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Isolation of indigenous microorganisms from soil contaminated with metal scraps for the uptake of selected heavy metals in constituted growth media

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Indigenous bacteria and fungi were isolated from soil obtained from a metal scrap dumpsite in Ibadan, Nigeria. Soil sample analyses showed presence of calcium, iron, magnesium, zinc, manganese and copper. A selective isolation of microorganisms was done using heavy metal constituted growth media at concentration of 25 mg/L. *Lactobacillus casei* was isolated from cadmium and lead composed growth media, *Corynebacterium xerosis* and *Corynebacterium kutsceri* from nickel composed growth media. *Aspergillus niger* and *Histoplasma capsulatum* were isolated from growth media composed of nickel and lead respectively at a 25 mg/L concentration. Growths were observed for all isolates at 50mg/L, 100mg/L and 400mg/L composed growth media. A 7 day bio-treatment process with isolates for uptake of heavy metals from growth media solution at 50 mg/L concentration of heavy metal was done. *Histoplasma capsulatum, Aspergillus fumigatus* and *Aspergillus niger* reduced the heavy metal concentrations in lead, nickel and cadmium to 6, 18.12 and 12.45 mg/L respectively. *Lactobacillus casei, and C. xerosis* reduced the heavy metal concentration of cadmium, lead and nickel to 13.55, 33, 25, 22.38, 15.45 and 29.11 mg/L respectively. These microorganisms reduced the initial concentration of heavy metals and could thus be used for bioremediation processes.

Key words: Bioremediation, biotreatment, bacteria, fungi.

INTRODUCTION

Heavy metals refer to any metallic element that has relatively high density and at low concentration is toxic or poisonous (Lenntech, 2004). The major functions of a soil are its ability to protect water and air quality, sustain plant and animal productivity, and to promote human health (Doran and Parkin, 1994; Chen and Mulla, 1999). Heavy metals, include lead (Pb), cadmium (Cd), Zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe), and the platinum group elements while cadmium and lead are not readily absorbed by

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microorganisms (Kris, 2012).

Heavy metals occur as natural constituents of the earth crust, and are persistent environmental contaminants since they cannot be easily degraded or destroyed and can enter into the body system through food, air and water, and bio-accumulate over a period of time (Lenntech, 2004; United Nations Environmental Protection/Global Program of Action, 2004; Draghici et al., 2010). Cadmium is released as a by- product of zinc (and occasionally lead) refining; lead is emitted during its mining and smelting activities, from automobile exhausts (by combustion of petroleum fuels treated with tetraethyl lead antiknock) and from old lead paints while mercury is emitted by the degassing of the earth's crust (Lenntech, 2004).

With the surface dumping of the metals to air and rain, acid mine drainage (AMD) can be generated. When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues (Trueby, 2003). Animals that graze on such contaminated plants and drink from polluted waters, as well as marine lives that breed in heavy metal polluted waters also accumulate such metals in their tissues, and milk, if lactating (Habashi, 1992; Garbarino et al., 1995; Horsfall and Spiff, 1999; Peplow, 1999). Lead is the most significant toxin of the heavy metals, and the inorganic forms are absorbed through ingestion by food, water, and inhalation (Ferner, 2001).

It is evident that the presence of heavy metals in our environment poses a great threat to life processes in the soil. The contamination of heavy metals in our environment is an unavoidable occurrence as a result of human activities. Although certain naturally occurring processes such as phytoremediation by plants and microbial processes can reduce their concentrations, it is therefore of utmost importance to study and understand these microorganisms. It is important to also note that certain microbes have evolved over time, capable of utilizing these metals more efficiently as nutrient source. The objectives of the study are to determine heavy metals present in the soil sample, isolate and identify bacteria and fungi and use these microorganisms for the uptake of selected heavy metals from compounded medium of nutrients and heavy metals.

MATERIALS AND METHODS

Collection of sample

Soil samples contaminated with heavy metal were collected from a scrap yard well over 40 years located at Gate, Ibadan, Oyo state, Nigeria in May, 2016 and brought to the laboratory for analysis

Identification of heavy metals present in the soil sample

The calibration plot method was used for the analysis of heavy metal concentration with the Atomic Absorption Spectroscopy (AAS). For each element, the instrument was auto-zeroed using the blank (distilled water) after which the standard was aspirated into the flame from the lowest to the highest concentration. The corresponding absorbance was obtained by the instrument and the graph of absorbance against concentration plotted. The samples were analyzed in duplicates with the concentration of the metals present being displayed in milligram per kilogram (mg/L) after extrapolation from the standard curve (Greenberg et al., 1985).

Isolation of fungi

This was carried out according to the method Joshi et al. (2011) with potato dextrose agar (PDA) containing 25 mg/L of Pb, Ni, Cr, and Cd, separately. The 1000 mg/L stock solutions of Pb, Ni, Cr and Cd were made in double distilled water using Pb $(NO_3)_2$, $NiCl_2{\cdot}6H_2O$, $CdCl_2$, and $K_2Cr_2O_7$. The stock solution of heavy metals were sterilized separately through bacteriological filters and added to sterilize PDA medium to reach a concentration of 25 mg/L.

Serial dilution of the sample was made up to 10^6 and 1 ml each of dilution 10^4 and 10^6 was added into sterilized Petri plates in duplicate. 20 ml of PDA medium containing 25 mg/L of, one of these heavy metals was poured in these Petri plates and incubated at 28°C for 72 h. The colonies of fungi were isolated and purified.

Identification of fungal isolates

The fungal isolates were identified according to Alexopoulus et al. (2002) by observing their morphology under x100 magnification of a compound microscope and their cultural characteristics.

Screening of fungal isolates for heavy metals tolerance

Further screening of the fungal isolates (25 mg/L) for heavy metals (Pb, Ni, Cr and Cd) tolerance were carried out using the following concentrations - 50, 100 and 400 mg/L in PDA medium. The fungal isolates alone were streaked on PDA medium and served as control. The plates were incubated for 72 h and afterwards observed for growth.

Isolation of bacteria

This was carried out according to the method by Joshi et al. (2011), using Nutrient agar (NA) containing 25 mg/L of Pb, Ni, Cr, and Cd separately. The 1000 mg/L stock solutions of Pb, Ni, Cr and Cd was made in double distilled water using Pb $(NO_3)_2$, $NiCl_2{\cdot}6H_2O$, $CdCl_2$, and $K_2Cr_2O_7$. The stock solution of heavy metals were sterilized separately through bacteriological filters and added to sterilized NA medium to make the concentration 25 mg/L.

A serial dilution of each sample was made up to 10^6 and 1 ml each of dilution 10^4 and 10^6 was added into sterilized Petri plates in duplicates. 20 ml of NA medium containing 25 mg/L of one of these heavy metals was poured in these Petri plates and incubated at 37°C for 48 h. The bacterial isolates were picked and purified.

Identification of bacterial isolates

Microscopic tests such as the Gram staining and spore staining tests were carried out. Some biochemical tests were also carried out on the isolates as described by Barrow and Feltham (1993) and identified following the Bergey's Manual of Determinative

Bacteriology according to Bergey and John (2000).

Screening of bacterial isolates for heavy metals tolerance

The further screening of bacterial isolates (25 mg/L) for heavy metals (Pb, Ni, Cr and Cd) tolerance were carried out using the following concentrations - 50, 100 and 400 mg/L in Nutrient Agar medium. The bacterial isolates alone were streaked on NA medium and served as control. The plates were incubated for 48 h at 37^0C and afterwards observed for growth.

Uptake of heavy metals in solution of growth media

This was done using the modified method Tsekova et al. (1998) by compounding the growth media for bacteria and fungi with specific heavy metals (cadmium, lead and nickel) of 25 mg/L. The solution was sterilized at 121°C for 15 min. Bacterial inoculum was incubated for growth at 37°C for 24 h and the fungal inoculum at 28°C for 72 h. The inocula were standardized with reference to the Mcfarland standard.

Statistical analysis

Statistical analysis was done using one way analysis variance (ANOVA) and significance difference accepted at P≤0.05.

RESULTS

10 metals were isolated from the soil sample obtained from the metal scrap dumpsite. Table 1 shows the analyses of the metals present in the soil sample which included calcium, iron, magnesium, nickel, cadmium, lead, zinc, magnesium and copper. The metal with the highest concentration was iron at 89,900.0 mg/L followed by magnesium - 12580.0 mg/L and calcium 9255.0 mg/L. Lead was 919.0 mg/L, copper 550 mg/L and manganese 535.0 mg/L. The metals with the lowest concentrations were cadmium and chromium at 0.60 and 0.20 mg/L, respectively.

Table 2 shows the bacteria isolated from the heavy metal constituted with nutrient media at 25 mg/L. *Lactobacillus casei* was isolated from cadmium constituted media and lead constituted growth media, *Corynebacterium xerosis* from lead and nickel constituted growth media while *Corynebacterium kutsceri* from nickel constituted media. The fungal isolates obtained at 25 mg/L of the respective heavy metals are shown in Table 3. The three fungi were *Aspergillus niger* from cadmium (CdF.1) which had septate hyphae with black spores and creamy white mycelium. The conidia were arranged in chains on the small vesicle with a row of phialides, *Aspergillus fumigatus* from nickel (N1F.1) and *H. capsulatum* from Lead (PbF.1), which was characterized by the presence of large, rounded and tubercula temacro conidia formed on short hyaline undifferentiated conidiophore with the mycelium showing white pink coloration.

Table 1. Analysis of metals present in the soil sample.

Metals	Concentration (mg/L)
Calcium	9255.00
Iron	89900.00
Magnesium	12580.00
Nickel	43.10
Cadmium	0.60
Lead	919.00
Chromium	0.20
Zinc	1065.00
Manganese	535.00
Copper	550.00

Table 2. Identification of bacterial isolates from selective growth media constituted with heavy metals at 25 mg/L.

Isolate code	Probable organism
Cd1	*Lactobacillus casei*
Pb1	*Lactobacillus casei*
Pb2	*Corynebacterium xerosis*
N1.1	*Corynebacterium xerosis*
N1.2	*Corynebacterium kutsceri*
N1.3	*Corynebacterium kutsceri*

Key: Cd1= Isolate from cadmium enriched media; Pb1-Pb2 = Isolates from lead enriched media; NI.1-NI.3 = Isolates from nickel enriched media.

The Table 4 shows the analyses of the final heavy metal concentrations, obtained after a 7 day bio-treatment of 50 mg/L concentration of the heavy metal in Nutrient medium and Potato dextrose agar by the indigenous bacterial and fungal isolates, respectively. *H. capsulatum, A. fumigatus* and *A. niger* were observed to reduce the heavy metal concentrations of lead, nickel and cadmium to 6.00, 18.12 and 12.45 mg/L, respectively. The bacterial isolates – *L. casei* reduced the initial heavy metal concentration (50 mg/L) of cadmium to 13.55 and 33.00 mg/L, respectively. *C. xerosis* reduced the initial 50 mg/L concentration of lead to 25.00 and 22.38 mg/L, respectively while *C. kutsceri* also reduced the initial heavy metal concentration (50 mg/L) nickel to 15.45 and 29.11 mg/L, respectively. This shows the uptake of the heavy metals by the bacterial isolates.

The bio-treatment of lead using a co-culture of *L. casei* and *C. xerosis* reduced the initial 50 mg/L concentration to 32.5 mg/L while another co-culture bio-treatment of nickel using *C. xerosis* and *C. kutsceri* reduced the initial 50 mg/L concentration to 0.20 mg/L. At P≤0.05 there was

Table 3. Identification of fungal isolates from soil sample at 25 mg/L heavy metal concentration.

Isolate code	Description	Probable organism
CdF.1	Septate hyphae with black spores and creamy-white mycelium. The conidia were arranged in chains on the small vesicle with a row of phialides.	*Aspergillus niger*
N1F.1	Septate hyphae with brownish spores, the conidia were columnar with uniseriate heads, the conidiophores were conical shaped bore on a small vesicle. The mycelium was observed to be creamy-white	*Aspergillus fumigatus*
PbF.1	Characterized by the presence of large, rounded, tuberculate macro conidia formed on short hyaline undifferentiated conidiophores. The Mycelium showed a white-pink coloration.	*Histoplasma capsulatum*

Key: CdF.1: Cadmium enriched media; N1F.1: Nickel enriched media; PbF.1: Lead enriched media.

Table 4. Analysis of heavy metal concentrations after treatment using indigenous bacterial and fungal isolates.

Isolate code	Initial concentration of heavy metal before treatment (mg/L)	Final concentration of heavy metal (mg/L)	Reduction (%)
Lead			
PbF.1	50±0.007	6.00±0.0000^{a}	88.00
Pb1,2	50±0.007	32.50±0.0707^{b}	35.00
Pb1	50±0.007	33.00±0.1414^{c}	34.00
Pb2	50±0.007	33.50±0.007^{d}	33.00
Cadmium			
Cd1	50±0.007	13.55±0.007^{b}	72.90
CdF.1	50±0.007	12.45±0.007^{a}	75.10
Nickel			
N1F.1	50±0.007	18.12±0.007^{c}	63.76
N1.1	50±0.007	22.38±0.007^{d}	55.24
N1.2	50±0.007	15.45±0.007^{b}	34.55
N1.3	50±0.007	29.11±0.007^{e}	41.78
N1.1, 1.2, 1.3	50±0.007	0.20±0.007^{a}	99.60

Key: a-e: superscripts indicating comparison of means; PbF.1= *H. capsulatum* used for biotreatment of lead constituted growth media; Pb1,2= *L. casei* and *C. xerosis* as a consortium for biotreatment of lead constituted media; Pb1 = *L. casei* used for biotreatment of lead constituted media; Pb2 = *C. xerosis* used for biotreatment of lead constituted media; CdF.1 = *A. niger* used for biotreatment of cadmium constituted media; Cd1 = *L. casei* used for biotreatment of cadmium constituted media ; N1F.1 = *A. fumigatus* used for biotreatment of nickel constituted media; N1.1, N1.2 and N1.3 = *C. xerosis* and *C. kutsceri* respectively used for biotreatment of nickel constituted media.

a statistical significant difference between the final concentrations observed in the bio-treatment of lead with bacterial and fungal isolates. Bio-treatment with *Histoplasma capsulatum* showed the highest reduction followed by *L. casei*, co-culture of *L.casei* and *C. xerosis* and lastly *C. xerosis*. At P≤0.05 there was a statistical significant difference between the final concentrations observed in the bio-treatment of cadmium with bacterial and fungal isolates.

Bio-treatment with *A. niger* showed the highest reduction followed by *L. casei*. At P≤0.05, there was a statistical significant difference between the final concentrations observed in the bio-treatment of nickel with the bacterial and fungal isolates. Bio-treatment with a co-culture of *C. xerosis* and *C. kutsceri* resulted in the highest reduction followed by *C. kutsceri*, (N1.2), *A. fumigatus, C. xerosis* and lastly *C. kutsceri* (N1.3).

DISCUSSION

Heavy metals can be found occurring naturally in the environment as they are by nature constituents of the environment. The indiscriminate use for human purposes

has altered their geochemical cycles and biochemical balance. This has however resulted in the excessive release of heavy metals such as cadmium, copper, lead, nickel, zinc etc. into natural resources like soil and even aquatic environments. A prolonged exposure and accumulation of such heavy metals can have deleterious health effects on human life and aquatic biota. The role of microorganisms in the biotransformation of heavy metals into non-toxic forms and the accumulation of heavy metals by bacteria has numerous applications for the bioremediation of heavy metal-contaminated sites (Ruchita et al., 2015).

Heavy metal ions are present in natural and industrial disposed wastewater. These metallic ions present on the surface and underground water resulted in soil contamination. Many conventional techniques have been employed to eliminate heavy metal ions including physical (membrane separation, ion exchange) and chemical (neutralization, precipitation) techniques (Yan and Viraraghavan, 2003). However, these methods are only efficient to eradicate mass of heavy metal present at high or moderate concentration but ineffective at diluted or low concentration of metal ions (Guibal et al., 1992).

According to Iram et al. (2013), the biotreatment of heavy metals would be possible with the aid of microorganisms (bacteria and fungi) isolated from metal contaminated soil. The isolation of *L. casei, C. xerosis* and *C. kutsceri* in this study, using cadmium, lead and nickel, respectively is supported by the study carried out by Sumaryati et al. (2015), who reported the bioremediation of cadmium with lactic acid bacteria. A review by Gosa (2015) reported species of *Corynebacterium* as, capable of the remediation process of heavy metals. Fungal isolates: *A. niger, A. fumigatus* and *H. capsulatum* were also isolated from growth media constituted with cadmium, nickel and lead, respectively which agrees with the results obtained from the study carried out by Barros et al. (2003), who reported the biosorption of cadmium using *A. niger.*

Iram et al. (2013) reported the potential of *A. fumigatus* in nickel bioremediation while Halttunen et al. (2007) reported the removal of cadmium by *L. casei* from 61.8±3.3 to 74.5±3.3% after 4 h of incubation and *L. fermentum* resulted in 81.2% reduction. This supports the finding of the study of cadmium reduction by *L. casei.* According to Halttunen et al. (2007) and Akhmetsadykova et al. (2013), there was a considerable reduction in the concentration of lead in solution after biotreatment with *L. casei*. Halttunen et al. (2007) reported up to 97% of lead removal from solution with an initial metal concentration of 100 and 1000 ug/L. The research findings indicate that bioremediation using screened indigenous microorganisms present in contaminated soil can reduce the concentration of heavy metals in given media. The research can be further implemented in a larger scale on site contaminated with heavy metals.

Conclusion

The isolation of authochthonous bacteria and fungi from soil contaminated with heavy metals showed that, these microorganisms may be capable of heavy metal bioremediation. The presence of some microbial species that are indigenous to the soil environment as revealed in this study shows adaptation of these organisms to the presence of pollutants such as heavy metals, and as a result have developed mechanisms to utilize them as part of their metabolism. Further studies needs to be carried out on the development of processes for the use of these species in the removal of heavy metals from the environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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