World Journal of Innovative Research (WJIR)
ISSN: 2454-8236, Volume-7, Issue-5, November 2019 Pages 113-127

Application of Heterotrophic Nitrifying Bacteria in Bioremediation of Heavy Metals

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***Abstract*— the study was undertaken to investigate thebiosorption 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 Microbial growth was observed in terms of CFU and O.D. The samples was withdrawn at day's interval, transferred to 10 ml vials and capped for AAS analysis.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 (NOB 5) bioaccumulated 97.74 % of nickel and the lowest amongst biosorption was carry out by Achromobacterxylosoxidans (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 (Landrigan*et 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-Ustun*et 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 (Hansda*et 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 (Srivastava*et 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 (Tchounwou*et 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) (Rial*et 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;Srivastava*et al.*, 2017). Metals are stable (Srivastava*et*

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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 (Hamsa*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 monooxygenase. Hydroxylamine (NH_2OH) 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, Nitrosospira, 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 *Candidatusnitrotoga* (Ma *et al*., 2014). Based on the 16S ribosomal DNA (rDNA) phylogenetic analysis results, *Nitrobacter, "Candidatusnitrotoga", Nitrococcus and Nitrospina* 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 (Daims*et al.*, 2015; Hanna *et al*, 2018).

Other organisms involved in nitrification are heterotrophic bacteria (*Arthrobacterglobiformis, Aerobacteraerogenes, Thiosphaerapantotropha, Streptomyces grisens,* various *Pseudomonas* spp, *Alcaligenesfaecalis* and *Achromobacterxylosoxidans*(Basha*et al.*,2018;Fitriyanto*et al.*,2017; Shoda and Ishikawa, 2014); *Rhodococcus* sp., *Diaphorobacter* sp., *Bacillus* sp., *Bacillus methylotrophicus., and* fungi (*Aspergillusflavus)* (Hamsa*et al.,* 2017). Recent research on the metabolic pathways of heterotrophic ammonia oxidation has been conducted using *Paracoccusdenitrificans*(Moir*et al.,* 1996b), *Alcaligenesfaecalis*(Joo*et al.,* 2005), *Acinetobactercalcoaceticus (*Zhao *et al.,* 2010), *Bacillus methylotrophicus*(Zhao *et al.,* 2012), *Pseudomonas stutzeri*(Zeng*et al*., 2011), *Pseudomonas putida*(Daum*et al.,* 1998), and a few other bacterial species (Hayatsu*et 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 (Naz*et al*, 2015).Various soil microorganisms have great potential for bioremediation (Banik*et al*, 2014; Jouteye*t 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, biomineralization, and biotransformation.; (c) metal entrapment in the extracellular capsules; (d) heavy metals precipitation; and (e) metal detoxification via oxidation–reduction (Zubair*et 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 (Ayangbenro 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.AkwaIbom State University, Obio-akpa in AkwaIbom, State,AdiasimIkotEssiendot, AkwaIbom State, University of Nigeria, Nsukka, Enugu State, University of Uyo, AkwaIbom, State and from solid waste disposal site in Uyo, AkwaIbom 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_2HPO_4 and 27 mg KH_2PO_4 per litre (pH 7.0) and shaken at 100 rpm for 2 h (Deni and Penninck, 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

suspension.

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: $(NH_4)_2SO_4$, 2.0; K_2HPO_4, 1; $MgSO_4.7H_2O$, 0.5; NaCl, 2.0; $FeSO_4.7H_2O$, 0.4 ; $CaCO_3$, 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: KNO_2, 0.1; Na_2CO_3, 1; NaCl 0.5; $FeSO_4.7H_2O$, 0.4. Each of ten test tubes was filled with 9 ml of the Winogradsky broth media 1 and 11, respectively, autoclaved at 121 ^{0}C 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 I and 11 was prepared by adding 15.0 g agar to 1000 ml of fresh broth and sterilized at 121 ^{0}C at 15 psi for 15 minutes and allowed to cool to about 45 ^{0}C 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 was incubated aerobically at room temperature (28 +2^{0}C) for 1week and examined for growth.

Purification of isolates

Discrete colonies that developed on Winogradsky agar media for nitrification phases 1 and 11 after 1week 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 ±2^{0}C) 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-napthylethylenediamine (NED) and an azo compound is formed as a result measured with spectrophometer at 540 nm.

Determination of nitrateusing Phenol disulphonic acid

Nitrate reacts with phenol disulphonic acid to give a yellow colour, absorbance measure at 410 nm using spectrophometer (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 25°C 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 defned 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:

$$q_e = \frac{(Ci - Ce)V}{M}$$

The percentbiosorption (R%) known as biosorption efficiency for the metal was evaluated from the following equation. Where qe is the amount of adsorbed metal ions of the adsorbent (mg g-1), Ci is the initial concentration of metal ion in the solution (mg L-1), Ce 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).

$$\%\text{Biosorption} = \frac{a-b}{a} x \frac{100}{1}$$

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 *Ralstoniametallidurans* or *Cupriavidusmetallidurans; Achromobacterinsolitus*; *Alcaligenesfaecalis*; *Lysinibacilluspakistanesis*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. *Achromobacter*xylosoxidans (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.

Achromobacterinsolitus (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.

Alcaligenesfaecalis (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.

Lysinibacilluspakistanesis(NOB 7) mitigation of copper range from 8.9 % to 48.3 %; 59 % to 76 % and 89.6 % at 1 day, 7 days 14 days, 21 days and 28 days respectively. NOB 7 biosorption of nickel was 10 % on day 1; 40.62 % on day 7; 79.86 % on day 14; 85.01 % on day 21 and 92.10 % on 28 days. NOB 7 mitigation of lead range from 18 % to 67.5 %; 77.9 % to 84 % and 95.05 % at 1 day, 7 days 14 days, 21 days and 28 days respectively. NOB 7 biosorption of cadmium was 6.5 % on day 1; 49 % on day 7; 52.85 % on day 14; 67.14 % on day 21 and 89.21 % on 28 days.

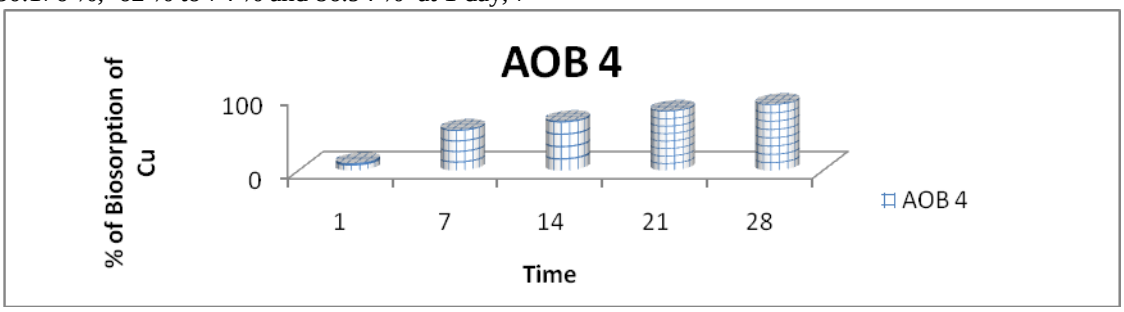


Figure 1: Biosorption of copper by ammonia oxidizing bacteria (AOB 4)

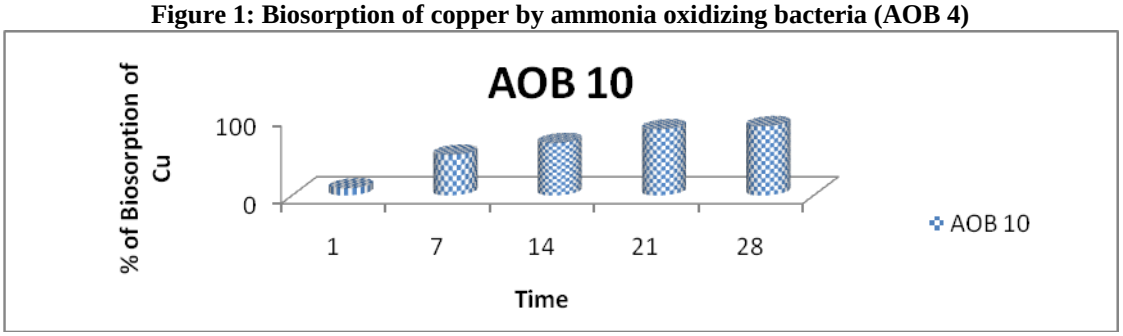


Figure 2: Biosorption of copper by ammonia oxidizing bacteria (AOB 10)

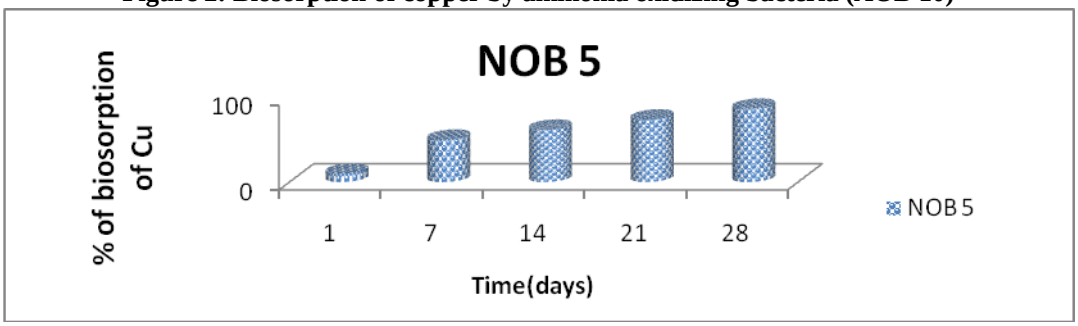


Figure 3: Biosorption of copper by nitrite oxidizing bacteria (NOB 5)

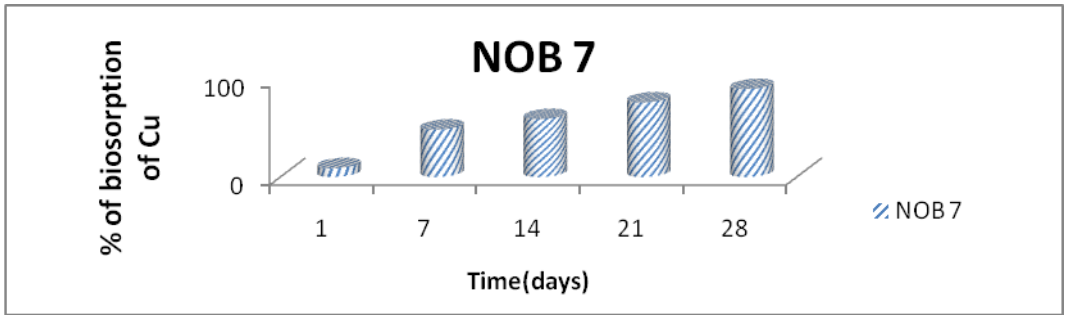


Figure 4: Biosorption of copper by nitrite oxidizing bacteria (NOB 7)

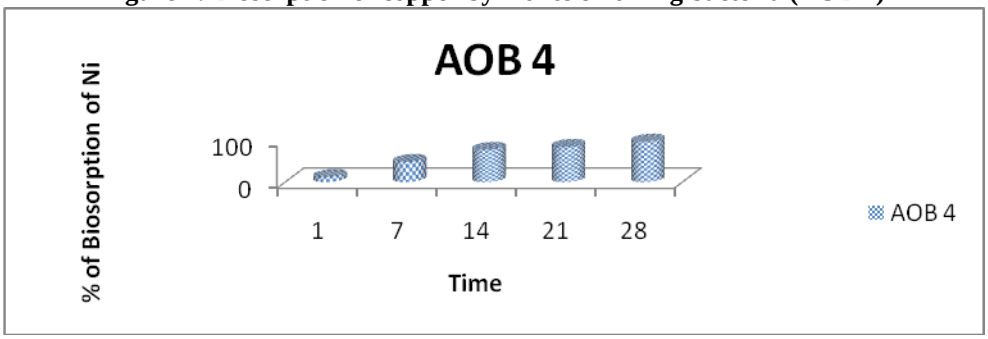


Figure 5: Biosorption of nickel by ammonia oxidizing bacteria (AOB 4)

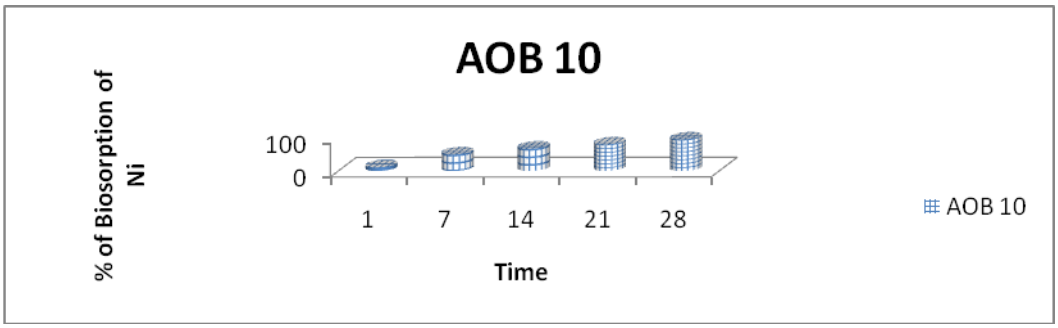


Figure 6: Biosorption of nickel by ammonia oxidizing bacteria (AOB 10)

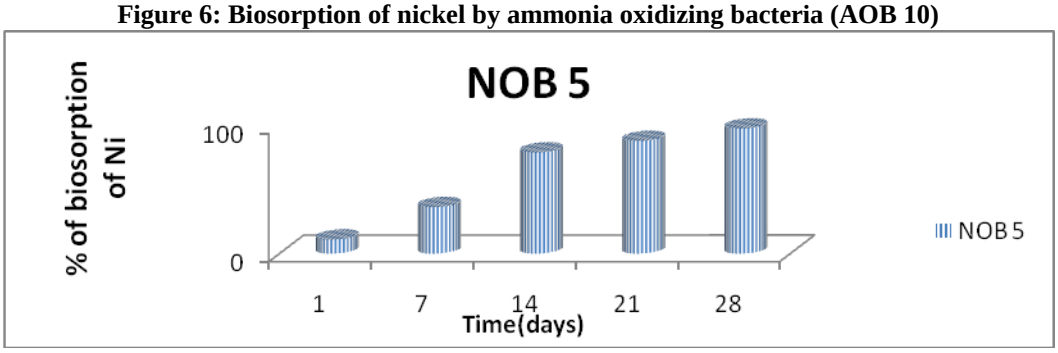


Figure 7: Biosorption of nickel by nitrite oxidizing bacteria (NOB 5)

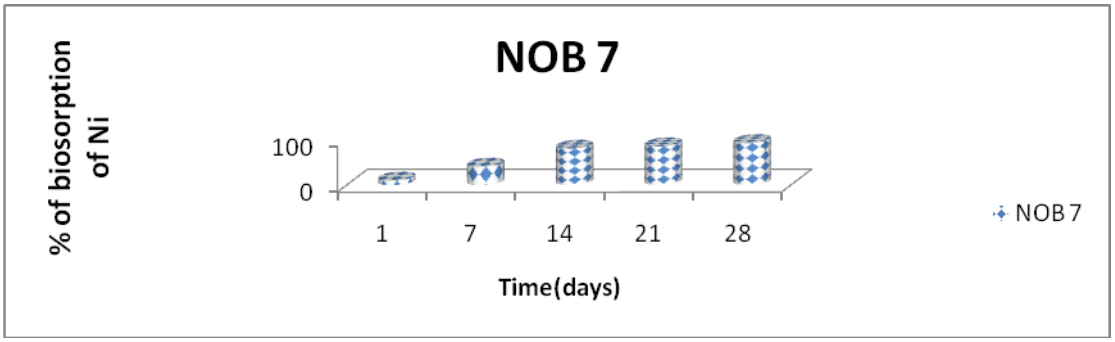


Figure 8: Biosorption of nickel by nitrite oxidizing bacteria (NOB 7)

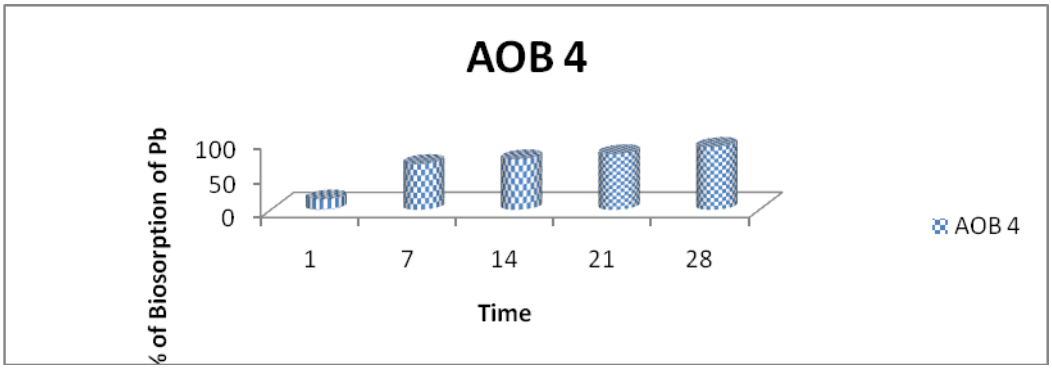


Figure 9: Biosorption of lead by ammonia oxidizing bacteria (AOB 4)

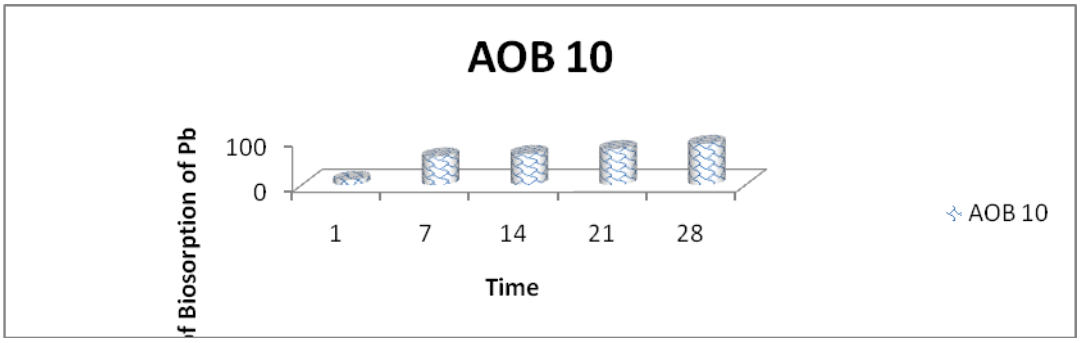


Figure 10: Biosorption of lead by ammonia oxidizing bacteria (AOB 10)

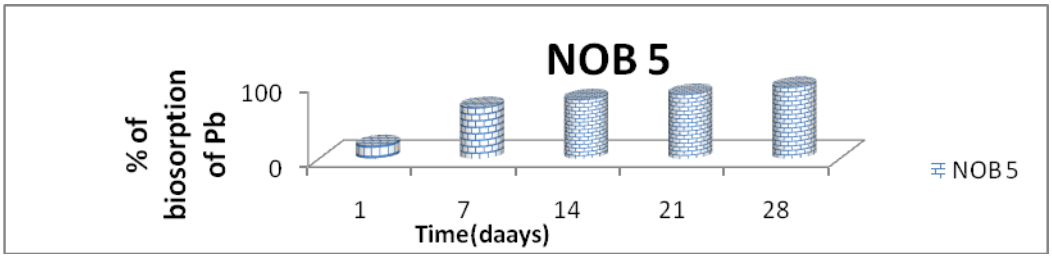


Figure11: Biosorption of lead by nitrite oxidizing bacteria (NOB 5)

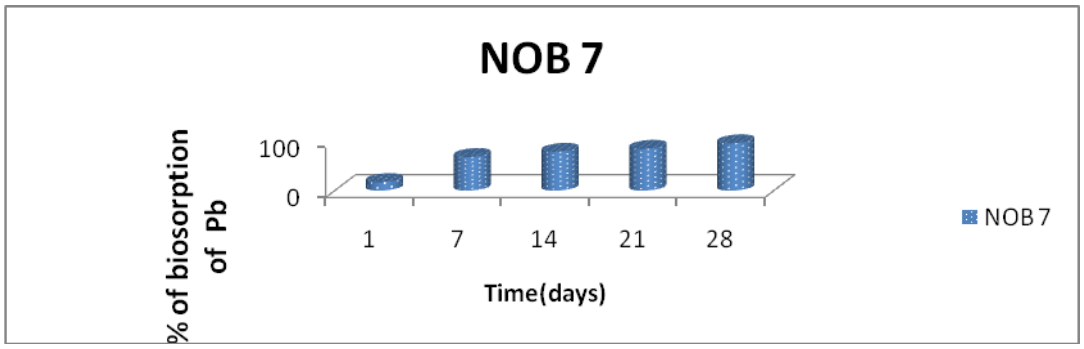


Figure 12: Biosorption of lead by nitrite oxidizing bacteria (NOB 7)

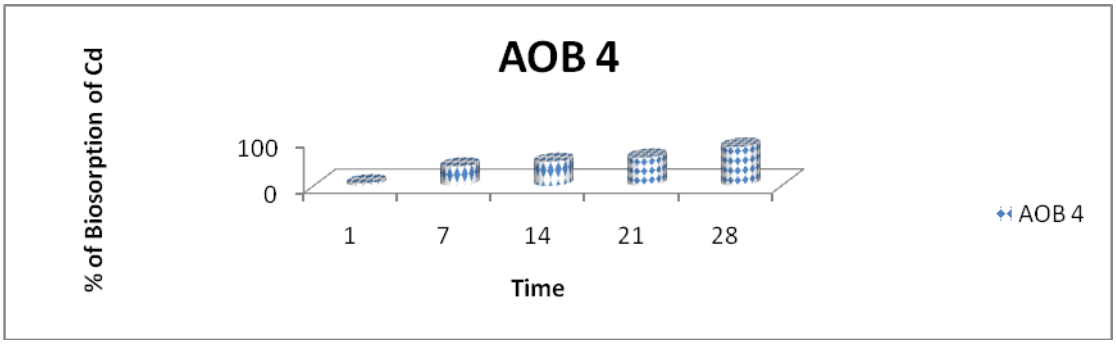


Figure 13: Biosorption of cadmium by ammonia oxidizing bacteria (AOB 4)

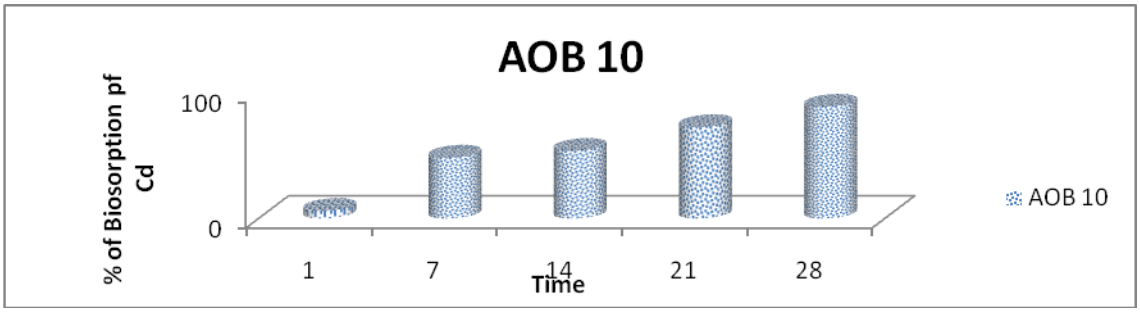


Figure 14: Biosorption of cadmium by ammonia oxidizing bacteria (AOB 10)

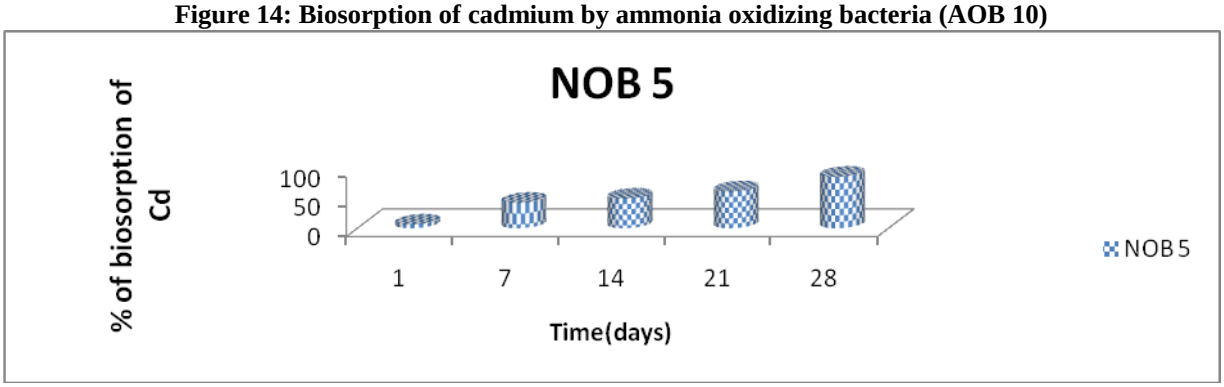


Figure 15: Biosorption of cadmium by nitrite oxidizing bacteria (NOB 5)

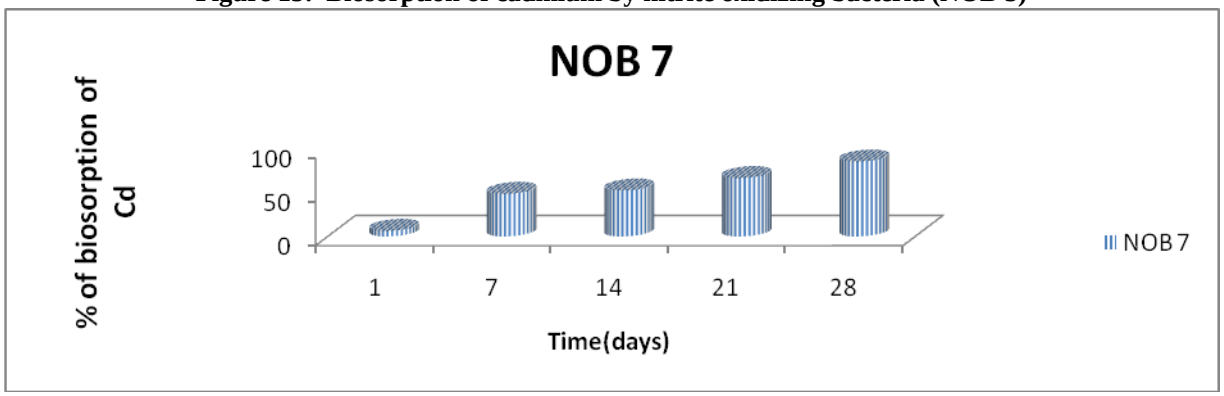


Figure 16: Biosorption of cadmium by nitrite oxidizing bacteria (NOB 7)

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); *Achromobacterinsolitus* (AOB 10 or A 10); *Alcaligenesfaecalis*; (NOB 5 or N 5) *Lysinibacilluspakistanesis*(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; 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 (NOB 5) bioaccumulated 97.74 % of nickel and the lowest amongst biosorption was carry 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.

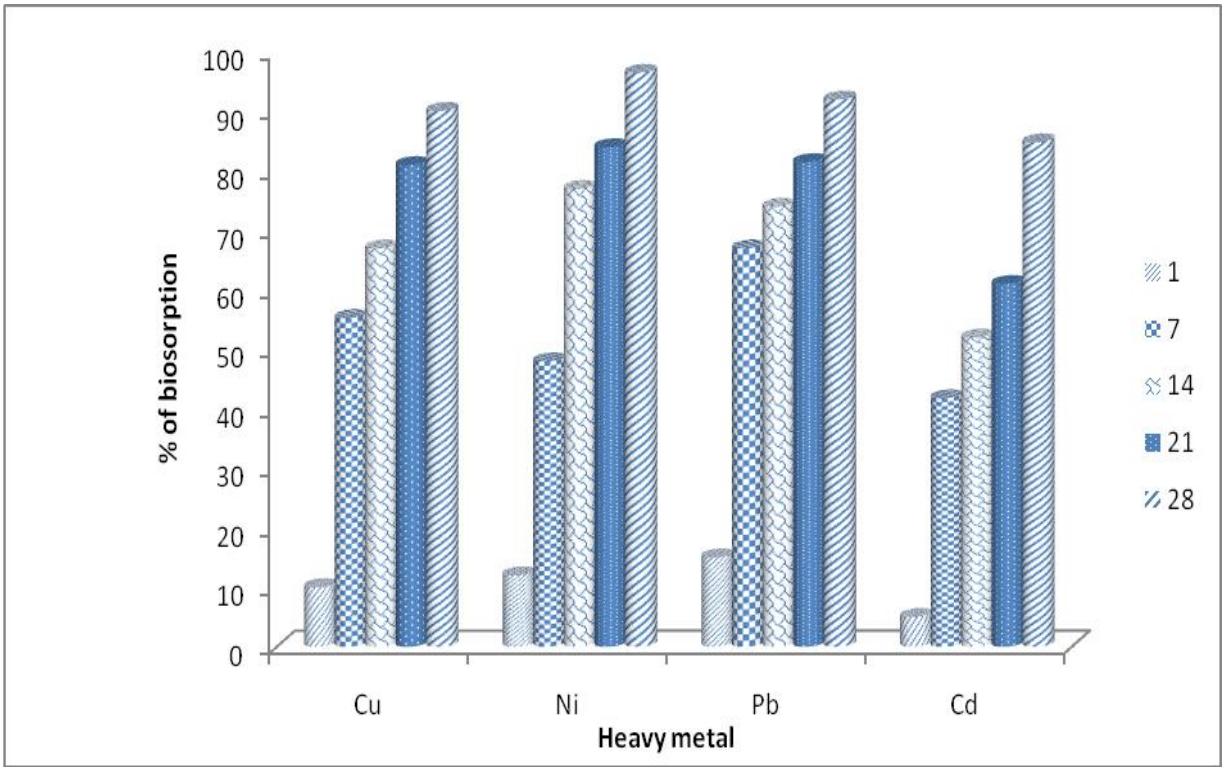


Figure 17:Biosorption of copper, nickel, lead and cadmium by ammonia oxidizing bacteria (AOB 4)

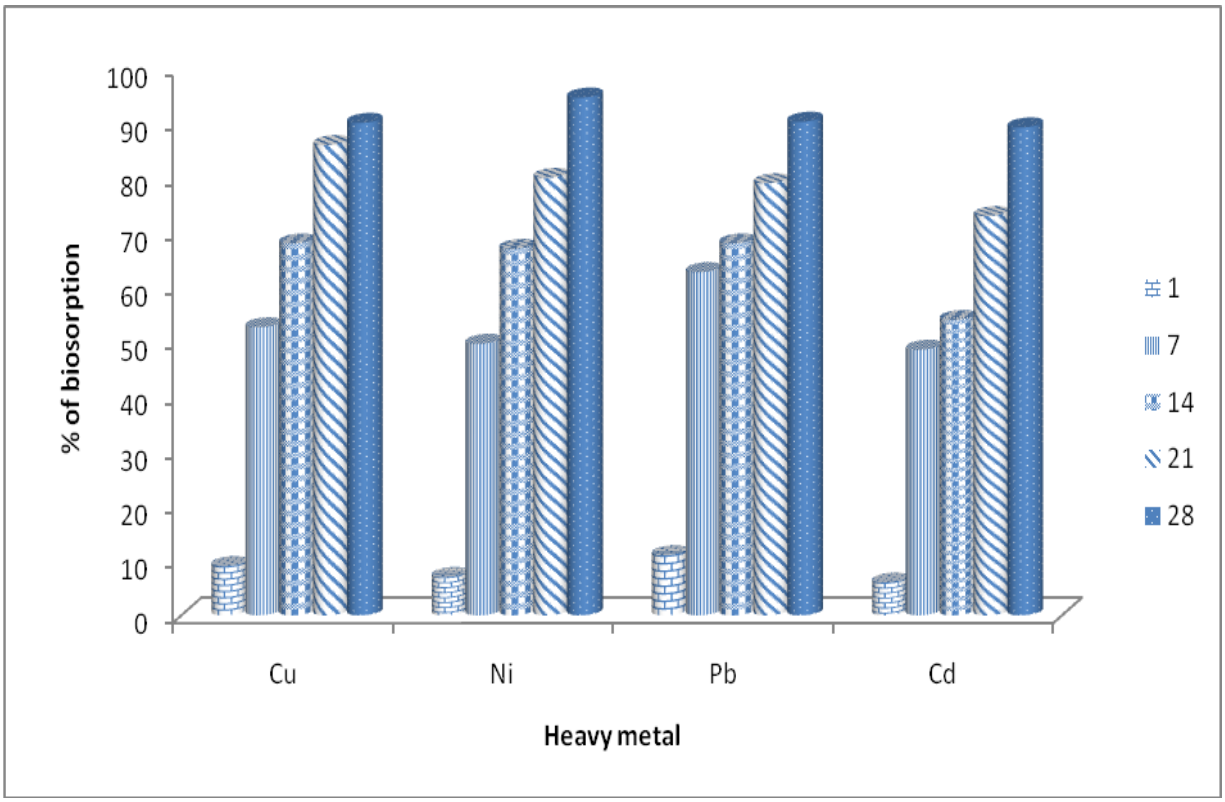


Figure 18: Biosorption of copper, nickel, lead and cadmium by ammonia oxidizing bacteria

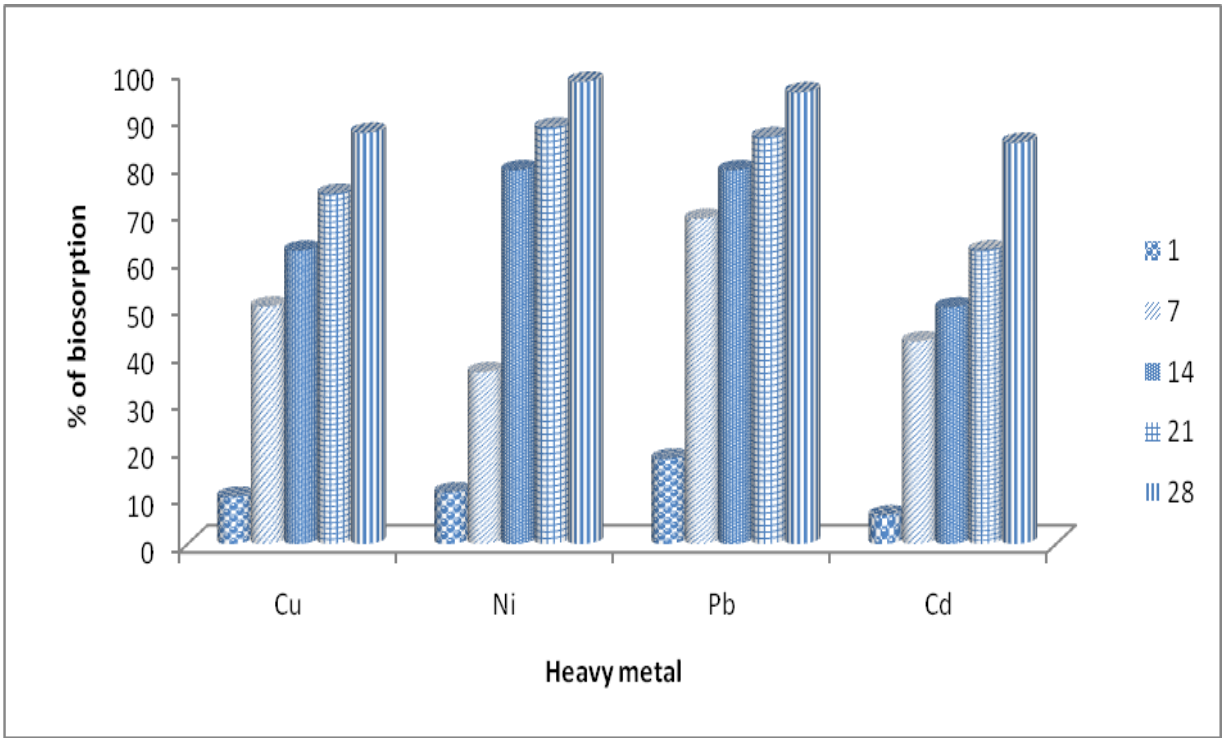


Figure 19: Biosorption of copper, nickel, lead and cadmium by nitrite oxidizing bacteria (NOB 5)

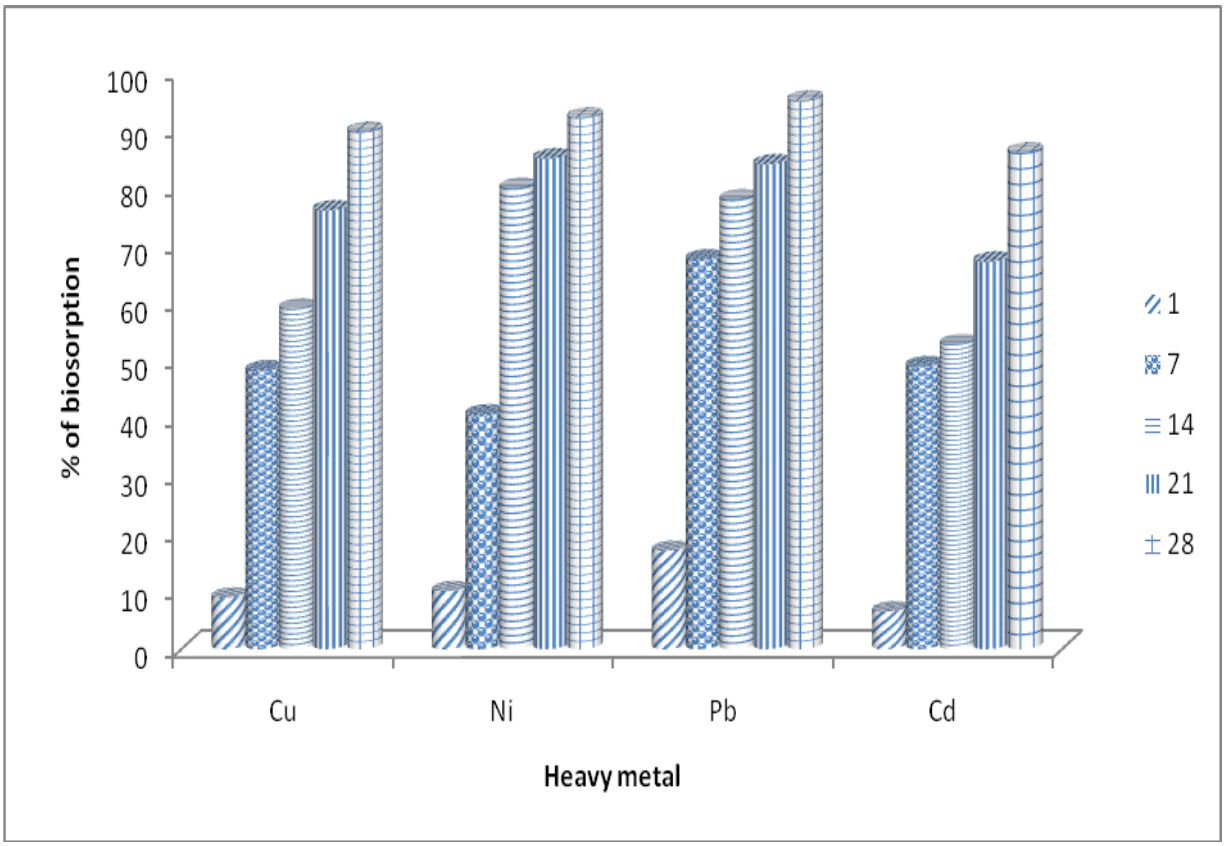


Figure 20: Biosorption of copper, nickel, lead and cadmium by nitrite oxidizing bacteria (NOB 7)

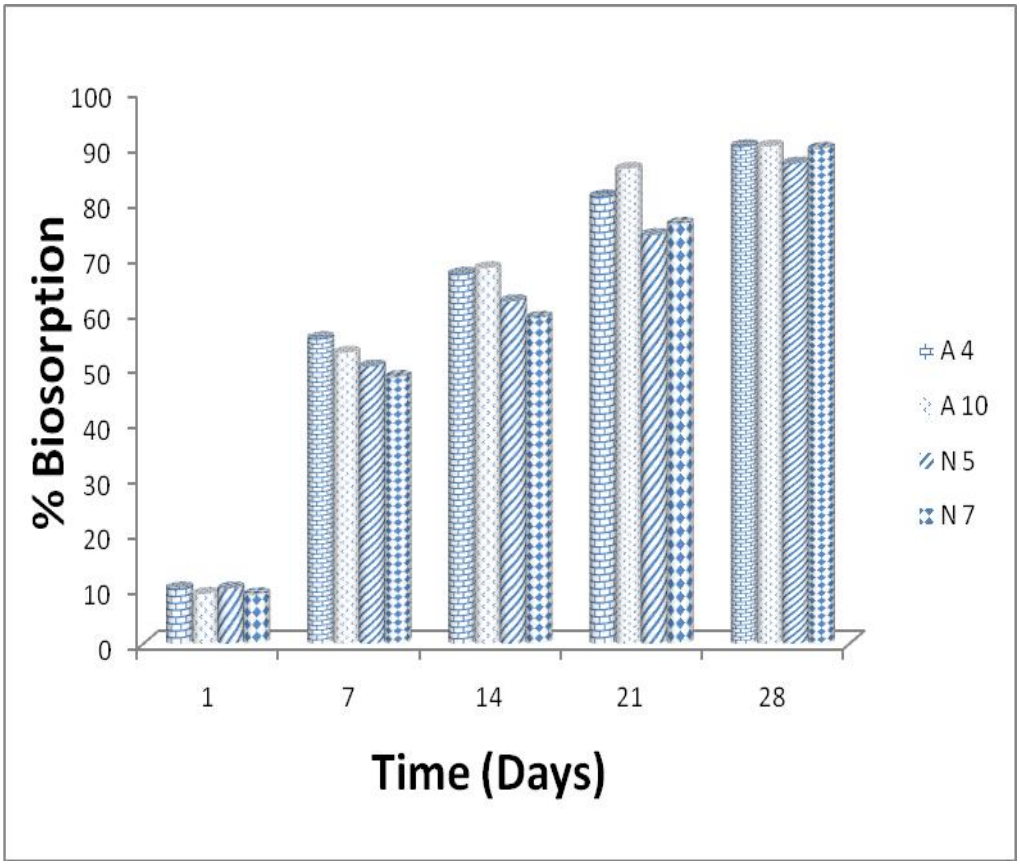


Figure 21: Biosorption of copper nitrifying bacteria

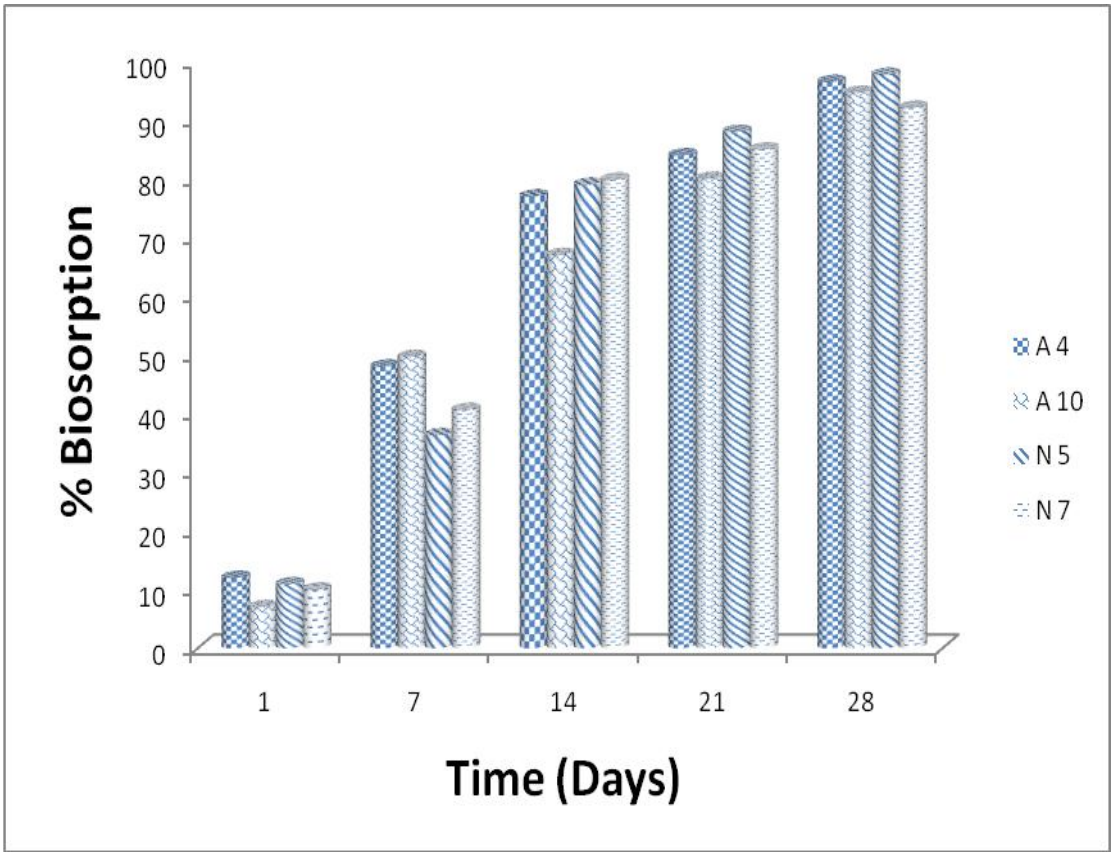


Figure 22: Biosorption of Nickel nitrifying bacteria

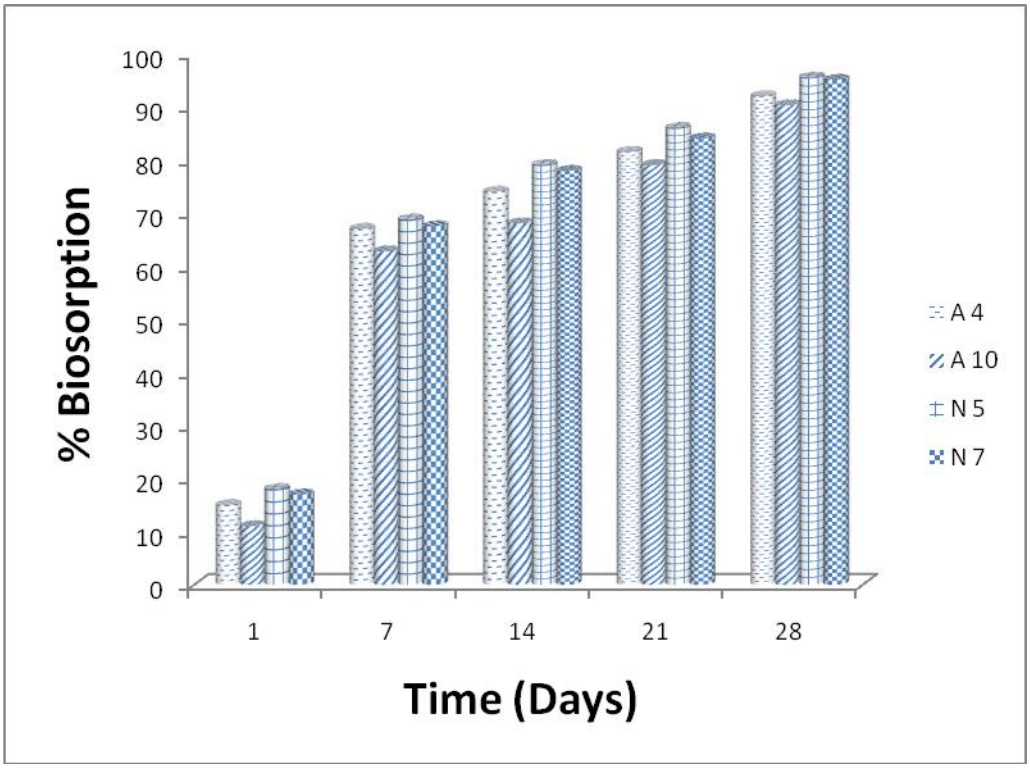


Figure 23: Biosorption of Lead nitrifying bacteria

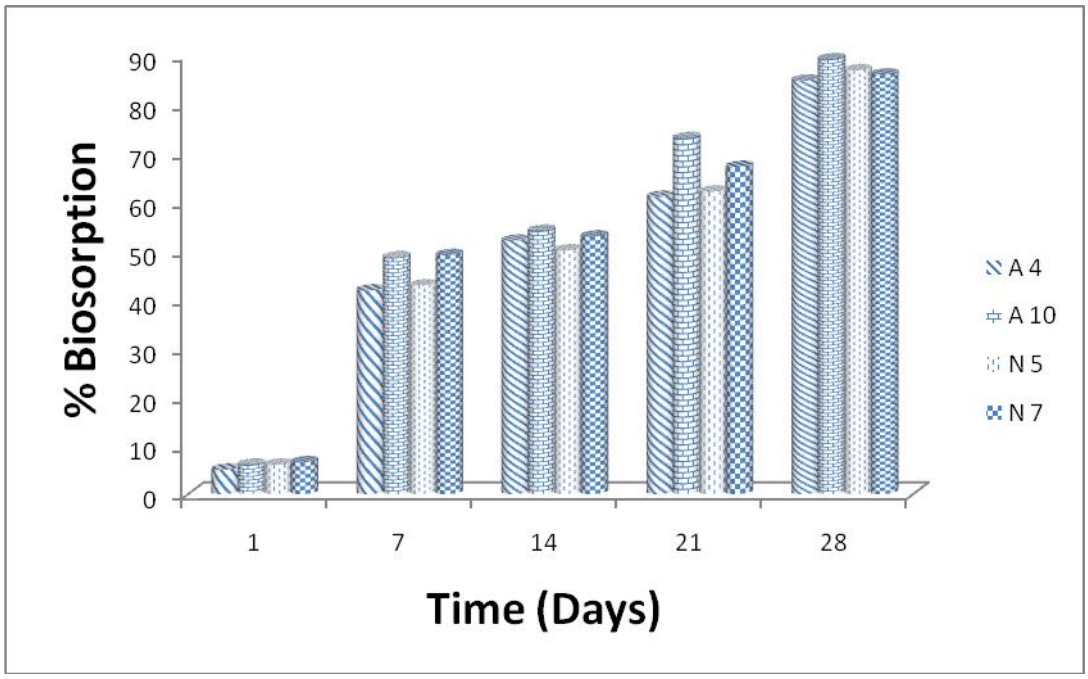


Figure 24: Biosorption of Cadmium nitrifying bacteria

Nitrifying bacteria (*Achromobacter*xylosoxidans*;* *Achromobacterinsolitus*; Alcaligenesfaecalis; Lysinibacilluspakistanesissp) were able to carry out biosorption of copper, nickel, lead and cadmium. *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 (NOB 5) bioaccumulated 97.74 % of nickel and the lowest amongst biosorption was carry 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*, 2015).

Various soil microorganisms have great potential for bioremediation (Banik*et al*, 2014; Jouteye*t 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 conditionsconditions including high metal concentrations.Heavy metals from contaminated soils by are remove by microorganims through the processes of precipitation, biosorption via sequestration byintracellular metal binding proteins (metallothioneins) and conversion of metals to innocous 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 (Wolejko*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; Lukina*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 (Sannisi*et al.*, 2010). Microorganisms play important role in stress environment and the derived ecosystem functions (Singh *et al.*, 2016a, b, c; Vimal*et al.*, 2017, Odokumaand Nrior (2015).). Microorganisms can mobilize or immobilize metals by biosorption, sequestration, production of chelating agents, chemoorganotrophic and autotrophic leaching, methylation 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

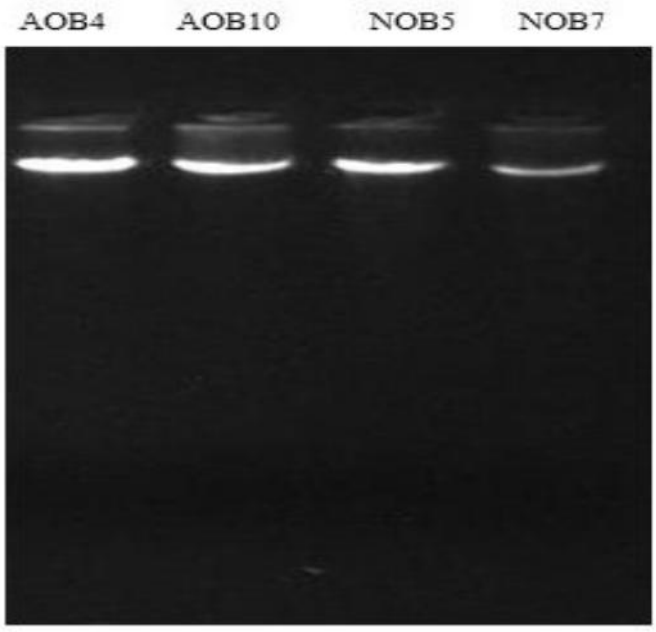
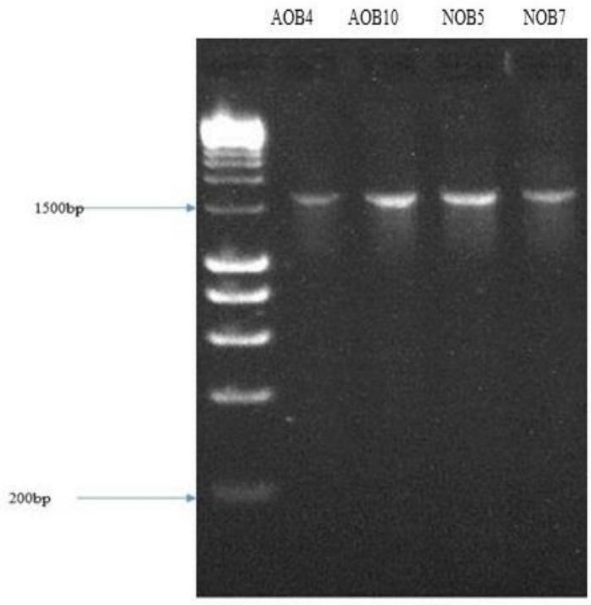


Image showing genomic DNA



M=Hyperladder molecular weight marker

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 areshown below:

GGGTATCTTATGAGATGTTCATGGCTCAGGTGCGCTGGGATC
ACCCCTTTATAGTTTGGCGGGCGGCTGAGTAAT
GTATAGGAACCTGCTAAAATAGCGGGGGATAACTACGCGAAAG
CGTAGCTAATACCGCATACGCCCTACGGGAG
AAAGTGGGGGATCTTCGGACTTGCACTATTGGAGCGAGCCGATA
TCGGATTAGCTAGTTGGTGGGGGTAACAGG
CTCTACCAAGGCGACGATCCGTAACTGGTTCTGAGAGGATGATC
AGCTCACACTGGAACTGAGACACGGCTCCAG
ACTCCTACGGGAGGCAGCAGTGGGGGAATTATTGGACAATGGG
GCGAAACGCCTGATCCAGCCATGCCGCGTGT
GCGATGAAGGCCTTCGGATTGTAAAGCACTTTTGGCTGGAAGGA

AGGGTCGCGGGTTAATACCCTTGCGAAACTT
GACGGTACCTGCAGAATAAGCACCGGCTAACTACGTGCCAGCA
GCCGCGGTAATACGTAGGGTGCAAGCGTTAAT
CGGAATTACTGGGCGTAAAGCGCGCGCAGGCGGTTCAGAAAGA
AAGATGTGAAATCCCCACGAGGCTTAACTCT
GGGAACTGCATTTTTAAACTACCTGAGCTAGAGTGTGTCAGAGG
GAGGTGGAATTCTCGCTGTGTAGCAGTGAAA
TGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAGCCTAC
CTGGGATAACTACTGACGCTCGATGCACCGA
AAGCCGTGGGGGAGCAAACAGGATTAGATACCCTGGTTAGTCC
ACGCCCGAAACGATGTCAGACTAGCCCGTTG
GGGCCCTCTGACATCTGTTAAGCGCAGCTCAACACGTGATGATG
ACAGCCTCTGGCAGTACAGTCGCACGATTATA
ACTCTCAAGTAATTGACGCGGGACCCCCACAAGCGCTGTGAT
GATGTGTGTTAAATCGATGAGACGCGCAGAAAA
CCCTTATCCTATCCCTTGTACATGTCTGAATGCTGTACCAGATAT
GCATGGCTCCGCTACGAGAACCTGAACACAGT
GCTTGCATGCCTGGTCGGTCAGCTCGGTCCGTGAGATGGTTGGG
TAAGGTCCGTAACTGAGCGCAACTGCCCTAG
TGCTACGAACGTGCAACTCGTCAATTGCACAAATATGC

• The isolate AOB10 has 96.9% pairwise identity with the organism Achromobacterinsolitus strainMa1Bc with NCBI accession number KY000495. The E-value is 0. The isolate sequences are shownbelow:
GGGGTATCTAAAGATGACATGGCTCAGGTGCGCTGGGACCCCCT
TAAAAGTTTGCCTCGGCCCAGAGGGGCGGG
AAAACCCCTTAAAATTTTGGGCGAGCCTACGCGAAAGCGTAACT
AATACCGAATACGCCCTACGGGGGAAAGCAG
GGGATCGCAAGACCTTGCACTATTGGAGCGGCCGATATCGGATT
AGCTAGTTGGTGGGGTAACGGCTCACCAAG
GCGACGATCCGTAGCTGGTTTGAGAGGACGACCAGCCACACTGG
GACTGAGACACGGCCCAGACTCCTACGGGA
GGCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCTGATCC
AGCCATCCCGCGTGTGCGATGAAGGCCTTCG
GGTTGTAAAGCACTTTTGGCAGGAAAGAAACGTCGTGGGTTAAT
ACCCCGCGAAACTGACGGTACCTGCAGAATA
AGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
GTGCAAGCGTTAATCGGAATTACTGGGCGTAA
AGCGTGCGCAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGA
GCTTAACTTTGGAACTGCATTTTTAACTACCG
AGCTAGAGTGTGTCAGAGGGAGGTGGAATTCCGCGTGTAGCAG
TGAAATGCGTAGATATGCGGAGGAACACCGA
TGGCGAAGGCAGCCTCCTGGGATAACACTGACGCTCATGCACGA
AAGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGCC
TTCGGGCCTTGGTAGCGCAGCTAACGCGTGA
AGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAA
GGAATTGACGGGGACCCGCACAAGCGGTGAT
GATGTGATTATTCGATGCAACGCGAAAACTTACCTACCCTTGAC
ATGTCTGGAATTCCGAGAGATTGGAAGTGCTC
GCAAGAGAGACCGGAACACAGGTGCTGCATGGCTGTCGTCAGC
TCGTGTCGTGAGATGTTGGGTTAGTCCCGCAA
CTGAGCGCCAACCATTGTCATTAGTGGCTACGAAGGCACTCTAA
TGAGAACTGCGGGTGACAAACGAAGACAGG
GGGGATGACGTCAGGTCCTCTCTATGGCCTTAAGGGAAAGGGGT
TCG

• The isolate NOB5 has 85.1% pairwise identity with the organism Alcaligenesfaecalis strain 10UPMR with NCBI accession number KJ748585. The E-value is 0. The isolate sequences are shownbelow:
TCTATGTTGATCATGGCTCAGGTGCGCGTTGGACACTTCTTTATA
GTTTGTCCTCGGCTCAGGGGGGGGGGAAACC
CCCTTTTAATTTTGTGCCGGCCCCAGGCTACTCGAAAAACTTACT
AAAAAAGCGCACGCCCTACAGGGGAGAGAGC
GGGATATCTAGAACTCTTGCTATTGGAGCTGCCGATATCGCATT
AGCTAGATGTTGGGGTACAGGCTCACCAAGG
CCCGATCCGTAGCTGGATTGAGAGGACGACCAGCGCACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGC
AGCAGTGTGGAATTTTGGACAATGAGGGGAGCCCCTGATCTCCC
CATCCCGCGTGTATGATGAAGGCCTTCTTGGT
TGTATAGTACTTTTGTTGGAGAAGAAAAGGTATCCCCTAATACG
ACGATACTGCTGACGGTATCTGCAGAATAAGC
TCACGCTAACTACGTGCCAGCAGCCGCGCGAATACGTAGGGCGC
AAGCGTTAATCGCAGTTACTGGGCGTAAAGC
GTGTGTAGGCGGATCGGAAAGAAAAAATGCGAAATCCCAGG
GCTCAACCTTGGAATTGCATTTTTAACTGTCGAG
CTAGAGTATGTCAGAGGGGGGTAGAATTCCTACGTGTAGCAGTG
AAATACGTAGATATGTGGAGGAATACCGAT
GGCGAAGGCAGCGACCTGCGGATGACACTGACGCTCAGACGCG
AAAGCGTGGGGAGCAATCAGTAGTCTATACC
CTGATAGTCCGACGCCGTAACGATGACAAGTAGCTGTTGTCGAC
GGCTTAGTCGTTACCACCGCAGCTAACCGATG
AAGTCGACCGCCTGACGAGTACAGTCCGAAGACTAAAACTAAT
AGTAACTGACTGGAACCCGGCCAAGTCGAGGC
ATGATGTGTATTAATTCGATGCAACGCATAACTTACCTTACCTGT
CATGTCTGCAAGCAATTAGACTTGGTCGGTGC
TGCGCATGATACCAGAGCAGATGCTGCTAGACTGCGTCAGCTTG
TCGGAGTTGAAGTAAGTCAATATCCGCTCGG
AACGGATACATGGACTGCATGAGCATAGCA

• The isolate NOB7 has 86.9% pairwise identity with the organism Lysinibacilluspakistanensisstrain NCCP-54 with NCBI accession number NR_113166. The E-value is 0. The isolate sequences areshown below:
GGAGGGGTACTGTATAGTTGTCTGGCTCAGGTGCGGTGGTACCT
CCTCTTAATACGTTAGCGGCGGACGGGTGAG
TAACACGTGGACAACCTACCCTATAGTTTGGGATAACTCCGGGA
AACCGGGGCTAATACCGAATAATCTATGTCAC
CTCATGGTGACATACTGAAAGACGGTTTCGGCTGTCGCTATAGG
ATGGGCCCGCGGGCGCATTAGCTAGTTTGGT
GAGGTAATGGCTCACAAAGGCGACGATGCGTAGCGGACCTGAG
AGGGGGATCGGCCCACACTGGGACTGAAAA
ACGGCCAAAACTCCTACGGGAAGGCAGCAGTAGGGAATCTTCC
ACAATGGGCGAAAGCCTGTTGGAGCACCCCCC
CGTGAAGTGAAGAAGGTTTTCGGATGGTAAAACTTTGGTGGTAG
GGAAAAAACAGTACGGTAGTAACTGGGCTG
TACCTTTGACGGTCCCTTATTAGGAAAGCCACGGCTTAATTACG
GGGCAACCAGCCGGGGTAATTACGTAGGGGG
GAAAGCGTTGTCCGGGAATTTATTGGGCCGTAAAGGCGCGCGCA
GGGGGGTCCTTTTAAGTCTGATGGGGAAGC
CCCCCGCCTCAACCGGGGGGGGGTCTTTGGAAACTGGGGGGACT
TGAGTGCAGAAGAAGGAAAGTGGATTTCCA
AGTGTACCGTTGAAAGCGTAAAGATTTGGAGGAACCCCGTGCCG
AAGCGACTTTCTGGTCTGTAATTGACGCTGA
GGCGCGAAACCGTGGGGAGCAAACAGGATTAGATACCCTGGGA
GTCCACGCCGTAACGATGAGTGCTACCTGTT
AAGGGGTTCCCCCCCCTTAGTGCTGCGCTAACGCATTAAGCACT
CCGCCTGGGGAGACGGCCGCTAGACTGAACC
CCAAGGAATTGGCGGGGGGGCCGCTCAAGCGGGGAACATGTGG
TTTATTCCGAGCACGCGAGAACCTTACCAGA
CATGATTCCCGTGACCACTATAGAGATTGGTTCCCCTTCGAGCC
ACGTTATAGTAGAGCATGTTAGCGTCAGCTCA
GTTCCTCAGATGTTGGTCTTACCTCCGCACGAGGCTTACACTCGA
CCTACGTGCCATCATCAGGTGGTCAGCTTAG
GTACTGCCCGATTATGTAGCAGAATGTACAGTC

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 pollutants from waters contaminated with pollutants that are not easily biodegradable, such as metals and dyes. Benefits of biosorption methods comprise: low cost; high efficiency; minimization of chemical and biological sludge; selectivity to specific metals; no additional nutrient requirement; regeneration of the bio sorbent; and the likelihood of metal recover.

Biosorption would offer an economically feasible technology

for efficient removal and recovery of metal(s) from aqueous solution

V. ACKNOWLEDGEMENTS

We thank the University of Nigeria Nsukka for giving us the opportunity to carry out the research and we also appreciate Rev. Fr. Prof. Vincent Nyoyoko, for funding the research.

VI. CONFLICT OF INTERESTS

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

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