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Research Article

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Bioremediation Efficiency of *Saccharomyces cerevisiae* on Cadmium and Lead from Groundwater Obtained from Mining Community

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Abstract

Water is a vital requirement for life and it is also an effective vehicle for the transmission of diseases if contaminated. Pollution caused by heavy metals is one of the major environmental problems that are imperative to be solved. Mining of solid minerals has been identified as an entry point of heavy metals into the environment consequently polluting various components of the environment such as soil and water. Bioremediation offers a promising means to reclaim such contaminated environment in an economical and eco friendly way. The focus of this study is to evaluate the bio sorption efficiency of cadmium and lead-resistant yeast from well water samples collected from Angwan Magiro, one of the lead-contaminated villages of Niger State, North Central Nigeria. Microbial enumeration of the water samples were carried out using pours plate technique, while physicochemical parameters were done by standard methods. Tolerance ability of the yeast isolates to the heavy metals was determined by cultivating on yeast broth supplemented with synthetic solutions of 1.50 mg/L cadmium concentration and 5.50 mg/L lead concentration. Based on the result of heavy metal tolerance assay, Saccharomyces cerevisiae was then selected to determine its efficiency in bio sorption of cadmium and lead in a rotary shaker incubated at an ambient temperature for a period of 28 days. Yeast cells were separated from solutions by centrifugation and the supernatants were analyzed for residual metals in solution using Atomic Absorption Spectrophotometer (AAS). Bio sorption experiment was carried out as function of solution pH. The results of this investigation reveal that Saccharomyces cerevisiae was efficient in the removal of lead with 99.54% and cadmium with 88.24% at pH 8.20. These findings suggest that Saccharomyces cerevisiae present in heavy metal-contaminated water could be an effective measure for remediation of the ecosystem.

Keywords: Heavy Metals; Bioremediation; Bio Sorption; *Saccharomyces Cerevisiae*

Introduction

Water is indispensable for life, as it's one of the vital components of all living creatures and of the physical environment. It makes up to 50-97% of plants and animals [1]. It is the second essential factor for life after oxygen. If this essential factor is not available, some organisms die early, some form resistance stage, while some others die late, and human beings are not excluded from this marvellous factor [2]. However, it is estimated that about 1.2 billion individuals worldwide do not have access to potable water [3]. In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities that depend on non-public water supply systems [4]. Increase in human population has exerted an enormous pressure on the provision of safe drinking water in developing countries [2].

Potable water is that water that is free from disease-producing microorganisms and chemical substances that are dangerous to health [5]. It is the water of sufficiently high quality that can be consumed or used with low risk of immediate or long-term harm [6,7]. WHO guidelines for drinking water quality defined safe (quality) drinking water as water that "does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages" [3]. The provision of potable water to the rural populace is necessary to prevent health hazards and many infectious diseases are transmitted by water through the faecal-oral route [8]. In Nigeria, majority of the rural populace do not have access to potable water and therefore depend on wells, streams and river water for domestic use [1].

Diseases contacted through drinking water kill about 5 million children annually and make 1/6 of the world population sick [9].

Drinking water is obtained from a variety of sources among which are streams, lakes, rivers, ponds, rain, springs and wells [10]. Groundwater represents an important source of drinking water and its quality is currently threatened by microbiological and chemical contaminants including heavy metals [11]. 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 [6].

The presence of heavy metals like; iron, copper, chromium, cobalt, manganese, nickel, zinc, lead, arsenic, cadmium and aluminium in high concentrations in groundwater can cause an adverse effect on human health and make that water not potable [12]. Water is considered polluted when it is altered in composition or condition directly or indirectly as a result of activities of man so that it becomes unsuitable or less suitable for any or all of the functions or purposes for which it would be suitable in its natural state [13].

Bioremediation utilizes microorganisms, green plants or their enzymes to return the natural environment altered by contaminants to its original condition [14]. Heavy metal bioremediation is termed bio sorption, which involves removal of heavy metals from wastewater and soil through metabolically-mediated or physicochemical pathways [15]. Microbial systems like fungi, bacteria and algae have been successfully used as adsorbing agents for removal of heavy metals [16]. Fungi, including yeasts, are excellent metal sorbers and can be grown using inexpensive growth media, they are ubiquitous, unicellular microorganisms in natural environments or in industrial effluents and exposed to a variety of environments with respect to nutrient availability, temperature, pH, osmotic pressure, access to oxygen and water, etc., all of which induce stress responses [17]. This study is therefore focused on evaluating the bio sorption efficiency of cadmium and lead-resistant yeast from well water samples collected from Angwan Magiro, one of the lead-contaminated villages of Niger State, North Central Nigeria. The effect of pH on the bioremediation process and tolerance to the heavy metals by the yeast isolate was also monitored.

Materials and Methods

Description of the Study Area

The study was conducted in Angwan Magiro, situated on the eastern flanks of Kagara town, the headquarters of Rafi Local Government Area, Niger State, Nigeria (Figure 1). 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 [18]. The people are predominately farmers and cattle rearers. The major sources of water supply in the area are ground water, which is obtained from hand-dug wells and borehole. 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 this community for over six 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).

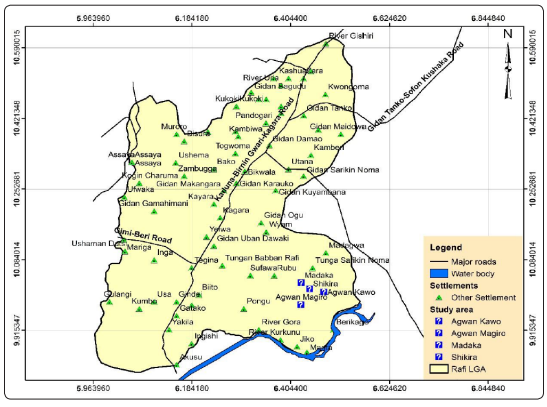

Figure 1: The study Area (Angwan Magiro) Rafi Local Government Area, Niger State, Nigeria.

Source: Department of Geography, Federal University of Technology, Minna (2018)

Plate: Gold Mining Site in Angwan Magiro (Field Photograph)

Collection of Water Samples

Water samples were collected from wells, which serves as the major source of drinking water in the study area. The two wells sampled were chosen based on the frequency of their usage and proximity to heavily populated areas. Water samples were collected for microbiological analysis, physiochemical analysis and bio sorption 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. Water samples were placed in ice pack to maintain temperature below 10oC and transported to the laboratory for analysis by standard methods as described in the American Public Health Association (1998) methods [19]. The preservation method for storage of the water samples was refrigeration.

Total Yeast Plate Count

The standard pour plate method was used for the enumeration of yeasts [19]. Serial dilution (10^{1}cfu/ml) of water sample was done and 1ml of the serially-diluted water samples was pipette into appropriately labelled petri dishes; freshly prepared Sabouraud dextrose agar (SDA), allowed to cool to 40°C was poured aseptically into the corresponding labelled Petri plates and mixed thoroughly. The Plates were allowed to solidify and incubated at room temperature (28±2°C) for 5 days. Colonies were counted and expressed as colony forming units per mL (cfu/mL) of water. Selected colonies were sub-cultured repeatedly on media used for primary isolation to obtain pure isolates, which were maintained on agar slants and kept in refrigerator until they were required for further characterization and identification [19].

Characterization and Identification of Yeast Isolates

The identification and characterization of yeasts was based on Gram's staining, morphology and sugar utilization profile. The biochemical

tests carried out were; sugar fermentation and assimilation tests and germ tube test. The yeast isolates were identified using the scheme of Barnett et al. [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 (50mL) of water sample was measured into a 100mL beaker using a pipette, and 10mL 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 10mL volume. Content was washed into a 50mL 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 Yeasts for Heavy Metal Tolerance

Two heavy metal salts were used for the screening of all yeast isolates viz. Lead nitrate, [$(Pb(NO_3)_2$] and cadmium sulphate ($CdSO_4$). Concentration of heavy metal solutions higher than initial concentrations obtained from water samples were prepared for the screening, using the broth dilution method [24,25].

Broth Method

Heavy metal tolerance capacity of the yeasts isolates was determined as described by Konopka and Zakharova [25]. Aqueous solutions of the metal salts; lead nitrate [$(Pb(NO_3)_2$] and cadmium sulphate ($CdSO_4$) were prepared separately in de-ionized water and left to stand for 24 hours for complete dissolution. Salts were prepared at concentrations higher than the initial concentrations of the heavy metals in the water samples being analysed. Yeast extract broth was prepared for the screening of yeast isolates. To each of the broth tubes, appropriate metal salt solutions were added. Media were sterilized by autoclaving at 121°C for 15 minutes and then allowed to cool. One millilitre (1mL) of respective standardized isolates' inoculum was then added to broth tubes and was incubated at ambient temperature for 5 days. 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 yeast 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]. The yeast isolate that recorded the highest turbidity was selected for bio sorption studies.

Biosorption Experiment

Bio sorption experiment was conducted using potato dextrose broth, following the procedure of Abioye et al. [27]. Potato dextrose broth was prepared using water samples under analyses containing known concentrations of the heavy metals; Pb and Cd. One hundred millilitres (100 ml) of potato dextrose broth (PDB) containing different water samples were dispensed in 250ml 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 (2ml) of 24 hours standardized isolates' inoculum of *Saccharomyces cerevisiae* was inoculated into 100ml of potato dextrose broth. Zero point five (0.5) McFarland standard was used for the standardization of inoculum. These experimental flasks with cultures were incubated at an ambient temperature. 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 cadmium concentrations in the supernatant were analyzed using Atomic Absorption Spectrophotometer (AAS) to determine the quantity of residual metals [19]. Controls without microorganisms were set up on each of the water samples using PDB, and incubation was done at room temperature. Experiment was performed in triplicates and average values were used in the results.

The sorption efficiency (%) (E) Was calculated using the following equation:

$$E = \left(\frac{C_i - C_f}{C_i}\right) \times 100$$

Where: C_i = initial concentration of the metallic ions (mg/L); C_f = final concentrate ion of metallic ions (mg/L) [28].

Effect of pH on bio sorption 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 (2mL) of inoculum was inoculated in 100mL each of the samples containing different concentrations of lead and cadmium. All the Erlenmeyer flasks were maintained at different pH values ranging from 1 to 9 for 28 days at ambient temperature. 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

Yeast Counts of the Water Samples Analyzed

The results of the yeast counts of the water samples collected from two wells in Angwan Magiro, Rafi Local Government Area, Niger State, Nigeria is presented in Figure 2. The mean total yeasts counts (TYC) in the well water samples ranged from 4.33±0.88 cfu/mL to 7.66±0.66 cfu/mL. The counts observed in both water samples were within the limit of 1.0x102 cfu/mL which is the standard limit of total yeast count for drinking water and there was, however, no significant difference ($P>0.05$) between the water samples analyzed [22].

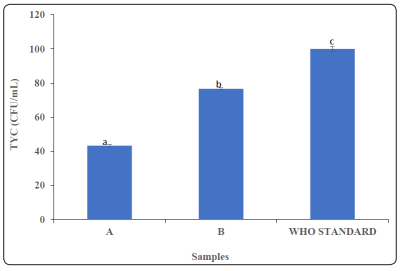

Figure 2: Yeast counts of water samples collected from wells in Angwan Magiro

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

Key: A & B = well water samples from Angwan Magiro. TYC = Total Yeast Count, cfu/mL = colony forming units per milliliter.

Occurrence of Yeasts Isolates in the water samples

The yeasts isolated from water samples in this study were identified as species of *Saccharomyces*, *Rhodotorula and Torulopsis*. The biochemical characteristics of the isolates are presented in Table 1. These organisms have been isolated from drinking water sources by other investigators [30,31].

Table 1: Morphological and Biochemical Characteristics of Yeasts isolates from Wells in Angwan Magiro

					Sugar Assimilation						Sugar Fermentation						
Gram Reaction	Shape	Colour	KNO3 Utilization	Germ tube test	Glucose	Galactose	Sacharose	Maltose	Lactose	Peptone	Glucose	Galactose	Sacharose	Maltose	Lactose	Peptone	Organism
+	O	Cream	-	-	+	+	+	+	-	-	+	+	+	+	-	-	*Saccharomyces cerevisiae*
+	O	Salmon Red	-	-	+	+	+	+	-	+	-	-	-	-	-	-	*Rhodotorula rubra*
+	O	Cream	-	-	+	-	-	-	+	+	+	-	-	-	-	-	*Torulopsis glabrata*
+	O	Cream	-	-	+	+	+	+	-	+	+	-	-	-	-	-	*Torulopsis dattila*
+	O	Grey-white	-	-	+	+	+	+	+	+	+	-	+	-	-	-	*Torulopsis candida*

O= Oval, + = Positive, - = Negative

The occurrence of yeasts in the water samples in this study could be due to the pH of the water samples, which was close to neutrality, which favours the growth of fungi species. This is contrary to the findings of Ayanbimpe et al. who recorded high occurrence of fungi in water used for domestic purposes, which was as a result of alkaline pH values of their water samples [30]. The presence of organisms such as *Torulopsis* sp in the well water samples demands attention because majority of residents not only bath with well water, but also drink without boiling it or treating it in any other way. Water has been identified as an avenue for spread of a number of fungal infections, particularly among hospital patients [32].

Physicochemical Parameters of the Water Samples

Table 2 shows the results of physicochemical analysis of well water samples in Angwan Magiro. Nitrate, chloride, conductivity, total dissolved solid, total hardness and phosphate, in both water samples were within the acceptable limit prescribed by Nigeria Standard for Drinking Water Quality and World Health Organization [22,33]. Both water samples recorded turbidity value above (5.0 NTU) limit as prescribed by NSDWQ [22,33]. The well water samples analyzed also recorded BOD5 values above the 10.00 mg/L limit prescribed by WHO, while well water sample A recorded pH value of 6.39 slightly below limits prescribed by NSDWQ and WHO [22,33]. Fluoride level of water sample B was slightly above the 1.5 mg/L limit prescribed by WHO [22].

Table 2: Physicochemical parameters of water samples collected from Angwan Magiro

Parameters	Water Samples		WHO Standard (mg/L)
	A	B	
Turbidity (NTU)	10.84±0.02^a	11.65±0.04^b	5
pH	6.39±0.03^a	6.62±0.02^b	6.5-8.50
BOD_5 (mg/L)	16.00±0.02^a	17.00±0.03^b	10
Fluoride (mg/L)	1.45±0.02^a	1.61±0.03^b	1.5
Phosphate (mg/L)	0.22±0.025^a	0.30±0.025^b	5
TDS (mg/L)	80.71±0.98^b	75.87±0.84^a	1000
Nitrates (mg/L)	16.38±1.10^b	15.74±1.20^a	50
Conductivity(µS/cm)	114.70±0.025^a	116.13±0.01^b	1000
T/hardness (mg/L)	27.46±0.27^a	27.43±0.31^a	200
Chlorides (mg/L)	1.27±0.03^a	1.84±0.02^b	250

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

Key: A & B = well water samples from Angwan Magiro. 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.

Water with BOD5 levels < 4 mg/L are deemed as clean while those >10 mg/L are considered polluted and unsafe [22]. Both water samples analyzed, recorded BOD levels >10 mg/L. This suggests that drinking water sources were polluted by organic matter. This may be due to the mixing of organic matter with the waterways. High values of BOD5 recorded in this study is similar to the values recorded from well water samples by Pratap-Chandran et al., 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 [11].

The high turbidity observed in the well water sample is due to contamination from soil runoff into the well water, which increases the cloudiness of the water as a result of particulate matters such as clay, silt and finely divided organic matter, being suspended within it [34].

Probable source of high fluoride in the well water sample could be as a result of weathering and circulation of water in rocks and soils, which will lead to fluorine being leached out and dissolved in ground water. Reda, while studying the physicochemical analysis of drinking water quality of Arbaminch town, also recorded high values of fluorine which ranged between 2.75-5.43 mg/L, which might be due to the presence of fluoride compounds like CaF_2, Na_3AlF_6 and $Ca_5(PO_4)_3F$ in the underground water [35].

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 [35]. 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., 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 [37].

Heavy metal contents of the water samples

The results of the concentrations of heavy metals analyzed in the water samples are presented in Table 3. 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.

Table 3: Heavy metal contents of the water samples

Heavy metals (mg/L)	Water Samples		WHO Standard (mg/L)
	A	B	
Lead (Pb)	4.36±0.11[b]	2.87±0.01[a]	0.01
Chromium (Cr)	2.23±0.01[b]	1.43±0.02[a]	0.05
Mercury (Hg)	0.002±0.0017[b]	0.002±0.001[a]	0.001
Zinc (Zn)	0.39±0.026[a]	0.47±0.026[b]	3.00
Cadmium (Cd)	0.34±0.02[a]	0.36±0.02[b]	0.003

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

Key: A & B = well water samples from Angwan Magiro

Well water samples under analysis showed severe contamination by lead (Pb), exceeding WHO maximum permissible limit of 0.01 mg/L for lead in drinking water [22]. The high contamination level of Pb could be attributed to the use of herbicides and other farming chemicals, which might have lead as impurity, and the artisanal gold mining activities taking place in Angwan Magiro, 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. Nuhu et al., recorded abnormal high contamination levels by lead in Bagega, due to the report that the gold bearing deposits in this region (Zamfara State) usually contained extremely high levels of lead [38]. Lead is bio accumulated in humans and can be transferred from mother to child during pregnancy. Bakare-Odunola, in a study, reported a lead concentration of 0.1 mg/L resulted in the development of neurological problems in fetuses and children [39]. High concentration in drinking water may result in lead poisoning that manifests in symptoms such as tiredness, lassitude, slight abdominal discomfort, irritation and anemia [40]. It has been reported to be a probable human carcinogen, damaging the nervous system causing brain disorder [41].

A safe limit of 0.003 mg/L has been recommended for Cd by WHO [22]. However, this limit was exceeded in all the water samples in the two settlements, which makes the water unsafe with respect to Cd contamination. Cadmium contamination of the water samples might be coming from other sources other than mining, as chemicals like fertilizers used in farmland could increase cadmium levels. This is in line with the findings of Wasiu et al. who in their study on heavy metal contamination in stream water and sediments of gold mining areas of South Western Nigeria, observed that the control site selected for their study, which was a farm land also had Cd concentrations higher than the recommended safe limit, clearly indicating that Cd contamination of the water samples might be coming from other sources [42]. High chromium concentration could be linked to the mining activities going on in the study area, as the waste rock left at the mining sites oxidizes to release free metals to the environment, which causes heavy metal poisoning resulting from disposal of waste chemicals into the environment which mistakenly get into the water bodies [23]. Inorganic mercury (metallic mercury and inorganic mercury compounds) enters the air from mining ore deposits, burning coal and waste, and from manufacturing plants. It

enters the water or soil from natural deposits, disposal of wastes, and volcanic activity [43]. Exposure to mercury occurs from breathing contaminated air, ingesting contaminated water and food, and having dental and medical treatments. Mercury, at high levels suppresses the central nervous system, thereby damaging the brain, kidneys, developing fetus and death [44].

Heavy metal tolerance by Yeast isolates

The result of the screening of yeast isolates for heavy metals tolerance is presented in Figure 3. It was observed that *Torulopsis glabrata* had the highest growth rate on yeast extract (broth) supplemented with lead nitrate [$(Pb(NO_3)_2$] having recorded an absorbance of 0.50±0.003, followed by *Saccharomyces cerevisiae* with mean absorbance of 0.42±0.003, *Rhodotorula rubra* had the lowest growth with a mean absorbance of 0.27±0.004. On the other hand, *Saccharomyces cerevisiae* had the highest growth rate on yeast extract (broth) supplemented with cadmium sulphate ($CdSO_4$), with a mean absorbance of 0.51±0.000, while *Torulopsis dattila* recorded the lowest growth rate with a mean absorbance of 0.16±0.002.

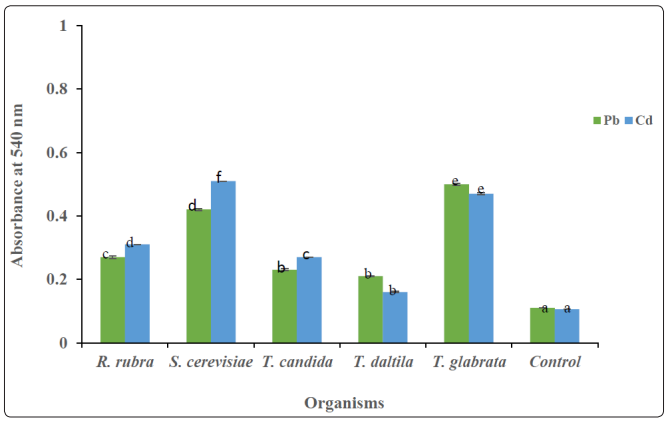

Figure 3: Heavy metal tolerance by Yeast isolates

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

The yeasts isolates when grown in media amended with varying concentrations of different heavy metals, showed a variable tolerance level to both tested metal salts (lead nitrate and cadmium sulphate). Due to their high level of metal tolerance (high growth rate) in all the two metal salts, as shown in Figures 3 above, *Saccharomyces cerevisiae* was selected for bio sorption studies, as it was identified as efficient organisms that were resistant to Pb and Cd. The result of this findings is similar to the findings of Johncy-Rani et al. 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 bio sorption approach" [26]. The identified efficient organisms were therefore selected for bio sorption studies.

Bio sorption of Pb and Cd by *Saccharomyces cerevisiae*

Figures 4 to 5 shows the accumulation of the heavy metals (Pb and Cd) by *Saccharomyces cerevisiae* in well water samples from Angwan Magiro, while Figure 6 shows the removal efficiency (%) of Pb and Cd by *Saccharomyces cerevisiae*. The initial concentration of Pb in the water samples had a mean value ranging from 2.87±0.01 mg/L to 4.36±0.11 mg/L, which was reduced to a mean concentration value which ranged from 0.02±0.00mg/L to 0.07±0.00 mg/L after 28 days of *Saccharomyces cerevisiae* inoculation (Figure 4), indicating bio sorption with a percentage mean range value of 97.56±1.87% to 99.54±1.66% (Figure 6), while the initial concentration of Cd in the water samples had a mean value ranging from 0.34±0.02 mg/L to 0.36±0.02 mg/L, which was reduced to a mean concentration value which ranged from 0.04±0.01 mg/L to 0.05±0.01 mg/L after 28 days of *Saccharomyces cerevisiae* inoculation (Figure 5), indicating bio sorption with a percentage mean range value of 86.11±0.62% to 88.24±0.30% (Figure 6).

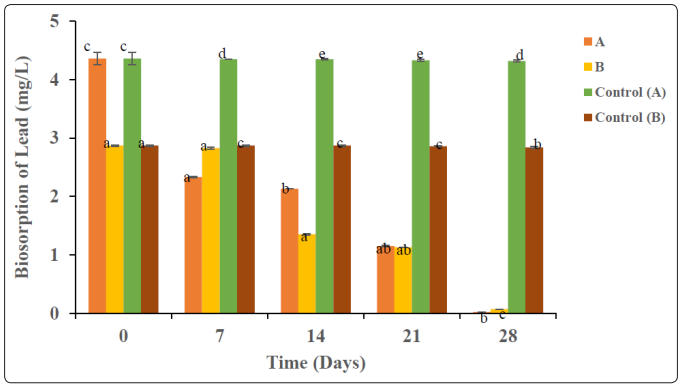

Figure 4: Biosorption of lead by *Saccharomyces cerevisiae*

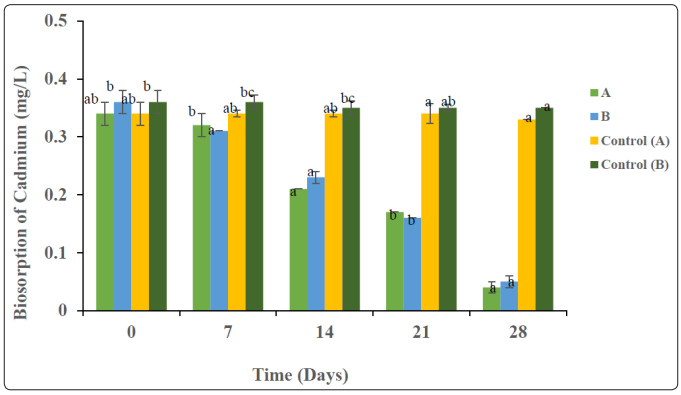

Figure 5: Biosorption of cadmium by *Saccharomyces cerevisiae*

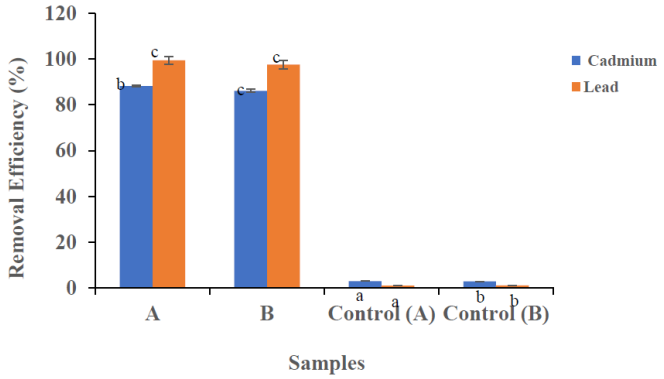

Figure 6: Removal Efficiency (%) of the heavy metals by *Saccharomyces cerevisiae*

There was a high percentage reduction in Pb concentrations (ranging between 97.56% - 99.54%) and Cd concentrations (ranging between

86.11% - 88.24%) in the well water samples after 28 days of *Saccharomyces cerevisiae* inoculation. This is similar to the reports of previous investigators. Zahira and Khalid recorded 96 -100% Pb removal in all water samples after 30 days of fungal inoculation [45]. Limin et al. studied the mechanism of Pb bio sorption by *Saccharomyces cerevisiae* and found that cell wall play an important role in adsorption due to many spots on it [46]. They stated that COOH, C=O, C-O and N-H are the main active binding sites for the adsorption of Pb, and that Pb combines with functional group containing C, N, O and P. They concluded that it mainly resulted from ion exchange and surface complexation. The higher affinity of *Saccharomyces cerevisiae* for lead in this result is therefore, in line with what has been reported in other studies [47,48]. The roles played by amines, carboxylic acids, phosphates, sulfhydryl group and lipids in lead biosorption have been studied by Parvathi et al. [49]. They concluded that electrostatic attraction may be the mechanism of bio sorption. Bashar et al., estimated that the maximum uptake capacity of Cd was close to 35 and 40 mg/L for *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* yeast cells, respectively [50]. Difference in removal of heavy metal might be due to the variation in binding sites available for heavy metal to bind or also it might be due to the difference in surface area of biosorbant material [45]. Microbes that survived on cadmium pollution could have developed a cadmium-resistant mechanism to tolerate cadmium [51]. Strains isolated from heavy metal polluted sites generally possess resistant to metals [52]. *Aspergillus, Penicillium, Alternaria, Geotrichum, Fusarium, Rhizopus, Monilia and Trichoderma* have been found resistance to cadmium [53].

Effect of pH on bio sorption of heavy metals by *Saccharomyces cerevisiae*

The water samples inoculated with *Saccharomyces cerevisiae* had an initial pH with a mean range of 6.39±0.50 to 6.62±0.05 which gradually decreased, and there was an increase again on day 21 until it got to pH with a mean range of 7.10±0.21 to 8.20±0.31 (on Day 28). The effect of pH on percentage bio sorption of lead and cadmium by *Saccharomyces cerevisiae* presented in Figure7. The optimum removal efficiency of lead and cadmium by *Saccharomyces cerevisiae* was 99.54% and 88.24% respectively at pH 8.20.

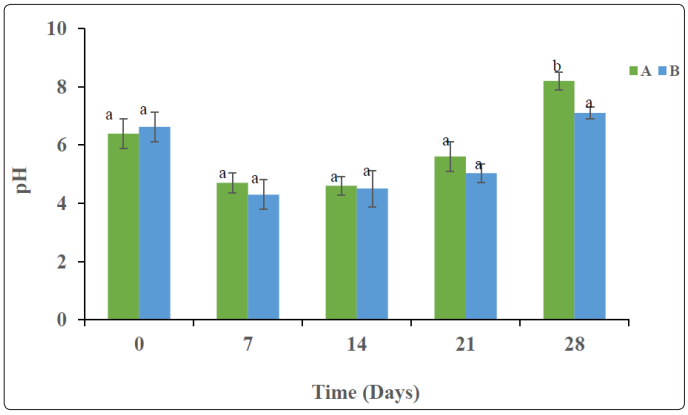

Figure 7: Effect of pH on bio sorption of the heavy metals by *Saccharomyces cerevisiae*

The percentage of bio sorption of the heavy metals (Pb and Cd) varied greatly along the given pH range 1-9. The most important single parameter influencing the bio sorption process is the pH of the adsorption medium [54]. There was an increase in the uptake capacity of *Saccharomyces cerevisiae* for the two metal ions with increasing pH during the bio sorption studies. The variation of adsorption of metal ions at various pH is on the basis of metal chemistry in solution and the surface chemistry of the sorbent [55]. Low pH limits the bio sorption of metal ions on fungal biomass surfaces, since at this pH the total surface charge of the cells becomes positive, and the competing metal cations and protons for binding sites on the cell wall consequently reduces metal adsorption [56]. It is believed that the existing groups in the cell wall have a high correlation with H3O+ which limits the adsorption of metal ions as a result of repulsive forces [57].

At high pH, the decrease in H+ ions causes the surface of the cell walls to be covered with negative charges. This then generates conductive conditions for carboxylic, phosphate, hydroxyl and amino acid groups. All of which easily react with metal ions therefore, resulting in the increased adsorption of metal ions [55,58]. Generally, an increase of pH causes deprotonation of metal ions binding sites exposed by cellular surface. However, a decrease of pH causes competition between protons and positively charged metal ions. However these rules concern only cations. Since biosorption is reversible process, decreasing pH would result in deprotonation. This property is used in regeneration of biosorbents [59]. The result of this study is similar to the findings of Maedeh and Habibollah who in their study on "bio sorption of ternary cadmium, nickel and cobalt ions from aqueous solution onto *Saccharomyces Cerevisiae* cells" observed that by increasing the pH from 2 to 8, the removal percentage was increased and reached 86.5%, 63.3% and 73.4% for cadmium, nickel and cobalt, respectively [55].

Conclusion

The results of this study showed contamination of the water samples by the presence of microbes, organic matter and some heavy metals. Thus the water does not meet the standard stipulated for drinking water by WHO and NSDWQ and serve as effective source of transmission of diseases. The bio sorption studies confirmed that the bio sorbent; *Saccharomyces cerevisiae*, have greater potential for the removal of Pb and Cd from heavy metal-contaminated water. The study showed that the pH of the solution has a great influence on the bio sorption 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.

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