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Bioremoval of Cadmium, Mercury, Nickel and Zinc from Leachate Sample Collected from Refuse Dump on Obiri Ikwerre / Air Port Link Road Using Living Cells of *Aspegillus Niger* and *Rhizopus Stolonifer*

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Abstract

Bioremoval of heavy metals from leachate sample collected from refused dump on Obiri Ikwerre / Air port link road was studied. Five treatment options were set up. These include Natural process (positive control), poisoned and filtered sample (negative control), Aspergillus niger, Rhizopus stolonifer and a mixed culture of the above microorganisms. The experiment was conducted at ambient temperature (30 ± 2^0C and pH range of 6.8 to 8.9 and incubated for 60days. Physicochemical analyses of the leachate on day zero revealed the presence of Cadmium (1.74mg/l), Mercury (0.91mg/l), Nickel (2.6mg/l) and Zinc (4.52mg/l) and was found to be relatively high in relation to WHO environmental standards. At the end of 60 days, the various treatment options (Natural process(positive control), poisoned and filtered sample(negative control), Rhizopus stolonifer, Aspergillus niger, and a mixed culture of the above microorganisms) were able to bioaccumulate 69.4%, 2.9%, 53.3%, 48.1% and 49% of Cadmium; 47.1%, 1.0%, 38.2%, 52.7% and 35.4% of Nickel; 63.7%, 1.2%, 48.6%, 49.8% and 72.4% of Zinc respectively. Mercury was bioremoved by only Positive Control, Rhizopus stolonifer and the mixed culture (2.1%, 2.8% and 2.3%). However, not all the heavy metal recovered from the sample was bioconcentrated. Aspergillus niger, Rhizopus stolonifer and the mixed culture bioconcentrated 33%, 22.1% and 41% of Cadmium; 22%, 18% and 28% of Nickel; and 40.7%, 32.9% and 58% of Zinc. None of the treatment options bioconcentrated Mercury. Analysis of variance of the variables (various treatment options) showed that Natural process (positive control), Aspergillus niger and the mixed culture showed significant difference ($P>0.05$) with time while there was no significant difference ($P<0.05$) for poisoned and filtered sample (negative control), and Rhizopus stolonife. This study has demonstrated the great potential of the above listed microorganisms to clean-up Cadmium, Nickel and Zinc impacted environment and can be employed in treatment of environments polluted with high levels of these metals.

Keywords: Aspergillus niger, bioremoval, heavy metals, leachate, rhizopus stolonifer.

Introduction

It has been shown that the release of toxic substances and their dispersal into the environment may be harmful on the exposed populations. Over the past few decades the huge increase in the use of heavy metals has resulted in an increased deposit of metallic substances into aquatic environment. These metals have the important characteristics of being persistent and most of their ions are toxic to living organisms. Therefore, in order to have a pollution-free environment, the toxic materials should be removed from wastewater before its disposal.

A number of studies on heavy metal removal from solution have been launched because of the ecological effects of toxic metals released into the environment[1,2]. Environmental pollution from man-made sources can easily create local conditions of elevated metal presence and this case could lead to some hazardous effects on animals and humans[1,3,4]. Mercury, lead, cadmium, arsenic, antimony, copper, zinc, nickel, cobalt etc. are toxic heavy metals coming from the various sources such as electroplating, plastic and paint manufacturing, mining, metallurgical process, petrochemical process, batteries, paper and pulp[5] are accumulated into the environment. Katarzyna C.[6] stated that domestic sources of heavy metals include antacids, baking powder, cigarette filters, dental amalgams, toothpaste, coloring agent in wall paper and toys, insecticides, rat poisons and cigarette smoke (1 cigarette contains 1-2 micrograms of Cadmium), incineration of tyres, rubber and plastic; vending machine, soft drinks, etc. Nickel containing effluents are common due to their large number of industrial uses ranging from electroplating to long-life batteries[7]. Elevated levels of nickel are harmful in the environment, and it has also carcinogenic effect[1]. The investigation of nickel removal from aqueous solution has major importance due to its toxicity[8]. There is, also significant interest regarding Zinc removal from wastewaters its toxicity for humans at levels s of 100-500 mg/Day[9]. World Health Organization (WHO) recommended the maximum acceptable concentration of Zinc in drinking water as 5.0 mg/l[10].

A number of methods based on ion exchange, electrochemical treatment, membrane technologies, evaporation recovery, chemical oxidation or reduction, and chemical precipitation for the removal of toxic metals from aqueous solutions are usually ineffective when the metals concentration are low (range of 1-100ppm)[5] or extremely expensive[1]. Therefore, new technologies that can concentrate and/or accumulate toxic metals at affordable costs are required hence bioremoval technology.

This study was done on solid wastes collected from a dump on Obiri Ikwerre / Airport road and was studied using a few microorganisms (living cells) isolated from environmental wastes *(Aspergillus niger and Rhizopus stolonifer* and *a mixed culture)* to bioremoval Zinc. The study is aimed at determining the physicochemical properties of the solid wastes and to explore the bioremoval potentials of *Rhizopus stolonifer, Aspergillus niger* and *Chlorella vulgaris* for the recovery of heavy metals such as Cadmium, Chromium and Zinc from the effluent discharge from Port Harcourt Refinery Company. It also studies the influence of some physicochemical parameters of the effluent (Total Organic Carbon, Nitrate and Phosphate) in the course of the bioremoval process. Finally, it examines the method employed by the test microorganisms in the recovery of these metals i.e, whether it is by biosorption, bioadsorption or both.

Material and Methods

Isolation of test microorganisms (Fungal spp): Culture madia used were prepared according to manufacturer's instruction (oxoid manual). Culture method was used to isolate the test organisms from environmental wastes. A total of 10 samples were analyzed. Each sample was suspend in normal saline and allowed for one hour. It was shaken by mechanical means and elute of each sample was poured into 10mls universal bottle. An aliquot of each sample inoculated in nutrient agar and potato dextrose agar and incubated at 35^0C for 48hours[11-15].

Sample Collection: A 10 litre container sterilized with 70% ethanol was filled with the effluent from the landfill located at Obiri Ikwerre / Airport link road at Aluu end and taken to the laboratory for analysis. The physicochemical parameters of the sample were measured almost immediately

Measurement of some physicochemical parameters of the liquid wastes: The following physicochemical parameters were determine, thus pH, temperature, total organic carbon, nitrate, phosphate, biochemical oxygen demand, total petroleum hydrocarbon and heavy metals such as cadmium, chromium, zinc, lead, cobalt, nickel and mercury. The heavy metals (Cadmium, Zinc, Lead, Mercury, Cobalt, Nickel, and Chromium) were determined using atomic absorption spectrophotometers (AAS) (Bulk Scientific 200A Mode). The values are expressed in mg/L of the liquid sample[13,16].

Bioconcentration and bioadsorption tests: The concentrations of the above heavy metals in the washed test microorganisms and the filtrate were determined respectively on the 60^{th} day using atomic absorption spectrophotometer. The bioconcentration factor was calculated.

Experimental Design: Five different treatment options were set up in 250mls conical flasks (Erlenmeyer) and labeled A-E. i. Flask A (Positive Control) contained 200mls of the untreated sample and was monitored for natural processes. ii. Flask B (Negative Control) contained 200mls of poisoned sample with 1% formalin solution. iii. Flask C contained 200mls of the filtered sample seeded with 10mls of pure culture of *Aspergillus niger,* iv. Flask D contained 200mls of the filtered sample seeded with 10mls of pure culture of *Rhizopus stolonifer.* v. Flask E contained 200mls of the filtered sample seeded with 2mls each of pure culture of *Rhizopus stolonifer and Aspergillus niger.*

The samples (A-E) were incubated at ambient temperature (30 ± 2^0C) for 60 days. 50mls of each sample was filtered to remove the cells and associated deposits and the concentrations of cadmium, nickel, and zinc in the medium were measured fortnightly.

The concentrations of the above heavy metals in the washed test microorganisms and the filtrate were determined respectively on the 60^{th} day using atomic absorption spectrophotometer.

Results and Discussion

Physicochemical analysis of the effluent: The values of the physicochemical analyses recorded in Tables 1 to 5 are mean of the duplicate analytical values. The analysis on Day zero showed that the sample contained high concentration of Nitrate (102.6mg/l), Phosphate (15.34mg/l), Cadmium (1.74mg/l), Mercury (0.91mg/l), Nickel (2.6mg/l), Chromium (3.5mg/l), Zinc (4.52mg/l), Lead (3.19mg/l), COD (800.8mg/l) BOD (396.6/mg/l), Conductivity ($55uscm^{-1}$) and total hydrocarbon (2.65mg/l) with temperature and pH of 36.5^0C and 8.6 respectively (table 1).

Percentage removal of materials from the leachate between day zero and day 60: The determination of Phosphate, Nitrate, Total Organic Carbon, Cadmium, Nickel, Zinc and Mercury concentration on the 14^{th} day for the different treatment options demonstrated an impressive performance (Table 2). Significant percentage of the heavy metals (except Mercury) where removed from the medium in the treatment options A, C, D and E. Option B did not show any evidence of heavy metal removal in the first 14 days. Treatment options A (natural processes) and B (poisoned sample) served as positive and negative controls respectively. In A, C, D and E; the percentage of Cadmium removed are 2.8mg/l, 1.9mg/l, 1.7mg/l and 2.7mg/l respectively.

Table 2 showed a steady rise in Nickel bioremoval for all the treatment options except for option B. Options A, C, D and E were able to bioremove 1.6mg/l, 0.9mg/l, 1.1mg/l and 1.2mg/l on the 14^{th} day. Also, there was a uniform rise in the potential of all the treatment options for the bioremoval of Zinc on the 14^{th} day with the exception of option B. there was no evidence of mercury removal except for treatment option A (0.1mg/l). There was a corresponding rise in the percentage of organic carbon, nitrate and phosphate utilization.

On day 28 (table 3), there was corresponding increase in percentage of heavy metals boiremoval (except mercury) for all the options. However, greater percentage removal was seen in options C and D than in E. The percent mercury lost was evident on day 28 for options C and E. No significant rate was observed for B and D with respect to Mercury lost in the medium.

On day 42, the increased rate of biorecovery continued for options all the treatment options for Cadmium and Zinc. Same was observed for Nickel but at a reduced rate. There was no evidence of Mercury removal by option C (table 4). Tables 2, 3, 4 and 5 showed corresponding increase in the rate of removal of Nitrate, Phosphate and Organic carbon from the leachate. Thus on the 60^{th} day, very high concentration of these compounds have been consumed in the different treatment options as shown in table 5.

On day 60, options A, C, D and E had bioremoved 69.4, 53.3, 48.1 and 49% of Cadmium; 47.1, 38.2, 52.7, and 35.4% of Nickel; 63.7, 48.6, 49.8 and 72.4% of Zinc respectively. Treatment options A, D and E showed low percentage loss of Mercury on the 60^{th} day; thus, 2.1, 2.8 and 2.3% respectively. The statistical analysis showed that the treatment options and time were significantly different at 0.05 probability levels while the analysis of covariance showed that the treatment option E has a negative covariance relationship (r= -1) with A, B, C and D. Other treatment options have positive covariance (r= +1) when paired with each other.

Bioconcentration and Bioadsorption of the metals under study: Table 6 showed the ability of the different test microorganisms to bioconcentate heavy metals being investigated. While all the treatment options showed ability to bioconcentate Cadmium, Nickel and Zinc, non showed bioconcentration for Mercury. Not all the heavy metals removed were bioconcentated, certain percentage of the metals were adsorbed to the cell surface and was wash off as filtrate during the cell wash. Options A, D and E demonstrated that the removal of Mercury was by bioadsorption. The percentage adsorbed to the cell surface is shown in table 7.

Bioconcentrarion factor: The bioconcentration factor of the different treatment option listed in Table 8 showed that option D has the highest factor for Cadmium with a recorded value of 0.45; E has the highest values for Nickel and Zinc (1.51 and 2.62 respectively).

Table-1
Physicochemical Parameters of effluent on day Zero

Parameter	Mean values
pH	8.6 (6.5-9.2)*
Temperature	36.5°c (21.8-33.4)*
Total organic carbon	800.8mg/l
Nitrate	102.6mg/l (50mg/l)*
Phosphate	15.34mg/l
Zinc	4.52 (3mg/l)*
Cadmium	1.74 (0.003mg/l)*
Lead	3.19 (0.001mg/l)*
Mercury	< 0.91 (0.001mg/l)*
Chromium	3.5 (0.05mg/l)*
Nickel	2.6mg/l
Biochemical Oxygen Demand	596.60mg/l
Total hydrocarbon	2.65mg/l

*Acceptable standards in (bracket) Nigeria (Clean water act, 2001)

Table-2
Percent removal on day 14

Sample Code	TOC	NO_3	$P0_4$	Cd	Ni	Zn	Hg
A	11.8	3.1	1.5	2.8	1.6	11.8	0.1
B	-	-	-	-	-	-	-
C	8.6	2.1	1.2	1.9	0.9	2.8	-
D	9.0	1.9	1.0	1.7	1.1	3.2	-
E	10.6	3,0	1.4	2.7	1.2	4.6	-

Table-3
Percent removal on day 28

Sample Code	TOC	NO	PO	Cd	Ni	Zn	Hg
A	25.6	20.2	18.6	10.1	8.6	21.7	0.6
B	0.2	0.01	-	-	-	-	-
C	18.8	12.9	17.3	15	11.8	20	0.2
D	17	10.9	12.5	13.8	21.9	17.6	-
E	28	19.6	18.2	12.6	16.1	25.4	0.4

Table-4
Percent removal on day 42

Sample Code	TOC	NO_3	PO_4	Cd	Ni	Zn	Hg
A	36	27	23.6	48.4	39.2	56.1	1.2
B	1.2	0.6	2.1	2.2	0.9	1.2	-
C	32.4	23	19.9	40	21	45	-
D	31.9	20	15	29	33.1	48	0.6
E	39.6	27.1	24.8	28.1	19.8	66.9	1.0

Table-5
Percent removal on day 60

Sample Code	TOC	NO_3	PO_4	Cd	Ni	Zn	Hg
A	56	40.1	27	69.4	47.1	63.7	2.1
B	1.8	1.0	2.6	2.9	1.0	1.2	-
C	40.1	39.5	25.7	53.3	38.2	48.6	-
D	56.2	31.6	20.8	48.1	52.7	49.8	2.8
E	63.8	43.4	37.6	49	35.4	72.4	2.3

Table-6
Percent Concentration of heavy metals in washed cell Samples on day 60 (Bioconcentration Test)

Sample code	Cd	Ni	Zn	Hg
C	33	22	40.7	ND
D	22.1	18	32.9	ND
E	41	28	58	ND

Note: ND=Not detected

Table-7
Percent Concentration of heavy metals in Filtrate on day 60 (Bioadsorption Test)

Sample code	Cd	Ni	Zn	Hg
C	20.3	16.2	8.6	-
D	26	34.7	16.9	2.7
E	8	7.4	14.4	2.3

Table-8
Bioconcentration factor of the heavy metals analysed

Sample code	Cd	Ni	Zn	Hg
C	0.35	0.57	1.84	-
D	0.45	0.47	1.49	-
E	0.14	1.51	2.62	-

Discussion: A number of studies on heavy metal removal from solution have been carried out because of the ecological effects of toxic metals released into the environment, essentially from industrial and domestic sources. In this study, emphasis was placed on heavy metals found at high levels in leachate from solid waste dump. Physicochemical analyses of the leachate revealed the presence of heavy metals such as Cadmium, Mercury, Nickel and Zinc (table 1). Microorganisms used in this study were *Aspergillus niger* and *Rhizopus stolonifer* and were represented as treatment options C, and D. Treatment option E is a mixed culture of the two test microorganisms. The physicochemical properties of the leachate were found to favour the growth of the test microorganisms (table 1). With optimum temperature (36.5^0C) and pH (8.6)[17,18] and sufficient concentration of organic carbon, Nitrate and Phosphate, the medium was able to sustain the growth of these organisms for the period under study. This is in line with Aksu Z. et al.[17] who postulated that metal removal by bioremoval from water and waste water is strongly influence by physicochemical parameters such as ionic strength, pH and concentration of competing organic and inorganic compounds. For instance, on the 60th day as shown in table 5, there were relatively high concentration of organic carbon, nitrate and phosphate left to support the growth of the test microorganisms and promote physicochemical interactions.

The activities in figure 1 demonstrated an excellent performance of *Aspergillus niger* (flask C) with a seemingly constant percent removal for 42 days followed by a slight reduction in percentage in the last 18 days. The increase may be attributed to cell growth and sufficient metal binding sites on the test microorganisms[19,20]. *Rhizopus stolonifer* (flask D) presented an interesting picture in its pattern of activity. Its first 14 days witnessed a low percentage loss of Cadmium, followed by a slight increase percent thereof till the 60th day. The slow rate of recovery the first 14 days may be that the microorganisms were adapting to the new environment or was at the preparatory stage for the production of appropriate enzyme system for biosorption of Cadmium. Besides, the production of appropriate enzyme system and increase in cell population may be responsible for increased evidence thereof. However, fungi are excellent source of biosorbent due to their fast growth rate, minimal nutrient requirement as well as large biomass production[1,21]. Gadd G.M. et al.[19] added that the composition of the fungal cell wall and the filamentous morphology of fungi may be added advantage. Similar pattern of evidence was seen in flask A (Positive control). The reduction in percentage removal between day 14 and 28 may not be unconnected with environmental changes in the medium or insufficient metal binding sites on the cell surfaces arising from inhibited cell growth and competition for the limited available metal binding sites[22]. However, very high evidence was observed in treatment option A. This may probably be due to the presence of different indigenous microorganisms in Flask A that may have greater recovery potential for Cadmium than the test microbes. Flask B (negative control) which contained poisoned sample showed some evidence, a probability that not all the microorganisms were eliminated by the poison.

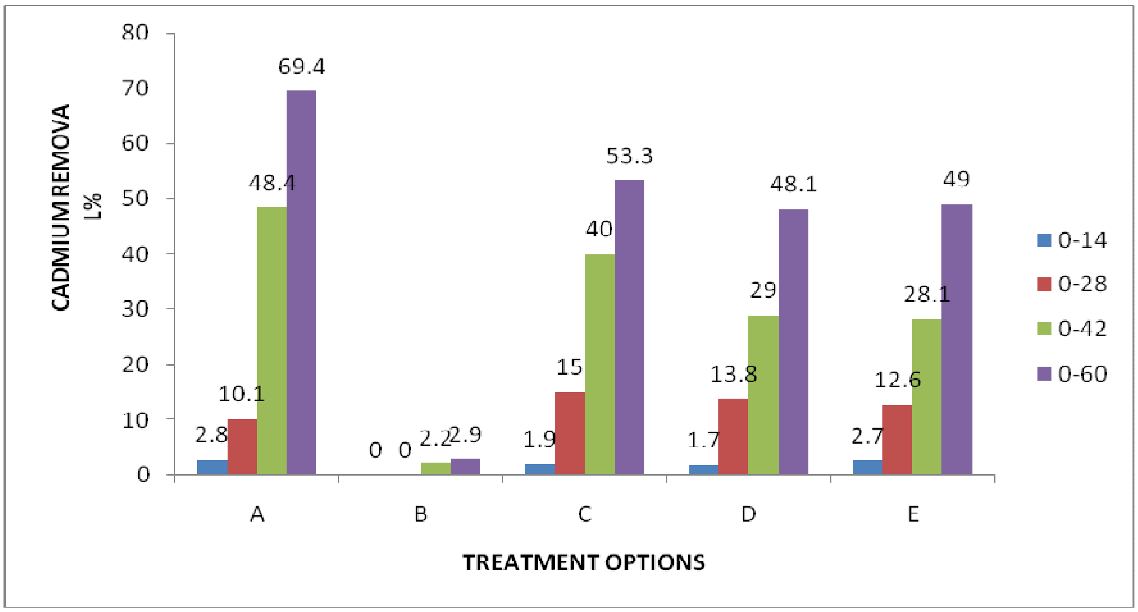

Figure-1

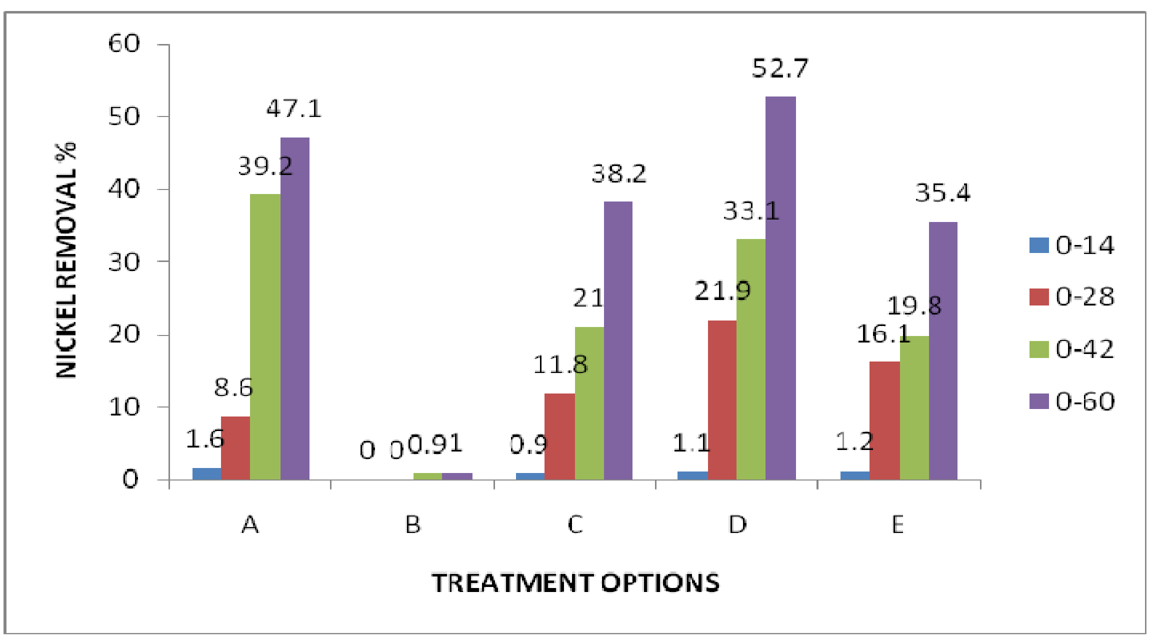

Figure-2

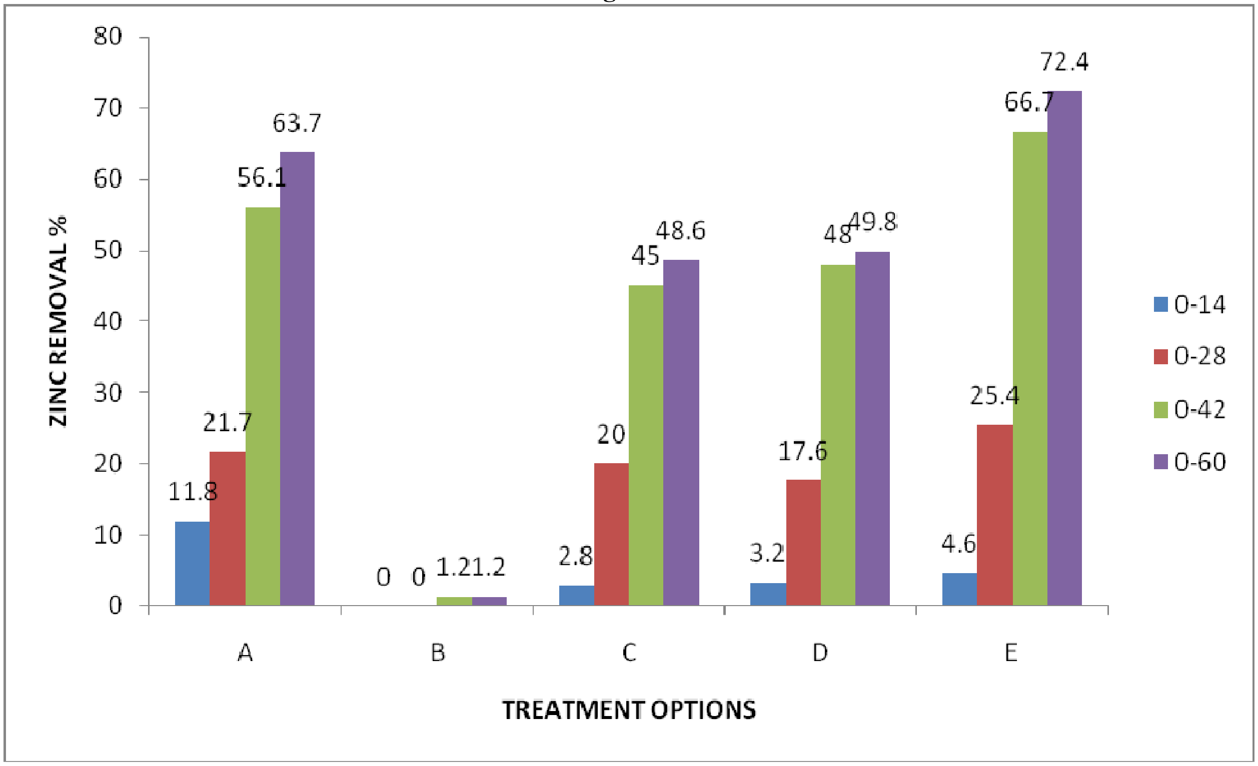

Figure-3

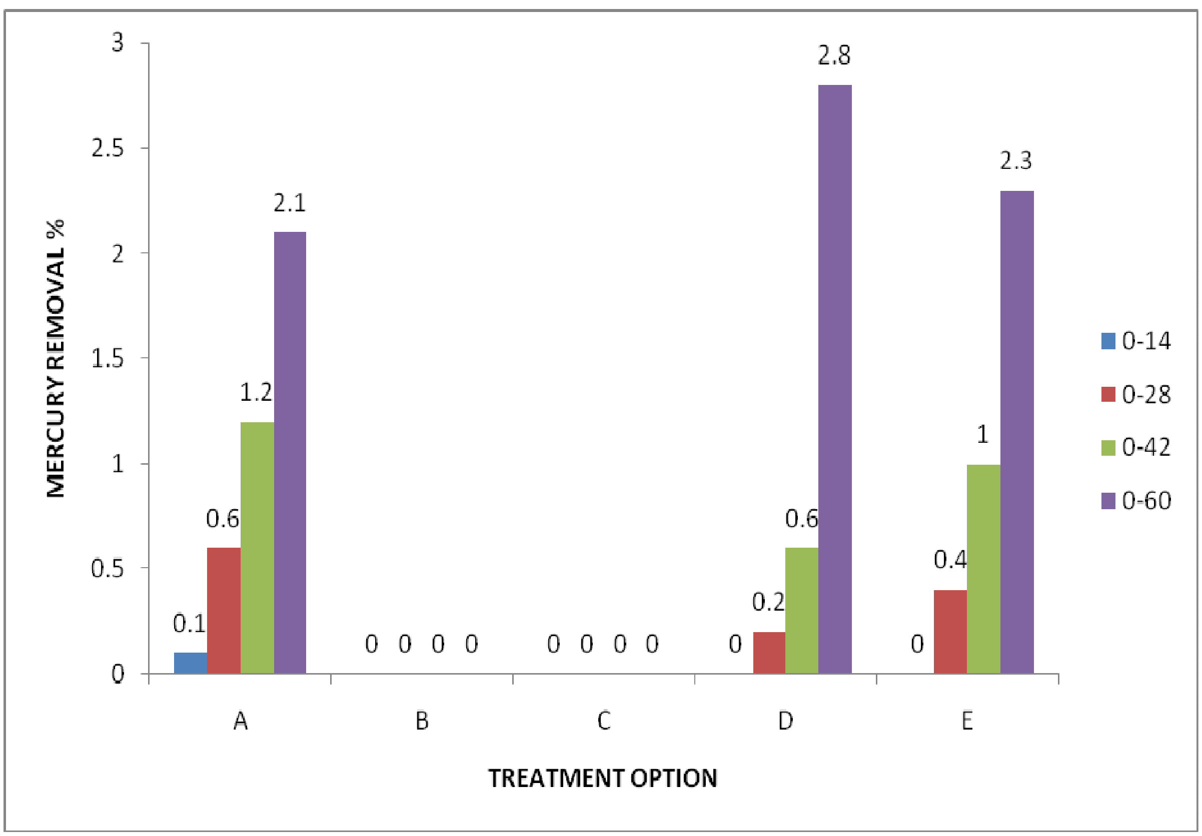

Figure-4

The bioremoval of Zinc by the test microorganisms as shown in figure 3 demonstrated that *Rhizopus stolonifer* bioaccumulated more Zinc than other test microorganisms with its peak performance on day 42. At the end of the study, *Aspergillus niger* and *Rhizopus stolonifer were able to* bioremoved 48.6 and 49.8% of Zinc. Also, flask A and B had an encouraging evidence recording a Zinc loss of 63.7 and 1.2% respectively. This showed that a large concentration of Zinc was lost through natural attenuation (flask A) and as little as 1.2% in the negative control (flask B).

Besides, the mixed culture of the test microorganisms was able to remove very high percentage of Cadmium present in the sample on day 60 (table 5). This efficiency is probably because the various organisms were able to optimize their different biorecovery potentials, and could co-exist, without toxic products from their metabolic activities (symbiosis). However, the low percent loss observered in the mixed culture for Nickel may be inhibition or production of intermediate metabolites that may compete for the Nickel binding site on the test microbes. Excellent activity was observed for treatment options Positive Control (A) and *Rhizopus stolonifer* the later showing greater evidence for Nickel removal.

However, not all the heavy metals recovered from the sample were bioconcentated. Some were adsorbed to the cell surfaces (table 7) and were washed off during cell wash. Table 6 showed the concentration of various heavy metals (studied) within the cells on day 60. The Fungal species demonstrated moderate evidence of bioconcentration of Cadmium, Nickel and Zinc but lacked capacity for bioconcentration of Mercury. [23] different microbes have been found to vary in their affinity for different heavy metal(s) and hence differ in their metal-binding capacities. This is evident in this study. Also, the removal of Cadmium, Nickel and Zinc by the test microorganisisms, in addition to adsorption, may also be by production of organic acids (e.g., citric, oxalic, gluonic, fumaric, lactic and malic acids), which may chelate toxic metals resulting in the formation of metalloorganic molecules [24]. It may form complex with carboxyl groups found in microbial polysaccharides and other polymers[24].

Basically, bioremoval by living organisms may be by metabolism independent binding where the metals are bound to the cell walls and/or metabolism dependent intracellular uptake whereby metal ions are transported across the cell membrane[25,19,26,8]. It is therefore not surprising that variations occurred between the concentration of the heavy metals in Tables 6 and 7, since the metals attached to the cell wall were not held strongly (van der waals force) and can easily be washed off or undergo reversible reaction. The ability of the mixed culture to show very high potential for bioconcentration of the metals may be synergistic. As individual microorganisms, *Aspergillus niger* showed the greatest potential for bioconcentration of Cadmium (53.3%), *Rhizopus stolonifer* did better for Nickel, Zinc and Mercury (52.7, 49.8 and 2.8% respectively). A very poor activity was observed in all the treatment options for Mercury, this may probably be due to its toxicity. Table 8 showed the different bioconcentration factor of the different test organisms for the different heavy metals under study. Apart from Mercury where no concentration was found in *Aspergillus, Rhizopus* and mixed culture, the organisms have higher concentration of the metals within the cell than was found in the environment on the 60th day. This intracellular concentration is by metabolic dependent intracellular uptake involving metal ion transport across the cell membrane[25,19,27,8]. With bioconcentration, the release of these metals back into the environment is minimal.

Generally, the microorganisms studied demonstrated low bioconcentration factor. This explains the availability of these metals for biosorption with Nickel and Zinc being more readily available to all the organisms than Cadmium. However, the metals were readily available to the mixed culture. The ability of these microbes to biorecover these metals from the effluent score them high and are recommended for use in designing a technology to be employed in remediating heavy metal impacted environment.

Conclusion

This study showed that leachate from wastes dump contained relatively high levels of Cadmium, Nickel, Mercury and Zinc. *Aspergillus niger* and *Rhizopus stolonifer* have showed great ability to bioremove these metals from the effluent. However, the study demonstrated that physicochemical parameters such as pH, Organic carbon, Nitrate and Phosphate have direct relationship with the ability of these microbes to recover these heavy metals. The bioremoval process is by both bioadsorption and biosorption. Bioremoval is an efficient system of clean-up and can be use to remediate heavy metal imparted environment.

Recommendations: i. Bioremoval of Cadmium, Nickel and Zinc can be achieved using *Aspergillus niger and Rhizopus stolonifer;* as such can complement the current treatment options for solid waste leachate. ii. The potentials of these microbes to biorecover these heavy metals from the leachate can be exploited in designing a new technology for remediating heavy metal impacted environment. iii. Since bioremoval is cost effective and does not leave toxic metabolic production, chemical or biological sludge and as such does not lead to secondary pollution, this area of research needs to be encouraged.

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