ASSESSMENT OF MICROBIAL ORGANISMS IN THE RIZOSPHERIC SOIL AND WATER FROM SCRAP METAL DUMP SITES IN EDO STATE NIGERIA

V. N. Chukwu
Environmental Biotechnology Sustainability Research Group
Department of Plant Biology and Biotechnology,
University of Benin, Benin City,
Edo State.

B. Ikhajiagbe
Environmental Biotechnology Sustainability Research Group
Department of Plant Biology and Biotechnology,
University of Benin, Benin City,
Edo State.

M. O. Aigbekaen
Environmental Biotechnology Sustainability Research Group
Department of Plant Biology and Biotechnology,
University of Benin, Benin City,
Edo State.

And

G. O. Anoliefo
Environmental Biotechnology Sustainability Research Group
Department of Plant Biology and Biotechnology,
University of Benin, Benin City,
Edo State.

Abstract

Research was carried out to assess the prevalence and distribution of microbial organisms in heavy metal polluted rhizospheric soils of four plants (Amarantus spinosus, Eleusine indica Andropogon tectorum and Peperonia pellucida) and water from around the nine scrap metal dump sites and also from a control site that has no trace of heavy metal pollution. The rhizospheric soil samples were taken from the four most prevalent plants present in the nine scrap metal dump sites and control sites. Water samples were taken from boreholes, wells, rivers nearest to the scrap metal dump sites and from water run offs at 3m and 5m from the scrap metal dump sites. A total of twenty-three (23) samples were assayed for microbial organisms. The research showed that the nine scrap metal dump sites understudied were highly polluted with Chromium(Cr) above the phytotoxicity limits and several other metals which includes Copper(Cu), Zinc(Zn), Cadmium(Cd) Lead(Pb) and Iron(Fe) were on the increase. The determination of the microbial colony size showed that the largest colony size for bacteria was 3.3 x 10^5 ctu/g at the 5m run off from the Christopher scrap metal dump site. For fungi, the largest colony was 7.5 x 10^4 ctu/g from the well

water sample close to Akugbe scrap metal dump site. The results also revealed that Mucor mucedo had the highest percentage occurrence of 83 percent followed by Penicillium species with 74 percent. Clostridium species had the least occurrence of 4 percent.

Keywords; Prevalence, rhizospheric soil, microbial organisms, heavy metals, scrap metals, isolates.

The study was conducted in Benin City, the capital of Edo State, Nigeria. It has an estimated population of 1,147,188 (Population Census, 2006). It is approximately 40 km (25 mi) north of the Benin River, and 320 km (200 mi) by road east of Lagos. Field investigation was carried out at nine different scrap metal dump sites within Benin City. The different scrap metal sites were accessed with the assistance of scrap metal dealers. Rhizospheric soil samples and water samples were obtained from the scrap metal dump sites. Samples were also collected from a control site that had no trace of heavy metal pollution.

Research conducted showed that scrap metal business is on the increase due to continuous growth in the population of man (Shen, Li, Wang, Chen & Chua, 2002; Chukwu, Anoliefo & Ikhajiagbe, 2017). Previous research has revealed that increase in scrap metal dump sites has resulted in the increase in heavy metal pollution to the environment Chukwu *et al.,* (2017). Chukwu *et al.* (2017) also, identified four most prevalent plants present in the nine scrap metal dump sites, which includes *Amarantus spinosus*, *Eleusine indica*, *Andropogon tectorum* and *Peperonia pellucida*. The survival of these four most prevalent plants in heavy metal polluted soils could be accrued to the prepondence of microbial organisms in the rhizospheric soil of the plants. Previous research has shown that microbial organisms in the rhizospheric soil of plants may not only serve as plant growth promoting rhizospheric organisms (PGPRs), but also help to protect plants against the toxicity of the heavy metals in the soil. These microbial organisms also have the ability to accumulate, degrade or use up the heavy metals in the soil (Sinha & Mukherjee, 2008; Ma, Rajkumar & Freitas, 2009a). This research therefore aims to identify and isolate culturable microbial organisms present in heavy metal polluted rhizospheric soil and water that could possibly serve as PGPRs and that could be used in phytoremediation.

Methodology

A total of twenty - three samples of soil and water from scrap metal dump sites were collected during the rainy and dry seasons. The collection points were assessed using a GPS device. Details of sample location were shown in previous study (Chukwu *et al.*, 2017).

Water sample collection methods

Water samples were collected in 500 ml sterilized plastic containers and were filled to the brim to exclude air. Water samples were carefully collected from accessible borehole, hand dug well, run-off at 5 m and 3 m and rivers closest to scrap metal dump sites.

Soil sample collection methods

Soil samples were obtained from the nine (09) scrap metal dump sites and a control site (with no history of industrial activities and scrap metal business) in Benin City. The rhizospheric soil of the most prevalent plants were collected using quadrats according to Anoliefo (2016).

Laboratory analysis of soil samples

Soil samples were analyzed for pH, Electrical Conductivity, and selected heavy metal content (Fe, Zn, Cu, Cr, Cd, and Pb) according to the method of Association of Official Analytical Chemists (AOAC), (2005).

Identification of Soil Microorganisms:

Isolation and characterization of bacterial and fungal isolates were carried out using the methods of (Calfee & Lee, 2014; Cheesebrough, 2001; Taiwo & Oso, 2004).

Results and discussion

This research identified from previous study that some species of plants could grow on scrap metal dump sites amongst which the four most prevalent plants in the nine scrap metal dump sites were identified (*Amarantus spinosus*, *Eleusine indica Andropogon tectorum* and *Peperonia pellucida*). Present research has shown that these scrap metal dump sites contain high concentration of heavy metals (Table 1). The concentration of the six heavy metals (Cu, Cr, Zn, Cd, Pb and Fe) in the rhizospheric soil varied Cu (0.00 - 1.35 ppm), Pb (0.14 - 1.18 ppm), Zn (0.00 – 1.05 ppm) Cr (0.17 – 3.30 ppm) and Cd (0.00 – 0.03 ppm). This gave different degrees of pollution of the rhizosheric soil samples under study. This results supports the findings of (Anoliefo, Ikhajiagbe, Okonofhua & Diafe, 2006; Anoliefo, Ikhajiagbe, Okonofhua, Edegbai & Obasuji, 2008; Adedeji, Olayinka & Nwanya, 2014). Chromium exceeded the phytotoxicity limits for all the rhizospheric soil samples investigated from all the sites except for rhizospheric soil of *E. indica* obtained from Evbwuotubu scrap metal dump site. The research agrees with the findings (Khan, Kuek, Chaundhry, Khoo & Hayes, 2000; Denton, 2007; Garbisu and Alkorta, 2001; Gisbert, Ros, Haro, Walker, Pilar, Serrano & Avino, 2003), that heavy metals are being released into the environment from various sources which includes scrap metals.

Table 1: Heavy metal concentration obtained from rhizospheric soil within 3 m radius of scrap metal dump sites

Sites	Plants	Heavy metals concentration (ppm)					
		Cu	Pb	Zn	Cr	Cd	Fe
	Ecotoxicity	200	50	50	1	4	200
A	*Eleusine indica*	0.30	0.62	0.00	2.40	0.00	14.10
B	*Amaranthus spinosus*	0.73	0.95	0.30	1.88	0.01	2.50
C	*Eleusine indica*	0.15	1.13	0.03	0.17	0.01	12.00
D	*Amaranthus spinosus*	0.40	0.56	0.02	1.94	0.01	10.70
E	*Andropogon tectorum*	0.06	0.42	1.05	1.53	0.00	1.60
F	*Amaranthus spinosus*	0.02	0.14	0.02	3.30	0.00	2.90
G	*Peperomia pellucida*	0.28	0.61	0.36	1.30	0.00	13.00
H	*Peperomia pellucida*	1.35	1.18	0.00	2.02	0.03	5.30
I	*Amaranthus spinosus*	0.05	0.38	0.12	1.41	0.01	13.4
J	*Andropogon tectorum*	0.00	0.32	0.00	0.00	0.00	2.70

Keys

A.) Osasogie Street by Total Filling Station, off Benin Sapele Road, Benin, B.) Omoragbon Agho, off Benin Sapele Road, Etete, Benin, C.) Evbuotubu Junction by Ekehuan Road Benin City, D.) Asoro Hill by Upper Ekehuan Road Benin, E.) Christopher Osewengie Street, Ekae Qtrs, off Sapele Road, Benin, F.) Akugbe Street, off Saint Saviour Road, Benin City, G.) Upper Iwehen, off Lagos Street, Oba Market, Benin City, H.) Evbienwen Street, off Wire Road, Benin City, I.) Iduowina Road, Iduowina Quarters, off Benin Auchi Road, Benin City, J.) Control Site; Reserved area, University of Benin, Benin City.

Table 2: Microbial count for bacteria and fungi in soil and water

The total microbial count of samples collected from the pilot study at different sites of collection for both water and rhizospheric soils have been presented in Table 2. The site with the highest colony count for bacteria was at Christopher from a run off at 5m from scrap metal dump site (3.3×10^5 cfu/g), followed by the site at Christopher from run off at 3m from scrap metal dump site, (2.0×10^5 cfu/g) and water sample collected from borehole near Osasogie scrap site (1.2×10^5 cfu/g) respectively, while the lowest colony counts were collected from soil sample attached to the root of *E. indica* at Akugbe scrap metal dump site (1.6×10^3 cfu/g), water sample collected from borehole at Evbuotubu (2.0×10^3 cfu/g) and soil sample attached to *A. tectorum* collected from Christopher (2.3×10^3 cfu/g). For fungi count the sample with the lowest colonies were water sample collected from bore hole at upper Iwehen (9.0×10^2 cfu/g) followed by soil attached to *A. spinosus* at Asoro scrap metal site (1.0×10^3 cfu/g) and from soil attached to *A. spinosus*

at Etete scrap metal site (2.5 x 10^3 cfu/g) respectively. Sites with the highest colonies were Christopher from run off at 3m from scrap metal dump site (7.5 x 10^4 cfu/g) and water sample collected from well at Akugbe (7.5 x 10^3 cfu/g), after which were followed by 5m run-off at Christopher scrap metal dump site (6.0 x 10^3 cfu/g), soil attached to *E. indica* at Evbuotubu (6.0 x 10^3 cfu/g) and water sample collected from the river at Uniben Reserved Area (control) (4.5 x 10^3 cfu/g) respectively. Therefore the research has shown that increased heavy metal pollution drastically reduced microbial activities. This agrees with the research carried out by Joner & Leyval, (2001);

Table 2: Microbial count for bacteria and fungi in soil and water

Treatments	**Microbial load (bacteria) (x 10^4 cfu/g)**	**Microbial load (Fungi) (x 10^4 cfu/g)**
A	1.0	4.5
B	3.1	0.09
C	0.31	0.35
D	5.0	1.5
E	2.0	7.5
F	1.1	0.50
G	0.20	0.50
H	0.50	0.65
I	0.70	0.30
J	0.68	0.40
K	12	0.50
L	20	7.5
M	33	0.60
N	2.8	0.55
O	6.0	4.0
P	4.2	0.25
Q	4.6	6.0
R	6.6	0.10
S	0.23	0.55
T	0.16	0.30
U	1.9	0.40
V	0.47	0.65
W	1.1	0.75

Table 3: Tabulation of the prevalence of microbial organisms from rhizospheric soil and water samples

MICROBIAL ORGANISMS SPECIES	SITES OF SAMPLING	NUMBER OF SITES DETECTED (PREVALENCE)	PERCENTAGE PREVALENCE
FUNGI			
Aspergillus niger	A, B, F, G, H, I, J, K, L, N, R, S, T, U, V, W	16	70
Penicillium spp.	A, C, D, E, F, H, I, J, M, O, P, Q, R, T, U, V, W	17	74
Mucor mucedo	C, D, E, F, G, H, I, J, K, M, O, P, Q, R, S, T, U, V, W	19	83
Trichoderma harzianum	B, D, E, G, H, I, J, M, Q, S, T, U, V, W	14	61
Aspergillus flavus	A, C, E, G, H, L, M, N, Q, S, T	11	48
Microsporus spp.	A, F, I, R, U, V, S	7	30
Rhizopus spp.	B, L, N, K	4	17
Fusarium solani	B, D, G, P	4	17
BACTERIA			
Bacillus substilis	A, B, C, D, E, F, H, I, J, K, L, M, N, P, Q, V	16	70
Staphylococcus epideymis	A, B, C, D, F, J, K, M, Q, R, S, T, U, V, W	15	65
Staphylococcus aureus	A, C, D, E, G, I, K, M, R, T, U	11	48
Pseudomonas aeruginosa	B, G, J, K, L, Q, R, T, U, V, W	11	48
Micrococcus letus	H, J, L, M, N, P, S	7	30
E. coli	E, K, W	3	13
Proteus mirabilis	O, T	2	9
Clostridium spp	R	1	4

Keys

A. Control site Uniben reserved area (water sample collected from river).
B. Upper Iwehen scrap metal site (water sample collected from borehole)
C. Evbuotubu scrap metal site (water run-off at 5m away from scrap metal site)
D. Evbienwen scrap metal site (water sample collected from borehole)
E. Akugbe scrap metal site (water sample collected from well)
F. Christopher scrap metal site (water sample collected from borehole)
G. Evbuotubu scrap metal site (water sample collected from borehole)

H. Iduowina scrap metal site (water sample collected from borehole)
I. Evbuotubu scrap metal site (water run-off at 3m away from scrap metal site)
J. Etete scrap metal site (water sample collected from borehole)
K. Osasogie scrap metal site (water sample collected from borehole)
L. Christopher scrap metal site (water run-off at 3m from scrap metal site)
M. Christopher scrap metal site (water run-off from scrap metal site)
N. Asoro scrap site (water sample from ogba river)
O. Osasogie scrap site (rhizosphere soil of *E. indica*)
P. Etete scrap metal site (rhizosphere soil of *A. spinosus*)
Q. Evbuotubu scrap metal site (rhizosphere soil of *E. indica*)
R. Asoro scrap metal site (rhizosphere soil of *A. spinosus*)
S. Christopher scrap metal site (soil attached to *A. tectorum*)
T. Akugbe scrap metal site (rhizosphere soil of *A. spinosus*)
U. Upper Iwehen scrap metal site (rhizosphere soils of *P. pellucida*)
V. Evbienwen scrap metal site (rhizosphere soils of *P. pellucida*)
W. Iduowina scrap metal site (rhizosphere soils of *A. spinosus*)

The microbial organisms were characterized and isolated to access the prevalence and distribution in the samples used for the study. Eight (8) bacteria species and eight (8) fungi species were isolated and their prevalence determined as a percentage of the number of sites they were detected in the total soil samples (Table 3). For the bacteria microbial organisms, *Bacillus substilis* had the greatest prevalence score of seventy (70) percent, *Staphylococcus epideymis* 65 %, *Staphylococcus aureus* 48 %, *Pseudomonas aureginosa* 48 %, *Micrococcus letus* 30 %, *E. coli* 13 %, *Protus mirabilis* 9 % and the least prevalence was *Clistridium* spp 4 %. The study also showed eight (8) fungi species were isolated and their degree of prevalence beginning from the highest to lowest is shown in Table 3 above. *Mucor mucedo* 83 % detected in 19 samples, *Penicillum* species 74 %, detected in 17 samples, and the *least Fusarium solani* 17 %, detected in four (4) samples. This agrees with the findings of (Rajkumar, Nagendran, Kui, Wang, & Sung, 2006; Sinha *et al*., 2008; Ma, Rajkumar & Freitas, 2009a).

Conclusion

The prepondence of the distribution of the various microbial organisms in the samples taken from boreholes, wells, rivers and water runoffs near the scrap metal dumpsites and rhizospheric soils from scrap metal dump sites is an evidence that the microbial organisms identified has specific features that helps it survive in heavy metal polluted soils and water, and could therefore serve as PGPRs. Some of the identified microbial organisms could also be applied in phytoremediation.

Recommendation

The research identified eight bacterial organisms and eight fungal species isolated from rhizospheric soil of the test plants (*Amaranthus spinosus, Eleusine indica, Andropogon tectorum* and *Peperomia pellucida*) in heavy metals polluted soil. The most prevalent organisms isolated from the nine metal scrap dump sites were identified as plant growth promoting rhizo-bacteria (PGPR) these included; *Bacillus substilis, Micrococcus luteus* and *Pseudomonas aeruginosa*, respectively. These organisms could also be applied in phytoremediation of heavy metal polluted soils.

References

Adedeji, O. H., Olayinka, O. O., & Nwanya., F. C., (2014). Soil and Water Pollution Levels in and around Urban Scrapyards Department of Environmental Management and Toxicology. *Journal of Environmental Science, Toxicology and Food* 8 (5): 60-68.

Anoliefo, G. O., (2016). *Man and his environment: Unsustainable exploitation of the land and the dileman of the dog. 172nd Inaugural lecture*, May 19, 2016, University of Benin, Benin City, Nigeria. Uniben Printing Press. Pp 59-60.

Anoliefo, G. O., Ikhajiagbe, B., Okonofhua, B. O. & Diafe, F. V., (2006). Eco-taxonomic distribution of plant species around motor mechanic workshops in Asaba and Benin City, Nigeria: Identification oil tolerant plant species. *African Journal of Biotechnology* 5(19), 1757-1762.

Anoliefo, G. O., Ikhajiagbe, B., Okonofhua, B. O., Edegbai B. O. & Obasuji, D. C., (2008). Metal Tolerant Species Distribution and Richness in and Around the Metal Based Industries: Possible Candidates for Phytoremediation. *African Journal of Biotechnology* 2 (11), 360-370.

Association of Official Analytical Chemists AOAC (2005). Methods of Analysis. *Association of Official Analytical Chemists*, Washington D. C. 326p.

Calfee, K. M. & Lee, M. W., (2014). Composite Sampling of a Bacillus anthracis Surrogate with Cellulose Sponge Surface Samplers from a Nonporous Surface. Plos One 9(12)

Cheesebrough, M., (2001). District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, Cambridge. 355p.

Chukwu, V. N., Anoliefo, G. O., Ikhajiagbe, B., (2017). Assessment of Plant Species Distribution within Scrap Metal Dump Sites in Benin City, Nigeria. *Nigerian Research Journal of Engineering and Environmental Sciences* 2(2) 2017 pp. 305-314

Denton, B., (2007) Advances in Phytoremediation of Heavy Metals Using Plant Growth Promoting bacteria and Fungi. *Basic Biotechnology journal* 3:1–5.

Garbisu, C. & Alkorta, I., (2001). Phytoextraction: A cost effective plant-based technology for the removal of metals from the environment. *Bio-resource technology*. 77(3):229–236.

Gisbert, C., Ros, R., Haro, A., Walker, D. J., Pilar, B. M., Serrano, R., & Avino, J. N., (2003). A plant genetically modified that accumulates Pb is especially promising for phytoremediation. *Biochemistry and Bio-physiology Research* 303(2):440–445.

Ikhajiagbe, B., & Anoliefo, G. O., (2012). Weed biodiversity studies of a waste engine oil-polluted soil exposed at different intervals of natural attenuation and substrate amendment. *Journal of Biological Sciences*, 12(5): 280-286.

Khan, A. G., Kuek, C., Chaundhry, T. M., Khoo, C. S., Hayes WJ (2000). Role of Plants, Mycorrhizae and Phytochelators in Heavy Metal Contaminated Land Remediation. *Chemosphere* 41:197–207.

Leyval, C., Joner, E. J., Del, Val, C. & Haselwandter & K., (2002). Potential of Arbuscular Mycorrhizal Fungi for Bioremediation. In: *Mycorrhiza Technology in Agriculture*. Birkhauser Verlag Basel, Switzerland, Pp 175–186.

Ma, Y., Rajkumar, M., & Freitas, H. (2009a). Improvement of Plant Growth and Nickel Uptake by Nickel Resistant-Plant-Growth Promoting Bacteria. *Journal Hazard Metal* 166:1154–1161.

Rajkumar, M., Nagendran, R., Kui, J. L., Wang, H. L., & Sung, S. K., (2006). Influence of plant growth promoting bacteria and Cr (VI) on the growth of Indian mustard. *Chemosphere*, 62: 741-748.

Shen, Z., Li, X., Wang, C., Chen, H. & Chua, H. (2002). Lead Phytoextraction from Contaminated Soil with High-Biomass Plant Species, *Journal of Environmental Quality*, 31(6): 1893–1900.

Sinha, S. & Mukherjee, S. K., (2008). Cadmium-Induced Siderophore Production by a High Cd-Resistant Bacteria Strain Relieved Cd Toxicity in Plants through Root Colonization. *Current Microbiology* 56:55–60.