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Research Article

Biosorption Potential of Bacteria on Lead and Chromium in Groundwater Obtained from Mining Community

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Abstract

The study was carried out to determine the metal biosorbing ability of lead and chromium-resistant bacteria isolated from groundwater sources collected from Shikira Community, Rafi Local Government Area, Niger State, Nigeria. Total viable counts and total coliform counts were done by the pour plate technique. Physicochemical properties of the water samples were determined using standard methods. Isolates were screened for heavy metal tolerance ability by cultivating on nutrient broth supplemented with 5.50 mg/L lead [$(Pb(NO_3)_2$] and 3.00 mg/L chromium ($K_2Cr_2O_7$) concentrations. *Pseudomonas aeruginosa* and *Micrococcus luteus* that showed high tolerance to the heavy metal salts were selected for biosorption studies which was conducted for a period of 28 days, after which the bacterial cells were separated from solutions by centrifugation and the supernatants were analyzed for residual metals in solution using Atomic Absorption Spectrophotometer (AAS). The effect of pH on the biosorption potential of the bacterial isolates was also determined. The optimum removal efficiency of lead and chromium by *Pseudomonas aeruginosa* was 99.73% and 95.84% at pH 2.70, while the optimum removal efficiency of lead and chromium by *Micrococcus luteus* was 98.21% and 90.13% at pH 4.20 and 4.70 respectively. The present study indicates that *Pseudomonas aeruginosa* and *Micrococcus luteus* removes lead and chromium efficiently from heavy metal-contaminated water, and therefore can be exploited for further research with reference to treatment of water contaminated with heavy metals.

Keywords: Heavy Metals; Biosorption; *Pseudomonas aeruginosa; Micrococcus luteus;* Spectrophotometer

Introduction

Water is indispensable for human existence and it is the second essential factor for life after oxygen [1]. Water that is wholesome and fit for drinking is said to be potable, and water described as "potable" must be free from disease-producing microorganisms and chemical substances that are dangerous to health, which must comply with certain physical, chemical and microbiological standards, which are designed to ensure that the water is potable and safe for drinking [2]. Drinking water could either be obtained from surface or underground sources; among which are streams, lakes, boreholes, rivers, ponds, rain, springs and wells [3]. In Nigeria, majority of the rural populace do not have access to potable water and therefore, depend on wells, streams, boreholes and river water for domestic use, majority of which contain pathogenic organisms or toxic chemicals that pose serious risks to human health [4].

Water pollutants mainly consist of microorganisms, heavy metals, fertilizers and thousands of toxic organic compounds [5]. Groundwater represents an important source of drinking water and its quality is currently threatened by microbiological and chemical contaminants including heavy metals [6]. The public health significance of water quality cannot be over emphasized, as many infectious diseases are transmitted by water through the faecal-oral route [7].

The quality of the atmosphere and water bodies are being threatened by industrial activities such as artisanal mining, which not only affects the productivity of crops, but also threatens the health and life of animals and human beings by way of the food chain [2]. The presence of heavy metals like; iron, copper, chromium, cobalt, manganese, nickel, zinc, lead, arsenic, cadmium and aluminum in high concentrations in groundwater can cause an

adverse effect on human health and make that water not potable [8]. Although some metals are necessary for biological processes, all of them are toxic at high concentrations. This is due to their oxidative capacity to form free radicals and their ability to replace essential metals in enzymes, interrupting their normal activity [6]. Other metals are not essential and accumulate in different organisms and because of this, they are toxic even at low concentrations. Mercury, chromium, lead, arsenic, copper, cadmium, cobalt, zinc, nickel, beryllium, manganese and tin are the most toxic heavy metals according to the United States Environmental Protection Agency [9]. The concentration at which a metal becomes toxic will vary between metals, environments and organisms. However, all of the toxic heavy metals become hazardous at relatively low concentrations. The World Health Organization and the Nigerian Standard for Drinking Water Quality (NSDWQ) stated that the maximum contaminant levels for lead (Pb) and chromium (Cr) in drinking water are 0.01 mgL^{-1} and 0.05 mg L^{-1} respectively [10,11]. Lead and chromium are toxic contaminants, even at very low concentrations. Routes of human exposure to these compounds include ingestion of food and water, inhalation of airborne particulates and contact with numerous manufactured items containing these compounds [12].

Biosorption using microbial biomass as the adsorbent has emerged as an alternative technique to the existing methods (e.g., chemical oxidation, chemical precipitation, ion exchange and filtration) for metal removal [13]. Microorganisms including bacteria, fungi and algae take up metals either actively (bioaccumulation) and/or passively (biosorption) [8]. Numerous microbial species are being researched upon for their ability to biosorb heavy metals especially for Pb and Cr remediation purposes [14] due to their small size, ubiquity, ability to grow under controlled conditions and resilience to a wide range of environmental conditions [15]. In this study, biosorption potential of bacteria on lead and chromium from groundwater sources collected from Shikira Community, Rafi LGA of Niger State, Nigeria was determined. The ability of the isolated bacterial strains towards remediation of these heavy metals was evaluated and effect of pH on the bioremediation process and tolerance to the heavy metals by the isolates was also monitored.

Materials and Methods

Description of study area

The study was conducted in Shikira Community Rafi Local Government Area, Niger State, Nigeria. Shikira community is about 40 kms from Kagara and situated on the eastern flanks of Kagara town, the headquarters of Rafi Local Government Area, Niger State, Nigeria (Figure 1). It is a herculean environment to access because of the difficult terrain. The road that opens up the area to the world is rugged, undulating, rocky and dirty. It snakes from Kagara through Madaka, the only sizeable town in the axis [16]. The people are predominately farmers and cattle rearers. The major sources of water supply in the area is ground water, which is obtained from hand-dug wells and borehole. Most residents in the area use pit latrines and open defecation. Waste disposal is also indiscriminately carried out. This heartland is also the hub of illegal artisanal mining activities in the eastern axis of the local government area. Mining activities have been going on in Shikira community for over eight years. Here and there, there are shallow pits and furrows, where small gold-bearing stones, called quartz, were extracted and then abandoned when they no longer yielded the gem stones. The gold prospectors then moved on to new minefields, which abound in the area (Plate).

Plate: Gold Mining Site in Shikira (Field Photograph).

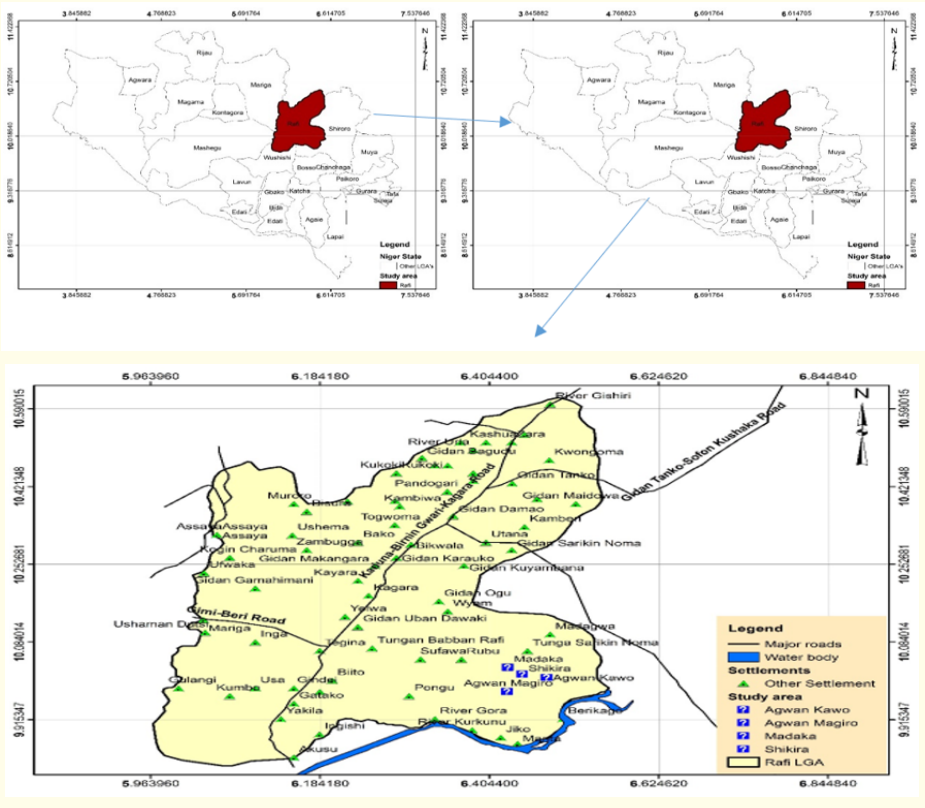


Figure 1: The Study Area (Shikira Community) Rafi LGA, Niger State, Nigeria.
Source: Department of Geography, Federal University of Technology, Minna, Nigeria (2016)

Collection of water samples

Water samples were collected from two sources (well and borehole), which are the major sources of drinking water in the study area. One borehole water sample and one well water sample were collected. The well was chosen based on the frequency of its usage and proximity to heavily populated areas while the borehole was the only source of borehole water in the community. Water samples were collected for both microbiological analysis, physiochemical analysis and biosorption studies.

Sampling methods

The water samples were collected with the use of sterile sample bottles. A strong thread was attached to the neck of a sterile bottle and gently released into the well. The opened bottle was allowed to sink below the water and was pulled up after observing there were no more bubbles from the bottle. The bottle was gently raised out of the well without allowing bottle to touch the sides of the well and the cap was carefully replaced, while the borehole water was first allowed to flow out for 1 minute, then water sample was collected aseptically in a sterile bottle and covered with a screw cap when no air bubbles were seen inside. Both water samples were placed in ice pack to maintain temperature below 10° C and transported to the laboratory for analysis by standard methods as described in the American Public Health Association [17] methods. The preservation method for storage of all water samples was refrigeration.

Bacteriological analysis of the water samples

Bacteriological characteristics were determined as described by Bezuidenhout., *et al*. [18]. Both water samples were analyzed bacteriologically for total coliform count using MacConkey agar and total viable count (total heterotrophic bacteria count) using nutrient agar. The serial dilution method was used for total viable count and total coliform count. The standard plate count technique

was used for isolation of bacterial isolates. Serial dilution (10^{-1} dilution) of water sample was done, and 1 mL each of serially-diluted samples was pipetted into appropriately labeled Petri dishes; freshly prepared media (Nutrient agar and MacConkey agar), allowed to cool were poured aseptically using the pour plate method into the correspondingly labeled petri plates and mixed thoroughly. Plates were allowed to solidify and were incubated at 37°C for 24 hours. Colonies were counted and counts recorded as colony forming units per mL (cfu/mL) of water. Pure cultures were maintained by repeated sub-culturing on fresh NA. The pure cultures were maintained on agar slants for further characterization and identification.

Total heterotrophic bacterial count

Water samples to be analyzed for quantitative bacterial analysis were plated on nutrient agar [17]. The plates were inverted and incubated at 37°C for 24 hours. Discrete colonies, which appeared on the plates at the end of incubation were counted and the results expressed as the number of bacteria (colony forming units) per milliliter of the water sample analyzed.

Total coliform counts

Total coliform count was done using MacConkey agar; for the differentiation of lactose fermenting and non-lactose fermenting enteric bacteria. Water samples were plated on MacConkey agar. The plates were inverted and incubated at 37°C for 24 hours. Discrete colonies which appeared on the plates at the end of incubation period were counted and the results expressed as the number of bacteria (colony forming units) per milliliter of the water sample analyzed [19].

Characterization and identification of bacterial isolates

The bacterial isolates were identified and characterized based on their Gram's reaction, colonial morphology of the isolates was examined and characteristics colonies were identified using microscopic technique and biochemical tests, while the identities of the bacteria isolates were known by comparing their characteristics with those of known taxa using Bergey's Manual of Systematic Bacteriology [20].

Physicochemical analysis of water samples

The physicochemical parameters of the water samples were determined using known standard methods [21]. The parameters analyzed were; pH, turbidity, conductivity, total dissolved solids, total hardness ($CaCO_3$), chlorides, flourides, nitrate (NO_3), phosphate (PO_4) and biochemical oxygen demand (BOD_5).

Determination of metals in water samples

This was carried out using atomic absorption spectrophotometer (AAS) [22]. Fifty milliliters (50 mL) of water sample was measured into a 100 mL beaker using a pipette, and 10 mL of nitric acid was added and beaker and content was placed on hot plate. Digestion was done till white fumes of nitric acid had escaped. Heating was continued until when content had reduced to 10 mL volume. Content was washed into a 50 mL volumetric flask and made to mark with distilled water.The digest was kept for determination of heavy metals (Cd, Cr, Pb, Zn and Hg) using Atomic Absorption Spectrophotometer (AAS 500 WIN) [23].

Screening of bacteria for heavy metal tolerance

Two heavy metal salts were used for the screening of all bacterial isolates viz. Lead nitrate, [$(Pb(NO_3)_2$] and potassium dichromate ($K_2Cr_2O_7$). Concentration of heavy metal solutions higher than initial concentrations obtained from water samples were prepared for the screening [24], using the broth dilution method [25].

Broth method

Heavy metal tolerance capacity of the bacterial isolates was determined as described by Konopka and Zakharova [25]. Aqueous solutions of the metal salts; lead nitrate [$(Pb(NO_3)_2$] and potassium dichromate ($K_2Cr_2O_7$) were prepared separately in de-ionized water and left to stand for 24 hours for complete dissolution. Salts were prepared at a concentrations higher than the initial concentrations of the heavy metals in the water samples being analysed. Nutrient broth (NB) was prepared for screening of bacteria isolates. To each of the broth tubes, appropriate metal salt solutions were added. Media were sterilized by autoclaving at 121°C for 15 minutes, then allowed to cool. One milliliter (1 mL) of respective standardized isolates' inoculum was then added to broth tubes and incubated immediately at 37°C for 48 hours. Controls which consisted of a metal-supplemented broth medium without the microorganisms were also incubated. The culture turbidity was observed for as an indication of bacterial growth against the controls, this was measured at 540 nm using a spectrophotometer. Growth was taken as the indication of heavy metal tolerance according to Johncy-Rani., *et al.* [26]. Two bacteria isolates that recorded the highest turbidity were then selected for biosorption studies.

Biosorption experiment

Biosorption experiment was conducted using nutrient broth, following the procedure of Abioye., *et al* [27]. Nutrient broth was prepared using water samples under analyses containing known concentrations of the heavy metals; Pb and Cr. One hundred millilitres (100 mL) of nutrient broth (NB) containing different water samples were dispensed in 250 mL Erlenmeyer flasks. Flasks were cotton plugged and covered with aluminium foil and sterilized at 121°C at 15 psi pressure for 20 minutes and allowed to cool to 40°C Two millilitres (2 mL) of 24 hours standardized isolates' inoculum of *Pseudomonas aeruginosa* was inoculated into 100 mL of nutrient broth (procedure was done for the other microorganism). Zero point five (0.5) McFarland standard was used for the standardization of inoculum. These experimental flasks with cultures were incubated at 37°C. All cultures were incubated in a rotary shaker for 28 days. Samples were drawn on the 7th, 14th, 21st and 28th day and the supernatant and residue were separated by centrifugation at 4000 rpm for 5 minutes. The biomass (residue) and the broth (supernatant) were digested separately with aqua regia (HCL: HNO_3 at 3:1 ratio). Lead and chromium concentrations in the supernatant were analyzed using Atomic Absorption Spectrophotometer (AAS) to determine the quantity of residual metals [17]. Controls without microorganisms were set up on each of the water samples using NB, and incubation was done at 37°C Experiment was performed in triplicates and average values was used in the results.

The sorption efficiency (%) (E) was calculated using the following equation [28]:

$$E = \left(\frac{c_i - c_f}{c_i}\right) \times 100$$

Where: C_i = initial concentration of the metallic ions (mg/L); C_f = final concentrate ion of metallic ions (mg/L).

Effect of pH on biosorption of Heavy metals

The metal sorption was monitored for pH range 1 to 9. 0.1 M of NaOH or 0.5M of HCl was used as pH regulators. Two milliliters (2 mL) of inoculum was inoculated in 100 mL each of the samples containing different concentrations of lead and chromium. All the Erlenmeyer flasks were maintained at different pH values ranging from 1 to 9 for 28 days in an incubator at 37°C. Solutions were centrifuged as above and supernatant was analyzed for the residual concentrations of the metal ions [27].

Statistical analysis

Data were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± SEM. Comparisons was between different groups was done using two-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $P<0.05$ were considered as statistically significant as described by Mahajan [29].

Results and Discussion

Microbial counts of the water samples analyzed

The results of the bacteriological analyses of water samples collected from borehole and well in Shikira Community in Rafi Local Government Area, Niger State, Nigeria is presented in Table 1. The mean total viable counts (TVC) of the water samples ranged from 6.33 ± 0.33 cfu/mL to 56.00 ± 1.73x10^1 cfu/mL. It was, however, observed that TVC in the samples were significantly different ($P<0.05$). Borehole water sample recorded a mean growth of 6.33 ± 0.33 cfu/mL, which is within the standard limit of total viable count for drinking water [11,22] while the well water sample analyzed recorded TVC counts above the limit of 1.0x10^2 cfu/mL, which is the standard limit of total viable count for drinking water (NSDWQ, 2007). The mean of total coliform counts (TCC) in the water samples ranged from 1.66 ± 0.33 cfu/mL to 14.33 ± 1.76 cfu/mL, with borehole water sample recording the least growth while well water sample had the highest total coliform counts, and there was however a significant difference ($P<0.05$) between the water samples analyzed. The TCC in the water samples analyzed exceeded the standard limits of 10 TCC per 100 mL set by the Nigerian Standard of Drinking Water Quality [11] and zero TCC per 100 mL of water set by the World Health Organization [22], and therefore not safe for drinking purposes.

Sample	TVC (cfu/mLx10^1)	TCC (cfu/mLx10^1)
Borehole water	6.33 ± 0.33^b	1.66 ± 0.33^b
Well water	56.00 ± 1.73^b	14.33 ± 1.7^{6}b
WHO STANDARD	1.0x10^2	0.00

Table 1: Bacterial counts of water samples analyzed.

Values are mean ± SEM of triplicate determinations. Values with different superscripts along a column are significantly ($p< 0.05$) different.

KEY: TVC = Total Viable Count, TCC = Total Coliform Count, cfu/mL = colony forming units per milliliter.

The bacteriological analysis of water determines the potability of water, and TVC and TCC have been used extensively as a basis for regulating the microbial quality of drinking water. All water samples analyzed exceeded the permissible limits of $1.0x10^2$ cfu/mL by WHO [22], and unfit for drinking purposes. The high TVC is an indication of the presence of high organic and dissolved salts in the water. Similar results have been reported by Agwu., *et al.* [30] for microbial analysis of drinking water sources in Aba metropolis, Abia State, Nigeria as well as Adegoke., *et al.* [31] on the bacteriological assessment of borehole water in Oyigbo town, Rivers State, Nigeria. The primary sources of these bacteria in water are animal and human wastes. These sources of bacterial contamination include surface runoff, pasture and other land areas where animal wastes are deposited.

The results of TCC obtained in this study are similar to that of Bello., *et al.* [32] who in their study on bacteriological and physicochemical analyses of borehole and well water sources in Ijebu-Ode, Southwestern Nigeria, reported the presence of total coliform outside the range allowed by WHO in over eighty percent of their samples. The presence of coliforms in the well water samples also suggests possible sources of contamination, which is potentially tied to livestock and human faeces that created a diffuse source of faecal contamination to water sources. This result also compares favourably with the report of Banwo [33], which indicates that the presence of bushes and shrubs makes it a likely possibility that smaller mammals may have been coming around these water bodies to drink water, thereby passing out faeces into the water. Well water contamination could also be due to poor hygiene (e.g., indiscriminate defecation) and sanitation practices that include laundry activities close to the well by households; and water sources being very near or down slope of latrines.

Occurrence of bacterial isolates in water samples

The bacterial isolates from borehole and well in this study were identified as species of *Escherichia, Salmonella, Streptococcus, Klebsiella, Bacillus, Micrococcus, Pseudomonas* and *Staphylococcus.* The biochemical characteristics of the bacterial isolates are presented in Table 2. These organisms have been isolated from drinking water sources by other investigators [2,3,30,32,34].

The isolation of potential pathogenic microorganisms such as *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli, Staphylococcus aureus, Micrococcus luteus, Streptococcus feacalis, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* is of high importance as it indicated that the drinking water analyzed is highly contaminated and unsafe for drinking. These are pathogenic organisms mainly of fecal origin. Any water source used for drinking or cleaning purpose should not contain any organism of feacal origin [2]. Although, presence of these bacteria in water may be unnoticed even in clear water, but may pose a potential risk to consumers, as the consumption of such contaminated water facilitates the widespread of infections which can ultimately lead to outbreak of an epidemic [3].

Physicochemical parameters of the water samples

Table 3 shows the results of physicochemical analysis of water samples in Shikira Community. Conductivity, total hardness, total dissolved solid, phosphate, chloride, flouride and nitrate in both water samples were within the acceptable limit prescribed by Nigeria Standard for Drinking Water Quality and World Health Organization [11,22]. Borehole water sample recorded turbidity value of 3.62 NTU, which is within the acceptable limit, while well water samples analyzed had a value of 10.84 NTU above limit (5.0 NTU) as prescribed by NSDWQ [11] and WHO [22]. The pH of the borehole water sample was within acceptable limit prescribed by NSDWQ [11] and WHO [22], while the well water sample recorded pH value of 6.39 slightly below limits prescribed by NSDWQ [11] and WHO [22]. Borehole water sample recorded a BOD_5 value of 3.00 mg/L, which is within the acceptable limit prescribed by WHO [22] while well water sample analyzed recorded BOD_5 value of 16.00 mg/L, above limit prescribed by WHO [22].

Turbidity represents an important aspect of water quality, as it is a measure of the clarity or cloudiness of water as a result of particulate matter being suspended within it [35]. The high turbidity observed in the well water sample could be as a result of contamination from soil runoff into the well water, thereby increasing the turbidity and cloudiness of the water as a result of particulate matters such as clay, silt and finely divided organic matter, being suspended within it [34]. These colloidal materials interfere with effective chlorination/disinfection, shield bacteria and provide adsorption sites for the bacteria that may cause undesirable tastes or odours in water samples [3]. The results obtained from this study agrees with the findings of Yasin., *et al.* [34] who worked on the physicochemical and bacteriological quality of drinking water of different sources in Jimma zone of Southwest Ethiopia, and reported high turbidity in the well water samples, stating therefore, that consumption of these highly turbid waters might constitute a health risk as excessive turbidity could protect pathogenic microorganisms from the effect of disinfectants and also stimulate the growth of microorganisms.

Gra's Reaction	Shape	Citrate	Catalase	Methyl Red	Voges Proskauer	Coagulase	Indole	MSA	Starch Hydrolysis	Oxidase	Slope	Butt	H_2S	SSA	Haemolysis	Urease	Bacteria
-	R	-	+	+	-	-	+	-	-	-	Y	+	-	-	γ	-	*Escherichia coli*
-	R	-	+	-	+	-	-	-	-	-	Y	+	+	+	γ	-	*Salmonella typhi*
+	C	-	-	-	-	-	-	-	-	-	Y	-	-	-	α	-	*Streptococcus pneumoniae*
+	C	+	-	-	-	-	+	-	-	-	R	+	-	-	α	-	*Streptococcus feacalis*
-	R	+	+	-	+	-	-	-	-	-	R	-	-	-	γ	+	*Klebsiella pneumoniae*
+	R	+	+	-	-	-	-	-	+	-	R	-	-	-	β	-	*Bacillus cereus*
+	R	+	+	-	-	-	-	-	-	-	R	-	-	-	β	-	*Bacillus subtilis*
+	C	-	+	-	-	-	-	-	-	-	R	-	-	-	γ	-	*Micrococcus luteus*
-	R	+	+	+	-	-	-	-	-	+	R	-	-	-	γ	-	*Pseudomonas aeruginosa*
+	C	+	+	-	-	+	-	+	-	-	R	-	-	-	γ	-	*Staphylococcus aureus*
+	C	-	+	-	-	-	-	+	-	-	R	-	-	-	γ	-	*Staphylococcus epidermidis*

Table 2: Morphological and Biochemical Characteristics of Bacterial Isolates in Borehole and Well from Shikira Community.

KEY; R= Rod, C= Cocci, - = Negative, + = Positive, MSA= Mannitol Salt Agar, H_2S= Hydrogen sulphide Production, SSA= Salmonella-Shigella Agar, Y= Yellow, R = Red.

Parameters	Water Samples		WHO Standard (mg/L)
	Borehole	Well	
Turbidity (NTU)	3.62 ± 0.02^a	10.84 ± 0.02^b	5
pH	6.54 ± 0.027^b	6.39 ± 0.03^a	6.5-8.50
BOD_5 (mg/L)	3.00 ± 0.025^a	16.00 ± 0.02^b	10
Fluoride (mg/L)	0.47 ± 0.01^a	1.45 ± 0.02^b	1.5
Phosphate (mg/L)	0.25 ± 0.024^b	0.22 ± 0.025^a	5
TDS (mg/L)	78.42 ± 1.27^a	80.71 ± 0.98^b	1000
Nitrates (mg/L)	14.55 ± 1.03^a	16.38 ± 1.10^b	50
Conductivity(µS/cm)	117.38 ± 0.03^b	114.70 ± 0.025^a	1000
T/hardness (mg/L)	27.91 ± 0.03^b	27.46 ± 0.27^a	200
Chlorides (mg/L)	0.95 ± 0.021^a	1.27 ± 0.03^b	250

Table 3: Physicochemical Parameters of Water Samples Collected from Shikira Community

Values are mean ± SEM of triplicate determinations. Values with different alphabets along a row are significantly ($p< 0.05$) different.

Key: Nephelometric Turbidity Unit (NTU), µS/cm = micro-Siemens per centimeter, mg/L = Milligram per litre, TDS = Total Dissolved Solids, BOD = Biochemical Oxygen Demand, T/hardness = Total hardness.

Hydrogen-ion concentration (pH) is an important parameter in evaluating the acid-base balance of water, it is the indicator of acidic or alkaline condition of water [36]. A sample is considered to be acidic if the pH is below 7.0. Meanwhile, it is alkaline if the pH is higher than 7.0 [37]. All samples had pH close to neutrality, which will allow the growth of most bacterial species. Bello., *et al.* [32] obtained similar pH range of 6.4 to 7.4 for borehole water samples and 6.3 to 7.5 for well water samples, stating that pH range is close to neutrality and would allow the growth of most bacterial species and consequently affect the bacterial counts. This slightly acidic trend could also be due to the geology of the area because the pH of water is generally influenced by the geology of the catchment area and buffering capacity of water, as reported by Agwu., *et al.* [30], who studied The assessment of drinking water sources in Aba metropolis, Abia State, Nigeria, and obtained similar acidic pH results which ranged between 6.29-6.45.

Biochemical oxygen demand (BOD_5) is used to measure oxygen used and equate it to the amount of organic matter within the water sample. BOD_5 measures the amount of oxygen used by microorganisms, in this case bacteria, to oxidize organic matter present within the water sample [23]. Water with BOD_5 levels < 4 mg/L are deemed as clean while those >10 mg/L are considered polluted and unsafe [22]. Borehole water sample recorded a BOD_5 level of 3.00 mg/L. This means that it is a clean water sample while well water sample analyzed, recorded BOD_5 levels >10 mg/L. This suggests that drinking water source was polluted by organic matter. This may be due to the mixing of organic matter with the waterways. High values of BOD_5 recorded in this study is similar to the values recorded from well water samples by Pratap-Chandran., *et al.* [6] who recorded BOD levels ranging from 4-12 mg/L, while assessing the physical and bacteriological quality of well water samples from Kanakkary Panchayath, Kottayam District, Kerala State, India, stating that water bodies receiving wastewater may have BOD values up to 10 mg/L or more, particularly near points of discharge.

Heavy metal contents of the water samples

The results of the concentrations of heavy metals analyzed in the water samples are presented in Figure 2. The levels of lead, cadmium, chromium and mercury were higher in both water samples analyzed, above the WHO permissible limit in drinking water, while the concentration of zinc in all the samples was within the recommended permissible limit.

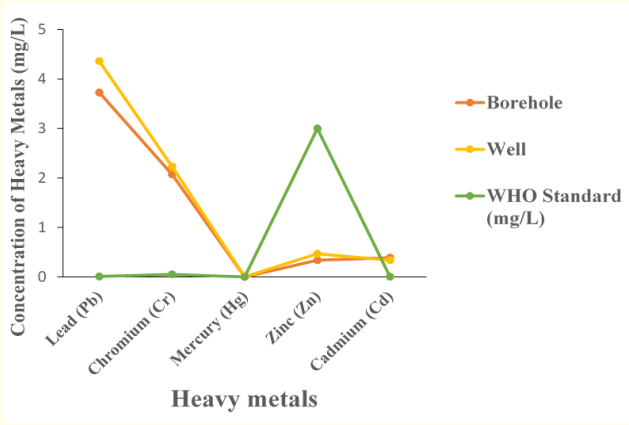


Figure 2: Heavy metal concentrations in the water samples analysed.

Water samples analyzed showed severe contamination by lead (Pb), cadmium (Cd), chromium (Cr) and mercury (Hg), exceeding WHO maximum permissible limits of 0.01, 0.003, 0.05 and 0.001 mg/L in drinking water respectively [22]. The high contamination levels of these metals could be attributed to the artisanal gold mining activities taking place in Shikira community, and due to the proximity of the water samples to active artisanal gold mining activities in the study area, leading to the formation of acid mine drainage. Lead in the compound form is soluble in water, and when it dissolves, it disperses into surrounding water bodies like well water as reported by Oladipo., *et al.* [38] who recorded high levels of lead in their study on heavy metal contaminations of drinking water sources due to illegal gold mining activities in Zamfara State, Nigeria.

High lead concentration in drinking water may result in lead poisoning that manifests in symptoms such as tiredness, lassitude, slight abdominal discomfort, irritation and anemia. Lead is bioaccumulated in humans and can be transferred from mother to child during pregnancy. It has been reported to be a probable human carcinogen, damaging the nervous system causing brain disorder [39]. Excessive lead also causes blood disorders in mammals. In comparison with a study by Bakare-Odunola [40] where a concentration of 0.1 mg/L had resulted in the development of neurological problems in fetuses and children.

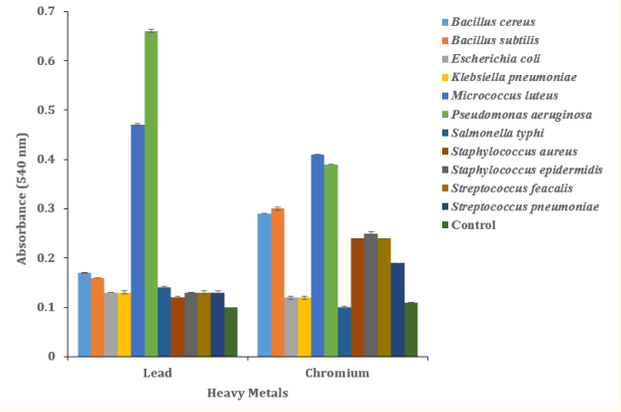
Geologic deposits of cadmium can also serve as sources to groundwater and surface water, especially when in contact with soft and acidic waters. This is similar to the findings of Oladipo., *et al*. [38] who also recorded high cadmium levels of 0.44-1.76 mg/L, in all water samples in a study conducted on heavy metal contaminations of drinking water sources due to illegal gold mining activities in Zamfara State, Nigeria. The use of herbicides, pesticides and artificial fertilizers could further increase the crude content of chromium, lead and cadmium in the soil while erosion would take off the top soil leading to mineral dissolution into water bodies. Chromium in excess is toxic thus leading to liver and kidney damage, internal hemorrhage, and respiratory disorders, as well as causing cancer in humans and animals through inhalation exposure [41].

The high concentration of mercury in water samples might be due to the use of the chemical, mercury by artisanal miners in the extraction of gold from ore during ore-processing of gold at the processing site. This is similar to the findings of Nuhu., *et al*. [42], who worked on heavy metal pollution: The environmental impact of artisanal gold mining on Bagega village of Zamfara State, Nigeria, and recorded 0.0010- 0.0007 mg/L mercury levels. The mercury used in gold mining gets into the soil, and through the process of erosion can make its way into wells, lakes and streams. This in turn can pollute local water supplies [38].

Heavy metal tolerance by bacterial isolates

The results of the screening of bacterial isolates for heavy metal tolerance is presented in Figure 3. *Pseudomonas aeruginosa* recorded the highest growth rate on nutrient broth supplemented with lead nitrate [$(Pb(NO_3)_2$], having recorded a mean absorbance of 0.66 ± 0.003, followed by *Micrococcus luteus* with a mean absorbance of 0.47 ± 0.003, while *Staphylococcus aureus* recorded the least growth with a mean absorbance of 0.12 ± 0.003. *Micrococcus luteus* recorded the highest mean absorbance for nutrient broth supplemented with potassium dichromate ($K_2Cr_2O_7$) and inoculated with bacterial isolates with a mean absorbance of 0.41 ± 0.000, *Pseudomonas aeruginosa* recorded a mean absorbance of 0.39 ± 0.000, while *Salmonella typhi* recorded the least with a mean absorbance of 0.10 ± 0.003.

The bacterial isolates when grown in media amended with varying concentrations of different heavy metals, showed a variable tolerance level to the two tested metal salts (lead nitrate and potassium dichromate). Due to their high level of metal tolerance (high growth rate) in the two metal salts, *Pseudomonas aeruginosa* and *Micrococcus luteus* were selected for biosorption studies, as they were identified as efficient organisms that were resistant to Pb and Cr respectively. Growth of the bacteria in nutrient broth medium supplemented with lead and chromium salts indicated the biosorption capacity of these organisms [27]. The result of this findings is similar to the findings of Johncy-Rani., *et al*. [26] who identified *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. as efficient strains that were resistant to Cu, Cd and Pb respectively having recorded absorbance that ranged between 0.44-0.69, in their study on "Comparative assessment of heavy metal removal by immobilized and dead bacterial cells: A biosorption approach". The identified efficient organisms were therefore selected for biosorption studies.



Each bar represents Mean ± SEM of triplicate determinations.

Figure 3: Tolerance of bacterial isolates to heavy metals.

Biosorption of Pb and Cr by bacterial species

Tables 4 to 7 shows the accumulation of the heavy metals (Pb and Cr) by the selected bacteria in both well and borehole water samples from Shikira Community, while Figures 4 to 5 shows the removal efficiency (%) of Pb and Cr by the bacterial isolates. In the present study, *Pseudomonas aeruginosa* and *Micrococcus luteus* were the bacterial isolates used as the biosorbents for the adsorption of Pb and Cr respectively.

The initial concentration of Pb in the water samples had a mean value ranging from 3.73 ± 0.11 mg/L to 4.36 ± 0.11 mg/L, which was reduced to a mean concentration value which ranged from 0.01 ± 0.00 mg/L to 1.24 ± 0.005 mg/L after 28 days of *Pseudomonas aeruginosa* inoculation (Table 4), indicating biosorption with a percentage mean range value of 71.56 ± 1.30% to 99.73 ± 1.43% (Figure 4), while *Micrococcus luteus* reduced Pb to a mean concentration value which ranged from 0.06 ± 0.00 mg/L to 1.17 ± 0.02 mg/L after 28 days of inoculation (Table 5), indicating biosorption with a percentage mean range value of 73.16 ± 1.98% to 98.21 ± 1.05% (Figure 4).

Sample	Time (Days) (mg/L)				
	0	7	14	21	28
Borehole	3.73 ± 0.11^{a}	3.60 ± 0.99^{b}	2.56 ± 0.01^{b}	2.05 ± 0.01^{b}	0.01 ± 0.00^{a}
Well	4.36 ± 0.11^{b}	2.94 ± 0.01^{a}	2.34 ± 0.01^{a}	1.96 ± 0.01^{a}	1.24 ± 0.005^{b}
Control (borehole)	3.73 ± 0.11^{a}	3.71 ± 0.006^{c}	3.68 ± 0.017^{c}	3.67 ± 0.003^{c}	3.65 ± 0.012^{c}
Control (well)	4.36 ± 0.11^{b}	4.36 ± 0.023^{d}	4.35 ± 0.012^{d}	4.35 ± 0.017^{d}	4.34 ± 0.006^{d}

Table 4: Biosorption of lead by *Pseudomonas aeruginosa*.

Values are mean ± SEM of triplicate determinations. Values with different superscripts along a column are significantly ($p < 0.05$) different.

Sample	Time (Days) (mg/L)				
	0	7	14	21	28
Borehole	3.73 ± 0.11^{a}	3.44 ± 0.01^{b}	2.19 ± 0.01^{a}	1.09 ± 0.01^{a}	0.06 ± 0.00^{a}
Well	4.36 ± 0.11^{b}	3.34 ± 0.01^{a}	2.22 ± 0.00^{b}	1.72 ± 0.01^{b}	1.17 ± 0.02^{b}
Control (borehole)	3.73 ± 0.11^{a}	3.71 ± 0.006^{c}	3.68 ± 0.017^{c}	3.67 ± 0.003^{c}	3.65 ± 0.012^{c}
Control (well)	4.36 ± 0.11^{b}	4.36 ± 0.023^{d}	4.35 ± 0.012^{d}	4.35 ± 0.017^{d}	4.34 ± 0.006^{d}

Table 5: Biosorption of lead by *Micrococcus luteus*

Values are mean ± SEM of triplicate determinations. Values with different superscripts along a column are significantly ($p < 0.05$) different.

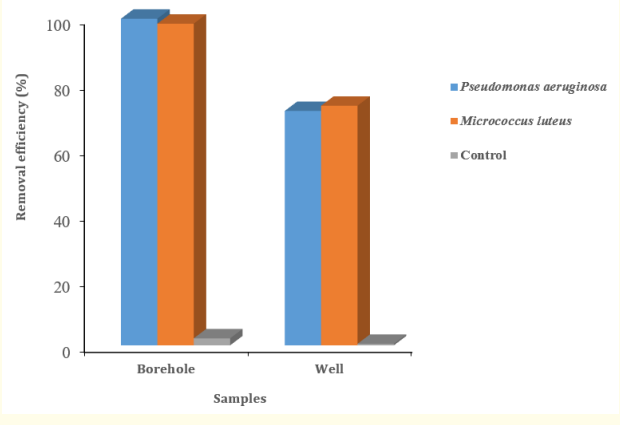


Figure 4: Removal efficiency of the bacterial isolates on lead.

The initial concentration of Cr in the water samples had a mean value which ranged from 2.07 ± 0.01 mg/L to 2.23 ± 0.01 mg/L, which was reduced to a mean concentration value which ranged from 0.10 ± 0.00 mg/L to 0.18 ± 0.00 mg/L after 28 days of *Pseudomonas aeruginosa* inoculation (Table 6), indicating biosorption with a percentage mean range value of 91.92 ± 0.68% to 95.84 ± 0.52% (Figure 5), while *Micrococcus luteus* reduced Cr to a mean concentration value which ranged from 0.22 ± 0.01 mg/L to 0.44 ± 0.01 mg/L after 28 days of inoculation (Table 7), indicating biosorption with a percentage mean which ranged from 78.67 ± 0.53% to 90.13 ± 0.72% (Figure 5).

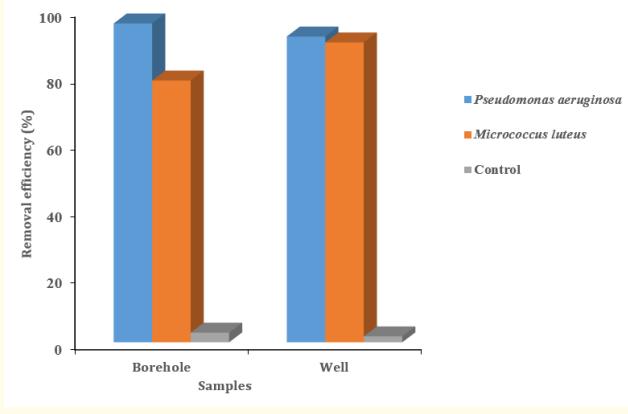


Figure 5: Removal efficiency of the bacterial isolates on chromium.

Sample	Time (Days) (mg/L)				
	0	7	14	21	28
Borehole	2.07 ± 0.01[a]	2.05 ± 0.01[a]	1.34 ± 0.01[a]	1.17 ± 0.00[a]	0.10 ± 0.00[a]
Well	2.23 ± 0.01[b]	2.20 ± 0.01[b]	1.80 ± 0.00[b]	1.20 ± 0.02[b]	0.18 ± 0.00[b]
Control (borehole)	2.07 ± 0.01[a]	2.07 ± 0.006[a]	2.06 ± 0.012[c]	2.05 ± 0.012[c]	2.01 ± 0.012[c]
Control (well)	2.23 ± 0.01[b]	2.23 ± 0.023[b]	2.22 ± 0.012[d]	2.20 ± 0.17[d]	2.19 ± 0.006[d]

Table 6: Biosorption of chromium by *Pseudomonas aeruginosa*.

Values are mean ± SEM of triplicate determinations. Values with different superscripts along a column are significantly ($p < 0.05$) different.

Sample	Time (Days) (mg/L)				
	0	7	14	21	28
Borehole	2.07 ± 0.01[a]	2.03 ± 0.00[a]	1.35 ± 0.01[a]	1.19 ± 0.01[a]	0.44 ± 0.01[b]
Well	2.23 ± 0.01[b]	2.22 ± 0.01[c]	1.51 ± 0.01[b]	1.25 ± 0.01[b]	0.22 ± 0.01[a]
Control (borehole)	2.07 ± 0.01[a]	2.07 ± 0.006[b]	2.06 ± 0.012[c]	2.05 ± 0.012[c]	2.01 ± 0.012[c]
Control (well)	2.23 ± 0.01[b]	2.23 ± 0.023[c]	2.22 ± 0.012[d]	2.20 ± 0.17[d]	2.19 ± 0.006[d]

Table 7: Biosorption of chromium by *Micrococcus luteus.*

Values are mean ± SEM of triplicate determinations. Values with different superscripts along a column are significantly ($p < 0.05$) different.

The results of biosorption studies revealed that the two bacterial isolates had the capability of accumulating these heavy metals (Pb and Cr). The primary goal of metal remediation is to remove the metals from the solution or to decrease metal mobility and toxicity within the sample. It is well recognized that microorganisms have affinity for metals and can accumulate heavy and toxic metals by a variety of mechanisms. Several principal sites of metal-complex formation in biological systems have been. These include accumulation in the cell wall, carbohydrate or protein polyphosphate complexes, and complexion with carboxyl groups of the peptidoglycan in the cell wall or entering into cells [43].

Pseudomonas aeruginosa and *Micrococcus luteus* were considered to be effective biosorbents because of their high adsorption capacity for the heavy metals. These organisms have earlier been reported to be involved in biosorption of heavy metals. Abioye., *et al*. [27] in a study on biosorption of chromium by *Bacillus subtilis* and *Pseudomonas aeruginosa* isolated from waste dump site, recorded an optimum chromium biosorption of 88.70% by *Pseudomonas aeruginosa*. Sethuraman and Balasubramanian [44] also noted maximum removal percentage of 78% for Cr (VI) by *Pseudomonas aeruginosa*. Zaied., *et al*. [45] also recorded 79.22% adsorption of Pb by *Micrococcus* sp. The differences in the percentage biosorption values between bacterial species in metal ion binding in this study, could be due to the properties of the metal sorbates or the properties of the microorganisms like functional groups, structure and surface [27].

The high percentage sorption uptake of the three metals by *Micrococcus luteus*, could also be due to the characteristic component of the Gram positive cell, such as teichoic acids associated with the cell wall, whose phosphate groups are key components for the uptake of metals [46]. Carboxyl groups are the main agents in the uptake of heavy metals.

Effect of pH on biosorption of heavy metals by bacterial isolates

The water samples inoculated with *Pseudomonas aeruginosa* had an initial pH with a mean range of 6.39 ± 0.50 to 6.54 ± 0.05 which gradually decreased until it got to pH with a mean range of 2.70 ± 0.05 to 4.34 ± 0.05 (on Day 28). The effect of pH on percentage biosorption of lead and chromium by *Pseudomonas aeruginosa* is presented in Figures 6 and 7. The optimum removal efficiency of lead and chromium by *Pseudomonas aeruginosa* was 99.73% and 95.84% at pH 2.70.

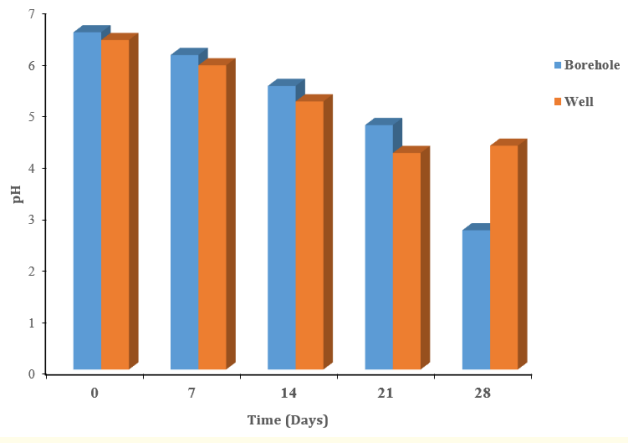


Figure 6: Effect of pH on biosorption of lead by *Pseudomonas aeruginosa.*

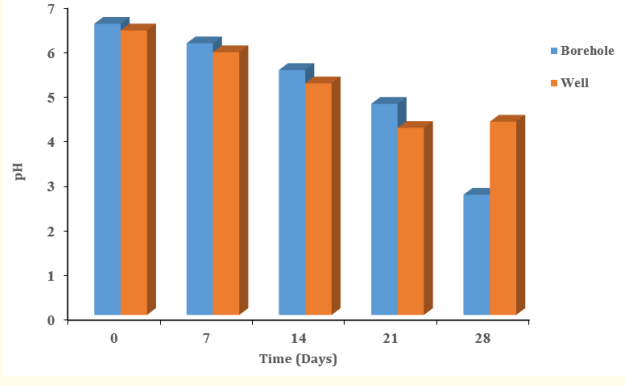


Figure 7: Effect of pH on biosorption of chromium by *Pseudomonas aeruginosa.*

The water samples inoculated with *Micrococcus luteus* had an initial pH with a mean range of 6.39 ± 0.50 to 6.54 ± 0.05 which gradually decreased until it got to pH with a mean range of 4.20 ± 0.05 to 4.73 ± 0.33 (on Day 28). The effect of pH on percentage biosorption of lead and chromium by *Micrococcus luteus* is presented in Figures 8 and 9 respectively. The optimum removal efficiency of lead and chromium by *Micrococcus luteus* was 98.21% and 90.13% at pH 4.20 and 4.70 respectively.

The percentage of biosorption of the heavy metals (Pb and Cr) varied greatly along the given pH range 1-9. The most important single parameter influencing the biosorption process is the pH of the adsorption medium [45]. The charge of the adsorbate and the adsorbent often depends on the pH of the solution. As the concentrations of the metal ions (Pb and Cr) in the water samples were decreasing, the pH of the solution mixture for the bacterial isolates was also decreasing. *Pseudomonas aeruginosa* recorded optimum sorption of Pb and Cr at pH 2.70, while *Micrococcus luteus* recorded optimum sorption of Pb and Cr at pH 4.20 and 4.70 respectively. This is similar to the results of Abioye., *et al*. [27] who recorded an optimum chromium removal at pH 4.0 by *Pseudomonas aeruginosa*. The decrease of pH resulted in an increased negative charge on the surface of the cell, which favoured electrochemical attraction and adsorption of metals [26,27]. The carboxylate and phosphate groups carry negative charges, hence it cannot bind effectively to a negatively charged functional group. However, as the pH decreases, the overall surface charge of cell surface will become positive, and the surface of sorbent would also be surrounded by the hydronium ions, which could enhance the metal ions interaction with binding sites of the biopolymers due to increased attractive forces [47].

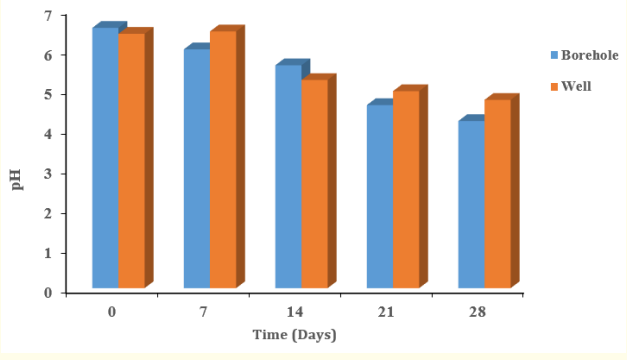


Figure 8: Effect of pH on biosorption of lead by Micrococcus luteus.

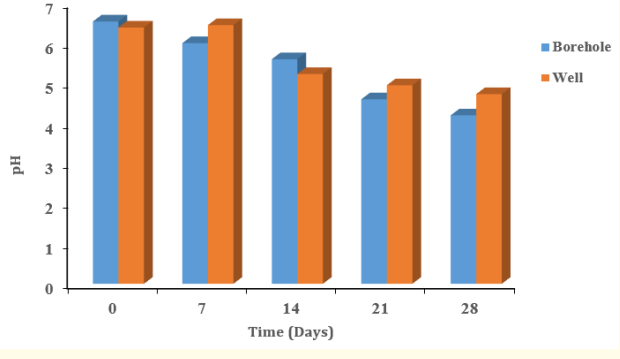


Figure 9: Effect of pH on biosorption of chromium by *Micrococcus luteus*.

Conclusion

The water samples analyzed showed contamination either by the presence of bacteria, organic matter, or some heavy metals. The bacteria isolated from the water samples were identified and screened for heavy metal tolerance ability, and two bacterial strains; *Pseudomonas aeruginosa* and *Micrococcus luteus*, that recorded high growth rate on media-supplemented with different concentrations of the two heavy metal salts (Pb and Cr) were selected for biosorption studies. The biosorption studies confirmed that the biosorbents have greater potential for the removal of Pb and Cr from heavy metal-contaminated water. The study showed that the pH of the solution has a great influence on the biosorption process, as these organisms have a remarkable metal adsorption capacity over a wide range of pH and therefore, could be employed in future for metal remediation from heavy metal-contaminated water.

Recommendations

- There should be regular monitoring of drinking water sources in Shikira Community to prevent water-related diseases and check the water resources from being polluted by human activities.
- Authorities should set up a small scale mining industry in this community with proper environmental monitoring and evaluation as well as a standard environmental impact assessment program be put in place in order to prevent outbreak of diseases and control the activities of the illegal mining.
- Remediation of water contaminated by heavy metals in the study areas is necessary in order to reduce the associated risks. It is clear from the result of this study that the organisms are good biosorbents that can be used for removal of heavy metals from the contaminated water.

Conflict of Interest

We hereby state clearly that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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