RESEARCH ARTICLE

Heavy metal tolerance of fungal and bacterial isolates, and their functional groups following biosorption

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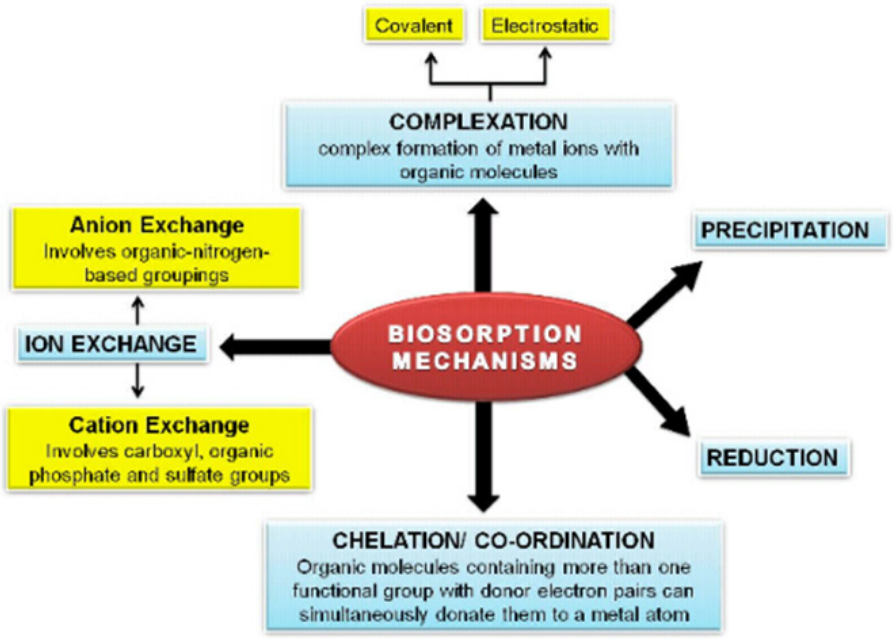

Highlights

- Heavy metals are released into the environment through different means.
- Some microorganisms, especially bacteria and fungi, have abilities to lessen heavy metal toxicity through sorption process.
- *Ochobactrum intermedium* and *Bacillus subtilis* as well as *Aspergillus niger* and *Cunninghamella bertholletiae* showed high sorption potentials for Cd(II), Ni(II), Pb(II) and Cr(III).
- The isolates tolerated Pb(II) and Cr(III) better than Ni(II) and Cd(II).
- Isolates elaborated certain functional groups due to the presence of heavy metals.

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Heavy metal tolerance of fungal and bacterial isolates, and their functional groups following biosorption

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Abstract: Heavy metal contamination still prevails due to improper discharge of effluents from industries, mining activities, agricultural and domestic sources. Hence, the objectives of this study were to isolate bacteria and fungi with high potentials for biosorption of Cd(II), Pb(II), Cr(III) and Ni(II) and to explore their functional groups. Bacteria and fungi able to grow in the presence of 0.1% of selected heavy metals were isolated and enumerated using nutrient agar and potato dextrose agar, respectively. The isolates were then screened for their abilities to withstand high concentrations of heavy metals. After the screening, the isolates with high potential were identified and used for percentage biosorption study. Fourier transform infrared spectroscopy was used to compare the spectra and functional groups elaborated by the control and the metal stressed isolates. Bacterial counts were higher than the fungal counts across the soil samples. Screening revealed that the isolates tolerated Pb(II) and Cr(III) better than Ni(II) and Cd(II). The isolates with high biosorption potentials were identified as *Ochobactrum intermedium, Bacillus subtilis, Aspergillus niger* and *Cunninghamella bertholletiae*. The functional groups common to the infrared spectra of the control flasks of *C. bertholletiae*, *A. niger* and *O. intermedium* were primary alcohol, aliphatic primary amine, aromatic amine, imine/oxime, thiol, sulfone and phenol while aliphatic ether, alkyl aryl ether, conjugated ketone, cyclic alkene, α, β-unsaturated ketone, sulfoxide, aromatic ester, isothiocyanate, secondary alcohol, tertiary alcohol, sulfonamide, sulfonate, allene, amine, conjugated acid and conjugated aldehyde were among the functional groups produced in the presence of heavy metals. The findings conclude that the isolates demonstrated a reasonable binding affinity for heavy metals and can be used in developing biosorbent at a commercial level for Cd(II), Pb(II), Cr(III) and Ni(II).

***Key words*:** Heavy metal, tolerance, sorption, microorganisms, functional groups

INTRODUCTION

Metals such as cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), arsenic (As), copper (Cu), zinc (Zn), and nickel (Ni) are continuously being added to soils through agricultural activities such as long-term application of sewage sludge, and industrial activities such as waste disposal, waste incineration etc. These sources cause the accumulation of metals and metalloids in soils and pose threats to food safety and public health due to soil-to-plant transfer of metals (Oyebanji *et al*., 2019).

Some of the methods commonly used for removing heavy metals include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, evaporative recovery, solvent extraction etc. (Fathollahi *et al*., 2021; Priyadarshanee and Das, 2021). However, these technologies have several disadvantages such as unpredictable rates of metal ion removal, high reagent, or energy requirements and/or generation of toxic sludge.

Use of microorganisms as biosorbents for heavy metals offers a potential alternative to existing methods (Benmalek and Fardeau, 2016). Among the various sources, both live and inactivated biomass of microorganisms (fungi, algae, bacteria, etc.) exhibit promising metal-binding capacities. Their complex cell walls contain high concentrations of functional groups including amine, amide, hydroxyl, carbonyl, carboxyl, imine, imidazole, sulfonate, thioether, sulfhydryl, phosphonate, phosphodiester, and phosphate, which have been associated with metal binding (Sher and Rehman, 2019). The importance of any given group for biosorption of a particular metal by a particular biomass depends on factors including the number of sites occurring within the biosorbent material, the accessibility of the sites, the chemical state of the site (availability); and affinity between site and metal (binding strength). Some prokaryotic (bacteria and archaea) and eukaryotic (algae and fungi) microorganisms produce or excrete extracellular polymeric substances (EPS) such as polysaccharides, glycoprotein, lipopolysaccharides, soluble peptides, etc. These substances possess a substantial quantity of anionic functional groups which can adsorb metal ions (Sher and Rehman, 2019).

Contamination of the environments with heavy metals is still persisting despite the challenges heavy metal posed to humans, animals, plants and microorganisms. Hence, this study intended to isolate bacteria and fungi with high efficiency to sorb heavy metals from waste dump sites and the rhizosphere of some plants. The objectives of this study were to determine the count of bacteria and fungi when grown in media amended with 0.1% of some heavy metals; screen the isolates for growth at different concentrations of the Pb(II), Ni(II), Cr(III) and Cd(II); determine the % growth and inhibition of fungal biomass at 0.1% concentration of the heavy metals; determine the functional groups utilized by the isolates in the presence of 0.1% of each heavy metal and control (without heavy

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metal); determine the percentage biosorption of 0.1% concentration of each heavy metal; and identify the isolates with good biosorption potentials.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected into sterile polythene bags using sterile hand trowels. The debris from the soil surfaces were removed before sampling. The samples were immediately taken to the laboratory for analysis (Oyewole *et al*., 2019).

Isolation of microorganisms from the soil in culture media containing 0.1% of heavy metals

Nutrient agar (NA) and potato dextrose agar (PDA) were amended with 0.1% w/v of each heavy metal and then sterilized by autoclaving. Ni(II), Pb(II), Cr(III) and Cd(II) were supplied as $NiSO_4.6H_2O$, $Pb(NO_3)_2$, $Cr(NO_3)_3.9H_2O$ and $Cd(NO_3)_2.4H_2O$ respectively. The media were poured into sterile plates and allowed to solidify. The soil sample was serially diluted up to 10^{-4} dilution and 0.1 mL of aliquot from the different dilutions were used to inoculate the plates and spread using sterile L-shaped glass rods. The NA and PDA plates were incubated at 37 °C and at room temperature respectively. The number of colonies on the NA and PDA plates were read at 48 and 72 hours respectively. The counts were expressed in cfu/g (Oyewole *et al*., 2019).

Purification and preservation of isolates

The colonies on the plates were sub-cultured on NA and PDA plates until pure cultures were obtained. The pure cultures were stocked on NA and PDA slants and stored in a refrigerator until they were needed (Manguilimotan and Bitacura, 2018).

Screening of the isolates for their ability to tolerate different concentrations of heavy metals

Different Czapek dox broths containing 0.2% w/v of Pb(II), Ni(II), Cr(III) and Cd (II) in 250 mL conical flasks were prepared. The broth medium (25 mL) each was dispensed into MacCartney bottles and sterilized by autoclaving. Inoculum plug (7 mm in diameter) was taken from the advanced edge of 72 hours old culture of each fungus. The cultures were placed on a shaker at 120 rpm and room temperature for 14 days. The extent of growth of the fungal mycelia in the broth was used to assess the rate of growth of each fungal isolate.

For the bacterial isolates, NA was prepared, sterilized, poured into sterile Petri dishes, and allowed to solidify. Twenty-four hours old culture of each bacterium was standardized using 0.5 Mc Farland's standard. The culture was used to inoculate set plates of NA using sterile swab stick. Cork borer of 7 mm in diameter was used to bore holes in the agar plates. The well or hole was filled with different concentrations (0.05, 0.1, 0.2, 0.5 and 1.0%) of each heavy metal. The plates were incubated at 37 °C for 24 hours. The diameter of zone of inhibition was measured in mm (Syed *et al*., 2021).

Determination of percentage biosorption of heavy metals

Two each of fungi and bacteria were selected for biosorption tests based on the screening conducted. Czapek dox broths were prepared and supplemented with 0.1% of each heavy metal in separate conical flask. Twenty-five milliliters of heavy-metal broth was dispensed into each conical flask and sterilized by autoclaving. Each of the flask was inoculated with the test fungal isolate as already described under screening test for fungi.

For the bacterial isolates, mineral salt medium (Bushnell Haas) broth was prepared and supplemented with 0.1% w/v of each metal. One gram of glucose was added per 100 mL of the broth. Twenty-five milliliters of each heavy- metal broth medium was dispensed into each conical flask and sterilized by autoclaving. Standardized inoculum, 1.5 mL (6%) was added to each flask.

Control experiments for each heavy metal were also set up but they were not inoculated with any fungus/bacterium. The inoculated conical flasks as well as the control flasks were placed on a shaker at room temperature and 120 rpm for 14 days. After the period of shaking, the cultures were filtered using Whatman filter No 42. The filtrates obtained were centrifuged at 3000 rpm for 30 minutes. Two mL of the supernatant obtained was digested with 8 mL of 2% Nitric acid (Oyewole *et al*., 2019). The concentration of each heavy metal in the supernatant was determined at the Central Research Laboratory, University of Ilorin, Ilorin, Nigeria using Buck Scientific ACCUSYS 211 Atomic Absorption Spectrophotometer.

$$\text{Biosorption of metal (\%)} = \frac{C_1 - C_2}{C_1} \times 100$$

Where:

C_1 = Initial concentration of heavy metal in the control broth

C_2 = Final concentration of heavy metal in the experimental broth (Oyekanmi *et al*., 2019).

Determination of fungal biomass at 0.1% concentration of each heavy metal

The fungal mycelia remaining in the conical flasks (obtained from the biosorption experiment above and control flasks lacking heavy metals) were rinsed with distilled water and poured into the folded Whatman filter No 42-filter paper (Abbas *et al.*, 2014). The filter paper was previously dried in an oven at 80 °C for 30 minutes and its initial was weight noted. The filter paper and its mycelial content was again dried in an oven at the same temperature and duration and its final weight were also noted.

$$\text{Fungal biomass growth (\%)} = \frac{X_2 - X_1}{Y_2 - Y_1} \times 100$$

Where:

X_1 = Weight of filter paper only for the experimental sample

X_2 = Final weight of mycelia and filter paper for the experimental sample

Y_1 = Weight of filter paper only for the control sample

Y_2 = Final weight of mycelia and filter paper for the control

sample

Determination of functional groups elaborated by the isolates due to heavy metal biosorption

The supernatant obtained from the biosorption flasks were used for Fourier Transform Infrared Spectroscopy. This was done for the control and experimental flasks for the selected fungal and bacterial isolates. The samples were mixed with KBr and the FTIR spectra were analyzed using Shimadzu FTIR 8400S spectrophotometer within the wavelength 400 – 4,000 cm^{-1}. The identity of the infrared spectrum of both the control (samples without heavy metal) and the experimental samples were compared in order to determine the functional groups exhibited by the isolates due to each heavy metal (Abuzar *et al.*, 2017; Hussain *et al*., 2020).

Identification of isolates

The DNA of the fungal and bacterial isolates were extracted using Zymogen DNA extraction kits. The PCR amplification of the bacterial DNA was done using forward and reverse primers, 27F: AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCCGCA respectively. ITS1: 5' TCCGTAGGTGAACCTGCGG 3' and ITS4: 5' TCCTCCGCTTATTGACATGS 3' primers were used for the forward and reverse reactions of the fungal DNA amplification.

The PCR cocktail mix consist of 2.5 of 10x PCR buffer, 1.0 µL of 0.025 M $MgCl_2$, 1.0 µL of each of forward primer and reverse primer, 1.0 µL of DMSO, 2.0 µL of 0.0025 M DNTPs, 0.1 µl of 5 µ/µl Taq DNA polymerase, and 3 µl of 10ng/µl DNA. The total reaction volume was made up of 25 µl using 13.4µl nuclease free water. Denaturation at 94 °C for 30 seconds, annealing at 56 °C for 30 seconds, elongation at 72 °C for 45 seconds. These processes were repeated for 36 cycles. Then, final elongation at 72 °C for 7 minutes and holding at 10 °C. The purified PCR product was subjected to sequencing. The nucleotide sequence was assessed on the website of NCBI in order to identify the isolates (Oladipo *et al*., 2018).

RESULTS AND DISCUSSION

Soil microbial counts on cultivation on media amended with 0.1% of heavy metals

The mean count of bacteria in media amended with 0.1% Cd(II), Ni(II), Pb(II) and Cr(III) was 1.92 x 10^4, 2.34 x 10^5, 2.5 x 10^5 and 2.62 x 10^5 cfu/g respectively. Similarly, the mean count of fungi in media amended with 0.1% Cd(II), Ni(II), Pb(II) and Cr(III) was 3.52 x 10^3, 4.98 x 10^3, 2.42 x 10^3, and 2.66 x 10^3 cfu/g respectively (Table 1). In this study, the bacterial counts were generally higher than the fungal counts across the soil samples.

Growth tolerance of fungal isolates in Czapek dox broth amended with 0.2% of heavy metals

All the fungal isolates grew profusely in the positive control Czapek dox broth since it does not contain any heavy metal. They were inhibited to different extent in the Czapek dox broths amended with 0.2 % of heavy metal with F4 (*Aspergillus niger*) and F5 (*Cunninghamella bertholletiae*) showing better tolerance than others (Table 2).

Growth tolerance of bacteria at different concentrations of heavy metals

All the bacterial isolates were not inhibited at 0.2, 0.5 and 1.0 % in media amended with lead. Nickel- amended media, 0.2 % did not inhibit all the bacteria while 0.5 % inhibited some of the bacterial isolates. One percent nickel- amended medium inhibited all the bacterial isolates. Chromium -amended media, both 0.2 and 0.5 % did not inhibit all the bacterial isolates, whereas 1.0 % inhibited some of the bacterial isolates. Cadmium amended media with 0.2 and 0.5 % inhibited all the bacterial isolates (Table 3).

Percentage of fungal biomass produced in 0.1 % heavy-metal Czapek dox broth amended media

The % of biomass produced by *A. niger* in 0.1 % heavy metal amended media followed this order: Pb(II) (100%) > Cr(III) (85.71%) > Cd(II) (60%) > Ni(II) (12.86%). The order for *C. bertholletiae* was Cr(III) (96.26%) > Pb(II) (92.52%) > Cd(II) (78.5%) > Ni(II) (6.54%) (Table 4).

Percentage biosorption of 0.1% heavy metals by the isolates

Ochobactrum intermedium sorbed 100% Cr(III) and Pb(II), 30.8% Cd(II) and 20.4% Ni(II) while *Bacillus subtilis* sorbed 100% Cr(III), 95.7% Pb(II), 48.9% Ni(II) and 48.7% Cd(II). For the fungal isolates, *A. niger* sorbed 100% Pb(II), 66.7% Cr(III), 41.1% Ni(II) and 13.5% Cd(II). In addition, *C. bertholletiae* sorbed 100% Cr(II), 97.9% Pb(II), 47.3% Ni(II) and 41.4% Cd(II) (Figure 1). Hegazy *et al*. (2023) found that the initial concentration of heavy metals played a significant role in removal of metals in a multi-component system. They reported that small ionic radius cations can be transferred to the adsorption sites more rapidly while high ionic radius cations may produce an immediate saturation of the absorbent due to steric effects with biosorption order of Fe(II) > Pb(II) > Cd(II) > Cu(II) > Ni(II).

Table 1: Counts of bacteria and fungi isolated from the soils when cultivated in media amended with 0.1 % of heavy metals.

Soil samples	Bacterial counts (cfu/g)				Fungal count (cfu/g)			
	Cd(II)	Ni(II)	Pb(II)	Cr(III)	Cd(II)	Ni(II)	Pb(II)	Cr(III)
A	9.0×10^3	7.0×10^4	3.0×10^5	3.0×10^5	2.2×10^3	8.0×10^2	2.2×10^3	4.7×10^3
B	4.0×10^3	2.2×10^5	3.0×10^5	3.0×10^5	2.0×10^3	1.8×10^3	1.4×10^3	5.3×10^3
C	3.0×10^3	2.8×10^5	3.0×10^5	3.0×10^5	2.3×10^3	5.0×10^3	2.4×10^3	1.3×10^3
D	4.0×10^4	3.0×10^5	1.5×10^5	1.52×10^5	9.5×10^3	1.1×10^4	2.7×10^3	1.8×10^3
E	4.0×10^4	3.0×10^5	2.0×10^5	2.6×10^5	1.6×10^3	6.3×10^3	3.4×10^3	2.0×10^2
Mean	1.92×10^4	2.35×10^5	2.50×10^5	2.62×10^5	3.52×10^3	4.98×10^3	2.42×10^3	2.66×10^3

A= Dump site; B= Rhizosphere of maize; C= Rhizosphere of cassava; D= Rhizosphere of rice; E= Rhizosphere of cowpea

Table 2: Growth tolerance of fungal isolates in Czapek dox broth amended with 0.2 % of heavy metals.

Fungal Isolates	Czapek dox Broth	Heavy metals			
		Pb(II)	Cr(III)	Ni(II)	Cd(II)
F1	++++	-	-	-	-
F2	++++	++	+	-	-
F3	++++	-	+++	+++	-
F4	++++	+++	+++	++	-
F5	++++	+++	+++	++	+++
F6	++++	-	-	+++	-
F7	++++	-	+	-	-
F8	++++	-	-	-	+

++++, Excellent growth; +++, Very good growth; ++, Moderate growth, +, little growth, -, No growth; F, Fungal isolates

Table 3: Minimum inhibitory concentration of heavy metals on the bacterial isolates.

Bacterial Isolates	Diameter of zone of inhibition (mm)										
	Pb(II)(%)			Ni(II)(%)			Cr(III) (%)			Cd(II)(%)	
	0.2	0.5	1.0	0.2	0.5	1.0	0.2	0.5	1.0	0.2	0.5
B1	-	-	-	-	17	22	-	-	-	30	45
B2	-	-	-	-	-	15	-	-	10	10	18
B3	-	-	-	-	-	19	-	-	-	22	30
B4	-	-	-	-	10	16	-	-	18	29	46
B5	-	-	-	-	-	15	-	-	-	30	40
B6	-	-	-	-	11	17	-	-	12	30	35
B7	-	-	-	-	-	15	-	-	12	-	16

- = No inhibition

Table 4: Percentage of fungal biomass when cultivated in Czapek dox broth amended with 0.1% of heavy metals.

Fungal isolates	Heavy metals	Parameter (%)	
		Growth	Inhibition
Aspergillus niger	Ni(II)	12.9	87.1
	Cr(III)	85.7	14.3
	Cd(II)	60.0	40.0
	Pb(II)	115.7	0.0
Cunninghamella bertholletiae	Ni(II)	6.54	93.5
	Cr(III)	96.3	3.74
	Cd(II)	78.5	21.5
	Pb(II)	92.5	7.48

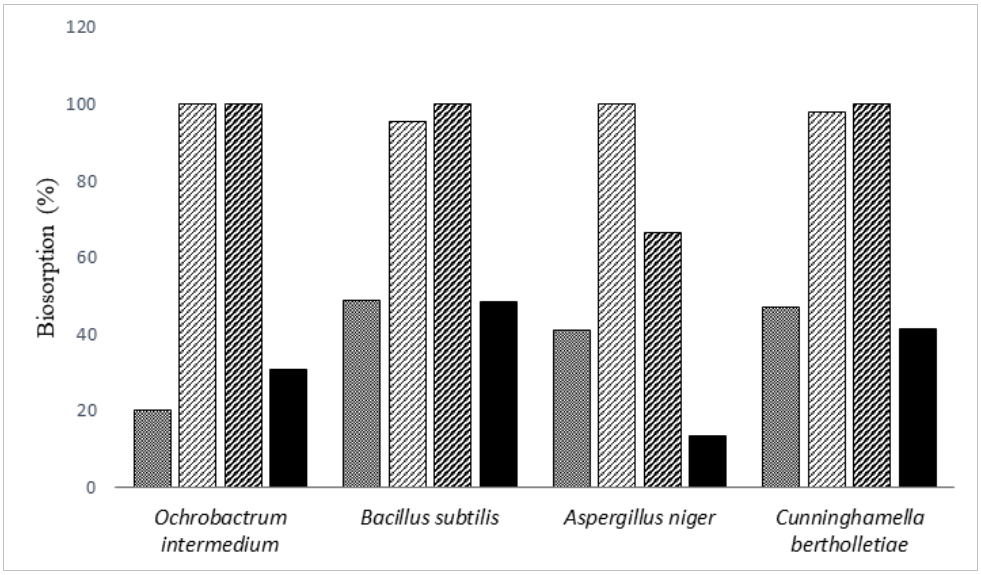

Figure 1: Percentage biosorption of 0.1 % heavy metals by the isolates.

Identified Isolates

In this study, the bacteria with better potentials to sorb heavy metals were identified as *O. intermedium* (B1) and *B. subtilis* (B6) while the fungi were *A. niger* (F4) and *Cunninghamella bertholletiae* (F5). Bacteria such as *Bacillus, Micrococcus, Streptococcus* etc. and fungi such as *A. niger*, *A. flavus* and *P. notatum* were isolated by Oyewole *et al*. (2019) in their study. Ahirwar *et al.* (2016) isolated heavy metal resistant bacteria, *P. fluorescens* and *B. cereus* in their study. Oyewole *et al*. (2019) observed that *Penicillium notatum* showed highest biosorption rate of 77.6% for cadmium at 10 ppm while *Aspergillus niger* showed the highest biosorption rate for nickel with 81.07% after 28 days of incubation.

Manguilimotan and Bitacura (2018) reported cadmium biosorption efficiency of 13.87% and 11.46% for *Aspergillus and Penicillium* in their study of biosorption by filamentous fungi isolated from coastal water and sediments. In this study *A. niger* produced the sorption of 13.5% for cadmium (II) while *Bacillus subtilis* produced the highest value of 48.7%.

In the present study, all the isolates had excellent biosorption of Pb(II) and Cr(III). The least sorption for Pb(II) and Cr(III) was 95.7% by *B. subtilis* and 66.7% by *A. niger,* respectively. Hussain *et al*. (2020) reported that *Penicillium digitatum* reduced the level of Pb(II) and Cr(III) by 84% and 70% at pH 5 after 24 hours. Abuzar *et al*. (2017) found that *Klebsiella variicola* reduced Ni by 49% and 68.6% after 24 and 48 hours respectively. In the present study, the sorption of nickel after 14 days of the experiment was 20.4, 48.9, 41.1 and 47.3% by *O. intermedium, B. subtilis, A. niger* and *C. bertholletiae,* respectively.

Functional groups produced by isolates for biosorption of heavy metals

In this study, the functional groups common to the infrared spectra of the control flasks of *C. bertholletiae*, *A. niger* and *O. intermedium* were noted as primary alcohol, aliphatic primary amine, aromatic amine, imine/ oxime, thiol, sulfone and phenol. Due to the presence of heavy metals, additional functional groups were produced by the fungal and bacterial isolates (Tables 5 – 7). The control sample demonstrated the presence of a number of absorption peaks and reflected the complex nature of the biomass.

The common functional group exhibited by *C. bertholletiae* for all the metals [Pb(II), Cd(II), Cr(III) and Ni(II)] was aliphatic ether. Common for Cd(II), Cr(III) and Ni(II) sorption was alkyl aryl ether. In addition, conjugated ketone and cyclic alkene were among the functional groups produced for Ni(II) and Pb(II) biosorption (Table 5). The FTIR spectra of *C. bertholletiae* without any metal and in the presence of the different heavy metals were given in Figures 2 to 6.

A. niger utilized α, β-unsaturated ketone and sulfoxide for biosorption of Cd(II). Common functional groups produced by *A. niger* for biosorption of Cr(III), Ni(II) and Pb(II) were aromatic ester, secondary alcohol, tertiary alcohol and isothiocyanate. Additional common functional groups produced for the sorption of Ni(II) and Pb(II) biosorption were aliphatic ether, sulfate, and sulfonyl chloride (Table 6). The FTIR spectra of *A. niger* in the absence and presence of the different heavy metals were shown in Figures 7 to 11.

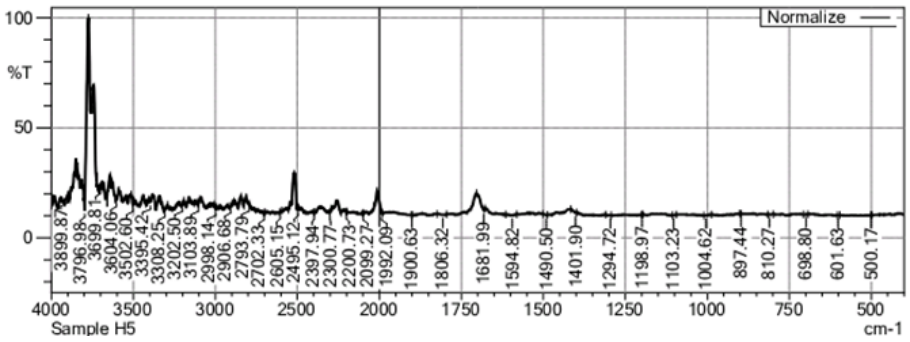

Figure 2: FTIR spectrum of *Cunninghamella bertholletiae* in the absence of any heavy metal.

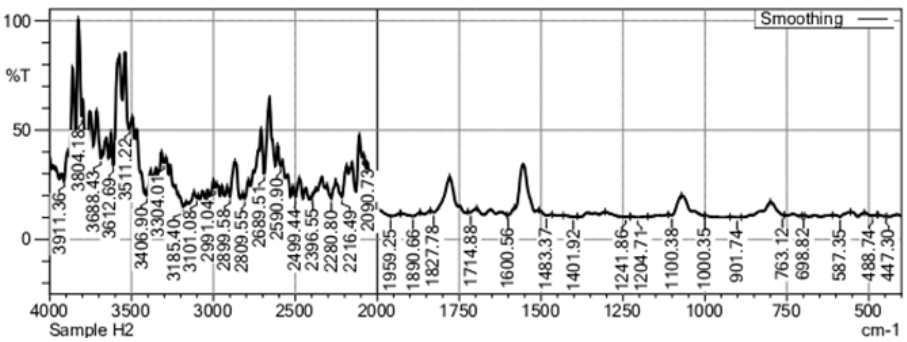

Figure 3: FTIR spectrum of *Cunninghamella bertholletiae* in the presence of Cadmium.

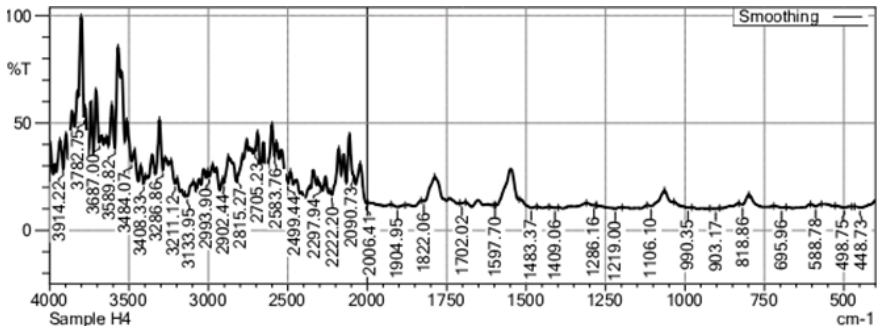

Figure 4: FTIR spectrum of *Cunninghamella bertholletiae* in the presence of Chromium.

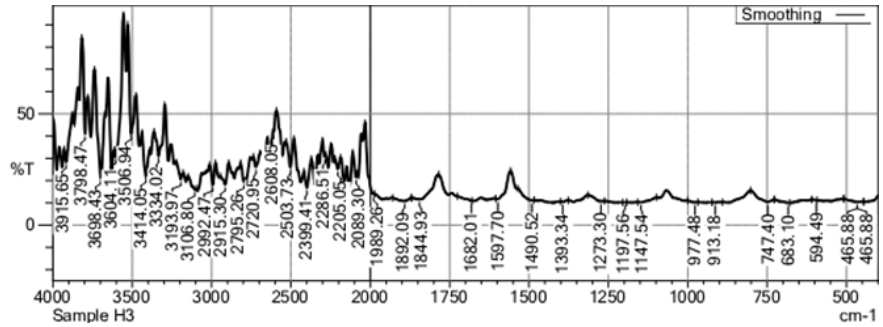

Figure 5: FTIR spectrum of *Cunninghamella bertholletiae* in the presence of Nickel.

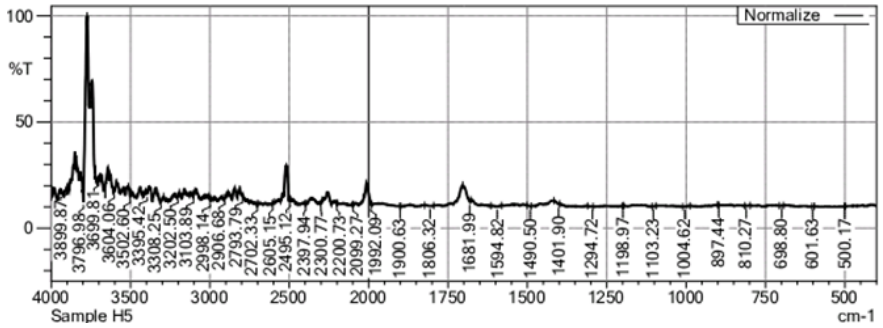

Figure 6: FTIR spectrum of *Cunninghamella bertholletiae* in the presence of Lead.

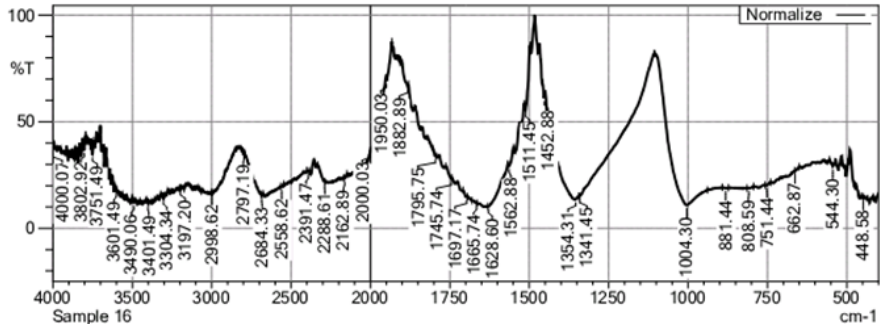

Figure 7: FTIR spectrum of *Aspergillus niger* in the absence of any metal.

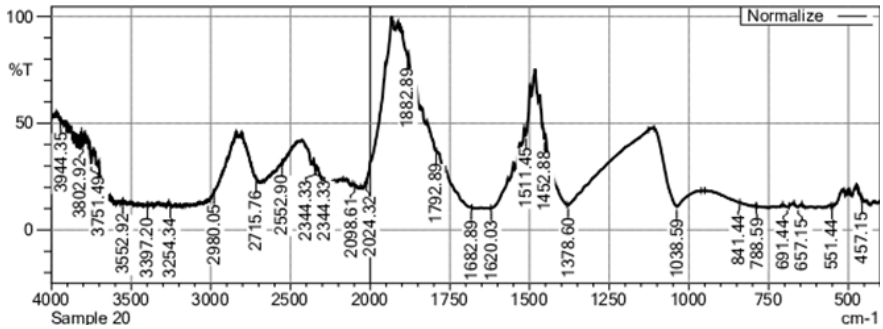

Figure 8: FTIR spectrum of *Aspergillus niger* in the presence of Cadmium.

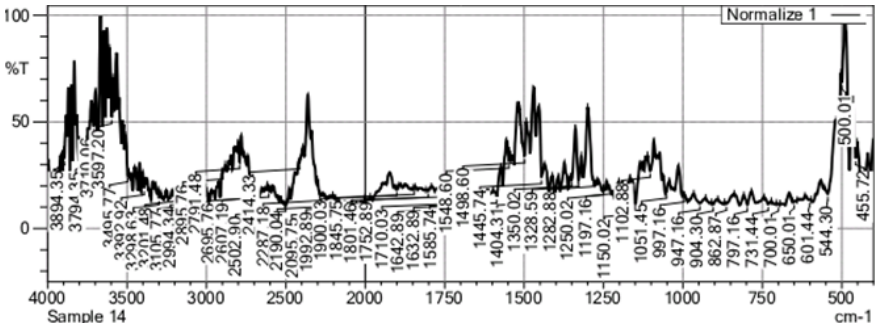

Figure 9: FTIR spectrum of *Aspergillus niger* in the presence of Chromium.

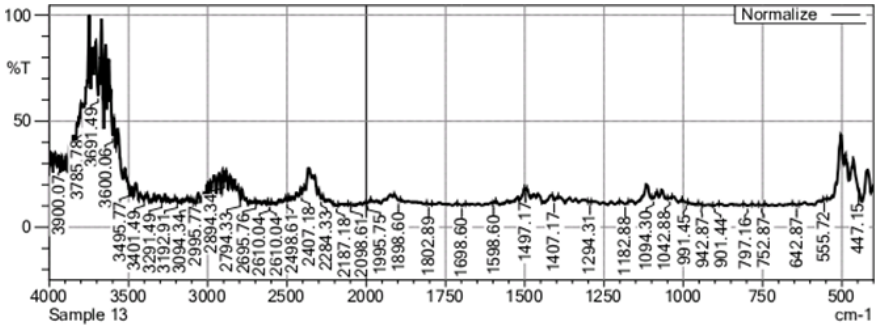

Figure 10: FTIR spectrum of *Aspergillus niger* in the presence of Nickel.

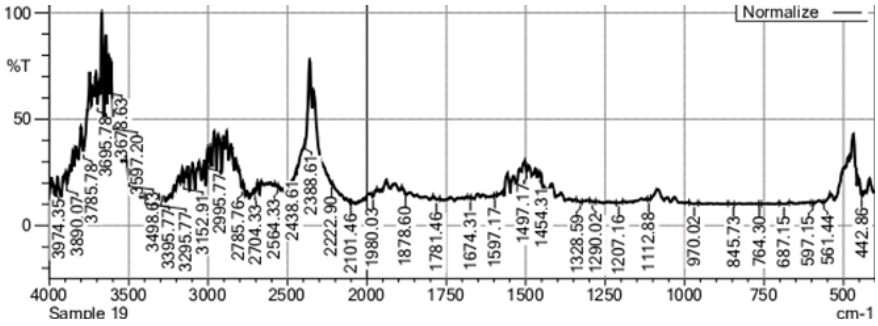

Figure 11: FTIR spectrum of *Aspergillus niger* in the presence of Lead.

Allene, amine, conjugated acid and conjugated aldehyde were among the common functional groups produced by *O. intermedium* for biosorption of Pb(II), Cr(III) and Cd(II). For Ni(II) biosorption, sulfonamide and sulfonate were detected in the infrared spectra of the supernatant of the isolate (Table 7). The spectra of *O. intermedium* with or without the different heavy metals were presented in Figures 12 to 16.

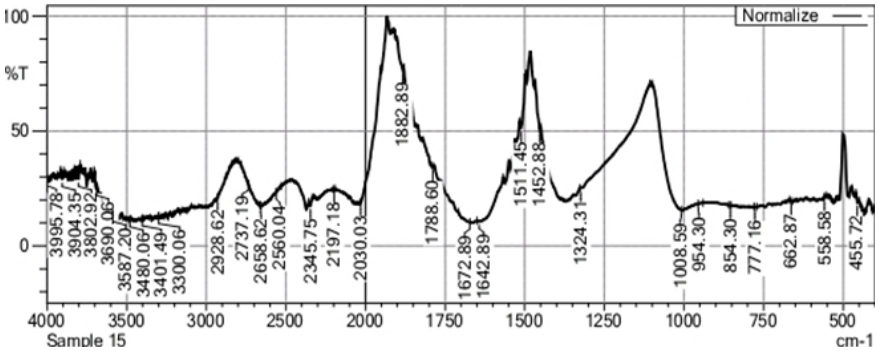

Figure 12: FTIR spectrum of *Ochrobactrum intermedium* in the absence of any metal.

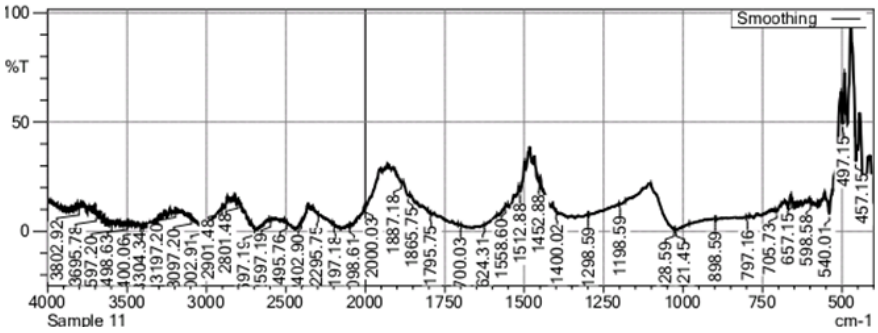

Figure 13: FTIR spectrum of *Ochrobactrum intermedium* in the presence of Cadmium

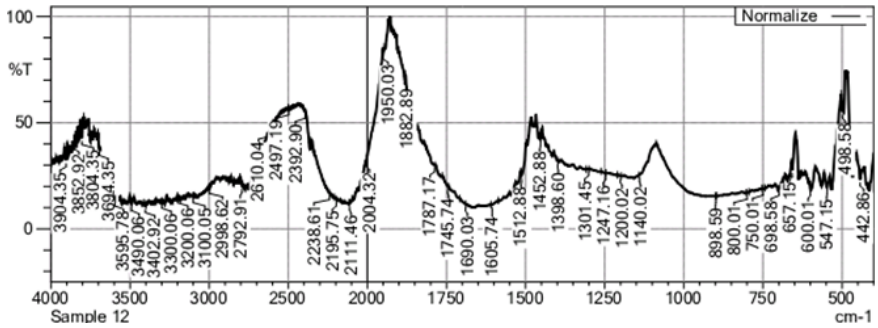

Figure 14: FTIR spectrum of *Ochrobactrum intermedium* in the presence of Chromium.

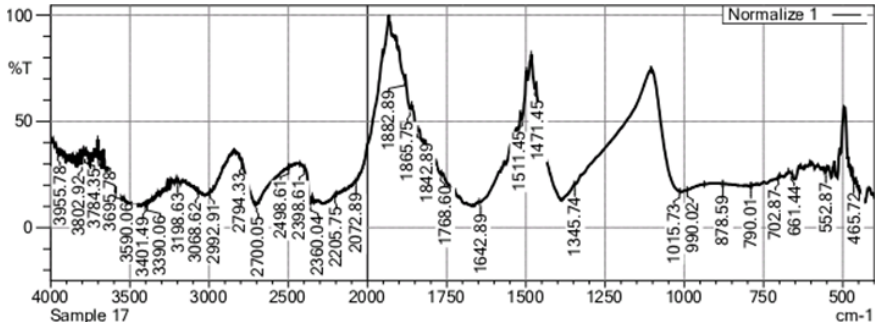

Figure 15: FTIR spectrum of *Ochrobactrum intermedium* in the presence of Nickel.

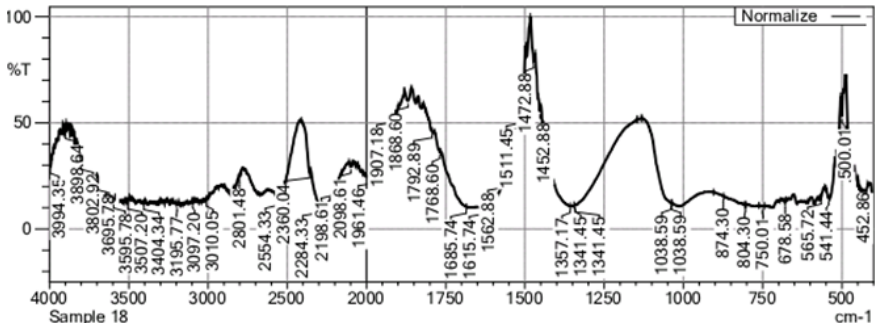

Figure 16: FTIR spectrum of *Ochrobactrum intermedium* in the presence of Lead.

Biosorption of metal is based on ions associating with the cell surface wherein ion exchange and complexation reaction with functional groups like carboxyl, amides, hydroxyl, phosphate and sulphydryl groups occur (Manguilimotan and Bitacura, 2018). Vale *et al*. (2016) showed that in a living fungal biomass of *A. niger*, amine and carboxyl groups were important functional groups involved in Cr(III) and Zn(II) biosorption. Abuzar *et al.* (2017) observed a change in absorption bands when the FTIR spectra of control and metal loaded biomass were compared. Some of the additional functional groups elaborated by their isolates in the presence of Ni(II) and Co(II) were amino, hydroxyl group and carboxylic group.

Table 5: Functional groups produced by *Cunninghamella bertholletiae* during growth in Czapek dox broth amended with 0.1 % of heavy metals

Functional groups	No metal	Cd(II)	Cr(III)	Ni(II)	Pb(II)
Acid halide	+	+	+	+	+
Alcohol	+	+	+	+	+
Aliphatic primary amine	+	+	-	+	+
Allene	+	+	+	+	+
Amine	+	+	+	+	+
Aromatic amine	+	-	+	+	+
Aromatic ester	+	-	+	+	+
Conjugated acid	+	-	+	+	+
Conjugated aldehyde	+	-	+	+	-
Imine/Oxime	+	-	-	+	+
Isocyanate	+	-	-	-	-
Isothiocyanate	+	+	+	+	-
Secondary alcohol	+	+	+	-	+
Sulfate	+	+	+	+	+
Sulfone	+	-	-	-	-
Sulfonyl chloride	+	+	+	+	+
Tertiary alcohol	+	+	+	+	+
Thiol	+	+	+	-	-
Phenol	+	-	-	-	-
Aliphatic ether	-	+	+	+	+
Aliphatic ketone	-	+		-	-
Alkyl aryl ether	-	+	+	+	-
Carboxylic acid	-	+	+	-	-
Conjugated alkene	-	+	-	-	-
Cyclic alkene	-	+	-	+	+
Tertiary amide	-	+	-	-	+
Vinyl ether	-	+	+	-	-
α, β-unsaturated ketone	-	+	-	-	-
Nitrile	-	-	+	-	-
Conjugated ketone	-	-	-	+	+

+ = Detected; - = Not detected

Table 6: Functional groups produced by *Aspergillus niger* during growth in Czapek dox broth amended with 0.1 % of heavy metals

Functional groups	No metal	Cd(II)	Cr(III)	Ni(II)	Pb(II)
Acid halide	+	+	+	+	-
Alcohol	+	+	+	+	+
Aliphatic primary amine	+	+	+	-	+
Allene	+	-	+	+	+
Amine	-	+	+	+	+
Aromatic amine	+	-	+	+	+
Conjugated acid	+	+	-	+	-
Conjugated acid halide	+	+	-	-	+
Conjugated aldehyde	+	-	+	+	-
Cyclic alkene	+	+	+	+	+
Esters	+	-	-	-	-
Imine/Oxime	+	+	+	-	+
Ketenimine	+	-	-	-	-
Phenol	+	+	+	-	+
Sulfonate	+	-	+	-	-
Sulfone	+	-	+	-	+
α-Lactone	+	-	-	-	-
Thiocyanate	+	-	-	-	-
Thiol	+	+	-	-	+
Sulfonamide	+	-	+	-	-

Conjugated alkene	+	-	-	-	-
Conjugated ketone	+	-	-	-	+
α, β-unsaturated ketone	-	+	-	-	-
Sulfoxide	-	+	+	-	-
Aliphatic ether	-	-	+	+	-
Alkyl aryl ether	-	-	+	-	+
Aromatic ester	-	-	+	+	+
Aliphatic ketone	-	-	+	-	-
Tertiary alcohol	-	-	+	+	+
Isothiocyanate	-	-	+	+	+
Secondary alcohol	-	-	+	+	+
Sulfate	-	-	+	+	-
Sulfonyl chloride	-	-	+	+	-
Carboxylic acid	-	-	-	+	-
Tertiary amide	-	-	-	+	+
Nitrile	-	-	-	-	+
Vinyl ether	-	-	-	-	+
Primary amide	-	-	-	-	+

+ = Detected; - = Not detected

Table 7: Functional groups produced by *Ochrobactrum intermedium* during growth in Czapek dox broth amended with 0.1 % of heavy metals.

Functional groups	**No metal**	**Pb(II)**	**Ni(II)**	**Cr(III)**	**Cd(II)**
Acid halide	+	+	-	+	+
Aliphatic primary amine	+	+	+	+	+
Aromatic amine	+	+	-	+	+
Conjugated acid halide	+	+	-	+	+
Conjugated alkene	+	+	+	+	+
Conjugated ketone	+	-	+	-	-
Cyclic alkene	+	+	+	+	+
Imine/Oxime	+	+	+	+	-
Isothiocyanate	+	-	+	+	+
Phenol	+	+	+	-	-
Sulfone	+	+	+	+	-
Thiol	+	+	-	-	+
Alcohol	+	-	+	+	+
Primary amide	+	-	+	-	-
Sulfonic acid	+	-	+	-	-
Allene	-	+	-	+	+
Amine	-	+	-	+	+
Conjugated acid	-	+	-	+	+
Conjugated aldehyde	-	+	-	+	+
Sulfonamide	-	+	+	-	-
Sulfonate	-	+	+	-	-
Sulfoxide	-	+	-	-	-
α, β-unsaturated ketone	-	+	-	-	-
Aliphatic ether	-	-	-	+	-
Alkyl aryl ether	-	-	-	+	-
Vinyl ether	-	-	-	+	-
Aromatic ether	-	-	-	+	+
Carboxylic acid	-	-	-	+	+
Sulfate	-	-	-	+	+
Nitrile	-	-	-	+	-
Sulfonyl chloride	-	-	-	+	+
Tertiary alcohol	-	-	-	+	+
Tertiary amide	-	-	-	-	+
Ketenimine	-	-	-	-	+

+ = Detected; - = Not detected

CONCLUSION

The study concludes that the *Ochobactrum intermedium, A. niger* and *C. bertholletiae* were able to tolerate and sorb Ni(II), Cr(III), Cd(II) and Pb(II) at different extents. The isolates can be used for developing biosorbent at a commercial level. Functional groups such as ether, ketone, carboxylic acid, amide, aldehyde, sulfoxide, alcohols (secondary and tertiary) etc were noted following the sorption of heavy metals.

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DECLARATION OF CONFLICT OF INTEREST

The authors have no conflict of interest.

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