British Microbiology Research Journal
13(6): 1-8, 2016, Article no.BMRJ.25128
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN ***international***
www.sciencedomain.org

Bioavailability and Toxicity of Plastic Contaminants to Soil and Soil Bacteria

E. I. Atuanya[1], U. Udochukwu[2*] and A. O. Dave-Omoregie[3]

[1]Department of Microbiology, University of Benin, Edo State, Nigeria.
[2]Department of Biosciences, Salem University, Lokoja, Kogi State, Nigeria.
[3]Biotechnology Unit, National Centre for Genetic Resources and Biotechnology, Moor Plantation, Ibadan, Nigeria.

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. The manuscript was written by author UU in complete agreement with all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/25128
Editor(s):
(1) Giuseppe Blaiotta, Department of Food Science, Via Universita, Italy.
Reviewers:
(1) Yordanka Tsankova Tasheva, University "Prof. Dr A.Zlatarov"- Burgas & Bulgaria, Bulgaria.
(2) Chen-Chin Chang, University of Kang Ning, Taiwan.
Complete Peer review History: http://sciencedomain.org/review-history/13860

Original Research Article

Received 19th February 2016
Accepted 8th March 2016
Published 25th March 2016

ABSTRACT

Aim: The bioavailability and toxicity of plastic contaminants to soil and soil bacteria was investigated to detect the presence of plastic contaminants in the soil and to evaluate the toxic effects of plastic contaminants to soil and soil bacteria.

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 together to form a composite sample. The physico-chemical characteristics of the soil samples were analysed. The soil was analysed for the presence of plastic components using the Perkin Elmer Gas Chromatograph model Auto-system XL. *Nitrobacter* acute toxicity test was carried out. The median effective concentrations (EC_{50}) and the median lethal concentration (LC_{50}) values were calculated using the probit analysis.

Results: The gas chromatography revealed that the control soil sample had zero concentration for chlorobenzene, dichlorobenzene, and benzene. The physico-chemical analysis for the plastic composted soil and the control soil had electrical conductivity 245.00, 61.00 us/cm, chloride 66.15,

**Corresponding author: E-mail: rev.dr.ud@gmail.com;*

16.00 mg/kg, potassium 171.50, 4.27 mg/kg, nickel 1.00, 0.25 mg/kg, vanadium 0.44, 0.23 mg/kg, and moisture 5.32, 7.21% and total organic carbon 5.26, 71.0.% respectively. The bacteriological analysis for the plastic composted soil and the control soil had growth ranging from 1.0x 10^1±0.16 to 4.0 x 10^2±0.11 cfu/g and 2.0 x 10^3±0.20 to 11.0 x 10^3±0.86 cfu/g respectively. The average turbidity result showed a normal bacteria growth curve when plotted for the control soil. There was significant difference ($P<0.05$) in the bacterial counts from the control soil sample. The toxicity analysis revealed higher percentage utilization of nitrite with EC_{50} values of 52.00, 81.72, 111.31 and 123.13 and higher bacteria inhibition with LC_{50} values of 25.04, 23. 93, 15.94 and 13.39.

Conclusion: The result obtained from this study suggest that autotrophic transformation by nitrifying bacteria which enhances soil fertility may be hindered in an ecosystem polluted with these plastics as nitrification process will be reduced.

Keywords: Bioavailability; plastic contaminant; toxicity; soil bacteria; soil.

1. INTRODUCTION

The term “plastics” includes materials composed of various elements such as carbon, hydrogen, oxygen, nitrogen, chlorine, and sulphur. Plastics typically have high molecular weight. Plastics also called polymers are produced by the conversion of natural products or by the synthesis from primary chemicals generally coming from oil, natural gas, or coal [1]. The bioavailable fraction of plastic contaminant is its pool that might be absorbed by plants or soil organisms [2]. Plastic components are present in soil in various forms with different solubility and availability. Plastics bioavailability depends on its chemical behaviour, soil properties and the individual characteristics of the receptor (organism or plant). Among soil properties pH, cation exchange capacity and redox potential play the most important role [3]. However, most conventional plastics such as polyethylene, polypropylene, polystyrene, poly (vinyl chloride) and poly (ethylene terephthalate) are non-biodegradable, and their increasing accumulation in the environment has been a threat to the planet. Bioavailability of plastic components can be evaluated with respect to their biodegradability. Microbes can only degrade a plastic contaminant that is available for their metabolism. The properties of plastics are associated with their bioavailability and biodegradability. Both the chemical and physical properties of plastics influence the mechanism of biodegradation. The surface conditions (surface area, hydrophilic, and hydrophobic properties), the first order structures (chemical structure, molecular weight and molecular weight distribution) and the high order structures (glass transition temperature, melting temperature, modulus of elasticity, crystallinity and crystal structure) of polymers play important roles in making it available for biodegradation processes [4]. Plastic debris in landfill also acts as a source for a number of secondary environmental pollutants [5]. Pollutants of note include volatile organics, such as benzene, toluene, xylenes, ethyl benzenes and trimethyl benzenes, released both as gases and contained in leachate [6]. Soil contamination with plastic contaminants commonly reduces the diversity or evenness (even distribution of species) of soil bacteria [7,8,9]. Plastic contaminants occupy spaces in the soil and the degradation of plastic components depletes oxygen in the soil [8]. Also during the degradation of these plastic components, toxic chemicals are released into the soil which alter soil physico-chemical properties and affect soil fertility. Plastic contaminants are able to immobilise nutrients in the soil thereby making it unavailable for plants and microbes in the soil. Plastic debris and contaminants of large sizes can block or prevent the numerous activities of soil animals which help to improve soil fertility [7,8].

2. MATERIALS AND METHODS

The aim of this research was to evaluate the bioavailability and toxicity of plastic contaminants to soil and soil bacteria. To detect the presence of degradation by-products of plastic contaminants in soil and to study the toxic effects of plastic contaminants to soil and soil bacteria. Five plastic composted soil samples (500 g each) were collected from different locations within the Edo State Waste Management site located at Iyowa in Benin City which were merged together to form a composite sample and another soil sample was collected in a farm land in Ekosodin community Ugbowo Benin-city which served as the control soil sample at a depth of 0-10 cm with a standard soil auger in plastic bags, tagged and was transported to the laboratory [10]. Ten grams (10 g) of each of the soil sample was dissolved in 90 ml mineral salt broth which was used to evaluate the turbidity for 7 days in a shaker incubator. Duplicate plates of nutrient agar were inoculated with each of the soil samples and bacteria counts were recorded every day for the 7 days. The physico-chemical parameters of the soil samples were analysed which include: pH, temperature, total organic carbon, silt and sand composition, nitrate, Phosphorus, calcium, magnesium, sulphate, potassium, vanadium and moisture content of the soil samples [11]. The soil was analysed for the presence of plastic components using the PerkinElmer Gas Chromatography model Auto-system XL [12]. For the acute toxicity test, concentrations of 100, 200, 300, 400 and 500 mg/l of the plastic composted soil were prepared for the determination of the median lethal concentration (LC_{50}) and 20, 40, 60, 80 and 100 mg/l were prepared for the determination of the median effective concentration (EC_{50}). A control experiment consisting of the $NaNO_2$ diluent only (without the plastic composted soil) was set up. *Nitrobacter* acute toxicity **t**est was carried out by inoculating 10 millilitres of *Nitrobacter* sp. into the test 250 ml volumetric flask containing the plastic composted soil. Nitrite concentration was determined and plates of Winograsky media were immediately inoculated by spread plate techniques. This was followed by nitrite determination from the various plastic composted soil concentrations after 1, 2, 3 and 4 h interval and was incubated. Percentage nitrite utilization for *Nitrobacter* sp. and percentage inhibition of bacteria growth was plotted against the test soil concentrations. The median effective concentrations (EC_{50}) and the median lethal concentration (LC_{50}) values were calculated using the probit analysis [13,14]. All results were subjected to the statistical analysis [15,16].

3. RESULTS AND DISCUSSION

The physico-chemical analysis of both soil samples had electrical conductivity 245.00, 61.00 us/cm, chloride 66.15, 16.00 mg/kg, , potassium 171.50, 4.27 mg/kg, nickel 1.00, 0.25 mg/kg, vanadium 0.44, 0.23 mg/kg, and percentage moisture 5.32, 7.21% and total organic carbon 5.26, 71.0.%, for the plastic composted soil and the control soil sample respectively (Table 3). The high electrical conductively recorded in the plastic composted soil sample could be as a result of the presence of lots of elements released into the soil samples from the degradation of plastic contaminants. The total organic carbon content recorded for the plastic composted soil was high also which is as a result of the release of carbon from carbon containing compounds from the plastic contaminants. The moisture content of the plastic composted soil sample recorded a low value; this is because soil water and oxygen are used up during combustion and degradation of these contaminants, thereby leaving the soil with low percentage moisture content [11]. It was observed that the plastic composted soil sample had a low pH value than the control soil sample [17]. The average turbidity result showed that there were growths recorded on the control soil sample with values ranging from 0.021±1.41 (ftu) to 1.061±2.30 (ftu) and the plastic composted soil sample had values ranging from 0.274±1.17 (ftu) to 1.682±0.16 (ftu) which seems to be higher than that of the control soil sample. This was because of the colour impartation from the plastic contaminants which affected the turbidity of the system. Each of the soil sample inoculated into the mineral salt broth were plated in nutrient agar plates for 7 days. The bacteria count obtained from the control soil sample follow a bacteria growth curve which corresponded with its turbidity values. The counts obtained from the plastic composted soil were few and did not really follow the bacteria growth curve pattern; the counts did not also correspond with its turbidity value (Fig. 3 and Table 4). Also the values were plotted and the growth from the control soil sample followed a bacteria growth curve pattern while the curve for the plastic composted soil did not really follow a normal bacteria growth curve (Fig. 3). T-test analysis was carried out on the turbidity values for the both soil samples and it was statistically significant ($P<0.05$). The gas chromatographic analysis revealed the presences of plastic contaminants in both soil sample (Figs. 4 and 5). The concentrations of the individual plastic contaminants were recorded and it showed that the control soil sample had zero concentrations for chlorobenzene, dichlorobenzene, and benzene (Table 1). Most of these compounds are in the degradation pathway of lots of plastic components like Bisphenol A, Polyvinyl chloride, Phthalates, Organotins, Alkyltins, PCBs and Alkyl phenols as reported by [18].

Plastic contaminants have been shown to have acute effects on the biotic and abiotic components of the terrestrial environment [19]. The toxicity analysis of the soil showed that bacteria growth was inhibited with increase in plastic contaminant concentration and time. Fig.

1 represents the bacteria growth inhibition by several concentrations of the plastic composted soil for 1, 2, 3 and 4 h time intervals. Also the bacteria ability to utilize nitrite from the test plastic composted soil was investigated. At varying concentration from 1 to 4 h, the bacteria showed an increase in nitrite utilization and began to decrease in nitrite utilization across the increasing plastic contaminant concentrations with time (Fig. 2). The toxicity analysis revealed that there were higher percentage utilization of nitrite with EC_{50} values of 52.00, 81.72, 111.31 and 123.13 and LC_{50} values of 25.04, 23. 93, 15.94 and 13.39 which decreased with increased

Table 1. Gas chromatography result for both soil samples

Parameters	Plastic composted soil	Control soil sample
Methylene	197.45	0.54
Hexane	87.45	0.26
Chloroform	21.56	0.31
Toluene	5.87	0.07
Tetrachloroethylene	1.48	0.01
Chlorobenzene	0.37	0.00
Dichlorobenzene	0.15	0.00
Benzene	0.11	0.00

Table 2. EC_{50} and LC_{50} values

Incubation time	EC_{50} for nitrite utilization	LC_{50} for 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. The physico-chemical analysis of both soil samples

Parameters	Plastic composted soil sample	Control soil sample
pH	4.60	5.
EC, us/cm	245	61
CL^-, mg/kg	66.15	16
SO_4^{2-}, mg/kg	2.7	0.67
NO_3^-, mg/kg	34.79	0.73
PO_4^{3-}, mg/kg	24.01	0.18
Na^+, mg/kg	93.1	2.32
K^+, mg/kg	171.5	4.27
Ca^{2+}, mg/kg	19.85	1.89
Mg^{2+}, mg/kg	24.26	4.94
Fe^{3+}, mg/kg	17.4	3.11
Zn^{2+}, mg/kg	2.7	0.67
Mn^{2+}, mg/kg	1.49	0.37
Cu^{2+}, mg/kg	2.21	0.12
Ni^{2+}, mg/kg	1.00	0.25
Cd^{2+}, mg/kg	0.76	0.19
V^{2+}, mg/kg	0.91	0.23
Cr^{6+}, mg/kg	1.3	0.32
Pb^{2+}, mg/kg	0.44	0.11
Sand, %	90	91
Silt, %	8	6
Clay, %	2	3
Total carbon, %	5.26	0.71
Total nitrogen, %	0.53	0.07
Moisture, %	5.32	7.21

Table 4. Bacteria counts for both soil samples

Time (Days)	Control soil sample	Plastic composted soil
1	9.0 $x10^3$±0.19	No growth
2	11.0 $x10^3$±0.54	2.0 $x10^2$±0.14
3	11.0 $x10^3$±0.34	1.0 $x10^2$±0.60
4	10.3 x 10^3±0.18	1.0 $x10^1$±0.16
5	8.0 x 10^3±0.34	No growth
6	8.0 x 10^3±0.11	No growth
7	6.8 x 10^3±0.10	No growth

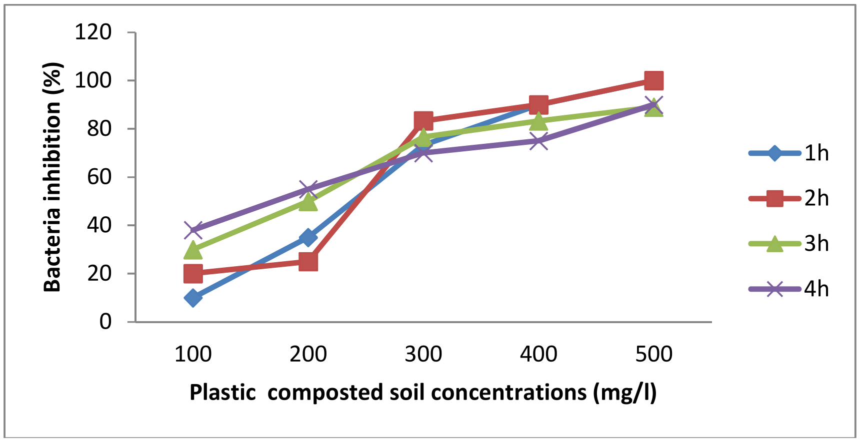

Fig. 1. Bacteria inhibition from 1 to 4 h

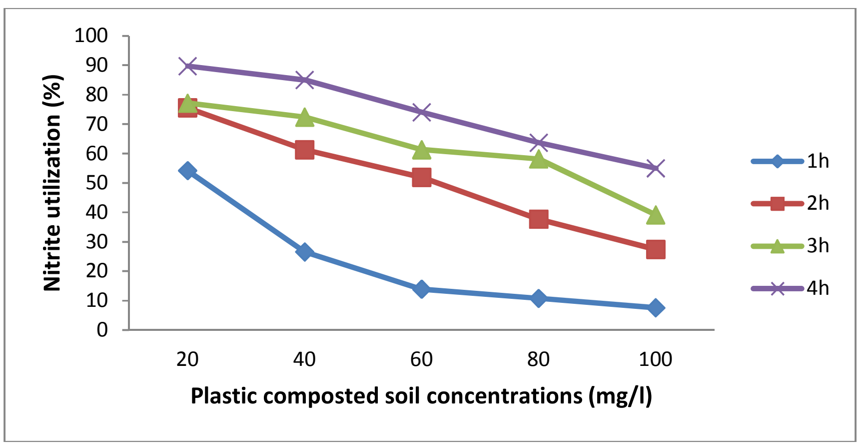

Fig. 2. Nitrite utilization from 1 to 4 h

exposure time and contaminant concentration showing a high inhibition of *Nitrobacter* growth. It was observed that through the process of plastic contaminants degradation, a lot of elements and harmful gases are released into the soil which adversely affects soil bacteria growth, soil properties and function. The acute toxicity effect of soil composted with plastics was conducted since the nitrification process is a function of enzyme activity and its measurement has been used as an indicator of pollution [20,21]. 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 [13]. The results of the acute toxicity studies showed that the toxicity of plastic composted soil on *Nitrobacter* sp. depended on the contact time and plastic composted soil concentration which was similarly reported by [11] who studied the toxicity of different insecticides concentrations on *Nitrobacter* sp. The EC_{50} values increased with increased in exposure time while the LC_{50} values decreased with increased exposure time (Table 2). This shows that at low plastic composted soil concentrations the bacteria was able to adapt and oxidize nitrite which increased with time (Fig. 2) and at higher plastic composted soil concentration, the bacteria growth and metabolism was reduced (Fig. 1) resulting to a decrease in the LC_{50} values. This is as a result of the inhibition of enzyme activities by the toxicant [22]. The comparison of the LC_{50} and EC_{50} values showed that the LC_{50} is a more sensitive criterion for the determination of the acute toxicity of plastic composted soil. The results obtained from this study suggest that autotrophic transformation by nitrifying bacteria may be hindered in an ecosystem polluted with these plastics as nitrification processes will be reduced.

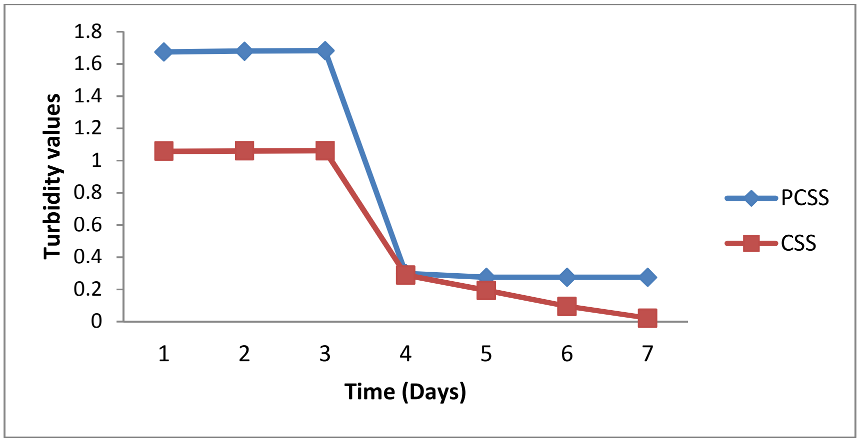

Fig. 3. Bacteria growth curve for Plastic Composted Soil Sample (PCSS) and the Control Soil Sample (CSS)

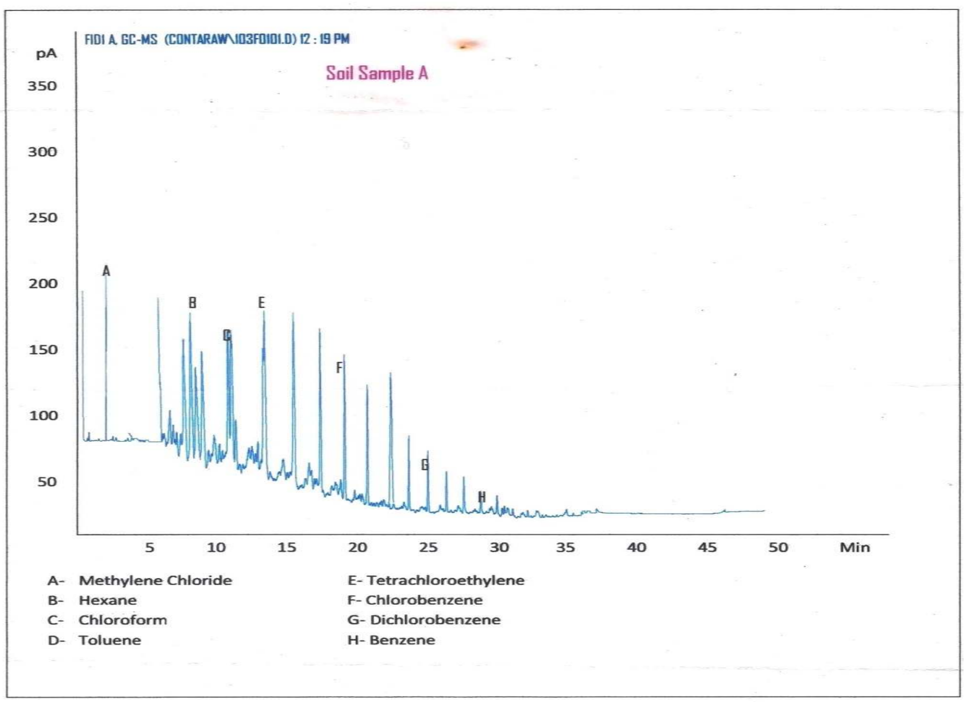

Fig. 4. Presence of plastic contaminants in the test soil

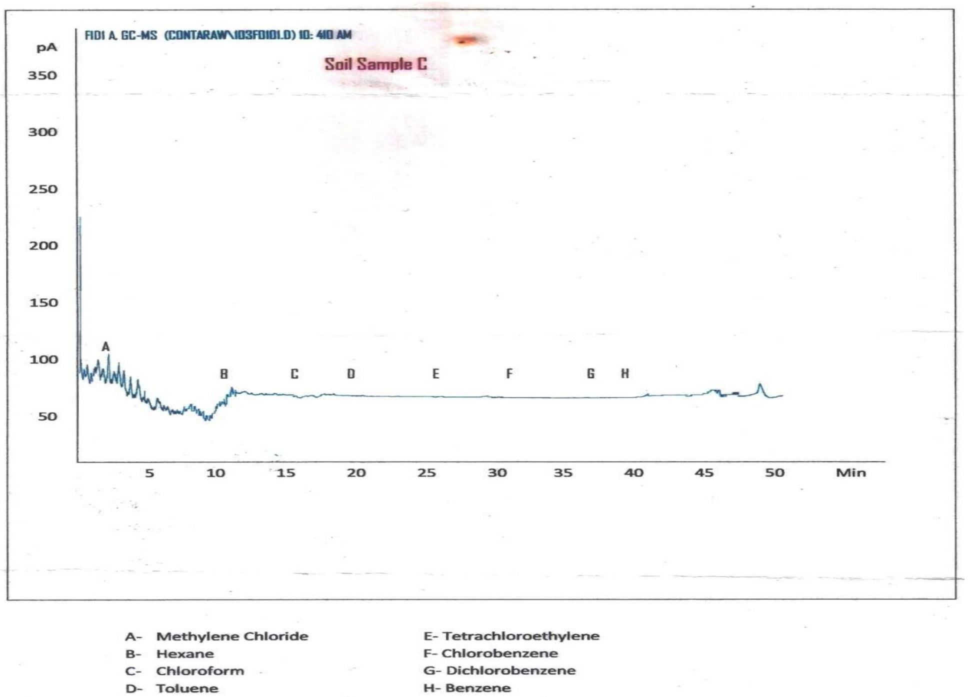

Fig. 5. Presence of plastic contaminants in the control soil sample

4. CONCLUSION

From this study, it was observed that plastic contaminants occupy space on land fill sites making the land unavailable for agricultural and other numerous purposes. It was also observed that plastics and its degradation by-products are greatly available and toxic to soil and soil bacteria. The results from this work indicates that uncontrolled plastic contaminants release into the soil environment will adversely affect soil nitrifying bacteria which will further alter the soil nitrification process and ultimately affect crop production. Further research into the production of biodegradable less toxic plastics, recycling and converting plastics waste into other useful areas will be a relief to the soil environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jonsson S, Ejlertsson J, Ledin A, Mersiowsky I, Svensson BH. Mono and diesters from *o*-phthalic acid in leachates from different European landfills. Water Resoureses. 2003b;37:609–617.
2. Mato Y, Takada H, Zakaria MP, Kuriyama Y, Kanehiro H. Toxic chemicals contained in plastic resin pellets in the marine environment-spatial difference in pollutant concentrations and the effects of resin type. Kankyo Kagakukaishi*.* 2002;15: 415–423.
3. Ng KL, Obbard JP. Prevalence of micro plastics in Singapore's coastal marine environment. Marine Pollution Bulletin*.* 2006;52:761–767.
4. Tokiwa Y, Calabia BP. Biodegradability and biodegradation of polyesters. Journal of Polymer Environment. 2007;15: 259–267.
5. 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.
6. Derraik JGB. Fate and biological treatment. Environmental Microbiology. 2002;76:3936.
7. Griffiths BS, Philippot L. Insights to resistance and resilience of the soil

microbial community. Microbiology Review. 2012;30:60–79.

8. Prosser JI. Differential effects of microorganism invertebrate interaction on benthic nitrogen cycling. Microbiology Ecology. 2012;81:507-516.
9. Van Elsas JD, Tam L, Finlay RD, Killham K, Trevors JT. In: van Elsas J.D, Jansson JK, Trevors J, (eds). Modern soil microbiology. 2nd edn. CRC, Bington, United Kingdom. 2007;177.
10. Ryan J. Soil and plant analysis in the Mediterranean region: Limitations and potential. Soil Science and Plant Analysis. 2000;31(11–14):2147–2154.
11. Atuanya E, 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.
12. Ibiene AA, Okpokwasili GSC. Comparative toxicities of three agro-insecticide formulations on nitrifying bacteria. Report and Opinion. 2011;3(12):14-17.
13. Okpokwasili GC, Odokuma LO. Response of *Nitrobacter* sp. to toxicity of drilling chemicals. Journal of Petroleum Science and Engineering. 1996a;16:81-87.
14. Kalra YP, Maynard DG. Manual for forest soil and plant analysis. Forestry Canada, Northwest Region, Edmonton, Alberta, Canada. Inf. Rep. NOR-X-319; 1991.
15. APHA. Standard methods for the examination of waters and wastewaters. APHAAWWA-WEF, Washington, DC; 1998.
16. Finney DJ. Statistical methods in biological assay. 3rd Edition, Charles Griffin, London; 1978.
17. Okpamen SU, Oviasogie PO, Ilori GE, Osayande PE, Uwubamwen IO. Remediation and management of acid sand soil in oil palm plantation by carbon. Greener Journal of Agricultureal Science. 2013;3(10):709–715.
18. Kolvenbach B, Schlach N, Raoui Z, Prell J, Zuhlke S, Schatter A, Guengerich FP, Corvine PF. Degradation pathway of bisphenol A: Does ipos substitution apply to phenols containing a quaternary alpha-carbon structure in the para position. Applied Environmental Microbiology. 2007; 73(15):4776-4784.
19. 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.
20. Williamson KJ, Johnson DG. A bacteria bioassay assessment of waste water toxicity. Water Resources. 1981;15: 383-390.
21. Wang W, Reed P. Nitrobacter as an indicator of toxicity in wastewater. Llinois Department of Energy and Natural Resources; 1983.
22. Jujena S, Dogra RC. Effect of Aldrin on growth and oxidative metabolism of Rhizobia. Journal of Applied Bacteriology. 1978;49:107-115.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13860