth of 10 meters below the surface from five distinct locations in River Benue, Makurdi, Benue state. They were then placed into sterile polythene bags and sent to the laboratory for examination for examination.

Experimental setup

The sediment samples are collected from five locations within Makurdi; waterworks JOSTUM, River Benue behind BSU, Old Bridge Makurdi, Wurukum under bridge, and Wadata River. These sediments were homogenized and allocated into 3 treatments labeled A, B, and C and amended with NPK (20:15:15). Treatment A underwent heat treatment, treatment B was taken as control, and TBT was added to treatment C. These treatments, which were made in triplicate, are described as follows:

Treatment A: 1kg NPK+ Heat treated Sediment+1000ml of water from the sample site.

Treatment B: 1kg NPK+ sediment as control +100mlof water from the sample site.

Treatment C: 3.0 mM TBT+1kg NPK+20kg Sediment+1000ml of water from the sample site.

Treatment choices were manually mixed twice a day to ensure adequate aeration, and samples were taken for examination on days 0, 14, 28, and 42, respectively.

Determination of total viable counts

The spread plate technique (Iqbal *et al.*, 2015) was used to determine total viable counts of bacteria that consume organotin TBT on Nutrient agar, MacConkey agar, and Manitol salt agar (MSA) supplemented with 3 mM TBT. A control sample was plated without TBT. In order to make a tenfold serial dilution of the samples, 1 g of sediment samples were diluted with 9 ml of physiological saline. The plates were spread-plated with 10^{-2} dilutions in duplicate, although dilution up to 10^{-3} was made. The plates were incubated at 28˚C room temperature for 48 hours. Colony forming units (CFU/g) were used to measure viability

Bacterial identification

The macroscopic characteristics of the bacteria on the plates included visible parameters such as colony color and shape. The isolates were identified by their microscopic, cultural, and biochemical traits (Hussain *et al.*, 2016; Iqbal *et al.*, 2015; Mohammad *et al.*, 2021; Saleem *et al.*, 2020).

Statistical analysis

Data was analyzed using SPSS version 20 software. Results were presented through tables. The statistical significance of means was measured by using the ANOVA. A ($P<0.05$) was considered as statistically significant. The means ± standard errors of the means were used to express results.

RESULTS

The total viable counts of freshwater sediments showed that the water-sediment A (heat treated) day 0,14th and 28th has the lowest total viable count of (0.00 CFU/g) while water sediment B (control) day 14th has the highest total viable count of ($6.1x10^1 \pm 3.5x10^1$ CFU/g), as shown in Table (1).

Table 1. Total viable count of bacterial isolates from freshwater sediments.

Samples/Sediments	Day 0 (CFU/g)	Day 14 (CFU/g)	Day 28(CFU/g)	Day 42 (CFU/g)
Sample A (heat treated)	0.00± 0.00	0.00± 0.00	0.00± 0.00	6.0x10±1.2x10
Sample B (control)	3.0x10±4.6x10	$6.1x10^1 \pm 3.5x10$	$1.10x10^2 \pm 2.3x10$	$1.0x10^1 \pm 1.7x10$
Sample C (TBT)	3.2x10±4.6x10	4.5x10±0.7x10	$1.5x10^1 \pm 0.7x10$	$3.0x10^1 \pm 2.8x10$

The bacteria isolated from the sediments were *Bacillus* spp., *Enterobacter* spp., *Shigella* spp., *Staphylococcus* species, *Pseudomonas* spp., and *Escherichia coli*. Table 2 displays the cultural, morphological, and biochemical traits of the isolates.

The percentage prevalence of the bacterial isolates across treatments, *Bacillus* spp. and *Pseudomonas* species had the highest percentage prevalence of 30.77% while *Shigella* spp. had the lowest of 3.85% across samples (Table 3).

Table 2: Cultural, morphological, and biochemical characteristics of bacterial isolates.

Colony Colour	Colony Shape	Morphology	Gram's Reaction	Motility	Catalase	Citrate	Urease	Indole	H_2S	Probable Isolates
Pale	Circular	Rods	-	+	+	+	-	-	-	*Enterobacter* spp.
Cream	Circular	Cocci	+	-	+	+	+	-	-	*Staphylococcus* spp.
Pale	Circular	Rods	-	+	+	-	-	+	-	*Escherichia coli*
White	Irregular	Rods	+	+	+	+	-	-	-	*Bacillus* spp.
Pale	Circular	Rods	-	-	+	-	-	-	-	*Shigella* spp.
Greenish	Circular	Rods	-	+	+	+	-	-	-	*Pseudomonas* spp.

KEY: + = Positive; - = Negative; H_2S = Hydrogen Sulphide

Table 3. Percentage prevalence of bacterial isolates across treatments.

Days	***Enterobacter* spp.**	***Staphylococcus* spp.**	***Escherichia coli***	***Bacillus* spp.**	***Shigella* spp.**	***Pseudomonas* spp.**	**Total**
Sample A (Heat treated)	0(0.00)	1(3.85)	0(0.00)	1(3.85)	0(0.00)	0(0.00)	2(7.69)
Sample B (Control)	2(7.69)	3(11.54)	3(11.54)	4(15.38)	1(3.85)	4(15.38)	17(65.38)
Sample C (Tributyltin)	0(0.00)	0(0.00)	0(0.00)	3(11.54)	0(0.00)	4(15.38)	7(26.92)
Total	2(7.69)	4(15.38)	3(11.54)	8(30.77)	1(3.85)	8(30.77)	26(100)

DISCUSSION

From the study carried out in this research it was seen that water sediment A (heat treated) days 0,14th and 28th has the lowest total viable count of (0.00), while the water-sediment B (control) day 14th has the highest total viable count of ($6.1 x 10^1 \pm 3.5 x 10^1$).

The bacteria isolated from the freshwater sediments were *Bacillus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Streptococcus* spp., *Staphylococcus* spp., *Escherichia coli,* and *Shigella* species. Our results are in agreement with a previous study (Ebah *et al.*, 2016).

Bacillus spp. and *Pseudomonas* species have the highest percentage prevalence of 30.77% and *Shigella* spp. having the lowest of 3.85% across samples. Thereby *Bacillus* spp. and *Pseudomonas* species have the highest occurrence because they have more degrading capacity than the rest, while *Shigella* spp. has the lesser degrading capacity to TBT.

The two isolates that exhibited unique growth in the presence of elevated TBT concentrations were determined to be *Pseudomonas* species and *Bacillus* spp. *Pseudomonas* and *Bacillus* species isolated from environmental matrices have been identified to have the capabilities to degrade polyethylene, polypropylene, polyvinyl chloride, polystyrene terephthalate, and other environmental organo-metals (Wilkes and Aristilde, 2017). According to their morphological and biochemical characteristics, these bacteria mostly obtain their carbon from TBT. Therefore, *Bacillus* and *Pseudomonas* species are able to degrade TBT into simpler carbon (Dubey and Roy, 2003).

This study clearly showed that some bacterial species are capable of degrading TBT. It's interesting to note that these bacterial isolates use TBT as their source of carbon thereby reducing the organotin to a less toxic substance that may pollute the environment.

CONCLUSION

Organotin chemicals contaminated the samples of freshwater sediment that were collected. The polluted sediments were successfully used to isolate TBT-degrading bacteria. Two TBT-degrading bacteria with high TBT resistivity were identified after examining the capacity of isolates. The six isolated bacteria were *Bacillus spp., Pseudomonas* spp., *Enterobacter* species, *Staphylococcus* spp., *Escherichia coli,* and *Shigella* species. TBT degradation efficiency and resistivity measured under the investigated conditions indicated that *Bacillus* and *Pseudomonas* were more efficient due to their high occurrence in the samples. The bacteria degrade the TBT into simpler carbon which serves as their main source of nutrients, thereby reducing the toxic content of the TBT to the environment.

RECOMMENDATIONS

- There is still a great deal of interest in using these organisms to remove heavy metals and harmful organic contaminants. Therefore, further studies should be performed on this, in order to use bacteria in controlling organotins (TBT) environmental pollution.

- The bacteria can be used as a source of degrading organotins (TBT) compounds in the environment.

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CONFLICT OF INTEREST

Authors hereby declare that they have no conflict of interest.

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