Advances in Research
8(4): 1-7, 2016; Article no.AIR.30536
ISSN: 2348-0394, NLM ID: 101666096

SCIENCEDOMAIN *international*
www.sciencedomain.org

Phytotoxicity, Bioload and Heavy Metal Evaluation of a Selected Municipal Dumpsite in Obosi, Anambra State, Nigeria

Martin O. Anagboso[1], Michael U.Orji[1], Amechi S. Nwankwegu[1*] and Fidelis Azi[2]

[1]Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.
[2]Department of Food Science and Technology, Faculty of Agriculture and Natural Resources Management, Ebonyi State University, P.O.Box 053, Abakaliki, Ebonyi State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2016/30536
Editor(s):
(1) Gulnihal Ozbay, Department of Agriculture & Natural Resources, Delaware State University, USA.
(2) Marco Trevisan, Faculty of Agricultural Sciences, Institute of Agricultural and Environmental Chemistry, Catholic University of the Sacred Heart, Italy.
(3) Francisco Marquez-Linares, Full Professor of Chemistry, Nanomaterials Research Group, School of Science and Technology, University of Turabo, USA.
Reviewers:
(1) Fuhua Wang, Public Monitoring Center for Agro-product of Guangdong Academy of Agricultural Sciences, China.
(2) Anonymous, University of Botswana, Botswana.
(3) Héctor Hernández Mendoza, Universidad Autónoma de San Luis Potosí, México.
Complete Peer review History: http://www.sciencedomain.org/review-history/17713

Original Research Article

Received 16th November 2016
Accepted 26th January 2017
Published 4th February 2017

ABSTRACT

Aims: To evaluate the pollution index of municipal solid waste dumpsite site located at Obosi metropolis of Anambra state, Nigeria.
Study Design: Study of the dump site bioload level using sabouraud dextrose agar and nutrient agar, heavy metal determination using atomic absorption spectrophotometry and assessment of the ecological impact of the dump using two selected agricultural plants maize (*Zea mays*) and beans (*Vicia faba*).
Place and Study Duration: Department of Applied Microbiology and Brewing Nnamdi Azikiwe University, PMB 5025, Awka, Anambra state, Nigeria between Februarys, 2014 to September, 2015.
Methodology: Sample collection from two locations in Obosi, Nigeria, the dumpsite and sample collected 1.10 km away from the dumpsite was used as control, determination of the

**Corresponding author: E-mail: smetchie@yahoo.com;*

physicochemical characteristics of the two soils, microbial enumeration, chemical analysis and growing of plants (phytotoxicity assay).

Results: Results showed that relatively alkaline pH was observed in the dumpsite soil (DSS) while slight acidity was reported in the control soil (CSS). The list of heavy metals and minerals quantified in this work via atomic absorption spectrophotometry (AAS) included mercury, lead, arsenic, cadmium zinc, chromium, aluminum, iron, manganese, magnesium, calcium, sodium and potassium. The heavy metal concentration of dumpsite was higher than that of control. The microorganisms isolated from the present study included; *Bacillus subtilis*, *Aspergillus candidus, and Aspergillus flavus*. In the phytotoxicity assessment, the maize grain and beans exhibited good germination conditions in CSS with an extremely poor germination performance in DSS due to heavy metal toxicity as well as alteration of other physicochemical status such as pH, texture and particle size configurations

In general, seed germination performance was higher in the maize (*Zea mays*) than beans (*Vicia faba*) with germination indices of 89.7% and 51.2% respectively. 2:1 DSS and CSS soil modification gave the best germination. Statistical analysis showed that there was a significant difference in the percentage seed germination at $p<0.05$.

Conclusion: Present study showed that Obosi dumpsite is polluted with heavy metals particularly mercury, chromium, lead and cadmium yielding very high in concentrations of heavy metals. If proper environment action is not carried out this will affect the surrounding agricultural soil and jeopardize human health in the area.

Keywords: Heavy metal; soil; pollution; municipal dumpsites; phytotoxicity.

1. INTRODUCTION

Indiscriminate disposal of domestic, industrial and municipal waste can potentially contribute to elevated levels of various heavy metals; iron (Fe), lead (Pb), zinc (Zn) chromium (Cr) and cadmium (Cd) in the soil ecosystem [1-3]. These metals are known to accumulate in soil and have long persistence time through interaction with soil component and consequently enter food chain through plants or animals .Similarly, continuous disposal of these wastes and particularly in unlined surfaces can enhance their mobility at environmentally hazardous levels [3,4]. The concentrations of heavy metals in soil and around dumps are influenced by type of wastes; topography, runoff and level of scavenging and are eventually introduced to the ecosystem through infiltration or disposal of leachates.

The presence of a poorly managed waste disposal scheme is often manifested through the use of conventional landfills and dumpsites owing to their accessibility, inexpensiveness, and convenience of methane gas recovery [5] although other options like composting and recycling are also available and are often practiced. The gradual increasing population and the proliferation of basic industrial processes particularly in major cities of the world in which Obosi metropolis is a typical example has led to emergency of civilization that have greater impact on the environment. The industrial revolution gave birth to environmental pollution and the large volume of industrial chemical discharges has added to the growing load of untreated domestic waste. The disposal of domestic, commercial and industrial garbage in the world is a problem that continues to grow with human civilization and no method so far is completely safe. Experience has shown that all forms of waste disposal have negative consequences on the environment, public health, and local economies. The aims of the present study therefore, were to ascertain the heavy metal concentration of Obosi dumpsite, bioload level status and determine the relative mobility of some potential heavy into some agricultural crops in the surrounding dumpsite environment.

2. MATERIALS AND METHODS

2.1 Sample Collection

1 kg of the soil (Dumpsite) was collected at a depth of 10 cm as measured with a metre rule after an excavator was used to remove the heaps of the solid waste before collecting the soil sample on the substratum. Equal quantity of soil sample was also collected from location away from the dumpsite (1.10 km) while ensuring the same depth and collection procedures and this however, served as experimental control. Both soil samples were collected into separate sampling bottles which were previously sterilized with cotton wool soaked in 70% alcohol [6] and

taken to the laboratory for analyses. Two plastic bowls were separately used to collect to the brims each of the soil samples for phytotoxicity assay.

2.2 Microbial Enumeration

The bioload levels (total microbial count) present in the two different experimental groups were determined. The spread plate methods on nutrient and sabouraud dextrose agar (Oxoid LTD, UK) were used for bacterial and fungal isolates. The dumpsite soil and control soil suspensions prepared and serially diluted to 10^{-5} via serial dilution with 1 g of each of the soils using distilled water as diluents. 0.1ml aliquots of the 10^{-5} dilutions were spread on triplicates of sterile agar plates. Dissolved nutrient and Sabouraud dextrose agar were amended with chloramphenicol and nystatin to exclude bacteria and fungi respectively. Plates were incubated for 18 – 24 hours for bacteria and 18 – 72 hours for fungi. Colonies were counted and expressed in colony forming unit per gram (CFU/g).

2.3 Characterization of Microbial Isolates

The identification of bacteria was based on morphological and biochemical characterization such as Gram staining, motility, sugar and alcohol fermentation tests, citrate, catalase, indole, methyl red, Voges Prauskauer, starch hydrolysis, and oxidase [7]. The identification of fungi was based on colony appearance, wet mount preparation, use of lactophenol blue and comparison with different fungal Atlases [8-10]. The isolates were further identified to species level at CABI Microbial Identification Services (United Kingdom, Bakem Lane, Egham Surrey TW20 9TY, UK) where partial 16S rDNA sequencing analysis was used for bacterial isolates and internally transcribed spacer (ITS), rDNA sequencing analyses used on fungal isolates.

Briefly, total of four (4) microbial isolates were submitted to CABI for microbial identification. A unique CABI reference number (IMI number) was assigned to each of the samples. Bacterial samples (both *Bacillus* species IMI504618, and IMI 504619 were processed using partial 16S rDNA sequencing analysis. Fungi samples IMI 504614 and IMI 504615 were processed using ITS rDNA sequencing analysis. All procedures were validated and processing undertaken in accordance with CABI's in-house methods as documented in TPs 61-68 and TP70 for bacteria and TPs 72-80 for fungi. Procedures involved the following steps: All original samples were subjected to a purity check. Molecular assays were carried out on each sample using nucleic acid as a template. A proprietary formulation [microLYSIS®-PLUS (MLP), Microzone, UK)] was subjected to the rapid heating and cooling of a thermal cycler, to lyse cells and release deoxyribonucleic acid (DNA). Following DNA extraction, Polymerase Chain Reaction (PCR) was employed to amplify copies of the partial 16S fragment of rDNA *in vitro* for bacteria and the ITS fragment of rDNA in vitro for fungi. The quality of the PCR product was assessed by undertaking gel electrophoresis. PCR purification step was carried out to remove unutilised dNTPs, primers, polymerase and other PCR mixture compounds and obtain a highly purified DNA template for sequencing. This procedure also allowed concentration of low yield amplicons. Sequencing reactions were undertaken using BigDye® Terminator v3.1 kit from Applied Biosystems (Life Technologies, UK) which utilises fluorescent labelling of the chain terminator ddNTPs, to permit sequencing. Removal of excess unincorporated dye terminators was carried out to ensure a problem-free electrophoresis of fluorescently labelled sequencing reaction products on the capillary array AB 3130 Genetic Analyzer (DS1) DyeEx™ 2.0 (Qiagen, UK). Modules containing prehydrated gel-filtration resin were optimized for clean-up of sequencing reactions containing BigDye® terminators. Dye removal was followed by suspension of the purified products in highly deionised formamide Hi-Di™ (Life Technologies, UK) to prevent rapid sample evaporation and secondary structure formation. Samples were loaded onto the AB 3130 Genetic Analyzer and sequencing undertaken to determine the order of the nucleotide bases, adenine, guanine, cytosine, and thymine in the DNA oligonucleotide. Following sequencing, identifications were undertaken by comparing the sequence obtained with those available in European Molecular Biology Laboratory (EMBL) via the European Bioinformatics Institute (EBI).

2.4 Heavy Metal Quantification

Heavy metal quantification of soils was done by atomic absorption spectrophotometry (AAS) and values expressed in microgram per gram (ug g-1). Series of standards of the element under analysis was run and a calibration curve was constructed by plotting the concentrations of the standards against the absorbance.

2.5 Phytotoxicity Assay

Agricultural performance of the two soils were evaluated using seed germination as monitoring tool to measure phytotoxic level of the dumpsite soil relative to the control. The method of seed germination and growth with little modification in temperature and time was employed [11] using two different agricultural crops; cereal grain of *Zea mays* species and beans seed Vicia *faba* species (Fabaceae). 10 g each of the two different experimental soils were placed in petri-dishes. Ten seeds each of *Zea mays* and *Vicia faba* respectively were distributed in the different dishes equally spaced. The plates were incubated for 4-5 days at room temperature. The soils containing the planted seeds were periodically moistened to check diffusional limitations of substrate supply and adverse physiological effect associated with cell dehydration as water penetrates the soil matrix and also to facilitate the swelling of the endosperm as well as the cotyledons and quicken germination. After this time, the number of germinated crops was counted and the elongations of the roots were measured from the transition point among the hypocolite to its extremity, root elongations and shoot lengths were also measured. The germination index (%IG) and percentage seed germination were calculated as thus:

$$\% IG = \frac{(\% SG) X (\% GR)}{100} \quad (1)$$

$$\% SG = \frac{(\% EG)}{(\% CG)} x \frac{100}{1} \quad (2)$$

$$\% GR = \frac{GERm}{GERCm} x \frac{100}{1} \quad (3)$$

Where % SG =Seed germination % GR =Growth of the roots, % EG=Germination in dumpsite soil % CG=Germination in control soil, GERm=Elongation of roots in dumpsite soil, GERCm = Elongation of roots in control soil.

2.6 Statistical Analysis

Each set of data in the experiments was collected in three replicates and the analytical result was calculated as the mean of three replicates. The standard deviations (or error bars) and statistical differences (5% level of significance) were analyzed by using GraphPad Prism 6® software (full version) (GraphPad Software, CA, USA).

3. RESULTS AND DISCUSSION

3.1 Soils Physicochemical and Microbiological Status

The physicochemical characteristics of the dumpsite soil (DSS) and the control soil (CSS) are shown in Table 1. The pH conditions of dumpsite soil and the control soil are 8.113 and 6.800 respectively.

Table 1. Physicochemical properties of the soil

Parameters	DSS	CSS
pH	8.10±0.02	6.80±0.33
Temperature (℃)	66.71±0.11	40.23±0.43
Nitrate (mg/l).	2.34±0.21	3.37±0.44
Density (g/ml)	1.53±0.31	1.36±0.32
Organic Carbon (%)	0.30±0.41	0.46±0.21
Electrical conductivity EC (µs/cm)	12.32±0.51	11.80±0.32
Silt (%)	9.00±0.33	20.32±0.04
Sand (%)	72.62±0.11	79.57±0.05
Clay (%)	18.40±0.22	0.12±0.01
Cation Exchang Capacity (cmol/kg)	0.12±0.03	0.55±0.07
Ca (cmol/kg)	0.08±0.14	0.07±0.08
Mg (cmol/kg)	0.030±0.31	0.45±0.09
K (cmol/kg)	0.130±0.15	0.01±0.01
Na (cmol/kg)	0.0204±0.23	0.02±0.71

[a]*DSS = Dumpsite Soil sample, CSS = Control Soil Sample*

3.2 Bioload Status

The result of microbiological enumeration of the different soils enabled us tracked the bioload levels. The microbial count of the dumpsite soil was found to be higher than the control soil (agricultural soil) and maintained 5.4±0.11 × 10^6 CFU/g and 1.9±0.21 × 10^6 CFU/g respectively for bacteria and 9.0±0.31 × 10^5 CFU/g and 5.0±0.12 × 10^5 CFU/g respectively for fungi on the point of collection (week 1). A sharp decline was observed on the dumpsite soil sample while the experiment lasted on the sixth week to 1.1±0.41× 10^6 CFU/g for bacteria and 4.0±0.33 × 10^5 CFU/g for fungi with temperature change from 66.71℃ to 42,35℃ and pH change from 8.113 to 6.251. There was no change in temperature and pH in CSS though, total microbial decline was reported but this was not as drastic as the DSS. The microbial counts on the sixth week were 1.6±0.23 × 10^6 CFU/g for bacteria and 4.0±0.34 × 10^5 CFU/g for the fungal isolates. We therefore, reportedthat changes in chemicals, physical

conditions (pH and dump pile temperature) and organic compositions of the soil microcosm withdrawn from the dumpsite led to a significant cell mass loses. Isolated bacterial species conformed to the works of previous inbestigators [12,13].

3.3 Molecular Analysis

Tables 3 and 4 below show the microbial characterization of isolates before molecular identification.

DSS1 IMI 504614 identified as *Aspergillus candidus.* This sample was identified by ITS rDNA sequencing analysis using the FASTA algorithm with the Fungus database from EBI and by examination of morphology. The sequence obtained from this sample showed top matches at 100% identity to multiple sequences of *Aspergillus candidus* including published sequences from reference cultures collection strains e.g. sequence JN942868 from DAOM 216320 published in Schoch C.L. et al. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences of the United States of America 109 (16):6241-6. Morphological features observed were consistent with published taxonomic descriptions of *A. candidus* e.g. Klich M. (2002) Identification of Common *Aspergillus* Species*.* Centraal Bureau voor Schimmelcultures, Utrecht, Netherlands.

CSS IMI 504615 identified as *Aspergillus flavus.* This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI and by examination of morphology. The sequence obtained from this sample showed 100% identity to ITS sequences described from multiple strains of *Aspergillus flavus* and several strains of *A. oryzae*. These included sequences from strainsreported in peer-reviewed literature e.g. *A. flavus* sequence JX502758 published in Hadrich I. et al. (2013) Microsatellite typing of *Aspergillus flavus* in patients with various clinical presentations of aspergillosis. *Medical Mycology* 51(6):586-91. Morphological features observed were consistent with published taxonomic descriptions of *A. flavus* e.g. Klich M. (2002) Identification of Common *Aspergillus* Species pp 46-47. Centraal Bureau voor Schimmelcultures, Utrecht, Netherlands. This species can be distinguished from *A. oryzae* based on conidial size. *A. flavus* has smaller conidia up to 6µm, whereas those of *A. oryzae* are larger, up to 8 µm.

DSSb IMI 504618 identified as *Bacillus subtilis:* This sample was identified by 16S rDNA sequence analysis using the FASTA algorithm with the Prokaryote database from EBI. The top 750 results gave matches of >99% to members of this species group which includes *B. subtilis*, *B. amyloliquefaciens*, *B. mojavensis* etc. The validated type strain sequence of *B. subtilis* gave amatch of 99.8% (AJ276351). Members of this species group are difficult to differentiate using this method.

DSSd IMI 504619 also identified as *Bacillus subtilis:* This sample was identified by 16S rDNA sequence analysis using the FASTA algorithm with the Prokaryote database from EBI. The top 750 results gave matches of >99% to members of this species group which includes *B. subtilis*, *B. amyloliquefaciens*, *B. mojavensis* etc. The validated type strain sequence of *B. subtilis* gave a match of 99.8% (AJ276351), and *B. mojavensis* (AB021191) gave a match of 99.6%. Members of this species group are difficult to differentiate using this method.

3.4 Heavy Metals and Mineral Quantification

The heavy metal and mineral quantification of the soil (Table 5) enabled us revealed the heavy metal status of the soils. Result showed that the overall concentration of the heavy metals was noticeably higher in the dumpsite soil especially the four potentially toxic ones including mercury, chromium, lead and cadmium which were quantified 12.49 ug g-1, 9.871 ug g-1, 11.30 ug g-1, and 6.207 ug g-1 respectively in the dumpsite and 0.00 ug g-1, 0.065 ug g-1, 0.970 ug g-1 and 0.140 ug g-1 respectively in the control soil.

Table 2. Microbial counts of soils

Parameter	DSS	CSS
Bacterial count on week one (CFU/g).	$5.4\pm0.11 \times 10^6$	$1.9\pm0.21 \times 10^6$
Fungal count on week one (CFU/g)	$9.0\pm0.31 \times 10^5$	$5.0\pm0.12 \times 10^5$
Bacterial count after 6 weeks (CFU/g)	$1.1\pm0.41\times 10^6$	$1.6\pm0.23 \times 10^6$
Fungal count after 6 weeks (CFU/g)	$4.0\pm0.33 \times 10^5$	$4.0\pm0.34 \times 10^5$

[b]Values are mean of triplicate analyses ±SD, DSS = Dumpsite Soil sample, CSS = Control Soil Sample, CFU/g = colony Forming Unit per gram

Table 3. Biochemical identities of bacterial isolates

S/ no	Isolates	Gram reaction	Motility	Citrate	Catalase	Indole	Methyl red	Voges Proskauer	Starch hydrolysis	Oxidase	Urease	Glucose	Lactose	Sucrose	Maltose	Xylose	Arabiose	Identity of bacterial species
1	DSSb	Rods +	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	*Bacillus subtilis*
2	DSSd	Rods +	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	*Bacillus subtilis*

[c]*DSSb and DSSd = Designation of bacterial isolates on plates, - = negative, + = positive without gas production*

Table 4. Identities of fungal isolates

S/no	Isolates	Cultural characteristics	Microscopic characteristics	Species
1	DSS1	Thick, dark brown and scattered surface of solid media	Septate and branched hypha, conidia in chains	*Aspergillus candidus*
2	CSS	Thick and flatty with pure green surface reverse yellow	Multi-segmented canoe-shaped and branched conidiophores	*Aspergillus flavus*

[e]*DSS1, DSS3 and CSS =designation of fungi isolates on plate*

Table 5. Heavy metal and mineral of the soil samples

Heavy Metal (ug g-1)	DSS	CSS
Mercury	12.49±0.10	0.00±0.13
Aluminium	5.61±0.11	5.10±0.22
Calcium	30.90±0.30	29.50±0.14
Potassium	20.60±0.22	9.50±0.12
Sodium	5.91±0.12	9.26±0.13
Magnesium	5.91±0.14	6.50±0.10
Arsenic	0.00±0.00	0.00±0.00
Chromium	9.89±0.31	0.07±0.01
Lead	11.30±0.33	0.97±0.13
Zinc	4.80±0.01	5.13±0.11
Iron	56.41±0.20	57.70±0.12
Cadmium	6.21±0.30	0.14±0.13
Manganese	4.20±0.21	7.90±0.54

[g]*DSS = Dumpsite Soil sample, CSS = Control Soil Sample*

Table 6. Heavy metal bioaccumulation of selected plants

Heavy metal/minerals (ug g^{-1})	A_1	A_2
Mercury	6.40±0.21	2.10±0.03
Lead	5.20±0.02	3.01±0.07
Arsenic	3.90±0.20	1.60±0.13
Iron	10.52±0.21	3.15±0.25
Copper	2.20±0.01	0.30±0.33
Chromium	13.20±0.41	6.02±0.41
Cadmium	7.20±0.56	2.70±0.17

[h]*Key: A_1 = Pawpaw leaves collected at the dumpsites, A_2 = Pawpaw leaves collected away (1.1 km) from the dumpsites*

The heavy metal concentration in the leaves of pawpaw (*Carica papaya*) plucked from the dumpsite was found to be far much higher than the leaves of pawpaw plucked 1.1 km away from the dumpsite (Table 6). Significant mobility of heavy metal to the surrounding agricultural plants was reported.The result of percentage seed germination (Table 7) was generally low in the dumpsite soil relative to the control soil for both *Vicia faba* and *Zea mays*. The modified soil type (1:2 ratio DSS : CSS) gave the best result in both plant materials thus, indicated that the heavy metals at these concentrations lost its phytotoxic effect and acted as trace elements. The germination index was 51.2% and 89.7% for *Vicia faba* and *Zea mays* respectively.

Table 7. Percentage seed germnation (%EG)

Sample	*Vicia faba*	*Zea mays*
DSS	10±0.03	50±0.14
CSS	70±0.11	70±0.32
1:1	30±0.22	80±0.44
1:2	50±0.13	90±0.12
Germination index (%IG)	51±0.25	89±0.46

[a]*Values are mean of triplicate analyses ±SD. DSS = Dumpsite soil sample, CSS = ControSoil Sample, 1:1 = one part of DSS to one part of CSS, 1:2 = one part of DSS to two parts of CSS*

4. CONCLUSION

The result of the present study has proven that Obosi dumpsite, Anambra state is polluted with heavy metals especially mercury, lead, chromium and cadmium. It is also observed that continuous indiscriminate waste disposal would lead to increased pollution and affect the surrounding agricultural lands in the dumpsite environment to a point that would result to total sterilization of the arable lands therein. The phytotoxicity assay also proved that the dumpsite soil itself at present has been rendered agriculturally irrelevant and most agricultural plants can no longer grow on it. Heavy metal contamination

can provide threat to human health and overall crop production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vandana P, Murthya N, Praveen R. Assessment of heavy metal contamination in soil around hazardous waste disposal sites in Hyderabad city (India): Natural and anthropogenic implications. Journal of Environmental Research and Management. 2011;2:27–34.
2. Ogundiran OO, Afolabi TA. Assessment of the physicochemical parameters and heavy metals toxicity of leachates from municpal solid waste open dumpsite. Int. J. Environ. Sci. Tech. 2008;5(2):243–250.
3. Uba S, Uzairu A, Harrison GFS, Balarabe ML, Okunola OJ. Assessment of heavy metals bioavailability in dumpsites of Zaria Metropolis, Nigeria. African Journal of Biotechnology. 2007;7(2):122–130.
4. Ebong G, Etuk H, Johnson A. Heavy metals accumulation by Talinum. Triangul are grown on waste dumpsites in Uyo metropolis Akwa Ibom State, Nigieria. J. Appl. Sci. 2007;7(10):1404–1409.
5. Alloway BJ, Ayres DC. Chemical principles of environmental pollution, 2nd edn. Chapman and Hall, UK; 1997.
6. Eziuzor CS, Okpokwasili GC. Bioremediation of hydrocarbon contaminated mangrove soil in a bioreactor. Nigerian Journal of Microbiology. 2009;23(1):1777–1791.
7. Ogbo FC. Basic microbiology: Fundamental and techniques. Cresco Printing and Publishers 2 Ikegbunne Street Ugbene Abakpa Nike Enugu. 2005;169–188
8. De Hoog GS, Guarro J, Gene J, Figueras MJA. Atlas of clinical fungi. Atlas Version 11; 2004.
9. Ellis D, Davis S, Alexiou H, Handke R, Bartley R. Description of medical. Secnd edition Nexus Print Solution Adelarde, Austalia; 2007.
10. Frey D, Oldfield RJ, Bridges RC. A colour Atlas of pathogenic fungi. Wolf medical Publications LTD London; 1979.
11. Nwankwegu AS, Orji MU, Onwosi CO. Studies on organic and in-organic biostimulants in bioremediation of diesel-contaminated arable soil. Chemosphere. 2016;162:148–156.
12. Anyanwu CU, Nwachukwu ON. Heavy metal resistance in bacteria isolated from contaminated and uncontaminated soil. International Journal of research and Environment. 2011;1:183–178.
13. Ogunmodede OT, Ajayi OO, Amoo IA, Adewole E. Characterization of dumpsite soil: A case study of Ado – Ekiti and Ijero – Ekiti Nigeria. 2013;3:43–50.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17713