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Toxicological Effects of Plastic Composted Soil on Nitrifying Bacteria

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Authors' contributions

This work was carried out in collaboration between all authors. The study idea was conceived by author EIA. Authors UU and AODO performed the laboratory work and run literature review. The manuscript was written by authors UU and JI in complete agreement with all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to evaluate the toxicological effects of plastic composted soil on some nitrifying bacteria which are *Nitrosomonas* sp. and *Nitrobacter* sp.
Methodology: Five plastic composted soil samples were collected from different locations within the Edo State Waste Management site located at Iyowa in Benin City which were merged to form a composite sample. *Nitrosomonas* sp. and *Nitrobacter* sp. were isolated from the soil samples. Plastic composted soil concentrations were prepared for LC_{50} and EC_{50} determination. *Nitrobacter* and *Nitrosomonas* acute toxicity test was carried out. Initial nitrite concentrations were determined and plates of Winograsky agar were immediately inoculated by spread plate techniques. Nitrite accumulation and utilization were also determined and inoculation by spread plate method was carried out from the various plastic composted soil concentrations after 1 h, 2 h, 3 h and 4 h time

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intervals. Plates were incubated at room temperature (28+/- 2℃) for 24 h. The percentage inhibition of bacteria (*Nitrosomonas* sp. *and Nitrobacter* sp.) was determined using the probit regression analysis in Excel Microsoft.

Results: The EC_{50} values for *Nitrosomonas* sp. are as follows; 0.52, 0.82, 1.11 and 1.23 with LC_{50} values of 27.47, 24.14, 19.74 and 16.73. It was observed that the EC_{50} values were very low which increased gradually with time, this suggest that there was a high percentage inhibition of *Nitrosomonas* sp. thereby altering and reducing the percentage nitrite accumulation by the bacteria. The EC_{50} values for *Nitrobacter* sp. are 52.00, 81.72, 111.31 and 123.13 and LC_{50} values are 25.04, 23.93, 15.94 and 13.39. The EC_{50} values for *Nitrobacter* sp. were high which indicates that there was high nitrite utilization by *Nitrobacter* sp. as the percentage inhibition gradually decreased with exposure time. The coefficient of determination showed that the bacteria response which could be either inhibition, utilization or accumulation greatly depends on the amount/concentration of the plastic contaminants present in the test soil sample.

Conclusion: The result from this study shows that the EC_{50} determination was more sensitive than the LC_{50} determination for *Nitrosomonas* sp. and that the LC_{50} determination was more sensitive than the EC_{50} determination for *Nitrobacter* sp. Also that the populations of *Nitrosomonas* sp. were more sensitive to the plastic contaminants than the populations of *Nitrobacter* sp. The results obtained from this study suggest that autotrophic transformation by nitrifying bacteria which enhances soil fertility may be hindered in an ecosystem polluted with plastics as nitrification processes will be altered.

Keywords: Toxicity; nitrifying bacteria; plastic composted soil; nitrite inhibition; nitrite utilization and accumulation.

1. INTRODUCTION

Since the beginning of industrialisation, a great variety of anthropogenic chemical compounds have been synthesised for countless uses. Some chemical groups, such as organochlorides and nitroaromatic compounds, are purposefully synthesised, while the production and incineration of some other commodities, such as polyvinyl chloride (PVC) plastic, create undesired toxic by-products. After the chemical products have served their purpose, they often end up in the environment. The microbes responsible for recycling these wastes are seldom well equipped to degrade the new types of molecules, which are therefore biodegraded slowly, if at all. The final destination of persistent contaminants is often the soil, or if they pass through a water treatment plant, either sewage sludge or sediment at the bottom of rivers, lakes or the sea, where they may accumulate, thereby rendering the environment hazardous to life [1,2]. Plastic debris in landfill also acts as a source for a number of secondary environmental pollutants [3]. Pollutants of note include volatile organics, such as benzene, toluene, xylenes, ethyl benzenes and trimethyl benzenes, released both as gases and contained in leachate [4]. Soil contamination with inorganic or organic pollutants commonly reduces the diversity or evenness of soil bacteria [5-7]. The first drawback associated with disposal of plastic waste is the fact that landfill facilities occupy space that could be utilised for more productive means, such as in agriculture [3]. Plastic components of landfill waste have been shown to persist for more than 20 years [8]. This is due to the limited availability of oxygen in landfills; the surrounding environment is essentially anaerobic [9,10]. Generally, a combination of multiple stressors, such as different pollutants or contamination exerts especially high pressure on soil communities, and the combined negative effect may not be additive but rather synergistic [11]. In the case of soil bacteria, this general ecological principle does not seem to hold; prior stress has been associated with both an increase in sensitivity and an increase in community resistance or resilience [5]. The production of persistent resting forms such as bacterial endospores under stressful conditions can result in increased resilience [5]. Dormancy in general, meaning minimal metabolic activity associated with minimal interaction with the environment, can deliver the same advantages and seems to be a common survival strategy for soil bacteria [12].

The genus *Nitrosomonas* and *Nitrobacter* belongs to a variety of nitrite-oxidizing bacteria which are responsible for the first and second

step of the nitrification process (oxidation of ammonia to nitrite and nitrite to nitrate). These bacteria were used to study the acute toxicity test of plastics composted soil on nitrogen transformation activities in the soil. Inhibition of these steps under uncontrolled conditions may lead to accumulation of ammonium and nitrite-nitrogen which is toxic [13]. Plastic contaminants have been shown to have acute effects on the biotic components of the terrestrial environment [14]. The acute toxicity effect of soil composted with plastics was conducted since the nitrification process is a function of enzyme activity (ammonia monooxygenase, hydroxylamine oxidoreductase and nitrite oxidoreductase) and its measurement has been used as an indicator of pollution [15,16]. The decline in the *Nitrobacter* sp. counts as the concentration of the plastic composted soil increased could be due to the toxic effect of plastic contaminants as earlier reported by [17]. [16] also noted that high concentrations of elements released from plastics inhibit microbial activities by causing damage or inactivating one or more critical enzymes. Toxicity studies have shown that the toxicity of plastic composted soil on nitrifying bacteria depended on the contact time and plastic composted soil concentrations which is in line with the toxicity evaluation of different insecticides concentrations on *Nitrobacter* sp. [18]. This means that at low plastic composted soil concentrations the bacteria was able to adapt and oxidize nitrite which increased with time. Also at higher plastic composted soil concentration, the bacteria growth count and metabolism was retarded even up to a hundred percent which is as a result of the inhibition of enzyme activities by the toxicant [19]. This suggest that autotrophic transformation by nitrifying bacteria may be hindered in an ecosystem polluted with these plastics as nitrification processes will be reduced.

2. MATERIALS AND METHODS

The toxicity of plastic contaminants to soil nitrifying bacteria was investigated to evaluate the toxic effects of plastic contaminants to nitrifying bacteria in the soil. Five plastic composted soil samples were collected from different locations within the Edo State Waste Management site located at Iyowa in Benin City which were merged to form a composite sample. *Nitrosomonas* sp. and *Nitrobacter* sp. were isolated from the soil samples using the methods employed by [17].

2.1 Preparation of Plastic Composted Soil Concentration for *Nitrosomonas* and *Nitrobacter* Toxicity Test

For the determination of the median lethal concentration (LC_{50}), plastic composted soil concentrations of 100, 200, 300, 400 and 500 mg/l. The median effective concentration (EC_{50}) of test soil was determined from soil concentrations of 20, 40, 60, 80 and 100 mg/l. A control experiment consisting of the $NaNO_2$ diluent only (without the plastic composted soil) was set up [18].

2.2 Nitrobacter and Nitrosomonas Acute Toxicity Test

Ninety millilitres of the test plastic composted soil was put in 250 ml volumetric flask. Ten millilitres of the bacteria standard inoculums was aseptically inoculated. Nitrite concentration was determined [20] and plates of Winograsky agar were immediately inoculated by spread plate techniques [17]. This was followed by nitrite determinations and spread plate inoculation from the various plastic composted soil concentrations after 1 h, 2 h, 3 h and 4 h time intervals. Plates were incubated at room temperature (28+/- 2℃) for 24 h. The percentage inhibitions of bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.) were determined using the formula below [21].

(%) Inhibition =

$$\frac{N_{(control)} - N_{(sample)}}{N_{(control)}} \times 100$$

$N_{(control)}$ = Number of colonies (cfu/ml) from the control sample.

$N_{(sample)}$ = Number of colonies (cfu/ml) from the test soil sample.

The percentage nitrite accumulation by *Nitrosomonas* sp. and percentage nitrite utilization by *Nitrobacter* sp. Were determined using the formula below.

(%) Accumulation or Utilization =

$$\frac{C_{(control)} - C_{(sample)}}{C_{(control)}} \times 100$$

$C_{(control)}$ = concentration of nitrite from the control sample.

$C_{(sample)}$ = concentration of nitrite from the test soil sample.

Using the probit regression analysis in excel Microsoft, the percentage inhibition values gotten for both bacteria were plotted against each of the test plastic contaminant concentration respectively. A dose response curve was prepared for the estimation of the lethal concentration (LC_{50}) which is the plastic contaminant concentration required to kill 50% of the total number of bacteria (cfu/ml) in the test system. Also, the percentage nitrite accumulation and utilization values for *Nitrosomonas* sp. and *Nitrobacter* sp. were plotted against each of the plastic contaminant concentration respectively. From the dose response curve, the effective concentration (EC_{50}) which is the plastic contaminant concentration that gives half (50%) of the maximum response was determined. The LC_{50} and EC_{50} values were calculated using the following equations generated by the values plotted, and each of these equations were selected depending on the one that best fits our dose response curve.

Equation (1): $Y = 1.485x + 1.466$

Equation (2): $Y = 4.097\ln(x)\ 2.173$

Equation (3): $Y = 2.547\ e^{0.249x}$

For LC_{50} calculation, the value for Y in the equations above is the 50% value of the total number of bacteria killed. For EC_{50}, calculation, the value for Y in the equations above is the 50% value of the total plastic contaminant concentration response. Each of these values was substituted for Y in the equations and the value gotten for X became our LC_{50} and EC_{50} values respectively [21]. All results were subjected to statistical analysis [22].

3. RESULTS AND DISCUSSION

The results from this toxicity studies showed that the toxicity of plastic contaminants on *Nitrosomonas* sp. and *Nitrobacter* sp. depended on the contact time and plastic contaminant concentration, It was observed that there was an increase in the percentage inhibition of both bacteria (Fig. 1A and B) with increased plastic contaminant concentration and exposure time. There was an increase in nitrite accumulation and nitrite utilization at lower plastic contaminant concentration which began to decrease as the plastic contaminant concentration increased with exposure time (Fig. 2A and B) The EC_{50} values for *Nitrosomonas* sp. are as follows; 0.52, 0.82, 1.11 and 1.23 with LC_{50} values of 27.47, 24.14, 19.74 and 16.73. It was observed that the EC_{50} values were very low which increased gradually with time, this suggests that there was a high percentage inhibition of *Nitrosomonas* sp. thereby altering and reducing the percentage nitrite accumulation by the bacteria (Table 1). The EC_{50} values for *Nitrobacter* sp. are 52.00, 81.72, 111.31 and 123.13 and LC_{50} values are 25.04, 23.93, 15.94 and 13.39. The EC_{50} values for *Nitrobacter* sp. were high which indicates that there was high nitrite utilization by *Nitrobacter* sp. as the percentage inhibition gradually decreased with exposure time. This means that *Nitrobacter* sp. could adapt to the plastic contaminant concentrations. The decrease of the LC_{50} values with exposure time shows that at the beginning there was a high inhibition of *Nitrobacter* sp. which corresponded to the low percentage nitrite utilization (Table 2). Increasing concentrations of organochlorine insecticides reduced total viable count of marine bacteria and treatment of *B. subtilis* with DDT resulted in alteration of membrane lipid composition [23,24]. The decrease in EC_{50} and LC_{50} with time may be attributed to increased water solubility with time. Aldrin at 100 µg/l and 200 µg/l inhibited the enzyme responsible for the metabolism of pentose and tricarboxylic acid cycle intermediates in *Rhizobium* sp. and nicotimamide adenine dinucleotide (NAD) dehydrogenase in *B. subtilis* and *E. coli* were inhibited by chlordane [19,25]. The coefficient of determination (r^2) which shows the relationship between the plastic contaminant concentrations and the bacteria response indicates that the bacteria response which could be either inhibition, utilization or accumulation greatly depends on the amount/concentration of the plastic contaminants present in the test soil sample (Tables 3 and 4).

Comparison of the LC_{50} values of *Nitrosomonas* sp. and *Nitrobacter* sp. suggests that the percentage inhibition of *Nitrosomonas* sp was higher than that of *Nitrobacter* sp. and the EC_{50} values of *Nitrobacter* sp. were higher than that of the *Nitrosomonas* sp. There was a higher percentage utilization of nitrite by *Nitrobacter* sp. compared to percentage nitrite accumulation by *Nitrosomonas* sp. Xylene was more toxic to *Nitrosomonas* sp. than *Nitrobacter* sp. Although both bacteria may have similar cell wall morphology as Gram-negative rods [26,27], the difference in response of these bacteria to plastic contaminants may be due to genetic differences [28]. Comparison of the LC_{50} and EC_{50} of plastic contaminants for *Nitrosomonas* sp. and *Nitrobacter* sp. revealed

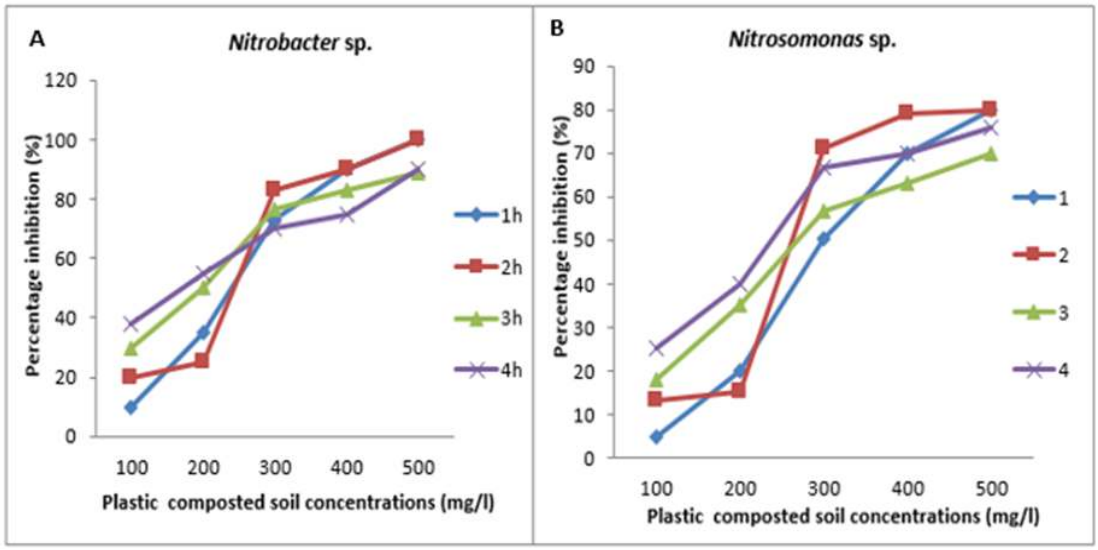

Fig. 1. Bacterial inhibition for four hours. (A) *Nitrobacter* sp. inhibition in plastic composted soil concentration (mg/l) from 1 to 4 hrs. (B) *Nitrosomonas* sp. inhibition in plastic composted soil concentration (mg/l) from 1 to 4 hrs

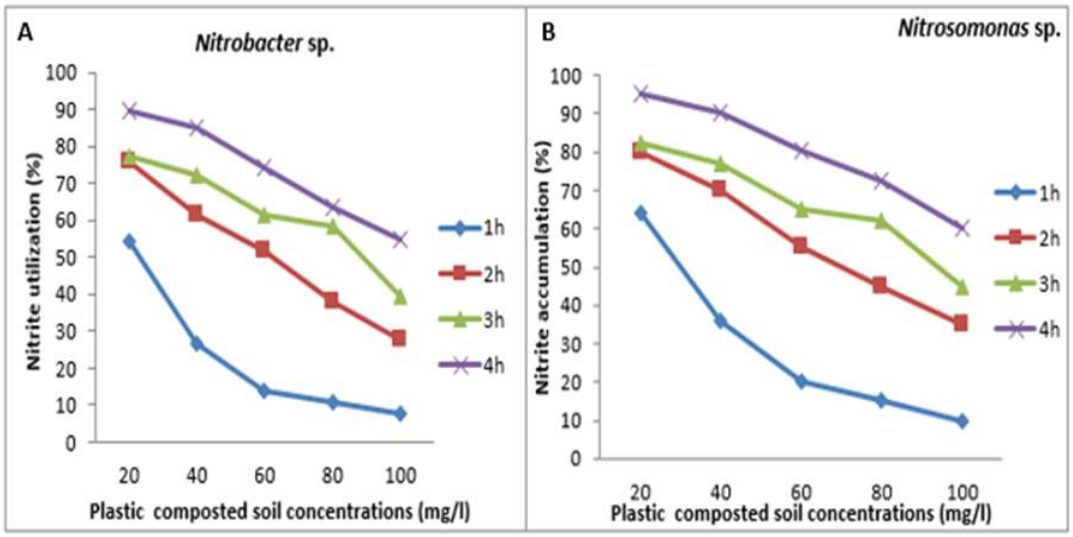

Fig. 2. Nitrite utilization/accumulation by the bacteria in four hours. (A) *Nitrobacter* sp. nitrite utilization in plastic composted soil concentration (mg/l) from 1 to 4 hrs. (B) *Nitrosomonas* sp. nitrite accumulation in plastic composted soil concentration (mg/l) from 1 to 4 hrs

that the EC_{50} values for *Nitrosomonas* sp. were significantly lower than its LC_{50} values. Also the LC_{50} values for *Nitrobacter* sp. were significantly lower than its EC_{50} values. This indicated that the EC_{50} determination was more sensitive than the LC_{50} determination for *Nitrosomonas* sp. which is in conformity with earlier reports of [17] and that the LC_{50} determination was more sensitive than the EC_{50} determination for *Nitrobacter* sp. [29]. This results shows that the populations of *Nitrosomonas* sp. were more sensitive to the plastic contaminants than the populations of *Nitrobacter* sp. [18].

Table 1. EC_{50} and LC_{50} values for *Nitrosomonas* sp.

Incubation time	EC_{50} (Nitrite accumulation)	LC_{50} (Percentage inhibition)
1 h	0.52	27.47
2 h	0.82	24.14
3 h	1.11	19.74
4 h	1.23	16.73

Table 2. EC_{50} and LC_{50} values for *Nitrobacter* sp.

Incubation time	EC_{50} (Nitrite utilization)	LC_{50} (Percentage inhibition)
1 h	52.00	25.04
2 h	81.72	23.93
3 h	111.31	15.94
4 h	123.13	13.39

Table 3. R^2 values for *Nitrosomonas* sp. regression equations

Incubation time	Nitrite accumulation	Percentage inhibition
1 h	0.816	0.974
2 h	0.996	0.823
3 h	0.930	0.941
4 h	0.987	0.905

Table 4. R^2 values for *Nitrobacter* sp. regression equations

Incubation time	Nitrite utilization	Percentage inhibition
1 h	0.816	0.954
2 h	0.996	0.872
3 h	0.930	0.918
4 h	0.987	0.974

4. CONCLUSION

Given that both bacteria are very important bacteria in the nitrification process, the results obtained from this study suggest that autotrophic transformation by nitrifying bacteria which enhances soil fertility may be hindered in an ecosystem polluted with plastics as nitrification processes will be altered.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Eljarrat E, Feo ML, Barcelo D. Degradation of brominated flamed retardants. In: Eljarrat E, Barcelo D, (Eds.), brominated flamed retardants. The Handbook of Environmental Chemistry Springer-Verlag Berlin Heidelberg, Germany. 2010;16:187-202.
2. Vilaplana F, Karlsson P, Ribes-Greus A, Ivarsson P, Karlsson S. Analysis of brominated flamed retardants in styrenic polymers. Comparison of the extraction efficiency of ultrasonication microwave-assisted extraction. J. Chromatogr A. 2008;1196-1197:139-146
3. Achten C, Hofmann T. Native polycyclic aromatic hydrocarbons (PAH) in coals – A hardly recognised source of environmental contamination. Science Total Environment. 2009;407(8):2461-2473.
4. Derraik JGB. The pollution of the marine environment by plastic debris. Marine Pollution Bulletin. 2002;44:842–852
5. Griffiths BS, Philippot L. Insights to resistance and resilience of the soil microbial community. Microbiology Review. 2012;30:60–79.
6. Prosser JI. Differential effects of micro-organism invertebrate interaction on benthic nitrogen cycling. Microbiology Ecology. 2012;81:507-516.
7. Van Elsas JD, Jansson JK, Trevors JT. Modern soil microbiology 2 edn. CRC Press: USA; 2007.
8. Ye J, Singh A, Ward OP. Biodegradation of nitroaromatics and other nitrogen-containing xenobiotics. World J. Microbiol. Biotechnol. 2004;20:117-135.
9. O'Hara K, Iudicello S, Bierce R. A citizen's guide to plastics in the ocean: More than a litter problem. Center for Marine Conservation, Washington, DC; 2004.
10. Goldberg ED. Diamonds and plastics are forever? Marine Pollution Bulletin. 1994;28:466.
11. Højer R, Bayley M, Damgaard CF, Holmstrup M. Stress synergy between drought and a common environmental contaminant: Studies with collembolan *Folsomia candida*. Global Change Biology. 2001;7:485-494.
12. Fierer N, Lennon JT. The generation and maintenance of diversity in microbial communities. American Journal of Botany. 2011;98(3):439–448.

13. Dokaniakis SN, Kornaros M, Lyberatos C. On the effect of xenobiotic bacterial nitrite oxidation. Proceedings of the 9 th International Conference on Environmental Sciences and Technology Rhodes Island, Greece; 2005.
14. Alonso-Magdalena P, Ropero AB, Soriano S, Garcia-Arevalo M, Ripoll C, Fuentes E, et al. Bisphenol A acts as a potent estrogen via non-classical estrogen triggered pathway. Molecular Cell Endocrinology. 2012;353:201-207.
15. Williamson KJ, Johnson DG. A bacteria bioassay assessment of waste water toxicity. Water Resources. 1981;15:383-390.
16. Wang W, Reed P. Nitrobacter as an indicator of toxicity in wastewater. Llinois Department of Energy and Natural Resources; 1983.
17. Okpokwasili GC, Odokuma LO. Response of *Nitrobacter sp.* to toxicity of drilling chemicals. Journal of Petroleum Science and Engineering. 1996a;16:81-87.
18. Ibiene AA, Okpokwasili GSC. Comparative toxicities of three agro-insecticide formulations on nitrifying bacteria. Report and Opinion. 2011;3(12):14-17.
19. Jujena S, Dogra RC. Effect of aldrin on growth and oxidative metabolism of rhizobia. J. Appl. Bacteriol. 1978;49:107-115.
20. APHA, Standard methods for the examination of waters and wastewaters. APHAAWWA-WEF, Washington, DC; 1998.
21. Atuanya EI, Tudararo-Aherobo L. Ecotoxicological effects of discharge of Nigerian petroleum refinery oily sludge on the biological sentinels. African Journal of Environmental Science and Technology. 2014;9(2):95-103.
22. Finney DJ. Statistical methods in biological assay. 3rd Edition, Charles Griffin, London; 1978.
23. Lal R, Saxena DM. Accumulation, metabolism and effects of organochlorine insecticides on microorganism. Microbiol. Rev. 1982;46(1):95-127.
24. Hicks GF, Corner TR. Location and consequence of 1,1,1, trichloro- 2,2-bis (p-chlorophenyl)-ethane uptake by *Bacillus megasterium.* Appl. Microbiol. 1973;25: 381-387.
25. Trudgll PW, Widdus R. Effects of Chlorinated insecticides on metabolic processes in bacteria. Biochem. J. 1970;118:48-49.
26. Odokuma LO, Oliwe SI. Toxicity of substituted benzene derivatives to four chemolithotrophic bacteria isolated from the New Calabar River. Global J. Pure and Appl. Sci. 2003;26:1-5.
27. Holt JG, Krieg NR, Sneath PHA, Stanley JF, Williams ST. Bergey's Manual of Determinative 19th edition. Williams and Wilkins's, Baltimore, Maryland, USA; 1994.
28. Patrick JE, Mang DT, Young LY. Degradation of toluene and m-xylene and transformation of o-xylene by denitrifying enrichment cultures. Appl. Environ. Microbiol. 1991;57:450-454.
29. Atuanya EI, Udochukwu U, Dave-Omoregie AO. Bioavailability and toxicity of plastic contaminants to soil and soil bacteria. British Microbiology Research Journal. 2016;13(6):1-8.

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