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Biodegradation of Combine Tributyltin and Diphenyltin by Bacteria in Freshwater Sediment

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Abstract:
This study investigated the inherent capability of bacterial isolates from River Benue to resist and degrade the combination of tributyltin (TBT) and diphenyltin (DPT) in aerobic conditions. TBT and DPT have been used as active ingredients in the formulation of biocides, fungicides, herbicides, and antifouling paints. Freshwater sediments were collected from 5 (five) different points of River Benue. The sediment samples were homogenized and allocated into 3 treatments labeled A, B, and C. Treatments A and B were amended with NPK, treatment B was heat treated, and treatment C was left unaltered. Treatment options were stirred manually once a day for proper aeration and were cultured using the pour plate method on days 0, 14, and 24, 42 respectively. Physiochemical analysis of freshwater sediments was conducted using the standard method. Bacterial strains were identified based on morphological and biochemical characteristics. The incidence of bacterial isolates was *Staphylococcus* spp. (10.53%), *Pseudomonas* spp. (42.11%), *Shigella* spp. (5.30%), *Escherichia coli* (10.53%), and *Bacillus* spp (31.58%). The results from this study showed that *Bacillus* spp. and *Pseudomonas* spp. were able to survive in the presence of TBT and DPT combined till day 42 having a percentage prevalence of 31.58% and 42.11% respectively. Findings from this study suggest that *Pseudomonas* spp. and *Bacillus* spp. hold significant potential for bioremediation of organotin-contaminated sites.

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INTRODUCTION

Organotin compounds (OTCs) are a broad class of organometallic compounds with at least one tin atom covalently bound to a carbon atom. The general formula for OTCs is 'RSnX,' and are classed as mono, di, tri, and tetra-organotin compounds (RSnX3, R2SnX2, R3SnX, and R4Sn) where 'R' represents an organic group, such as phenyl, alkyl, and 'X' is an anion, such as oxide, fluoride, or chloride, etc. (Arraq and Hadi, 2023; Barbosa *et al.*, 2022). Organotin compounds (OTCs) have been detected in various water settings such as ports, harbors, and coastal regions (Concha-Graña *et al.*, 2021). OTCs are widely used and resistant to deterioration, which means they stay in the environment for a very long period. OTCs are pollutants in various habitats owing to their large annual production volumes, widespread usage, and great stability in marine water (Cole *et al.*, 2018; Dai *et al.*, 2022).

Additionally, in aquatic settings, OT chemicals interact with conventional pollutants such as metal/metalloid ions, nanomaterials, and organic contaminants (OCs), which can be hazardous to species when combined. There is a major risk to both human health and the ecology from heavy metal poisoning in the soil (Iqbal *et al.*, 2020; Iqbal *et al.*, 2019). Rapid urbanization and industrialization have made organotin chemicals in the soil a severe hazard to many communities, particularly in developing nations, exposing many people to harmful ecological and health concerns (Landrigan *et al.*, 2020; Tudi *et al.*, 2022).

Certain types of organotin are found in soil naturally as a result of parent material weathering processes, but at trace (<1000 mg kg^{-1}) and seldom harmful amounts. The majority of soils in rural and urban environments may accumulate one or more organotins above specified thresholds that are high enough to pose risks to human health, plants, animals, ecosystems, or other media (de Carvalho Oliveira and Santelli, 2010). In gastropods, organotins such as tributyltin (TBT) and diphenytin (DPT) cause sexual aberrations (Heindel *et al.*, 2017). Bonefish will exhibit a decrease in phagocytic activity when exposed to highly immunotoxic dibutyl phthalate (DBP) (Zhang *et al.*, 2021). Many plant species have the potential to remove metal compounds from the soil and water sources (Ashraf *et al.*, 2021; Iqbal and Ashraf, 2018; Sattar *et al.*, 2018; Sultana *et al.*, 2019a; Sultana *et al.*, 2019b).

Human exposure to TBT can occur through the consumption of tainted drinking water, beverages, and seafood in particular (Borghi and Porte, 2002). There have been reports of high OTC concentrations in products derived from marine fisheries. As a result, it is anticipated that the human diet will contain certain OTCs that will leave residues in human tissue and blood (Okoro *et al.*, 2011; Zhang *et al.*, 2008).

The use of organometallic compounds in the environment is persistently increasing with advances in science and technology. However, these compounds are hard to break down or dissolve spontaneously since they are rather persistent. Bacteria play important roles in degrading OTC most especially TBT and DPT (Shah and Dahanukar, 2012). There are reports about the isolation of Tributyltin tolerant strains from the genus *Pseudomonas* among others (Bernat *et al.*, 2014). This study aimed to investigate the biodegradation of organotins combination of TBT and DPT by bacteria in freshwater sediment.

MATERIALS AND METHODS

Study Area

This study was conducted in Makurdi, a town, the capital of Benue state, north-central Nigeria (Figure 1). Located on latitude and longitude 7^o 55’27.96” N, 8^o 40’43.56” E. It is situated on the south bank of the Benue River. Founded about 1927 when the railroad from Port Harcourt (279 miles [449 km] south-southwest) was stretched to Jos and Kaduna, Makurdi rapidly established into a transportation and market center (Britannica, 2023).

The Makurdi people are predominantly an agricultural population, raising cash crops like rice, cotton, yams, soybeans, sesame seeds, and shea nuts. As staple foods, yams, sorghum, millet, peanuts (groundnuts), and cassava are cultivated (Britannica, 2023).

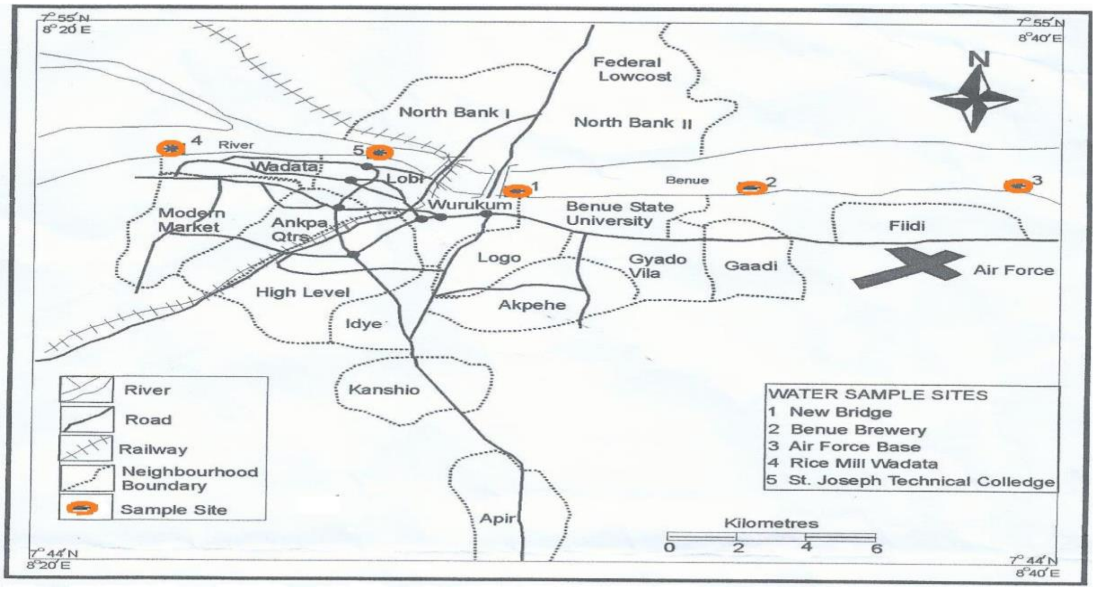

Fig. 1. Map of Makurdi Showing Sample Sites. Sourced from the Ministry of Lands and Survey Makurdi.

Sample Collection

Freshwater sediment was collected from five different points of River Benue, in Makurdi, Benue State, Nigeria. They include waterworks UAM, behind BSU, Old Bridge, Wurukum under the new bridge, and Wadata.

Sediment samples were collected from a depth of 10m from the surface with hands covered with safety gloves into a sterile polythene bag and transported immediately to the Microbiology laboratory for analysis (Ebah *et al.*, 2016; Prasad and Aranda, 2018).

Physicochemical Analysis of Sediment

Freshwater sediment (1kg) was sent to the Department of Fishery and Aquaculture, Joseph Sarwuan Tarka University, Makurdi Benue State, Nigeria, to ascertain the measure of the following physicochemical characteristics, such as pH, dissolved oxygen, total dissolved solids, electrical conductivity, and temperature following previous studies (Adesuyi *et al.*, 2015; Adowei and Bale, 2023).

Experimental Set-Up

All chemicals were used without additional purity following a previous study (Ebah *et al.*, 2016). TBT (96% purity) and DPT (96% purity). NPK fertilizer was added to the sediment to boost the microbial activities in the soil.

The sediment samples were homogenized and divided into 3 treatments labeled A, B, and C. Treatments A and B were amended with NPK, treatment B was heat treated, and treatment C was left in its natural state. These treatments were prepared in triplicate and listed as follows:

Treatment A: 1.5mm TBT + 1.5mm DPT + 20g NPK + 1kg of heat-treated sediment +1000ml freshwater.

Treatment B:1.5mm TBT + 1.5mm DPT + 1kg sediment +1000ml freshwater as control.

Treatment C: 1.5mm TBT + 1.5mm DPT + 20g NPK+1 kg Sediment + 1000 ml freshwater.

Treatment options were stirred manually once a day for proper aeration and sampling was done on days 0, 14, and 24, 42 for analysis respectively.

Preparation of Media

The various media used for this work which include Nutrient Agar, MacConkey Agar, and Mineral Salt Agar were prepared according to the manufacturer's instructions. The media was sterilized by autoclaving at 121°C for 15 minutes and when cooled poured into sterile petri dishes.

Identification and Characterization of Isolates

Characteristics of bacteria growth on the plate were macroscopically observed for visible features like the color of colonies and the shape formed. The isolates were identified using their microscopic, cultural, and biochemical characteristics (Hussain *et al.*, 2016; Iqbal *et al.*, 2016; Khalid *et al.*, 2016; Mohammad *et al.*, 2021; Saleem *et al.*, 2018).

Enumeration of Bacteria

The Spread plate method was used for the enumeration of bacteria for colony count from freshwater sediment samples. After overnight incubation colonies were counted and colony-forming units per milliliter (CFU/mL) were determined (Iqbal *et al.*, 2015).

Statistics Analysis

Data was entered and analyzed using statistics package for social science version software (20). The result was presented through tables; the statistical significance of means was measured by using the ANOVA. A ($p<0.05$) was considered as statistic significant. All results are expressed as the mean ± standard error of the mean.

RESULTS

The results of physicochemical parameters of freshwater sediment completed from 5 different points of Rivers Benue in Makurdi town are presented in Table 1. Sample UAM had the highest pH of 8.5, dissolved oxygen of 7.4 ml/dl, total dissolved Solid of 63 g/l, electrical conductivity of 109 s/m, and temperature of 29 °C while Sample BSU had the pH of 7.9, dissolved oxygen of 3.4 ml/dl, and total dissolved solid of 30 g/l, the electrical conductivity of BSU sample was 56 s/m with the temperature of 29.1°C. Sample OLD BRIDGE has pH 7.5, with dissolved oxygen of 5.2 ml/dl, and total dissolved solid of 29 g/l, the electrical conductivity of the OLD BRIDGE sample was 58 s/m with the temperature of 29.01°C. Sample WURUKUM has the pH of 7.7, dissolved oxygen of 5.5 ml/dl, and total dissolved solid of 40 g/l, the electrical conductivity of the WRUKUM sample was 81 s/m with the temperature of 28.9 °C. Sample WADATA has the pH of 7.8, dissolved oxygen of 5.8 ml/dl, and total dissolved solid of 29 g/l, the electrical conductivity of the WADATA sample was 60 s/m with the temperature of 28.9 °C respectively.

Table 1. Physicochemical Parameters of Freshwater Sediment.

SAMPLE	pH	Dissolved Oxygen (Mg/l)	Total dissolved Solid (Mg/l)	Electrical Conductivity (μs/cm)	Temperature (°C)
UAM	8.5	7.4	63	109	29.0
BSU	7.9	3.4	30	56	29.1
OLD BRIDGE	7.5	5.2	29	58	29.1
WURUKUM	7.7	5.5	40	81	28.9
WADATA	7.8	5.8	29	60	28.9

Table 2 showed the colony count of treated samples. Treatment A, has the lowest colony count of $0.0x10^1$ cfu/g, on days 0, 14, and 28, however in day 42 growth was observed with colony count of $1.4x10^4$ cfu/g also Sample B has the colony count of$1.41 \ x10^1$ cfu/g in day 0, $2.82 \ x10^1$ cfu/gin day 14, $2.82 \ x10^1$ cfu/g in day 28 and $2.12 \ x10^3$ cfu/g in day 42. Sample C was

5.66 $x10^1$ cfu/g, 4.24 $x10^1$ cfu/g, 2.82 $x10^1$ cfu/g, and 2.12 $x10^3$ cfu/g on days 0, 14, 28 and 42 respectively. Growth was observed on day 42 this may be due to contamination in the process of stirring the treatment. The highest growth was observed in sample C as 5.66 $x10^1$cfu/g, 4.24 $x10^1$ cfu/g, 2.82 $x10^1$cfu/g, and 2.12 $x10^3$cfu/g on days 0, 14, 28, and 42 respectively. There was a significant increase ($p < 0.05$) in colony counts of treatments with time (Figure 2).

Table 2. Colony count of bacteria isolates from freshwater sediment.

	DAY0 $x10^1$cfu/g	DAY14 $x10^1$cfu/g	DAY28 $x10^1$cfu/g	DAY42 $x10^3$cfu/g
A	0.00∓	0.00∓	0.00∓	1.41∓
B	1.41∓	2.82∓	2.82∓	2.12∓
C	5.66∓	4.24∓	2.82∓	2.12∓
p-value	0.00	0.00	0.00	0.02

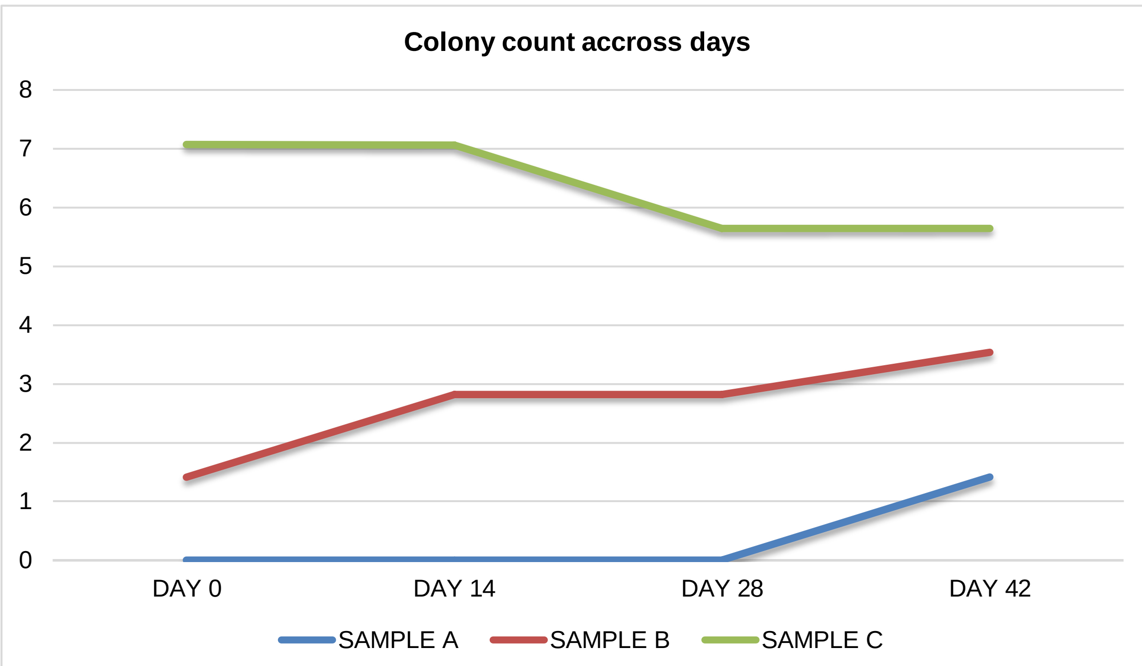

Fig. 2. A graph showing the colony count of treatment across days.

Table 3 showed the biochemical test of bacteria isolates. *Bacillus spp*. was identified with a yellow colony color, circular colony shape, and rod morphology, the gram stain test, catalase test, hydrogen sulfide test, and citrate test were positive (+) while urease test, indole test, and sulfur indole and motility test were negative (-). *Staphylococcus spp.* was also identified, with yellow colony color, round colony shape, and cocci morphology, the gram stain test, catalase test, urease test, and indole test were positive (+), hydrogen sulfide test and citrate test, and sulfur indole and motility test were negative (-). *Pseudomonas spp*. was also identified with green colony color, and irregular and rod morphology, the gram stain test was negative (-), catalase test and citrate test were positive (+), while the urease test, indole test, hydrogen sulfide test, and sulfur indole and motility test were negative (-). *Shigella spp.* was also identified with a pale colony color, circular colony shape, and rod morphology. the gram stain test was negative (+), the catalase test and indole test were also positive (+), while the citrate test, urease test, hydrogen sulfide test and sulfur indole and motility test were negative (-).*E. coli* was also identified with grayish-white colony shape, circle colony shape and rod morphology, the gram stain test was negative (-), catalase test, indole test and hydrogen sulfide test were positive (+), while citrate test, urease test, and sulfur indole and motility test were negative (-).

Table 3. Biochemical characters of isolated bacteria strains from freshwater sediment.

COC	COS	MPH	GMT	CAT	CIT	URT	IDO	H2S	SIM	BSP
Yellow	round	cocci	+	+	+	+	+	-	-	*Staphylococcus* spp.
Green	Irregular	Rod	-	+	+	-	-	-	-	*Pseudomonas* spp.
Pale	circular	Rod	+	+	-	-	+	-	-	*Shigella* spp.
Grayish white	Circular	Rod	-	+	-	-	+	+	-	*E. coli*
Yellow	Circular	Rod	+	+	+	-	-	+	-	*Bacillus* spp.

Table key: Colony Colour = COC, Colony Shape = COS, Morphology = MPH, Gram Stain Test = GMT, Catalase Test = CAT, Citrate Test = CIT, Urease Test = URT, Indole Test = IDO, Hydrogen Sulfide Test = H2S, Sulphide, Indole, Motility Test =SIM, Bacteria Specie = BSP.

Table 4 showed the percentage prevalence of bacteria isolates across treatments. It was observed that treatment A has a total percentage prevalence of 5.30% the lowest among the treatments, B and C have a total percentage prevalence of 68.42% and 26.32% respectively.

Table 5 showed the percentage prevalence across days. Day 0 has the highest prevalence rate of 36.84%. The lowest percentage prevalence count was 15.79% recorded on Day 28 of the experiment. With *Pseudomonas* spp. having a total percentage prevalence of 42.11% which is the highest among the bacteria isolates.

Table 4. Percentage prevalence of bacteria isolates across treatments.

TREATMENT	*Staphylococcus* spp	*E. coli*	*Bacillus* spp	*Pseudomonas* spp	*Shigella* spp	**TOTAL**
A	0.00%	0.00%	5.30%	0.00%	0.00%	5.30%
B	10.53%	10.53%	21.05%	21.05%	5.30%	68.42%
C	0.00%	0.00%	5.30%	21.05%	0.00%	26.32%
TOTAL	10.53%	10.53%	31.58%	42.11%	5.30%	100%

Table 5. Percentage prevalence of bacteria isolates across days.

DAY	*Staphylococcus* spp	*E. coli*	*Bacillus* spp	*Pseudomonas* spp	*Shigella* spp	**TOTAL**
DAY 0	5.30%	5.30%	10.53%	10.53%	5.30%	36.84%
DAY 14	5.30%	5.30%	5.30%	10.53%	0.00%	26.32%
DAY 28	0.00%	0.00%	5.30%	10.53%	0.00%	15.79%
DAY 42	0.00%	0.00%	10.53%	10.53%	0.00%	21.05%
TOTAL	10.53%	10.53%	31.58%	42.11%	5.30%	100%

DISCUSSION

The present study investigated the physiochemical parameters of freshwater sediment, the results showed that sample UAM has the highest pH, Dissolved Oxygen, Total dissolved Solid, and Electrical Conductivity but with a temperature of 29.0^{o}C. This may be due to waste inflow from the waterworks of Joseph Sarwuan Tarka, Makurdi which is about 1km away from the sample site according to previous studies, wastewater has the potential to increase the pH of freshwater sediment and other physicochemical parameters (Cohen and Kirchmann, 2004; Lemessa *et al.*, 2023; Rahman *et al.*, 2021). The mean of these parameters also indicates the freshwater sediment has the appropriate conditions necessary for the survival of mesophilic bacteria species such as *E. coli, Bacillus* spp*.,* and *Pseudomonas* spp*.* (Kim and Wuertz, 2015; Simões *et al.*, 2023).

A gradual reduction in the total viable count of Treatment C was observed from 5.66 x10^{1}cfu/g on day 0 to 2.12 x10^{1}cfu/g on day 42, this suggests that had affected the bacteria isolates, the surviving bacteria isolates might have the inherent capability to utilize TBT and DPT combine as their source of carbon. Furthermore, isolates were sub-cultured. Out of 5 bacteria isolates 2 isolates showed consistent growth in the presence of 3.0mm of TBT and DPT combined with an optimum physiochemical condition for growth; temperature of 28.9°C, pH 7.8, total dissolved oxygen of 5.8ml/dl, total dissolved solid of 60g/l and electrical conductivity of 60 s/m under aerobic condition. Further investigation was carried out to identify the bacteria species that possess the inherent capability to resist and degrade the combination of TBT and DPT. A series of biochemical tests were carried out on the bacteria isolates. *Escherichia coli, Staphylococus* spp*., Shigella* spp*., Bacillus* spp*.* and *Pseudomonas* spp*.* were identified. This agrees with previous studies that reported the bacteria species that can resist and degrade the combination of TBT and DPT (Ebah *et al.*, 2016; Polrot *et al.*, 2022).

The percentage prevalence of bacteria isolates across the day, *Escherichia coli* and *Staphylococcus* spp. were prevalent in the treatment from DAY 0 to DAY 14 but absent in DAY 28 and 42, this indicates that *E. coli* and *Staphylococcus* spp*.* could not survive the combination of TBT and DPT across the 42 days of this study. *Bacillus* spp. and *Pseudomonas* spp*.* having the highest percentage prevalence of 31.58% and 42.11% respectively, and were prevalence from DAY 0 to DAY 42 which is the last day of this experiment. This is also in line with previous studies in which *Pseudomonas* spp. and *Bacillus spp.* were also identified to have inherent capability of degrading TBT (Ebah *et al.*, 2016; Simões *et al.*, 2023). However, the combination of TBT and DPT in this present study holds a huge significance.

CONCLUSION

The findings presented in this study demonstrate that *Pseudomonas* spp*.* and *Bacillus* spp*.* were able to tolerate 3.0mm of TBT and DPT combined in freshwater sediment over a period of 42 days. These microorganisms might hold significant potential for the bioremediation of organotin-contaminated sites. However, it is important to note that the actual process of bioremediation on a large commercial scale remains a distant reality as the biochemical mechanisms involved in organotin biodegradation are not yet fully understood.

Comparing the treatments, it was observed that NPK-treated sediments had a higher rate of degradation compared to the unamended treatments or those treated with no nutrient amendments. Therefore, the use of nutrient amendments, such as NPK, is crucial for the efficient degradation of organotins such as TBT and DPT.

RECOMMENDATIONS

Based on the results and findings from this study, it is recommended that:

1. Further research should be conducted to investigate the biochemical mechanisms involved in the biodegradation of organotins by microorganisms such as *Pseudomonas* spp*.* and

Bacillus spp. This will provide a better understanding of the processes involved and enable the development of more efficient bioremediation strategies for organotin-contaminated sites.

2. That biostimulation or nutrient amendment, such as NPK, can be used for the degradation of organotins such as tributyltin and diphenyltin.

3. Further study should be conducted to understand metabolic pathway of biodegradation of organotin by bacteria; this will give more insight towards genetic modification to bacteria to improve bioremediation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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