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The use of *Pseudomonas* species AGAINST HEAVY METAL POLLUTANTS IN THE SOIL SAMPLES FROM SELECTED MECHANIC GARAGES IN BENUE

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Abstract: **Biological processes have been used to remove environmental pollutants like heavy metals and hydrocarbons through the process of Bioremediation. Bioremediation can either be ex-situ or in-situ depending on the requirement of the condition. Industrialization and urbanization has been a major cause for the increase in the number pollutants in the soil. The research was aimed at quantifying the combined activities of Pseudomonas species isolated from rice fields in reducing the amount of heavy metals (Cu, Pb, Ni, Cr and Cd). The research covered three senatorial districts of Benue State Nigeria (Zone A; Katsina-Ala and Zakibiam. Zone B; Gboko and Aper. Zone C; Otukpo and Ugbokolo). The samples were treated with the bacteria isolated at 24 hours, 48 hours and 72 hours. The results obtained were expressed in simple percentage which showed that the organism has the highest activity at 48 hours with 100% efficacy of Pb and in Zone A. In Zone B, Cr and Ni were degraded at 100% while Zone C has Pb and Cr degraded at 100%. The lowest heavy metal degradation was observed in Zone A at 17.31%, Zone B at 21.74% and Zone C at 25.49%. The findings in this study show that a combined effect of three Pseudomonas species has the best activity on the removal of heavy metals from polluted soils at 48 hours incubation period.**

***Keywords: Pseudomonas species*, Bioremediation, Heavy metals, Pollutants.**

1. INTRODUCTION

The use of microorganisms to remove or detoxify the contaminants of soil, water, or sediment otherwise termed "pollutants" which may threaten public health, is known as bioremediation [11], [13]. In this case suitable microorganisms with tolerance towards toxic levels of heavy metals are used to take-up metals through the acquisition of specific resistance systems such as efflux and uptake mechanisms, extracellular precipitation [3]. It is a general concept that includes all those processes and actions that take place in order to biotransform an environment, already altered by contaminants, to its original status [19]. There are Ex situ and In situ bioremediation [4]. The aim of bioremediation is to minimize these environmental pollutants [8]. Pollution is the introduction of contaminants into the natural environment that causes adverse change in environment. It can take the form of chemical substances or energy, such as noise, heat or light. Its issues escalated as population growth far exceeded view ability of neighborhoods to handle their waste problem. Reformers began to demand sewer systems, and clean water [6]. Heavy metal is any relatively dense metal or metalloid that is noted for its potential toxicity, especially in environmental contexts [2]. According to [10] heavy metals are normally regarded as metals with an atomic number 22 to 92 in all groups from period 3 to 7 in the periodic table. Some of the metals such as Cu, Zn, Cd, Pb, Fe, Cr, Co, Ni, Mn and Se are essential in trace quantities for the general wellbeing of living organism but an excess of these can be lethal.

Pseudomonas species are characterized as a group of Gram-negative rod shaped bacteria measuring 0.5 to 0.8 μm by 1.5 to 3.0 μm. Almost all strains are motile by means of a single polar flagellum. The bacteria thrive in soil and water, and on surfaces that are in contact with soil or water. Its metabolism is respiratory and never fermentative, but it grows in the

absence of O_2, if NO_3 is available as a respiratory electron acceptor. It has minimal nutritional needs [12]. This experiment was aimed at isolating, characterizing and using *Pseudomonas* species to reduce heavy metal pollutants in the soil samples in mechanic garages across Benue state of Nigeria.

2. MATERIALS AND METHOD

Study Area and Sample Collection

The study covered the three senatorial districts in Benue state. Zone A: kastina-Ala (longitude 7.1658°E, latitude 9.2841°N) and Zakibiam (longitude 7.5006°E, latitude 9.6063°N). Zone B: Gboko (longitude 7.3368°E, latitude 9.0018°N) and Apir (longitude 7.7338°E, latitude 8.5214°N) and Zone C: Ugbokolo (longitude 7.1587°E, latitude 9.3529 ^{0}N) and Otukpo (longitude 7.1982°E, latitude 8.1393°N). 40 g each of soil samples was collected randomly from each of these 6 mechanic garages of the target study. The soil samples were collected and sterilized. For the isolation of *P.* species, soil sample was taken at random from various points in the University of Agriculture Makurdi rice farm and bulked. The samples were collected in a sterile container and taken to biological sciences research laboratory for analysis.

Microbial isolation

This was carried according to the method described by [17].

Biochemical and morphological characterization

This was done according to the method described by [7]

Experimental Details

- 10 g each of the mechanic garage soil samples was weighed into 4 different foil papers.
- The first 10 g served as a control (not incubated on a media inoculated with *pseudomonas* spp)
- The 2nd 10 g was incubated on King's B media inoculated with *P.* spp for 24 hrs.
- The 3rd was incubated for 48 hrs while the 4th was incubated for 72 hrs each in a separate conical flask.
- After each incubation, the media were sterilized and the soil samples were regenerated by evaporating the media to dryness using heating mantle.

Determination of the heavy metals

The heavy metals determined are: lead, cadmium, chromium, nickel and copper.

Atomic Absorption Spectrophotometric analysis

After the microbial treatment (incubating the soil samples on a media containing the *P.* spp), the soil samples were digested by adding 20 ml of H_2SO_4 and 10 mls of HNO_3 in the ratio of 2:1. The mixture was heated on a Bunsen burner until the brown fumes subsides. 10 ml of HNO_3 was added continuously at the interval of 10 minutes and heated until the solution turned colourless.The digested samples were then analysed using Atomic Absorption Spectrophotometre.

3. RESULTS

The results were presented in the tables below.

Table 1: presents the Cultural and Biochemical Characterization of *Pseudomonas* Species used

S/NO	COLOUR OF COLONY	SHAPE OF COLONY	GRAM REACTION	MORPHOLOGY	CATALASE	UREASE	OXIDASE	CIRTATE	MANNITOL	MALTOSE	LACTOSE	ORGANISM
1	Cream	Irregular	-ve	Rod	+ve	-ve	+ve	+ve	+ve	-ve	-ve	*P.aeruginosa*
2	Cream	Irregular	-ve	Rod	+ve	-ve	+ve	+ve	+ve	+ve	+ve	*P.fluorescens*
3	Cream	Irregular		Rod	+ve	-ve	-ve	-ve	-ve	-ve	-ve	*P. putida*

Key: +ve = positive to the test
-ve = negative to the test

Table 2: shows Descriptive statistics of treated heavy metal by location

Element	Sampling unit	Control	Treatment mean	SEM	CV
Cu	Otukpo	1.27	0.98	0.12	20.38
	Ugbokolo	1.04	0.89	0.02	4.66
	Apir	1.12	0.92	0.05	9.29
	Gboko	1.02	0.85	0.07	13.57
	Katsina- Ala	1.52	0.92	0.08	15.22
	Zaki-Biam	1.22	0.80	0.36	77.55
Pb	Otukpo	0.02	0.00	0.00	173.21
	Ugbokolo	0.08	0.06	0.01	16.67
	Apir	0.08	0.05	0.01	28.64
	Gboko	0.06	0.02	0.01	124.90
	Katsina- Ala	0.07	0.07	0.01	20.83
	Zaki-Biam	0.11	0.01	0.01	173.21
Cr	Otukpo	0.00	0.00	0.00	-
	Ugbokolo	0.98	0.79	0.04	8.93
	Apir	0.92	0.78	0.04	9.25
	Gboko	0.91	0.04	0.04	173.21
	Katsina- Ala	0. 81	0.03	0.06	13.35
	Zaki-Biam	1.01	0.01	0.01	100.00
Ni	Otukpo	0.34	0.23	0.03	20.85
	Ugbokolo	0.02	0.00	0.00	173.21
	Apir	0.32	0.26	0.02	13.34
	Gboko	0.02	0.01	0.00	86.60
	Katsina' Ala	0.54	0.19	0.02	20.28
	Zaki-Biam	0.28	0.27	0.07	46.26
Cd	Otukpo	0.01	0.00	0.00	-
	Ugbokolo	0.08	0.04	0.01	43.30
	Apir	0.07	0.04	0.01	35.25
	Gboko	0.06	0.02	0.01	86.60
	Katsina- Ala	0.05	0.01	0.01	28.64
	Zaki-Biam	0.09	0.00	0.00	173.21
Sample size		1	3	3	3

SEM= Standard error of mean CV= Coefficient of variation

Table 3: presents the percentage degradation of the heavy metals from Zone A (Otukpo and Ugbokolo) mechanic garages (Values in Part Per Million)

Cr is not found in the soil in Otukpo mechanic village and no degradation. Cd is completely degraded throughout the period of treatment while Pb is degraded completely only at 48 and 72 hours. Cu presented the least value and Ni has a fairly percentage degradation value. Cu has the least percentage degradation value starting from the 24 hours through to 72 hours. Ni is the most degraded having 100 percent degradation values at 48 and 72 hours of treatment and 50 percent at 24 hours while Cd has the same value of percentage degradation at 48 and 72 hours respectively.

Incubation period → / Metal ↓	24hr			48hrs			72hr		
Change in heavy metal degradation	Crude	Treated	% degradation	Crude	Treated	% degradation	Crude	treated	% degradation
Cu	1.12	1.01	9.82	1.12	0.84	25.00	1.12	0.91	18.75
Pb	0.08	0.07	12.50	0.08	0.04	50.00	0.08	0.05	37.50
Cr	0.92	0.86	6.52	0.92	0.72	21.74	0.92	0.76	17.39
Ni	0.32	0.30	6.25	0.32	0.23	28.13	0.32	0.26	18.75
Cd	0.07	0.06	14.29	0.07	0.03	57.14	0.07	O.04	42.86

Ugbokolo									
	Crude	treated	% degradation	Crude	Treated	% degradation	Crude	Treated	% degradation
Cu	1.02	0.98	3.92	1.02	0.76	25.49	1.02	0.81	20.59
Pb	0.06	0.04	33.33	0.06	0.01	83.33	0.06	0.00	100.00
Cr	0.91	0.11	87.91	0.91	0.00	100.00	0.91	0.00	100.00
Ni	0.02	0.01	50.00	0.02	0.00	100.00	0.02	0.01	50.00
Cd	0.06	0.04	33.33	0.06	0.01	83.33	0.06	0.01	83.33

Table 4: presents the percentage degradation of the heavy metals from Zone B mechanic garage (Apir and Gboko) (Values in Part Per Million)

Change in percentage degradation in Ni is in the ratio of 1 :2 :1 (50 : 100 :50) within the periods considered. Cr has the highest percentage while Cu has the least in all the period of treatment. Cd and Pb have the same values at 24 and 48 hours but at 72, lead increased to 100 while Cd remained the same (83.33). Cd has the highest value of 57.14 at 48hour. The value decreased to 42.86 at 72 hours while Ni has the least value of 6.25 at 24 hours which increased up to a factor greater than 4 at 48 hours and then decreased again at 72 hours. Cu at 48 hours has a value that is half the figure observed for in Pb at same period of incubation.

Incubation period → / Metal ↓	24hr			48hrs			72hr		
Change in heavy metal degradation	Crude	Treated	% degradation	Crude	Treated	% degradation	Crude	treated	% degradation
Cu	1.12	1.01	9.82	1.12	0.84	25.00	1.12	0.91	18.75
Pb	0.08	0.07	12.50	0.08	0.04	50.00	0.08	0.05	37.50
Cr	0.92	0.86	6.52	0.92	0.72	21.74	0.92	0.76	17.39
Ni	0.32	0.30	6.25	0.32	0.23	28.13	0.32	0.26	18.75
Cd	0.07	0.06	14.29	0.07	0.03	57.14	0.07	O.04	42.86

Gboko									
	Crude	treated	% degradation	Crude	Treated	% degradation	Crude	Treated	% degradation
Cu	1.02	0.98	3.92	1.02	0.76	25.49	1.02	0.81	20.59
Pb	0.06	0.04	33.33	0.06	0.01	83.33	0.06	0.00	100.00
Cr	0.91	0.11	87.91	0.91	0.00	100.00	0.91	0.00	100.00
Ni	0.02	0.01	50.00	0.02	0.00	100.00	0.02	0.01	50.00
Cd	0.06	0.04	33.33	0.06	0.01	83.33	0.06	0.01	83.33

Table 5: presents the percentage degradation of the heavy metals from Zone C (Zaki-Biam and Katsina Ala) mechanic garages (Values in Part Per Million)

This table shows that Cr has the highest percentage of 91.79 within the 24 hrs of treatment followed by Cu with 33.48%. Ni and Pb have the least recorded 17.86 and 18.18% respectively. Cr continued to decrease as the period increased while other heavy metals fluctuated in their values. Cd and Pb have 100% change in degradation at the 48 and 72 hours while Cr is only at 48 hour. Same Cd was not degraded at all at the 24 hours while Cu has the least value at 24 hours.

Incubation Period → / Metal ↓	24hr			48hrs			72hr		
	Crude	treated	% degradation	Crude	Treated	% degradation	Crude	Treated	% degradation
Cu	1.22	1.08	33.48	1.22	0.82	83.61	1.22	0.86	29.51
Pb	0.11	0.09	18.18	0.11	0.06	45.45	0.11	0.07	36.36
Cr	1.01	0.93	91.79	1.01	0.72	28.71	1.01	0.78	22.77
Ni	0.28	0.23	17.86	0.28	0.16	42.86	0.28	0.17	39.29
Cd	0.09	0.07	22.22	0.09	0.04	55.55	0.09	0.05	44.44

Katsina- Ala									
	Crude	Treated	% degradation	Crude	Treated	% degradation	Crude	Treated	% degradation
Cu	1.52	1.51	0.66	1.52	0.43	71.71	1.52	0.45	70.39
Pb	0.03	0.02	33.33	0.03	0.00	100.00	0.03	0.00	100.00
Cr	0.03	0.01	66.66	0.03	0.00	100.00	0.03	0.02	33.33
Ni	0.54	0.41	24.07	0.54	0.17	68.52	0.54	0.23	57.41
Cd	0.01	0.01	0.00	0.01	0.00	100.00	0.01	0.00	100.00

4. DISCUSSION

The results of the cultural, morphological and biochemical characteristics of *Pseudomonas* species isolated is similar to studies carried out by [14] except in their Indole production test where they reported *P. fluorescens* to be Indole positive whereas the results of this work shows negative. The percentage of heavy metal remediation reveals that a significant percentage of the heavy metals in the soil were removed by this method of remediation giving credence to this employed method. The control (unremediated) soil from the same locations is higher in their content of heavy metals than the treated soils. This could be as a result of stress arising from accumulation of secondary metabolite produced in the media by the activities of the microbes or the exhaustion of the nutrient in the earlier days. This is in consistence with the research done by [5]. Comparing to the treatment, the media used as control has higher population of the microbes throughout the days of the study. This could be that the heavy metals interfere with the metabolic activities of the bacteria in the treated soil samples. The effect of sampling location on heavy metals in zone A (Kastina-Ala and Zakibiam) shows a significant difference in lead, chromium, and cadmium between these two places but no difference in copper and nickel at $p< 0.05$. In zone B (Gboko and Apir), only chromium and nickel differ significantly while there is no difference between the lead, copper and cadmium. In zone C (Otukpo and Ugbokolo), only copper and lead differ significantly. The difference could be attributed to the geological and anthropological factors in these zones. The geological factor is natural and varies from place to place [16]. Difference in industrial activities and urban development could be another factor. The effect of incubation period on heavy metals shows that for all the samples used, there is significant difference in the means of heavy metal concentration between the control and the 24 hours of incubation but a significant difference exists between the control and the 48 hours only for copper and cadmium. This may mean that in exhaustion of other nutrients, the heavy metals became toxic to the microbes such that it could not take it any more rather it goes on to produce the secondary metabolite. This agrees with the work done by [5]. This work supports the findings of [8] and [5] however, in the works done by these authors the species were inoculated at a strain level while three species were used for this study.

5. CONCLUSION

There is a low concentration of heavy metals from the sampled locations which in general implies low industrialization and urbanization in these locations. A combination of three *Pseudomonas* species *(Auriginosa, Putida and Flourescence)* can remediate the heavy metals in the soil to a significant percentage. These species are one of the organisms that are effective in bioremediation of heavy metals; high concentrations of some of these heavy metals affect gelatinase expression and pigment formation. The microorganism is most effective at the 48 hours of treatment and hence a combination of these organisms could successfully be exploited biotechnologically for the bioremediation of; Pd, Cu, Cd, Ni and Cr in contaminated soils and other industrial processes such as biomining.

REFERENCES

[1] Adhikari, T., Manna, M. C., Singh, M. V. and Wanjari, R.H. (2004). Bioremediation measure minimize heavy metals accumulation in soils and crops irrigated with city effluent. *Food, agriculture and environment*. Pp 266-270.

[2] Bánfalvi, G. (2011). "Heavy Metals, Trace Elements and their Cellular Effects". In Bánfalvi, G. Cellular Effects of Heavy Metals. Springer. pp. 3–28. ISBN 9789400704275.

[3] Cánovas, D., Durán, C., Rodríguez, N., Amils, R. and de Lorenzo, V. (2003). Testing the limits of biological tolerance to arsenic in a fungus isolated from the River Tinto. *Environmental Microbioiology* **5:** 133-138.

[4] Evans, G. M. and Furlong, J. C. (2003). Environmental Biotechnology Theory and Application. John Wiley and Sons,West Sussex, England.

[5] Garima, A., Anjali, C., Rachna, C and Jyoti, P. (2015). Study on Role of *Pseudomonas aeruginosa* on Heavy Metal Bioremediation. *Int. J. Pure App. Biosci.* 3 (4): 92-100 (2015)

[6] Lee Jackson, (2014). Dirty Old London: The Victorian Fight Against Filth. Cited in David Clay Large, *Berlin* (2000) pp 17-18

[7] Monica Cheesbrough (2010). District Laboratory Practice in Tropical Countries. 5th printing 2010. pp. 62-70

[8] Paul, D., Pandey, G., Pandey, J. and Jain, R. K. (2005). Accessing microbial diversity for bioremediation and environmental restoration. Trends in Biotechnology, 23(3),135–142. perspectives. Gene, 480(1–2), 1–9.

[9] Rajendran, P., Muthukrishnan, J., and Gunasekaran, P. (2003). Microbes in heavy metal remediation. Indian Journal of Experimental Biology, 41(9), 935–944.

[10] Sharma and Rehman, M. B. (2009). Laboratory scale approach, Indian Journal of Microbiology, 48: 142-146.

[11] Talley, J. (2005). Introduction of recalcitrant compounds. In W. Jaferey and L. Talley (Eds.), Bioremediation of recalcitrant compounds (pp. 1–9). The Effect of Long-Term Exposure to Particulate Pollution on the Lung Function of Teheranian and Zanjanian Students, *Pakistan Journal of Physiology*. 3(2), pp. 1-5.

[12] Todar, K. (2011). Textbook of Bacteriology. 1st ed. **11:** 1425-1426.

[13] Wasi, S., Jeelani, G. and Ahmad, M. (2008) Biochemical characterization of a multiple heavy metal pesticides and phenol resistant *Pseudomonas fluorescens* strain. Chemosphere 71: 1348–1355.25.

[14] Wasi, S., Tabres, S. and Ahmed, M. (2010). Isolation and characterization of pseudomonas *flourescens* strain tolerant to major India water pollutants. Journal of Bioremediation and Biodegradation 1:101. Doi:10.4172/2155-6199.1000101

[15] Agostinho, A., de Lima, S., Márcia, A., Ribeiro, de Carvalho, Sérgio, A. L., de Souza, Patrícia, M., Teixeira Dias, Renato, G., da Silva Filho, Carmen, S., de Meirelles Saramago, Cleonice, A., de Melo Bento and Ernesto Hofer (2012) heavy metal tolerance (cr, ag and hg) in bacteria isolated from sewage. *Brazilian Journal of Microbiology* (2012): 1620-1631 ISSN 1517- 8382

[16] Gadepalle, V. P., Ouki, S. K. Van Herwijnen, R. and Hutchings, T. (2008). Effects of amended compost on mobility and uptake of arsenic by rye grass in contaminated soil. Chemosphere, 72, 1056 – 1061.

[17] Onyeze, R.C., Udeh, S.M.C., Okwor, M.C. and Ugwu, O.P.C. (2013). Isolation And Characterization Of Bacteria That Are Associated With The Production and Spoilage of Ogi (Akamu). International journal of pharma medicine and biological science. Vol.2 No.3,July 2013