Application of Heterotrophic Nitrifying Bacteria in Bioremediation of Heavy Metals

Nyoyoko Veronica Fabian, Anyanwu Chukwudi U, Dibua Esther

Abstract— the study was undertaken to investigate the biosorption of the selected heavy metals by different nitrifying bacteria isolates. Microbial growth was observed in terms of CFU and O.D. The samples was withdrawn at day’s interval, transferred to 10 ml vials and capped for AAS analysis. Copper at concentration of 100ppm was bioaccumulated 90.1%, 90.04%, 86.9%, 89.62% after a period of 28 days by AOB 4, AOB 10; AOB 5; AOB 7 respectively. Nickel at concentration of 100ppm was bioaccumulated 96.51%, 94.67%, 97.74 %, 92.1% after a period of 28 days by AOB 4, AOB 10; AOB 5; AOB 7 respectively. Lead at concentration of 100ppm was bioaccumulated 92%, 90.25%, 95.5 %, 95.05% after a period of 28 days by AOB 4, AOB 10; NOB 5; NOB 7 respectively. Cadmium at concentration of 100ppm was bioaccumulated 84.82 %, 89.21%, 86.95%, 86.07% after a period of 28 days by AOB 4, AOB 10; NOB 5; NOB 7 respectively. Achromobacterinsolitus (AOB 10) has the highest biosorption capacity of copper, bioaccumulated 90.04 % of copper after the period of 28 days. Alcaligenesfaecalis (NOB 5) has the highest biosorption capacity of nickel, bioaccumulated 97.74 % of nickel after the period of 28 days. Alcaligenesfaecalis (NOB 5) has the highest biosorption capacity of lead, bioaccumulated 95.5 % of nickel after the period of 28 days. Achromobacterinsolitus (AOB 10) has the highest biosorption capacity of cadmium, bioaccumulated 89.21% of cadmium after the period of 28 days. The highest biosorption was carry out by Alcaligenesfaecalis (AOB 5) bioaccumulated 97.74 % of nickel and the lowest amongst biosorption was carry out by Achromobacterxyllosoxidans (AOB 4) bioaccumulated 84.82 % of cadmium. Remediation of pollutant using microbial process (bioremediation) has proven effective and reliable due to its eco-friendly features.

Index Terms— Bioremediation, Biosorption, Heavy metals, Nitrifying bacteria, Pollution.

I. INTRODUCTION

Highlight In the recent years, the world is witnessing various kinds of pollutions that threaten human life and at times makes inhabitable. Cause one in six premature deaths that has killed 9 million people worldwide in 2015 (Landriganet al., 2018). The World Health Organization (WHO) has estimated that 4.9 million deaths (8.3 per cent of total mortality worldwide) are attributable to environmental exposure and inappropriate serious management of toxic chemicals (Pruss-Ustunet al., 2011). Environmental pollution has been on the rise in the past few decades owing to increased human activities on energy reservoirs, unsafe agricultural practices and rapid industrialization (Hadia and Ahmed, 2018). Industrial development has improved the living conditions but has also affected the basic amenities of life, such as air, soil and water (Hansdaet al., 2015). Amongst the pollutants that are of environmental and public health concerns due to their toxicities are: heavy metals, nuclear wastes, pesticides, greenhouse gases, and hydrocarbons. Toxic metals apply their toxicity in the displacement of essential metals from their normal binding sites of biological molecules, inhibition of enzymatic functioning and disruption of nucleic acid structure, oxidation stress, genotoxicity and interfering with signalling pathways (Srivastavaet al., 2017). Ecologically, the accumulation of heavy metals in soils is extremely hazardous because soil is a major link in the natural cycling of chemical elements; it is also a primary component of the trophic chain (Liu et al., 2012; Sagi and Yigit, 2012; Wyszkowska, 2013). The danger of heavy metals is intensified by their almost indefinite persistence in the environment due to their absolute nature which cannot be degraded (Gupta et al., 2016). Metals are non-biodegradable but can be transformed through sorption, methylation, complexation and changes in valence state (Anyanwu et al., 2011).

Remediation of heavy metal contaminated soils and water is necessary to reduce the associated risks, make the land resource available for agricultural production, enhance food security and scale down land tenure problems arising from changes in the land use pattern. Microbe-metal interaction in soil/waste disposal is of interest to environmentalists in order to use adapted microorganisms as a source of biomass for bioremediation of heavy metals (Sharma, 2016 Singh et al., 2016a, b, c ). Autochthonous (indigenous) microorganisms present in polluted environments hold the key to solving most of the challenges associated with biodegradation and bioremediation of polluting substances (Verma and Jaiswal, 2016).

Heavy Metals particularly in biological sense are often used for those metals and semimetals with potential human or environmental toxicity (Tchounwouet al., 2012). Heavy metals can also be classified depending on whether they have a biological role for microorganisms; essential (e.g. Co, Ni, Cu) or non-essential (e.g. Cd, Hg, Pb) (Rialet al., 2011). The main heavy metals associated with environmental contamination, and which offer potential danger to the ecosystem, are copper (Cu), zinc (Zn), silver (Ag), lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), strontium (Sr), cesium (Cs), cobalt (Co), nickel (Ni), thallium (Tl), tin (Sn) and vanadium (V) (Wang & Chen, 2008; Srivastavaet al., 2017). Metals are stable (Srivastavaet al.

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al., 2017) and non-biodegradable, but can be transformed through sorption, methylation, complexation and changes in valence state Anyanwu et al. (2011). Unlike organic contaminants which can be converted to nontoxic compounds, metals are intrinsically stable in nature (Bruins et al., 2000).

Nitrification describes the oxidation of ammonium (NH$_4^+$) or ammonia (NH$_3$) to nitrate by living organisms and is a primary activity within the nitrogen (N) cycle. Nitrification is carried out by nitrifying microorganism (Hamsaat et al., 2017). The oxidation of ammonium to nitrate is a two-step process involving the transformation of ammonia or ammonium to nitrite and the conversion of nitrite to nitrate. The first and rate limiting step of nitrification is the oxidation of ammonia to nitrite. In the first step of nitrification, ammonia is converted into hydroxylamine by the enzyme ammonia monoxygenase. Hydroxylamine (NH$_2$OH) is then converted to nitrite by the enzyme hydroxylamine oxidoreductase. Nitrite-oxidizing bacteria that produce the enzyme nitrite oxidoreductase aid in the conversion of nitrite to nitrate (Kitzinger et al., 2018). The first type of reaction is the oxidation of ammonia to nitrite by ammonium-oxidizing bacteria (AOB) which include Nitrosomonas, Nitrosococcus, Nitrospira, Nitrosolobus, and Nitrosovibrio or ammonia-oxidizing archaea (AOA).

The second type of reaction involves the oxidation of nitrite to nitrate by nitrite-oxidizing bacteria (NOB) include Nitrobacter, Nitrococcus, Nitrospina, Nitrospira and the newly discovered Candidatus nitrotoga (Ma et al., 2014). Based on the 16S ribosomal DNA (rDNA) phylogenetic analysis results, Nitrobacter, “Candidatus nitrotoga”, Nitrococcus and Nitrospira belong to a, b, g and d classes of Proteobacteria, respectively. Nitrospira belongs to phylum Nitrospira (Ma et al., 2014; Hoang et al., 2016). Nitrospira in the NOB group have been reported as complete ammonia oxidizing bacteria (comammox) that perform the complete nitrification of ammonia to nitrate (Daimset et al., 2015; Hanna et al., 2018).

Other organisms involved in nitrification are heterotrophic bacteria (Arthrobacterglobiformis, Aerobacteraerogenes, Thiosphearatmotropha, Streptomycyris grisen, various Pseudomonas spp., Alcaligenesfaecalis and Achromobacterxylooxidans (Basha et al., 2018; Fritiyaneto et al., 2017; Shoda and Ishikawa, 2014); Rhodococcus sp., Diaphorobacter sp., Bacillus sp., Bacillus methylotrophicus, and fungi (Aspergillusflavus) (Hamsaat et al., 2017). Recent research on the metabolic pathways of heterotrophic ammonia oxidation has been conducted using Paracoccusdenitrificans (Moir et al., 1996b), Alcaligenesfaeacilis (Jo et al., 2005), Acinetobactercalcoaceticus (Zhao et al., 2010), Bacillus methylotrophicus (Zhao et al., 2012), Pseudomonas stutzeri (Zeng et al., 2011), Pseudomonas putidat (Daum et al., 1998), and a few other bacterial species (Hayatsuet al., 2008).

Some studies have suggested that the biochemical mechanisms of heterotrophic nitrification differ among strains. The two main genera of microbes involved in nitrification have been identified in many studies and are the aerobic; gram negative, chemoautotrophic Nitrosomonas and Nitrobacter (Hoang et al., 2016). Most nitrifying bacteria thrive in the temperature range of 25-30° C, and require a neutral pH

Microorganism-based remediation is the use of microorganism and their product example enzyme and bio surfactant in ecosystem restoration. Microorganisms possess astonishing metabolic pathways which utilize various toxic compounds as a source of energy for growth and development, through respiration, fermentation, and co-metabolism. Due to their characteristic degradative enzymes for a particular contaminant, they have evolved diverse mechanisms for maintaining homeostasis and resistance to heavy metals, in order to adapt to toxic metals in the ecosystem. It depends on the resistance of the utilized microbe to the pollutant (heavy metal) that is either activated independently or through pollutant(metal) stress (Nazeet al., 2015). Various soil microorganisms have great potential for bioremediation (Banik et al., 2014; Joutey et al. 2015). Microorganisms are essential in remediation of heavy-metal-contaminated environments as they have a variety of ways to endure metal toxicity (Ojuederie and Babalola, 2017).

Strategies developed by microorganisms of agronomic importance for continued existence in heavy metal polluted environments, or to avoid heavy metal stress include mechanisms such as bioaccumulation, (a) transport of metals across cytoplasmic membrane; (b) biosorption and bioaccumulation to the cell walls, mineralization, and biotransformation; (c) metal entrapment in the extracellular capsules; (d) heavy metals precipitation; and (e) metal detoxification via oxidation–reduction (Zubairet al., 2016). These mechanisms are exploited for in situ (treatment at the site of contamination), or ex situ (the contaminated site can be excavated or pumped and treated away from the point of contamination), remediation (Ayangelowo and Babalola, 2017).

II. MATERIALS AND METHODS

Sample collection
Surface soil samples at depth of 0-15 cm were collected at random from five different sites. Akwalmob State University, Ohio-akpa in Akwalmob, State, AdiasimIkotEssiendot, Akwalmob State, University of Nigeria, Nsukka, Enugu State, University of Uyo, Akwalmob, State and from solid waste disposal site in Uyo, Akwalmob State. The soil was collected using sterile auger borer and into sterile polyethylene bag, merged to form a composite soil sample and transferred to the laboratory for analysis.

Preparation of samples for analyses:
Precisely, 5 g of the sieved soil sample was suspended in 45 ml of sterile phosphate buffer containing 139 mg of K$_2$HPO$_4$ and 27 mg KH$_2$PO$_4$ per litre (pH 7.0) and shaken at 100 rpm for 2 h (Deni and Peninnck, 1999; John and Okpokwasili, 2012) in order to liberate the organisms into the liquid medium.

Preparation of media
Media preparation was carried out using Winogradsky broth medium for serial dilution of soil samples and Winogradsky solid medium for the inoculation of serially diluted soil
Preparation of Winogradsky broth
Winogradsky broth medium phase 1 (used for the isolation of nitrifying bacteria responsible for oxidizing ammonium to nitrite) was prepared with the following composition (g/l) in sterile distilled water: (NH₄)₂SO₄, 2.0; K₂HPO₄, 1; MgSO₄.7H₂O, 0.5; NaCl, 2.0; FeSO₄.7H₂O, 0.4 ; CaCO₃, 0.01. Winogradsky broth medium phase 11 (used for the isolation of nitrifying bacteria responsible for oxidizing nitrite to nitrate) was prepared with the following composition (g/l) in sterile distilled water: KNO₃, 0.1; Na₂CO₃, 1; NaCl 0.5; FeSO₄.7H₂O, 0.4. Each of ten test tubes was filled with 9 ml of the Winogradsky broth media 1 and 11, respectively, autoclaved at 121 ⁰C at 15 psi for 15 minutes and allowed to cool. The test tube used to carry out ten-fold serial dilutions of the soil suspension (John and Okpokwasili, 2012).

Preparation of Winogradsky agar media
Winogradsky agar media for nitrification phases 1 and 11 was prepared by adding 15.0 g agar to 1000 ml of fresh broth and sterilized at 121 ⁰C at 15 psi for 15 minutes and allowed to cool to about 45 ⁰C before dispersing into sterile Petri dishes (John and Okpokwasili, 2012).

Isolation of nitrifying bacteria from soil sample
All the plates will be aseptically inoculated with 0.1 ml of the appropriate dilution of the soil suspension using spread plate technique. All the inoculated Petri dishes were incubated aerobically at room temperature (28 ±2⁰C) for 1 week and examined for growth.

Purification of isolates
Discrete colonies that developed on Winogradsky agar media for nitrification phases 1 and 11 after 1 week of incubation was aseptically sub-cultured repeatedly on corresponding freshly prepared Winogradsky agar medium. All the inoculated Petri dishes were incubated aerobically at room temperature (28 ±2⁰C) for 3 - 5 days. The pure isolates was transferred to Winogradsky agar slants and stored in the refrigerator for further use.

Identification of isolates
Pure isolates from the corresponding agar slants was characterized and identified using morphological (cell and colonial morphology, shape, motility, and gram reaction), biochemical and physiology attributes (Holt et al. 1994; Cheesbrough, 2006). The molecular characterization was based on 16SrDNA sequencing (Saha et al., 2013).

Screening the isolates for nitrification ability of isolates
Ammonium oxidation: Nitrite determination by Griess Method
(Bhaskar and Charyulu, 2005).
Sulfanilamide (SA), reacted with nitrite in acidic media to form a diazonium salt. This intermediate reacts with N-naphthylethenediamine (NED) and an azo compound is formed as a result measured with spectrophotometer at 540 nm.

Determination of nitrate reducing Phenol disulphonic acid
Nitrate reacts with phenol disulphonic acid to give a yellow colour, absorbance measure at 410 nm using spectrophotometer (Jagessar and Sooknundun, 2011).

Experimental Set-up for Bioremediation of Heavy Metals
Bioremediation of selected heavy metals was carried out in a flask. Analytical grades of metal salts were used to prepare stock solutions. The mineral salt medium for ammonia oxidizing bacteria and nitrite oxidizing bacteria was amended with the appropriate aliquot of the stock solution of the metal salt.

Bioremediation of Copper, Nickel, Cadmium and Lead
Bioremediation of the selected heavy metals by isolates was carried out in a 250ml Erlenmeyer flask containing sterile minimal salt medium. In mitigation experiment with 100 mg/L concentration of the metals was taken into 100 ml minimal salt medium. The experiment was conducted on a shaker incubator at 25⁰C and continuous shaking at 130rpm. Mitigation was assessed by comparing the disappearance of the metals in the sample and controls over the period of microbial growth. The metal concentrations were monitored over a time to compare lag periods and bioaccumulation rates for different concentrations. The lag period was determined as the time during which the metal concentrations remained relatively constant. Microbial growth was observed in terms of CFU and O.D. (John and Okpokwasili, 2012). The samples (5ml) was withdrawn hourly from 0 to 6 h and then every 24 h for 14 days … Samples was transferred to 10 ml vials and capped for AAS analysis. The physicochemical parameters such as pH, temperature will be observed (Sharma, 2016). Biosorption capacity (mg/g) of the biosorbent can be defined as the amount of biosorbate (metal ions) biosorbed per unit weight of the biosorbent and can be expressed by using the following mass balance equation:

\[ q_e = \frac{(C_i - C_e)V}{M} \]

where \( q_e \) is the amount of adsorbed metal ions of the adsorbent (mg g⁻¹), \( C_i \) is the initial concentration of metal ion in the solution (mg L⁻¹), \( C_e \) is the equilibrium concentration of metal ion in the solution (mg L⁻¹), \( V \) is the volume of the medium (L), and \( M \) is the amount of the biomass used in the adsorption process (g).

\[ \%\text{Biosorption} = \frac{a-b}{a} \times 100 \]

where \( a \) is the weight of heavy metal in before incubation control; \( b \) is the weight of heavy metal in the each case after incubation.

III. RESULTS AND DISCUSSION
Biosorption of copper, nickel, lead and cadmium by different Nitrifying bacteria
Biosorption of copper, nickel, lead and cadmium by different Nitrifying bacteria Achromobacter xylosoxidans also called Ralstonia metalidurans or Cupriavidus metallidurans; Achromobacteriolsius; Alcaligenesfaecalis; Lysinibacillusus pakistaniensis sp. novel candidatus. The four isolates is represent as AOB 4; AOB 10; AOB 5; AOB 7 respectively. Samples were assessed for biosorption of selected heavy Metals at intervals of 1 day, 7 days 14 days, 21 days and 28 days using shake flask method under controlled environmental condition. Achromobacterxylosoxidans (AOB
4) mitigation of copper range from 10% to 55.5%; 67% to 81% and 90.1% at 1 day, 7 days 14 days, 21 days and 28 days respectively. AOB 4 biosorption of nickel was 12% on day 1; 48% on day 7; 77% on day 14; 84% on day 21 and 96.51% on 28 days. AOB 4 mitigation of lead range from 15% to 67%; 74% to 81.5% and 92% at 1 day, 7 days 14 days, 21 days and 28 days respectively. AOB 4 biosorption of cadmium was 5% on day 1; 41.796% on day 7; 52% on day 14; 61% on day 21 and 84.82% on 28 days. 

*Achromobacter insolitus* (AOB 10) mitigation of copper range from 9% to 52.67%; 68% to 86% and 90.04% at 1 day, 7 days 14 days, 21 days and 28 days respectively. AOB 10 biosorption of nickel was 7% on day 1; 49.64% on day 7; 67% on day 14; 80% on day 21 and 94.67% on 28 days. AOB 10 mitigation of lead range from 11% to 62.75%; 68% to 79% and 90.25% at 1 day, 7 days 14 days, 21 days and 28 days respectively. AOB 10 biosorption of cadmium was 6% on day 1; 48.56% on day 7; 54% on day 14; 73% on day 21 and 89.21% on 28 days.

*Lysinibacillus paniscus* (NOB 7) mitigation of copper range from 9.97% to 50.176%; 62% to 74% and 86.94% at 1 day, 7 days 14 days, 21 days and 28 days respectively. NOB 7 biosorption of nickel was 10.9% on day 1; 36.31% on day 7; 79% on day 14; 88% on day 21 and 97.74% on 28 days. NOB 7 mitigation of lead range from 18% to 68.75%; 79% to 86% and 95.5% at 1 day, 7 days 14 days, 21 days and 28 days respectively. NOB 7 biosorption of cadmium was 6% on day 1; 42.8% on day 7; 50% on day 14; 62.06% on day 21 and 86.95% on 28 days.

*Alcaligenes faecalis* (NOB 5) mitigation of copper range from 9.97% to 50.176%; 62% to 74% and 86.94% at 1 day, 7 days 14 days, 21 days and 28 days respectively. NOB 5 biosorption of nickel was 10.9% on day 1; 36.31% on day 7; 79% on day 14; 88% on day 21 and 97.74% on 28 days. NOB 5 mitigation of lead range from 18% to 68.75%; 79% to 86% and 95.5% at 1 day, 7 days 14 days, 21 days and 28 days respectively. NOB 5 biosorption of cadmium was 6% on day 1; 42.8% on day 7; 50% on day 14; 62.06% on day 21 and 86.95% on 28 days.
Figure 4: Biosorption of copper by nitrite oxidizing bacteria (NOB 7)

Figure 5: Biosorption of nickel by ammonia oxidizing bacteria (AOB 4)

Figure 6: Biosorption of nickel by ammonia oxidizing bacteria (AOB 10)

Figure 7: Biosorption of nickel by nitrite oxidizing bacteria (NOB 5)

Figure 8: Biosorption of nickel by nitrite oxidizing bacteria (NOB 7)
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Figure 9: Biosorption of lead by ammonia oxidizing bacteria (AOB 4)

Figure 10: Biosorption of lead by ammonia oxidizing bacteria (AOB 10)

Figure 11: Biosorption of lead by nitrite oxidizing bacteria (NOB 5)

Figure 12: Biosorption of lead by nitrite oxidizing bacteria (NOB 7)

Figure 13: Biosorption of cadmium by ammonia oxidizing bacteria (AOB 4)
Comparative study of bioremediation of selective heavy metals (copper, nickel, lead and cadmium) by four nitrifying bacteria

Comparative study of bioremediation of selective heavy metals (copper, nickel, lead and cadmium) by four nitrifying bacteria: *Achromobacter xylosoxidans* (AOB 4 or A 4); *Achromobacter insolitus* (AOB 10 or A 10); *Alcaligenes faecalis*; (NOB 5 or N 5) *Lysinibacillus pakistanensis* (NOB 7 or N 7).

Copper at concentration of 100ppm was bioaccumulated 90.1%, 90.04%, 86.9%, 89.62% after a period of 28 days by AOB 4, AOB 10; AOB 5; AOB 7 respectively. Nickel at concentration of 100ppm was bioaccumulated 96.51%, 94.67%, 97.74 %, 92.1% after a period of 28 days by AOB 4, AOB 10; AOB 5; AOB 7 respectively. Lead at concentration of 100ppm was bioaccumulated 92%, 90.25%, 95.5 %, 95.05% after a period of 28 days by AOB 4, AOB 10; NOB 5; AOB 7 respectively. Cadmium at concentration of 100ppm was bioaccumulated 84.82 %, 89.21%, 86.95%, 86.07% after a period of 28 days by AOB 4, AOB 10; NOB 5; NOB 7 respectively. *Achromobacter insolitus* (AOB 10) has the highest biosorption capacity of copper, bioaccumulated 90.04 % of copper after the period of 28 days. *Alcaligenes faecalis* (NOB 5) has the highest biosorption capacity of nickel, bioaccumulated 97.74 % of nickel after the period of 28 days. *Alcaligenes faecalis* (NOB 5) has the highest biosorption capacity of lead, bioaccumulated 95.5 % of nickel after the period of 28 days. *Achromobacter insolitus* (AOB 10) has the highest biosorption capacity of cadmium, bioaccumulated 89.21% of cadmium after the period of 28 days.

The highest biosorption was carried out by *Alcaligenes faecalis* (NOB 5) bioaccumulated 97.74 % of nickel and the lowest amongst biosorption was carried out by *Achromobacter xylosoxidans* (AOB 4) bioaccumulated 84.82 % of cadmium. Biosorption of copper by nitrifying bacteria was in the order of AOB 10 > AOB 4 > NOB 7 > NOB 5. Biosorption of nickel by nitrifying bacteria was in the order of NOB 5 > AOB 4 > AOB 10 > NOB 7. Biosorption of lead by nitrifying bacteria was in the order of NOB 5 > NOB 7 > AOB 4 > AOB 10. Biosorption of cadmium by nitrifying bacteria was in the order of AOB 10 > NOB 5 > NOB 7 > AOB 4.
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Figure 17: Biosorption of copper, nickel, lead and cadmium by ammonia oxidizing bacteria (AOB 4)

Figure 18: Biosorption of copper, nickel, lead and cadmium by ammonia oxidizing bacteria
Figure 19: Biosorption of copper, nickel, lead and cadmium by nitrite oxidizing bacteria (NOB 5)

Figure 20: Biosorption of copper, nickel, lead and cadmium by nitrite oxidizing bacteria (NOB 7)
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Figure 21: Biosorption of copper nitrifying bacteria

Figure 22: Biosorption of Nickel nitrifying bacteria
Nitrifying bacteria (Achromobacter xylosoxidans; Achromobacter insolitus; Alcaligenes faecalis; Lysinibacillus pakistanensis sp) were able to carry out biosorption of copper, nickel, lead and cadmium. Achromobacter insolitus (AOB 10) has the highest biosorption capacity of copper, bioaccumulated 90.04% of copper after the period of 28 days. Alcaligenes faecalis (NOB 5) has the highest biosorption capacity of nickel, bioaccumulated 97.74% of nickel after the period of 28 days. Alcaligenes faecalis (NOB 5) has the highest biosorption capacity of lead, bioaccumulated 95.5% of lead after the period of 28 days. Alcaligenes faecalis (NOB 5) has the highest biosorption capacity of cadmium, bioaccumulated 89.21% of cadmium after the period of 28 days.

The highest biosorption was carried out by Alcaligenes faecalis (NOB 5) bioaccumulated 97.74% of nickel and the lowest amongst biosorption was carried out by Achromobacter xylosoxidans (AOB 4) bioaccumulated 84.82% of cadmium.

Biosorption of copper by nitrifying bacteria was in the order of AOB 10 > AOB 4 > NOB 7 > NOB 5. Biosorption of nickel by nitrifying bacteria was in the order of NOB 5 > AOB 4 > AOB 10 > NOB 7. Biosorption of lead by nitrifying bacteria
was in the order of NOB 5 > NOB 7 > AOB 4 > AOB 10. Biosorption of cadmium by nitrifying bacteria was in the order of AOB 10 > NOB 5 > NOB 7 > AOB 4. Statistical analysis ascertain that there is a significant difference (P<0.05) in biosorption rates between medium with bacteria isolate and control. Biosorption of heavy by nitrifying bacteria shows a positive result. Biosorption of heavy metals by different nitrifying bacteria depended on pollutant (heavy metal) (Naz et al., 2017).

Various soil microorganisms have great potential for bioremediation (Banik et al., 2014; Joutey et al., 2015). Microorganisms are essential in remediation of heavy-metal-contaminated environments as they have a variety of ways to endure metal toxicity (Ojuederie and Babalola, 2017). Microorganisms are very sensitive; they react quickly to any kind of changes (natural and anthropogenic) in the environment, and quickly adapt themselves to new conditions including high metal concentrations. Heavy metals from contaminated soils by are remove by microorganisms through the processes of precipitation, biosorption via sequestration, and conversion of metals to innocuous forms by enzymes (enzymatic transformation) (Ojuederie and Babalola, 2017).

Microorganisms take heavy metals into the cell in significant amounts. This phenomenon leads to the intracellular accumulation of metal cations of the environment and is defined as bioaccumulation (Woleko et al., 2016). Some bacterial plasmids contain specific genes for resistance to toxic heavy metal ions (Liu et al., 2018; Pacwa-Plociniczak et al., 2018; Lukin et al., 2016; Sharma, 2016), ability to produce sidophore, and ability to solubilize phosphate (biofertilizers) (Ibiene et al., 2012; Gupta et al., 2014). Some microorganisms can adjust their metabolic activity or community structure to adapt to the harmful shock loadings (Sannisiet al., 2010). Microorganisms play an important role in stress environment and the derived ecosystem functions (Singh et al., 2016a, b, c; Vimal et al., 2017, Odokuma and Nrior, 2015). Microorganisms can mobilize or immobilize metals by biosorption, production, production of chelating agents, chemoorganotrophic and autotrophic leaching, and redox transformations. These mechanisms stem from prior exposure of microorganisms to metals which enable them to develop the resistance and tolerance useful for biological treatment (Viti et al., 2003; Velasquez and Dussan, 2009).

### Molecular Characterization of Nitrifying Bacteria Isolates

**Image showing genomic DNA**

**16SrRNA gene amplification results**

The isolate AOB4 has 88.5% pairwise identity with the organism Achromobacterxylosoxidansstrain ChemUPES_3 with NCBI accession number MK281584. The E-value is 0. The isolate sequences are shown below:

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GGGTATCTTATGAGATGTTCTAGTGGCCTAGGCGCTGGGATC
ACCCTTTATATGTTTGCGGCGGCGCTGAATAT
GTATAAGAACCTGCTAAAATAGCGGGGGATAACTACGCGAAAG
CGTAGCTAATACCCGATACCCCTACGGGGAG
AAATGTCGGGATCTTCCGACCTGACTATTTGAGCCAGCCGATATCGGATTAGCTAGTTGGTGGGGGTAACAGG
CTCTACCAAGGCGACATCCGTAACCTGTTGTAGGAGGATGATC
AGCTCACACTGGAACCTGAGACACGGGCTCAG
ACTCTCACGGAGGAGGACGACGAGAGCTGAGGAGGATGATC
GCAGAAGGCTGTAGCCATGCGGCGCGTTG
CGATGAAAGCCCTTCGAGATTGTAAGACACTTCTTGGCTGGAAGGA
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AGGGTGC GG TGGTAAATACCC CTTCGCAAACTTT
GACGACTTGCGAAGACCATCGCAGTAACGGTCCGCAC
CGCGCGCTAGCTGCGAACGAGGATGCTG
CGGAA GAGGGCGCAGCGGAGGTAGAATTCCTACCGA
CGGTTCTCGCATCAGCTGCTTACTGCTGT
GGTGCGAGCGGGTTGAGAGGCGGACTGCCCT

The isolate AOB10 has 96.1% pairwise identity with the organism Achromobacter insolitus strain Ma1Bc with NCBI accession number AB254670. The isolate sequences are shown below:

- The isolate NOB7 has 86.9% pairwise identity with the organism Lysinibacillus pakistanensis strain NCCP 54 with NCBI accession number KY000495. The E-value is 0. The isolate sequences are shown below:

- The isolate NOB8 has 86.9% pairwise identity with the organism Lysinibacillus pakistanensis strain NCCP 54 with NCBI accession number KY000495. The E-value is 0. The isolate sequences are shown below:

### IV. CONCLUSION

The organisms were able to carry out bio sorption of copper, nickel, lead and cadmium. The organisms remain attractive potential candidates for further investigations regarding their ability to remove heavy metal in bioremediation. It may be a good option for bioremediation of environment: Soil, aqua culture and waste since it is regarded as an eco-friendly and efficient. Bacterial biosorption can be used for the removal of specific metals; no additional nutrient requirement; and the likelihood of metal regeneration of the bio sorbent; and the likelihood of metal recovery. Biosorption would offer an economically feasible technology."
for efficient removal and recovery of metal(s) from aqueous solution

V. ACKNOWLEDGEMENTS

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VI. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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