Bioleaching of Heavy Metals from Hospital Sewage Sludge using Cassava Peels Fermentation Extracts

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Abstract

The geometric increase in urbanisation cum industrialization has resulted in drastic increase in the volume of wastewater and sludge which contain heavy metals which deteriorate the soil and ground water quality and bioaccumulate in food chains. The use of strain-specific microbial fermentation in the production of organic acids towards the removal of heavy metals has been extensively studied. However, the use of indigenous micro flora toward the bioleaching of heavy metals has not been well researched. This study assessed the bioleaching of heavy metals from hospital sewage sludge using cassava peels fermentation extracts. Sewage sludge sample was collected from the University College Hospital sewage treatment plant, Ibadan, Oyo State, Nigeria and analysed for selected heavy metal concentrations such as Copper (Cu), Zinc (Zn), Chromium (Cr), Nickel (Ni), Cadmium (Cd) and Lead (Pb) by Atomic Absorption Spectrophotometry using standard methods. Source-segregated cassava peels were collected from a market in Ibadan. *Aspergillus tereus* Fermentation Extract (ATFE) and Crude Fermentation Extract (CFE) were obtained each from 50g of CPs after 13-day fermentation using acid-producing strain of *Aspergillus tereus* and indigenous microbial populations respectively while the Commercial-grade Itaconic Acid (CIA) served as control. The batch study was performed by adding 10 ml of the treatment (extracts and controls) at room temperature (RT) and elevated temperature (ET) at the varied pH (3– 5) to 3g of the sample each. The mixture was centrifuged after a leaching time of 1-12 days at 1000 rpm for 1hour. The filtrate was analysed for heavy metals concentrations and compared with the standards. Data were analyzed using descriptive statistics, paired t-test and ANOVA at $p>0.05$. The mean heavy metal concentration (mg/kg) in the sludge were Zn(52.3±0.1), Ni(5.6±0.01), Cu (2.22±0.2), Cr (1.46±0.1) and Pb(1.9±0.1) and were below the permissible limits (mg/Kg) set by national standards. Optimum heavy metals removal for ATFE at room temperature was achieved on day 12 at pH 3.5 for Ni (78.4%) and at elevated temperature was achieved on day 1 at pH 4.0 for Zn (72.3%). The optimum pH for CFE lies between 3 – 4.5 for Ni (76.2%) at room temperature and Cr (76.6%) at elevated temperature. ATFE showed higher removal when compared with control, except Cr and Pb. Crude fermentation extract of cassava peel was found to be effective in removing the heavy metals from sewage sludge. Therefore, its use could be embraced and promoted for removing heavy metals from sewage sludge, thus safe disposal could be achieved.

Keywords: *Aspergillus tereus* Fermentation Extract, Crude Fermentation Extract, Hospital sewage sludge, Cassava peels.

Introduction

The geometric increase in the urbanization coupled with industrialization has brought about the generation of wastes different forms in the environment. These wastes have posed a lot of threat to both man and the environment. This is due to industrialization, increasing population density and high urbanized societies [1,2]. Sewage sludge is the solid, semi-solid or liquid residue generated during the treatment of sewage in a treatment works. Sludge generated at these plants contains heavy metals at relatively high concentrations, which may vary considerably with time and mostly depend on industrial activities. Heavy metals such as Lead (Pb), Chromium (Cr), Nickel (Ni) and Cadmium (Cd) constitute toxic pollutants found in sewage sludge [3-5]. Heavy metals accumulate in soil and in plants when sludge is applied as fertilizer and eventually produce harmful effects in animals and humans. Due to the high level of awareness of the negative impacts of high concentration of heavy metals to the environment, stringent guidelines and verifications have been designed to limit the application of sewage sludge to agricultural soils [6].

In order to use sewage sludge as soil conditioner, it is necessary to remove or minimise the amount of heavy metals in sludge to prevent biomagnification and bioaccumulation. Inorganic chelating agents which are the most popular extracting reagents for heavy metal removal have been proved to be very efficient as they form stable complexes with most heavy metals over a broad pH range. Although, the use of inorganic and synthetic chelating agents for heavy metal removal tends to decrease soil productivity and impaired the chemical and physical structure of soils due to mineral dissolution. These compounds are very stable and could remain adsorbed in the soil after extraction, thus making the soil unfit for further use [3,7].

In bioleaching of metals from sewage sludge, organic acids are more promising than inorganic chelating agents, since extraction can be at mildly acidic condition and are biodegradable, hence low environmental impact. Also, the use of organic acids as a better option for heavy metal solubilisation from sewage sludge have been explored by several authors to have low effectiveness compared to the synthetic chelates [8]. The use of strain-specific microbial fermentation in the production of organic acids has been studied [9,10]. However, the use of indigenous microflora towards the removal of heavy metals has not well been researched. Therefore, this study assessed the bioleaching of heavy metals from hospital sewage sludge using cassava peels fermentation extracts.

Materials and Methods

Study area

University College Hospital (UCH), Sewage Treatment Plant located in the University College Hospital (UCH), Ibadan, Oyo State, Nigeria (Fig 1). The Sewage Treatment Plant involved the conversion of the organic matter into inorganic matter before discharging the treated effluent into the recipient environment while the sludge is processed into organic manure for agricultural purposes.

Collection and Analysis of dewatered sewage sludge

Sewage sludge sample was collected from the University College Hospital (UCH) Sewage Treatment Plant, Ibadan, Oyo State. The sample was prepared by drying and homogenised through a 0.2 mm stainless steel sieve, digested and analysed for the heavy metal content (Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), Lead (Pb) and Zinc (Zn)) according to A.O.A.C [11].

Production of fermentation extracts (extractants)

Itaconic acid producing strain of *Aspergillus tereus* was isolated from the soil using the pour plate method and screened using Czepak-Dox Agar. The *A. tereus* fermentation extract and crude fermentation extract were obtained from fermentation of 50g of cassava peels for 13 days using acids-producing strain of *A. tereus* as inoculum and no inoculation was carried out on the cassava peels for natural/crude fermentation. Therefore, the substrate (cassava peels) still retains its indigenous microflora which is needed for crude fermentation [12,13]. The *A. tereus* and crude fermentation extracts were used for the removal of heavy metals from sewage sludge while the commercial itaconic acid served as the control.

Extraction studies

Extraction of heavy metal was carried out to determine the efficacy of extractants using varied optimum conditions (pH, temperature and contact time) as modified by Okareh and Enesi [14]. The unadjusted and adjusted pH (3, 3.5, 4, 4.5 and 5) were used to determine the effect of progressive acidification on the removal of heavy metals using the fermentation extracts.. The adjustment of pH was done using 1M HCl. The extraction was carried out at contact time of 1, 3, 6, 9 and 12 days at ambient/room temperature (28^{o}C) and the elevated temperature (45^{o}C) for each extractant using 10ml for each extractant. Each tube containing 3 g of sieved sewage sludge in 25ml centrifuge tubes was filled with varied doses (ml) of the extractants. The tubes were stirred continuously on a rotary shaker at 150 rpm. Each tube was centrifuged at 1000 rpm for 1hour. The supernatant was decanted and filtered through a filter paper. The concentrations of heavy metals in the final solutions (filtrate) were determined by an Atomic Absorption Spectrometer.

Data Management

The obtained data was subjected to statistical analysis using Statistical Package for Social Science (SPSS 21) to evaluate statistically significant effects. Adsorption/leaching model was used to calculate the amount of metal ion leached and percentage removal of heavy metals from the sewage sludge.

Removal efficiency:

$$\text{Efficiency (\%)}\ \frac{(C1\,V1)}{Cs\,Ms}\ x\ 100$$

Where Q_e is the metal uptake (mg/g); C_1 and C_s are the concentrations of the metal in supernatant (in mg/L) and sludge (mg/kg) respectively, M is the mass of the sewage sludge (kg) and V is the volume of the extractant (l).

Results

Heavy metal contents of Dewatered Sewage Sludge

The selected heavy metal content of copper, zinc, chromium, nickel, cadmium and lead are presented in mg/kg in Table 1.

Table 1. Physico-chemical properties of dewatered sewage sludge

Parameter	Unit	Values	NESREA	WHO
pH		6.20±0.2	6-9	6.5-8.5
Copper (Cu)	mg/KgDM	2.22± 0.2	100	15.0
Zinc (Zn)	mg/KgDM	52.33±0.1	421	1.0
Chromium (Cr)	mg/KgDM	1.46±0.1	100	0.05
Nickel (Ni)	mg/KgDM	5.63±0.01	70	NA
Cadmium (Cd)	mg/KgDM	ND	03	0.01
Lead (Pb)	mg/KgDM	1.92± 0.1	164	0.01

ND = Not detected, NA = Not available

The heavy metals concentrations in the sewage sludge were compared with the national and international maximum permissible standards.

Efficacy of the fermentation extracts for heavy metal removal

Effects of Temperature on the heavy metal removal from the dewatered sludge

Optimum heavy metal removal of the fermentation extracts at room and elevated temperature (45^{o}C) at different pH values of day 1 to day 12. At the room temperature, *A. tereus* fermentation extract had the highest optimum heavy metal removal of 78.4% and 73.2% for nickel and copper respectively; crude fermentation extract had the highest optimum heavy metal removal of 76.2% and 74.8% for nickel and zinc respectively; while commercial itaconic acid had the highest optimum heavy metal removal of 73.1% and 68.9% for copper and zinc respectively as shown in Table 2. At the elevated temperature, *A. tereus* fermentation extract had the highest optimum heavy metal removal of 72.3% and 70.9% for zinc and copper respectively; crude fermentation extract had the highest optimum heavy metal removal of 76.6% and 76.3% for chromium and lead respectively; while commercial itaconic acid had the highest optimum heavy metal removal of 70.8% and 66.5% for copper, zinc and chromium respectively as shown in Table 3.

Table 2: Removal of heavy metals at room temperature for *Aspergillus tereus* extract

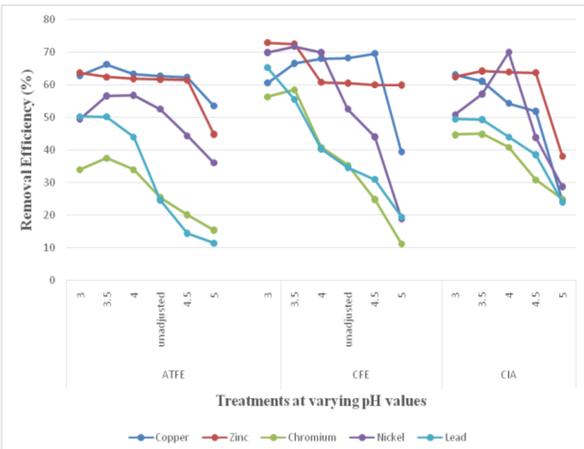
Heavy metals		Extractant		
		Aspergillus Tereus fermentation extract	Crude fermentation Extract	Itaconic acid
Copper	Extraction Efficiency (%)	73.2	74.4	73.1
	pH	3.0	3.5	3.0
	Contact time	12	9	12
Zinc	Extraction Efficiency (%)	69.3	74.8	68.9
	pH	3.0	3.5	3.0
	Contact time	9	12	9
Chromium	Extraction Efficiency (%)	51.6	70.5	58.8
	pH	4.0	3.0	4.0
	Contact time	12	12	12
Nickel	Extraction Efficiency (%)	78.4	76.2	67.8
	pH	3.5	4.0	3.5
	Contact time	12	12	3
Lead	Extraction Efficiency (%)	71.1	71.6	67.8
	pH	4.0	3.0	4.0
	Contact time	3	12	3

Table 3: Removal of heavy metals at elevated temperature for *Aspergillus tereus* extract

Heavy metals		Extractant		
		Aspergillus tereus fermentation extract	Crude fermentation Extract	Itaconic acid
Copper	Extraction Efficiency (%)	70.9	75.6	70.8
	pH	3.5	3.5	3.5
	Contact time	12	12	12
Zinc	Extraction Efficiency (%)	72.3	75.1	66.5
	pH	4.0	3.5	3.5
	Contact time	1	12	12
Chromium	Extraction Efficiency (%)	65.7	76.6	66.5
	pH	3.0	3.5	3.0
	Contact time	3	6	3
Nickel	Extraction Efficiency (%)	68.4	75.2	64.8
	pH	3.5	3.5	3.5
	Contact time	12	1	12
Lead	Extraction Efficiency (%)	53.2	76.3	53.8
	pH	3.0	3.0	3.0
	Contact time	9	1	9

Effects of pH on the heavy metal removal from the dewatered sludge

The heavy metal removal efficiency of the fermentation extracts and the control increases with increase in the pH values from 3 to 4.5, then decreased as pH increases towards 5. In few, there was decrease in removal efficiency with increase in the pH values at different temperatures and contact times as presented in Fig.2 – 6.



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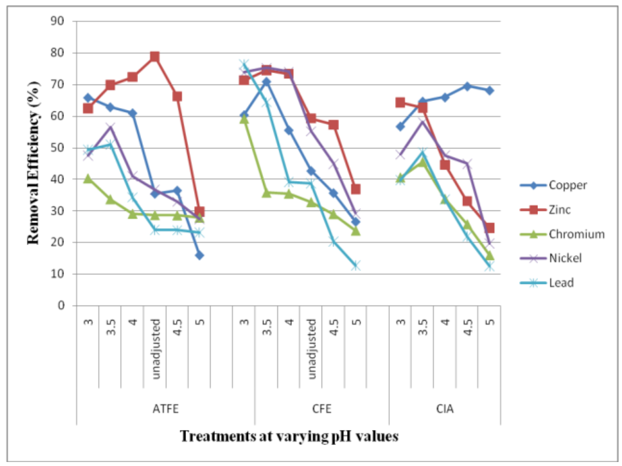

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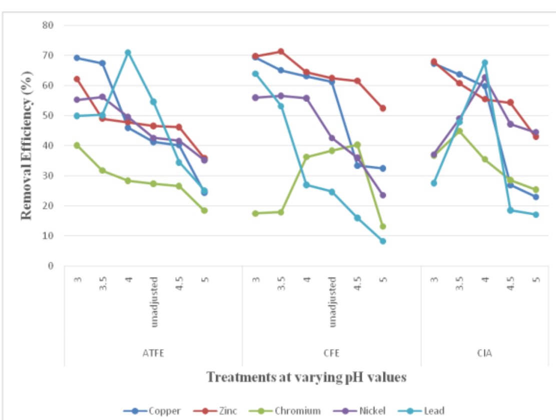
Fig. 2a: Effect of pH on metal removal at room temp. on day 1 Fig. 2b: Effect of pH on metal removal at elevated temp. on day 1

ATFE – *Aspergillus tereus* fermentation extract

CFE – Crude fermentation extract

CIA – Commercial-grade itaconic acid



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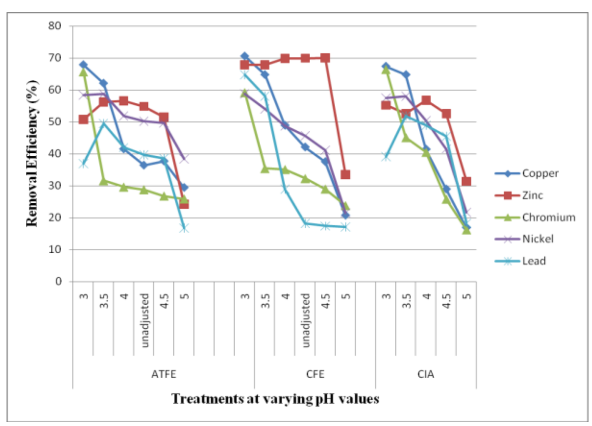

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Fig. 3a: Effect of pH on metal removal at room temp. on day 3 Fig. 3b: Effect of pH on metal removal at elevated temp. on day 3

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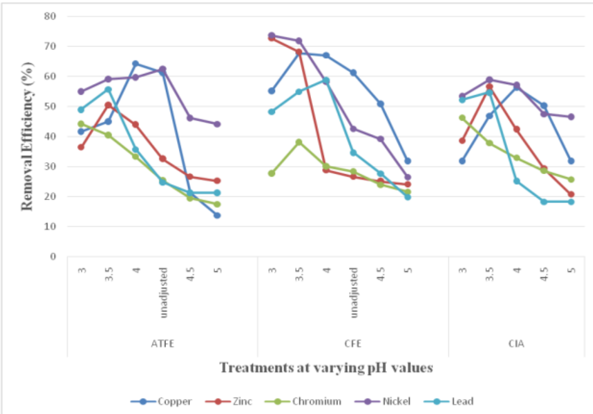

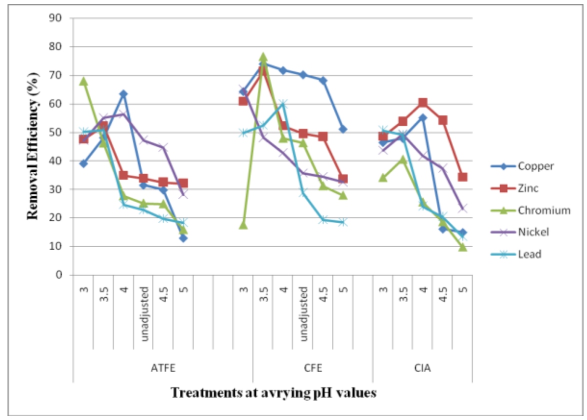

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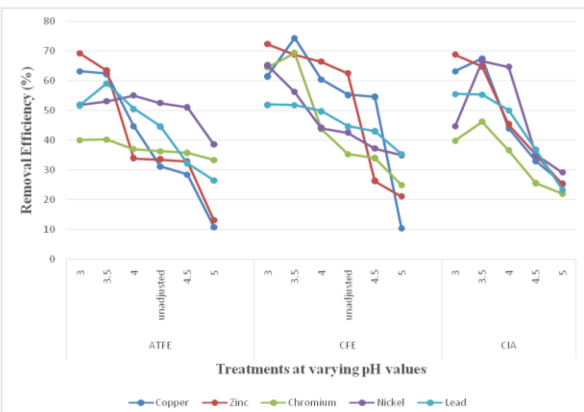
Fig. 4a: Effect of pH on metal removal at room temp. on day 6 Fig. 4b: Effect of pH on metal removal at elevated temp. on day 6



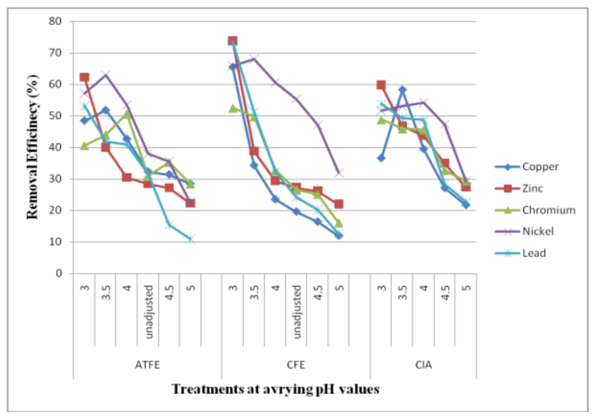


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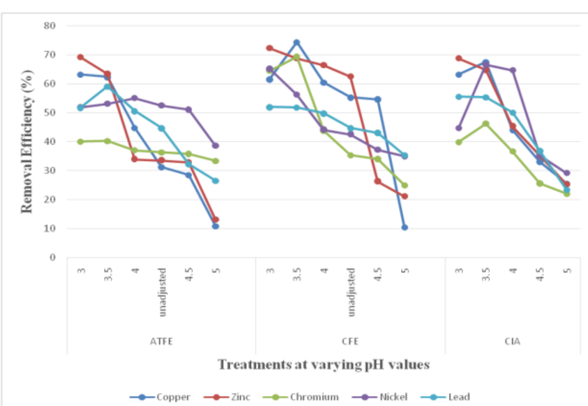
Fig. 5a: Effect of pH on metal removal at room temp. on day 9 Fig. 5b: Effect of pH on metal removal at elevated temp. on day 9



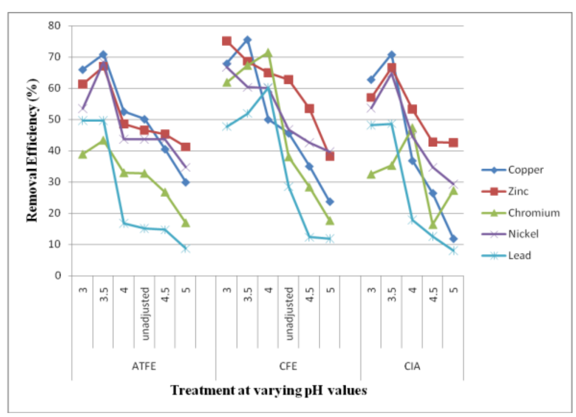


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Fig. 6a: Effect of pH on metal removal at room temp. on day 12 Fig. 3b: Effect of pH on metal removal at elevated temp. on day 12

Effects of contact time on the heavy metal removal from the dewatered sludge

Generally, there were initial increases in removal efficiency with corresponding increase in contact time from day 1 to 9, then decreased to day 12. In few, there was corresponding increase in removal efficiency as the contact time increases while there was downward trend in removal efficiency as contact time increases in others. The effects of contact time on the heavy metals are represented in Fig.7-11.

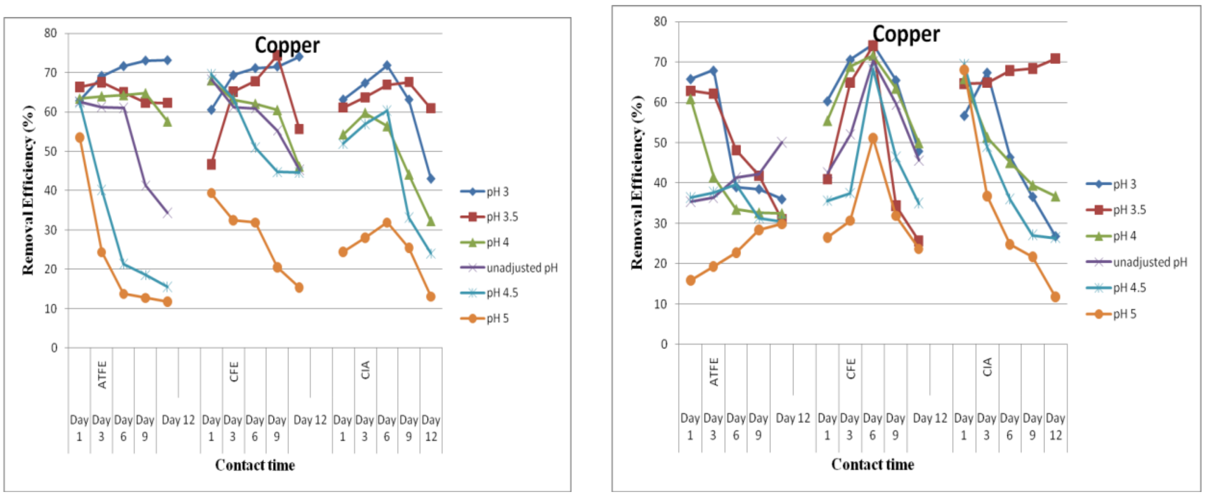

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Fig. 7a: Effect of contact time on Copper removal at room temp. Fig. 7b: Effect of contact time on Copper removal elevated temperature

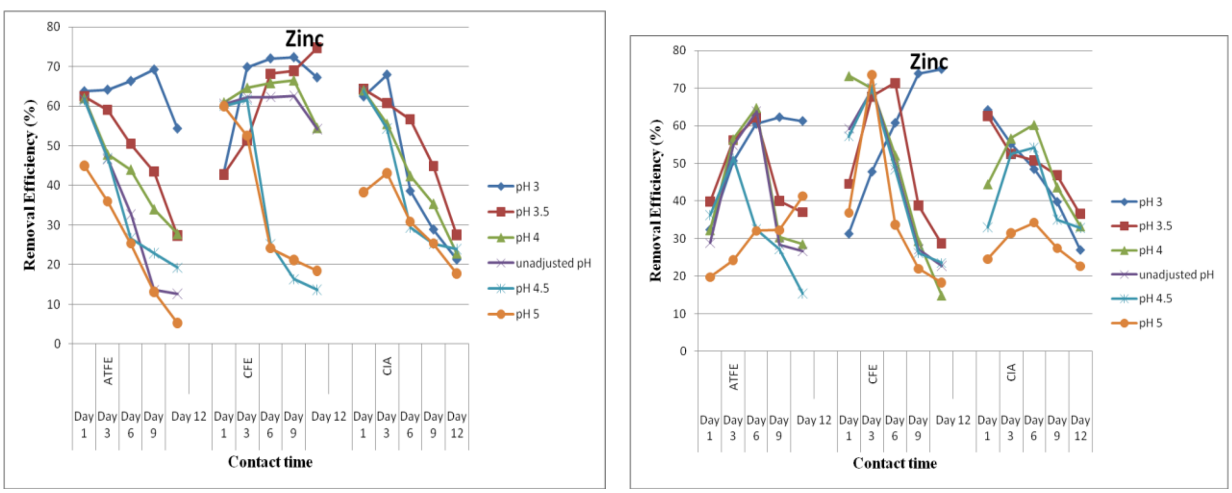

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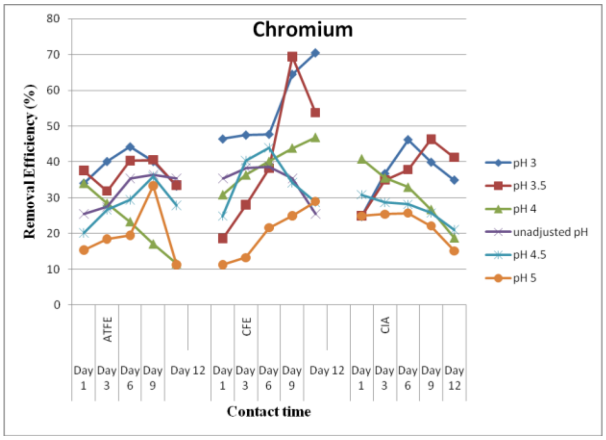
Fig. 8a: Effect of contact time on Zinc removal at room temp. Fig. 8b: Effect of contact time on Zinc removal elevated temperature



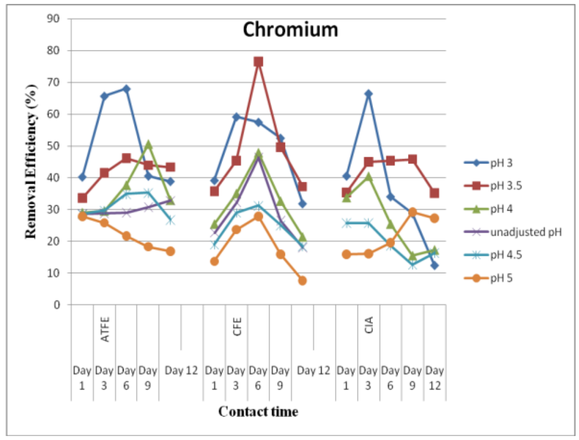


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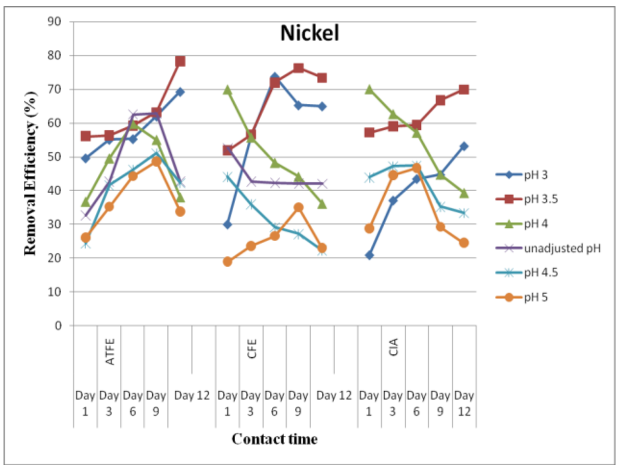
Fig. 9a: Effect of contact time on Chromium removal at room temp. Fig. 9b: Effect of contact time on Chromium removal elevated temperature



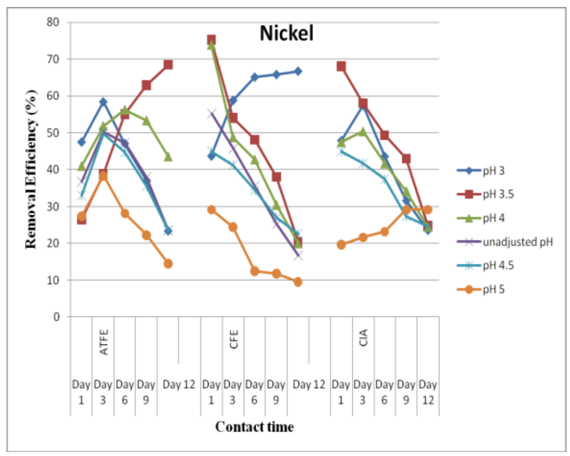


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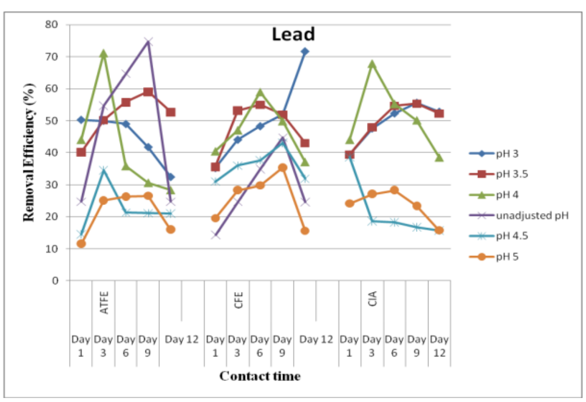
Fig. 10a: Effect of contact time on Nickel removal at room temp. Fig. 10b: Effect of contact time on Nickel removal elevated temperature



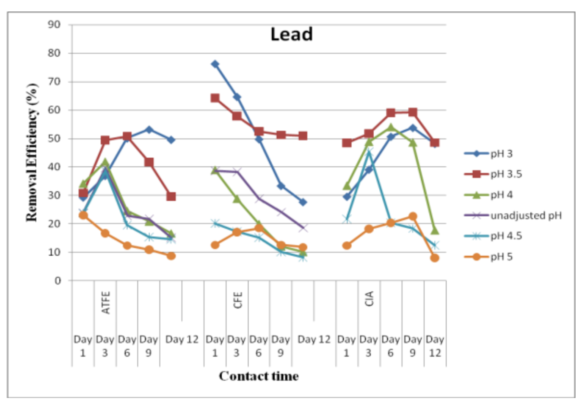


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Fig. 11a: Effect of contact time on Lead removal at room temp. Fig. 10b: Effect of contact time on Lead removal elevated temperature

Discussions

Heavy metal concentrations of sewage sludge

The high concentrations of Zn in this study is consistent with the work of Tolosana and Erhlich [15] which showed that effluent from medical institutions in South Africa had high levels of Zn and Cu. The metal concentrations were below the permissible limits for the safe disposal of sludge. However, their removal is recommended due to bioaccumulation which constitutes serious threats to the environment, groundwater and food chain [16].

Effects of Treatment/ Extractants on the heavy metal removal from the dewatered sludge

Aspergillus tereus fermentation extract was more effective in the extraction of heavy metals from sewage sludge at room temperature compared with commercial itaconic acid except for Cr while the extraction is more effective at elevated temperature when compared with commercial itaconic acid except the Cr and Pb. The extraction efficiency of crude fermentation extract is more desirable at elevated temperature because it showed appreciably higher extraction efficiency for the heavy metals assayed. Some organic acids had demonstrated their ability to solubilize some metals at higher concentration [8]. Zn showed the greatest solubility by the *A. tereus* fermentation extracts at elevated temperature. These findings confirmed the work of Enesi [17] which reported that zinc exhibits the same degree of mobility in sewage sludge. Chromium showed the least solubility by the *A. tereus* fermentation extracts at both temperature. These findings were conformed with Jakubus and Czekala [18] who reported that, irrespective of the oxidation degree, the dominant part of Cr (80 – 90 %) was bound firmly, difficult to dissolve in the soil and sewage sludge.

Effects of pH on the heavy metal removal from the dewatered sludge

The optimum pH for extraction lies between 3 – 4.5 which was most effective for the removal of Ni (78.4%) for *A. tereus* fermentation. Crude fermentation extract was effective for the removal of Ni (76.2%) at room temperature and Cr (76.6%) at elevated temperature. It should be noted that after pH 4, there was a decrease in the removal efficiency, which may be due to the formation of soluble complexes of filling up of the binding sites. At low pH values, the surface of the extractant would be closely associated with hydroxonium ions (H_3O^-) by repulsive forces, to the surface functional groups, consequently decreasing the percentage removal of metal. As the solution pH increases, the onset of the metal hydrolysis and the precipitation began. When the pH of the extractant was increased from 3 to 4, there was a corresponding increase in deprotonation of the extractant's surface, leading to a decrease in H^+ ion on the surface. This creates more negative charges on the extractant's surface, which favours extraction of positively charge species [16,19,20].

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Effect of contact time on the heavy metal removal from the dewatered sludge

There were initial increases in removal efficiency with corresponding increase from day 1 to 9. These findings showed a great disparity with Stylianou *et al*., [16], which reported that using synthetic inorganic chelating agents, maximum heavy metal removal efficiency is attained in less than 24 hours. It is worthy to know that the initial faster rate was due to the availability of the uncovered surface area of the extractants since the kinetics depends on the surface area of the extractants. The extraction takes place at the more active binding sites and as these sites are progressively filled, the extraction process tends to be more unfavourable.

Conclusion

This study which determined the efficacy of two fermentation extracts of cassava peel wastes in removing heavy metals from sewage sludge indicated that crude fermentation extract of cassava peel waste can remove heavy metals from sewage sludge. The fermentation extract showed appreciable heavy metal removal efficiency when compared with commercial-grade organic acids. The cheaply available substrate for fermentation and the low cost of the fermentation process in obtaining the crude fermentation extract makes its use economically acceptable.

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