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[1]Department of Microbiology, Faculty of Science, Sokoto State University, Nigeria.
[2]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria.
[3]Department of Chemistry, Faculty of Physical Science, Ahmadu Bello University, Zaria, Nigeria.

***Corresponding author's email:**
h_hauwau@yahoo.com

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Biosorption of Heavy Metals (Chromium and Copper) using *Bacillus Firmus* isolated from contaminated gold mining soil of Anka in Zamfara State, Nigeria

Hauwa Hussain[1], Clement M. Z. Whong[2], Saleh A. Ado[2], Nuhu A. Abdulmumin[3]

Soil pollution is among the major global concerns of environmental contamination; the release of contaminants into the environment by human activities has increased over the past decades. Soil contamination by mining activities has attracted considerable public attention and the magnitude of the problem in the sampling site calls for immediate action. This research investigates the potentials of *Bacillus firmus* for the removal of heavy metals from contaminated mining soils. This bacterial species was isolated from the mining soil and the soil was analyzed for its heavy metal content. The toxic metal of interest for biosorption in this work are copper and chromium and the biosorbent used was *Bacillus firmus*. The biosorption ability was also studied for 21 days and was calculated using Beer- Lambert's law. The bacterial species was tested for its ability to remove heavy metal concentration. It was observed from the result that copper was highly absorbed at day 21 with 98.2% removal and 96% on day 1 and 7 respectively. The least result was observed on day 14 at 78%. Chromium was also removed most at day 21 with 90.9%, 84.9% at day 7 and the least recorded was at day 1 with 39.3%. This study suggest that this approach could be a very important technology for heavy metal bioremediation at cheaper cost.

Keywords: Biosorption, Heavy metals, *Bacillus firmus*, Mining

1. Introduction

Metals play an important role in the metabolic processes of the biota. Some heavy metals are essential and are needed by the organisms as micro nutrients (cobalt, chromium, nickel, iron, manganese and zinc, among others) and are known as trace elements (Bruins *et al.,* 2000). They function in redox processes, in order to stabilize molecules through electrostatic interactions, as catalysts in enzymatic reactions, and regulating the osmotic balance (Nies, 1999 and Hussein *et al*., 2005).

While some heavy metals are required in large quantity as nutrients, they become strongly inhibitory for microorganisms at relatively high concentrations (Atlas and Bartha, 1993).Their toxicity occurs through the displacement of essential metals from their native binding sites or through ligand interactions (Sevgi*et al.*, 2010).

High concentration and accumulation of these heavy metals in soils may cause reduction in soil fertility through their adverse impact on heterogeneous microbial communities inhabiting the soils. Heavy metals are also known to cause toxic effect on both plants and animals *via* food chains (Dutton and Fisher 2011 andTakahash*et al*., 2012). Most heavy metals are hazardous to humans and they include lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), copper (Cu), zinc (Zn), and chromium (Cr). Such metals are found naturally in soil in large amounts and when concentrated in particular area, they present a danger. Arsenic and cadmium can cause cancer, mercury can cause mutations and genetic damage while lead, copper and mercury can cause brain and bone damages.

Anthropogenic activities such as gold mining and processing, petroleum refining, tailings and other industrial activities have been the main sources of heavy metal contamination in the environment (Garbarino*et al.,* 1995; Duruibe*et al*., 2007; Boamponsem*et al.,* 2010 andGirigisu*et al.,* 2012). When ores are processed for gold, poisonous substances such as oxides and sulfides of heavy metals are released into the environment as pollutants (Boamponsem*et al.,* 2010). These heavy metals are among the pollutants that cause severe threats to humans and the environment and have begun to cause concern in most countries, due to the possibilities of their geoaccumulation, bioaccumulation and biomagnifications in ecosystems (Adelakan and Abegunde, 2011).

Several approaches have been attempted, for the removal of heavy metals from contaminated environment. Among the various methods exploited, conventional strategies like filtration, flocculation, adsorption, and ion exchange chemical precipitation, and electrochemical treatments are ineffective especially when metal ion concentration are in aqueous solution lower than 50mg/L. These methods produce large amount of sludge which is treated with great difficulties. Ion exchange, membrane technologies and activated carbon adsorption process are extremely expensive, disruptive and less practical under natural environmental conditions. Biosorption which is a relatively new, inexpensive and socially acceptable technology involves the use of renewable resources like microbes and plants to tackle heavy metal problems and subsequently to restore the lost fertility of soils (Nies. 1999).

The major advantages of biosorption over conventional treatment methods include low cost, high efficiency, minimization of chemical and biological sludge, and regeneration of biosorbent and possibility of metal recovery (Kratchovil, 1998). The process of biosorption is a complicated process. Therefore this study aims at isolating and exploiting bacterial species at the same time determining the potential of the species for biosorption of the heavy metals in the contaminated soil of a mining area.

2. Study Area

Zamfara State is located in North-Western Nigeria, and it is largely inhabited by Hausa and Fulani tribes. It has a total of 14 Local Governments Areas. Zamfara State has an area of 39,762 square km, and it has a population of 3,602,356 according to the 1991 census. The state is bordered in the north by Niger Republic, and in the south by Kaduna State. In the east it is bordered by Katsina State and in the west by Sokoto and Niger States. The climate of Zamfara is warm tropical with temperature rising up to 38^{o}C between March and May. Rainy season is in late May to September while the cold season known as Harmattan lasts from December to February. Agriculture is the most predominant occupation of the people of the State. Abare mining site is located inAnka Local Government of Zamfara State and was used for this study. It lies between 5.45^{o}E to 6.22^{o}E longitude and 11.3^{o} N to 12.21^{o}N.

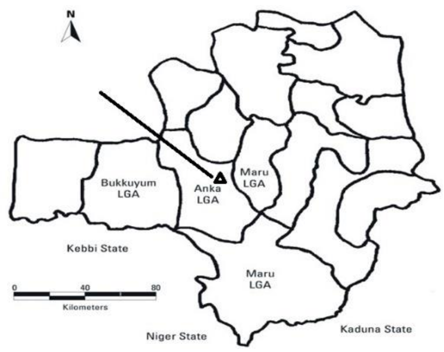

Figure 1. Schematic map showing artisanal gold mining region in Anka Local Government Area, Zamfara State, Nigeria.

3. Materials and Methods

3.1 Sample Collection

Soil sample was collected in a sterile polyethylene bag with the use of soil auger from the surface of the contaminated mining site and was taken straight to the microbiology laboratory of Ahmadu Bello University, Zaria for further evaluation.

3.2 Isolation and characterization of *Bacillus firmus* from the soil sample

The soil samples were homogeneously mixed and then sieved with the use of 2.0mm sieve to remove unwanted soil debris. One (1) gram of the soil was weighed into test tube containing 9mL of sterile distilled water, and agitated for a minute. Serial dilution of the soil was made up to10^{-5} dilutions. Aliquot of 0.1mL of the prepared dilution was aseptically transferred onto the surface of solidified Nutrient agar for the isolation of *Bacillusfirmus*. It was spread well with the use of a sterile bent glass rod. Plates were prepared in duplicates and incubated at 37^{o}C for 18-48 h and were observed for bacterial growth. Different colonies observed were then purified by repeated streaking for each distinct colony on nutrient agar until pure colony was obtained. The purified bacterial isolates were transferred on sterile nutrient agar slants and stored for further identification. Isolates were identified using the identification scheme provided in Bergy's Manual of Determinative Bacteriology (1997), based on staining and biochemical reactions, such as Gram staining, motility, oxidase, catalase, coagulase, MR-VP etc. Microgen biochemical tests kit was used according to manufacturer's instruction to identify the isolates to species level.

3.3 Preparation of stock solutions of the metal

Stock solutions of $CrCl_2$, and $CuSO_4$were prepared by dissolving 0.1g of the heavy metal salts in 1000mL of distilled water. The flasks were shaken vigorously to obtain a clear solution which was sterilized by autoclaving at 121 ^{0}C for 15min.The solutions were stored in refrigerator for further use.

3.4 Assessment of the Biosorption potentials of the isolates

The experiment was carried out in250mL flasks to which 50g of the soil sample was weighed in two different conical flask containing 100mL of sterile nutrient broth, and contaminated with heavy metals(Cr, and Cu)separately having initial concentration of 50ppm. Samples were inoculated with 15 mL of bacterial biomass from exponential phase. A control containing heavy metal without biomass was set up and incubated at room temperature, at pH 7. 15 mL of sample was collected at time interval of 7, 14, and day 21), and was centrifuged at 4000 rpm for 10 min. The remaining concentrations of metals were analyzed by Flame Atomic Absorbance Spectrophotometer AA (Varian). Each experiment was carried out twice and the mean values were recorded. $\left(\frac{C1-C2}{c1}\ x\ 100\right)$

4. Results and Discussion

4.1 Results

The results for the physicochemical and heavy metal analysis of the soil samples are presented in table 1 while the results of biosorption studies on the organism is presented on Figure 1.

Table 1. Physicochemical parameters and heavy metal content of the soil sample from the mining site

Parameter	Value
pH	7.79
Electrical conductivity (µs/cm)	0.0085
Organic matter (%)	1.14
Nitrogen content (%)	0.525
Moisture content (%)	0.39
Phosphorus (ppm)	15.452
Chromium (ppm)	0.302
Gold (ppm)	0.359
Lead (ppm)	42.474
Cadmium (ppm)	-
Zinc (ppm)	0.318
Copper (ppm)	5.498

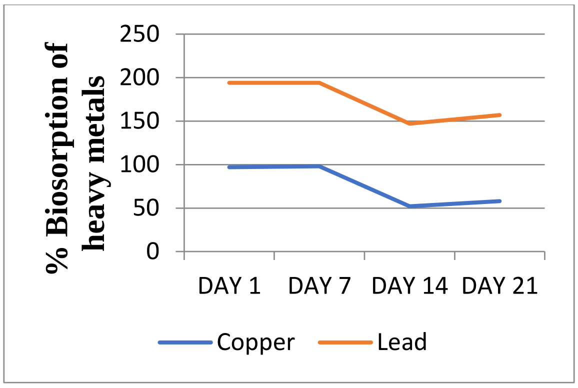

Figure 2. Percentage Biosorption potential of *Bacillus Firmus*

Plate 1. Biosorption of chromium by *Bacillus firmus* after 21 days

Plate 2. Biosorption of Cupper by *Bacillus firmus* after 21 days

4.2 Discussion

Table 1 shows the results of physico-chemical parameters of the soil sample. pH value is an important parameter of soil, which has a great impact on chemical reactions of soils.It plays an important role in complexation, redox-coordination, mineralization reactions and a leading role in precipitation, and desorption of soils. The pH of the soils in this study was slightly basic which suggest neutral to slightly sub-alkaline conditions. Alkaline soils can lead to stunted plants, poor growths and reduced yields, in some crops and pastures, due to its sulfur content. The ideal soil pH for plants ranges from 5-8with optimum of 7.5 for some crops and pastures. This result agrees with the report of Rafiel*et al*. (2010) who reported the pH of Soil in Dashkasan gold mine of Iran to be slightly basic

(7.15 and 7.75) and similar to the findings of Ruqia, Nazir *et al*. (2015) who reported an alkaline soil in the study of soil and water collected from Tanda Dam Kohat Pakistan. And it disagrees with the work of Itumoh*et al*. (2011) who reported a pH of 6.7 in an analysis of soil from a lime stone mining soil in Sokoto Nigeria. And also contrary to the report of Ibrahim *et al*. (2014), who reported a pH of 6.8 in microbial and heavy metal qualities of agricultural soil of Tsafe in Zamfara State, Nigeria. The electrical conductivity of the soil sample is0.0085 µs/cm. This implies that the soils have tendency for high salt that may impact negativity on water and nutrient uptake by crops. The percentage organic matter in the soil is 1.14%,and phosphorus was found in abundant, 15.452%. This is contrary to the report of Wild (1995), and Dutta and Agrawal (2002), which stated that low content of phosphorus in the mining soil may be attributed to inherent soil characteristics such as high calcium content across the study area which could potentially render phosphorus through fixation, and also through vegetation and top soil removal around the mining area(Agboola, 1982 and Salami *et al*., 2002). Heavy metal content of the soil was very minimal with the exception of lead and copper which has 42.474 and 5.498 respectively. This result is almost similar to the report of Majiya *et al.* (2014) who reported a value of 197.07mg/kg at the Bagegaore processing site. But contrary with another report of Majiya *et al.*(2014) who reported a value of 46.09mg/kg at the 10-20m deep of the ore processing site of Bagega in Anka Zamfara State, Nigeria. Cadmium was completely absent in the sample and this agrees with the report of Majiya *et al.* (2014) who reported the same value at 0 – 5cm at the top soil in Bagega ore processing site, 0.035mg/kg, and 0.03mg/kg in 10-15cm and 20 – 30cm at the top soil of the mining site respectively. Gold and chromium were also found in little quantity. The basis for biosorption of metals is the metal binding abilities of various biological materials, which have been proven to be potential metal biosorbents, due to the nature of the cellular components.Several functional groups of the cell wall, including carboxyl, phosphate, amine, and hydroxyl groups. In the biosorption process, bacterial cell wall plays a key role. The cell walls of Gram-positive bacteria naturally carry a negative charge due to the presence of phosphate group and teichoic acid that binds and regulate the movements of cations across the membranes, because of the cell surface of bacteria, it can adsorb appreciable quantities of positively charged cationic metals eg Pb (Parungao*etal*., 2007).

Beer-Lambert's law was used in determining percentage biosorption and the result of this study was consistent with the report of Tarangini andSatpathy (2009). However, the high biosorption potential of *Bacillus firmus*on copper might be as a result of rapid synthesis of enzyme that is responsible for the metabolism of Cu. This also agrees with the work of Onwurh (2001) who reported that Bacteria species from soil can metabolize heavy metals concentration from the soil leading to its degradation. Lovely (1993)reported that *Bacillus Sp* can enzymatically reduce some metals in metabolic processes. The maximum biosorption of copper by *Bacillus firmus* was at day 1and day 7 with 96.37%, and 97.80respectively. Biosorption was decreased by day 14 with 56.80% and increases a little by day 21 with 60.67%.Chromium was also absorbed the most at day 21 with 90.95% and 84.9% at day 7, the least absorbed was at day 1 with only 39.3% removal ability by *Bacillus firmus*. This result agrees with the work of Tarangini (2009)who reported a maximum chromium biosorption around 86% by Bacillus species. Biosorption was slow at the beginning after day1, but there was sudden increase with increasing time possibly due to the availability of binding site on the biomass. In general, the biosorption capacity increase with increasing time. Biosorption of copper was rapid at the initial time with 96%, but this slowly decreases with increase in time. The change in the rate of removal might be due to the fact that initially all sorbent siteswere vacant and also the solute concentration gradient was high. The rate was high at the beginning due to the high affinity of free metal ion binding sites on biosorbents but after 14 days of incubation, the rate of biosorption reached equilibrium at day 21. Zoubolis *et al*. (2004) and Volesky (1990) have also observed that the initial shortest time period of sorption processes is important for a high rate of metal sorption. Similar results have been reported by Gabr*et al*. (2008) that the biosorption of heavy metals depends on the higher heavy metal solution taken for the study.

5. Conclusion

The results of this study have shown that *Bacillus firmus* can be used to remove heavy metal contamination from contaminated environments. The effectiveness of this bacterial species to do this was observed through the biosorption experiment carried out. The maximum percentage removal observed for these metals were 98% and 90% for copper and chromium respectively. Thus, confirming the bioremediation potential of this organism for a cleaner and healthier environment for man.

Conflict of Interest

The authors declare no conflict of interest.

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