Optimization of Sorption of Lead and Copper from Lake Gerio Water Sample by *Aspergillus niger*

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Abstract: Forefront among those to be affected by significant copper (Cu) and lead (Pb) contamination of Lake Gerio are fish within, crops whose irrigation source is water from the lake and ultimately humans who may intuitively find it drinkable. By employing Minitab central composite design (CCD) in which temperature, contact time and *Aspergillus niger* biosorbent dosage were varied from 25-50℃, 20-180 min and 5-15g, respectively, this study hopes to optimize Cu and Pb ions sorption, in order to find the best remediation condition for the raw water. Ab initio, *Aspergillus niger* isolated from the soil was added to water sample from the lake (kept at pH = 6) to formulate a 0.02 L aqueous solution. Atomic Adsorption Spectroscopy (AAS) revealed the presence of Cu^{2+} and Pb^{2+} in the mixture before and after sorption while Energy-Dispersive X-ray (EDX) analysis gives the composition of other heavy metals before sorption and in low concentrations after adsorption. Twenty runs response surface methodology CCD, generated important statistical metrics to assess the quadratic model for adsorptive capacity (q_e) and removal efficiency (RE) of both Cu and Pb. Optimum q_e for Pb^{2+} sorption is 0.000191 mg/g (100% RE) at 20 min, 25℃ & 5g while that of Cu^{2+} is 0.0006965 mg/g at 180 min, 37.12℃ & 5g and 0.000571 mg/g (98.051% RE) at 50 min, 50℃ and 5g dosage. These optimal combinations occurred at $\cong$ 1.0 desirability at R^2 values of 0.7987 & 0.4925 (q_e and RE: Pb) and 0.8932 & 0.6757 (q_e and RE: Cu). However, the fungi best adsorb Cu based on good actual model obtained compared to Pb, even though Pb optimal sorption duration (20 min) is faster/lower. Though sorption is achieved, showing the promise held by *Aspergillus niger* in this study, model improvement is still needed in order to increase precision of estimates (especially for $q_{e\,Pb}$), closeness of fits and in achieving a better 2-factor combinations from 3D surface plots. If *Aspergillus niger* can be harnessed for large-scale water treatment, it could contribute to mitigating the impact of heavy metal contamination in aquatic ecosystems.

1. Introduction

Lake Gerio is located in Northwestern part of Jimeta, in Northeastern Nigeria between longitude 12°26'55'' E and Latitude 9°18'29'' N (Barde *et al.*, 2020). Irrigation schemes had been exploited to study the growth of tomato (Oke, 2015) and rice (Jamala *et al.*, 2011; Usman, 2012) using the

natural lake water. Presence of microbial loads in Lake Gerio (Adeyemo *et al.*, 2021; Idowu *et al.*, 2022; Onyia *et al.*, 2011) as well as parasitic infections (Amos *et al.*, 2018) and heavy metals (Bawuro *et al.*, 2018; Ogwu & Kwabuge, 2019); especially in tissues of fishes in the lake which will make or mar fish production and consumption have all been investigated (Abiodun & Miller, 2007; Ekundayo *et al.*, 2012; Karim *et al.,* 2016). Oil from boats, urine, faeces, pesticides and fertilizers washed into the lake from nearby irrigated farms are some of the imminent pollutants (Ahmad & Galtima, 2011; Shinggu *et al.*, 2015). Thus, in order to assess the water quality of the lake for drinking and irrigation, Ogwu & Kwabuge (2019), Tya & Abbayero (2017) and Barde *et al.* (2020) carried out a coliform, conductivity, transparency, temperature, heavy metal level, total dissolved solids, total nitrogen and pH tests on sample of the water body. As a mitigating move, Luka *et al.* (2017) investigated the possibility of using mixed culture of *Bacillus subtilis* and *Pseudomonas alcaligenes* to reduce pollution of the lake. Accidental consumption of heavy metals such as lead (Pb) in water causes neurological damage, joint and muscle pain (feebleness in wrists, fingers and ankles), behavioral disorders, impaired blood synthesis, anemia, kidney damage, reproductive issues, hypertension, severe stomachache gastrointestinal distress, impaired learning and IQ and peripheral neuropathy (Bayuo *et al.*, 2022; Chokor, 2023). Copper (Cu) intake in contaminated water causes almost similar disease and symptoms with additional liver damage, oxidative stress, gastroenteritis and Wilson's disease (Albesa II *et al.*, 2019). Adequate literature search during this investigation discloses the presence of both Pb and Cu in Lake Gerio. Their toxicity to humans and animals is one of the strong reasons leading to numerous studies regarding their removal through biosorption.

Sorption is a simple, cheaper and environmentally friendly technique used to remove heavy metals from contaminated water using agricultural biomass, nanoparticles and microorganisms. For instance, Cu or Pb or both were previously removed using *Casuarina* leave powder (Srinivasa *et al.*, 2013), phenothiazine-imine-chitosan (Bejan & Marin, 2023), local marine seaweed (Ng, 2022), onion skin (Waweru *et al.*, 2016), mucuna shell, Africa elemi seed, oyster shell (Okolo *et al.*, 2020), magnetic iron nanoparticles (Al Shammari *et al.*, 2023), *Pinus radiata* bark resin (Aranda *et al.*, 2022), orange peel (Afolabi *et al.*, 2022; Surovka & Pertile, 2017), sawdust (Al-Janabi *et al.*, 2023), *Gmelina arborea* leaves (Tijani *et al.*, 2011), kaolinite clay (Ndiritu *et al.*, 2017), hydroxyapatite (El Hammari *et al.*, 2023), rubber tires (Cherono *et al.*, 2021), sago waste (Quek *et al.*, 1998), tea waste (Wan *et al.*, 2014), Algerian diatomite (Safa *et al.*, 2012), fennel seeds (Mabungela *et al.*, 2022), citric acid modified wood (Low *et al.*, 2004) and doum palm kernel (Gutti *et al.*, 2015) from aqueous solutions of water and wastewater. Several other metals present in water bodies such as molybdenum (Mb), zinc (Zn), iron (Fe), mercury (Hg), boron (B), calcium (Ca), cadmium (Cd), nickel (Ni), manganese (Mn), cobalt (Co), antimony (Sb), chromium (Cr), silver (Ag) and arsenic (As) are hazardous in certain concentrations and must be eliminated (Qasem *et al.*, 2021). Hence, this study task involves finding the Cu and Pb levels in Lake Gerio by carrying out Atomic Absorption Spectrometer (AAS) detection, normally employed in the literature (Elkhatat *et al.*, 2021) and Energy-Dispersive X-ray (EDX) analysis of collected water sample; preparation of *Aspergillus niger* culture and using it as adsorbent to examine the effect of varying contact time, temperature and dosage on the two elements' sorption from the contaminated water; studying the adsorbent effect after adsorption by running a Scanning Electron Microscopy (SEM) and Fourier Transform Infrared

Spectroscopy (FTIR) analysis and; lastly, extracting the best parameter combinations for maximum Cu and Pb percent removal and uptake using Minitab software.

Alternative study of the effects of varying time, pH, salinity, temperature adsorbent dosage and metal ion concentration in batch mode (Bhateria & Dhaka, 2019; Jonglertjunya, 2008; Musumba *et al.*, 2020; Somsani, 2012; Sravani *et al.*, 2012), on microbial-adsorbent usage and other organic waste activated carbons to remove heavy metals are countless in the literature. These parameters are best optimized using suitable design of experiment (DOE) response surface methodology (RSM) tool. But RSM optimization studies on hundreds of existing fungi and algae is still not exhausted. John (2012) looked at the potentials of Cd, Pb and Cu removal from contaminated water using algae; Sharif *et al.* (2022) utilized *Eucheuma denticulatum* for Pb sorption; while Singh *et al.* (2007) employed *Spirogyra neglecta* to adsorb Cu and Pb. Countless *Aspergillus* species, including *Lepiota hystrix* (Kariuki *et al.*, 2017), *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus versicolor* (Gazem & Nazareth, 2013; Iram *et al.*, 2015), *Lentinula edodes*, *Agaricus bisporus* (Castanho *et al.*, 2021), *Aspergillus terreus*, *Aspergillus hiratsukae* (Abou-Taleb *et al.*, 2017; Palanivel *et al.*, 2023), *Aspergillus neoalliaceus* (Aracagok, 2022), *Aspergillus oryzae* (Chatterjee & Abraham, 2016) and *Penicillium sp.* (Dusengemungu *et al.*, 2020; Ezzouhri *et al.*, 2010; Price, 2000), had also been researched, without analyzing their chosen experimental parameter/conditions performance based on selected designs like central composite design (CCD), Box-Behnken design (BBD), Taguchi designs, Plackett-Burman design (PBD) and full factorial designs. Use of CCD RSM subset is rampant for similar study (Afolabi *et al.*, 2021; Amini *et al.*, 2008; Ghosh *et al.*, 2013; Kaur *et al.*, 2019; Yusuff, 2017), due to its efficiency, flexibility, precise estimation of response surfaces with relatively fewer experimental runs, inclusion of star or axial points that allows for exploring curvature of the response surface and high resolution offer in terms of factor levels for investigating both linear and quadratic effects over other designs. In view of this comparative advantage, CCD is adopted for this study to visualize the 3D response-factor relationship.

2. Methodology

2.1. Water Sample Collection

Sampling was carried out along the mainstream drainage channel of Lake Gerio. Water samples were randomly collected upstream. Water samples were collected in nitric acid (HNO₃) pretreated 1.5 L polypropylene bottles for metal detection and in 500 mL amber colored bottles for physico-chemical assessment. These bottles were labelled and stored in a cooler for onward transfer to the laboratory.

2.2. Fungal Culture

Aspergillus niger used in the present study was isolated from soil as reported by David *et al.* (2020) and Palanivel *et al.* (2023). Soil was collected from a refuse dump in Doubeli Junction area of Jimeta Yola, Adamawa State, Nigeria. After microscopic confirmation, the fungus was grown on potato dextrose agar at 25℃ for 48–72 h in accordance with Shrivastava & Gupta (2021) and Abdoljabar *et al.* (2021). Pure cultures of *Aspergillus niger* were maintained on potato dextrose agar slants. Other authors utilized the fermentation means of citric acid production to isolate *Aspergillus niger* (Spanelova *et al.*, 2003). To identify the fungus, a small portion of the pure isolate was teased with a

sterile inoculating loop into 2 drops of lacto phenol in cotton blue on a clean slide and a cover slip was placed on it. This was examined under a light microscope. Identification of fungus was done using morphological parameters. It includes, the examination of the size, shape, color, spore formation, number of days for the fungus to reach 9 cm maximum diameter of the petri dish and the texture of the fungal growth.

2.3. SEM, AAS, EDX and FTIR of Aqueous Solution

Cu and Pb heavy metals present in the digested water samples were quantified in flame of UNICAM 969 AAS, also used by Klos (2018) and Uzun & Guzel (2000). Availability and distribution of the heavy metals in Lake Gerio water was evaluated by comparing the statistical mean levels of the metals in the water samples relative to each other. A constant initial Pb and Cu concentrations in the lake was recorded following the APHA 1998 standard test method (Lipps *et al.*, 2023). At specified contact time, *Aspergillus niger* dosage and temperature based on 20 runs, an AAS was carried out to determine the final concentration of the metals after sorption. The analysis was carried out at Central Multi-Purpose Geo-Physics and Geo-Chemical Laboratory Units at Modibbo Adama University, Nigeria. Alternatively, EDX analysis was carried out on the Lake water sample before and after Cu and Pb adsorption to determine the entirety of the metals present.

Surface modifications of the biosorbent before and after encounter with Pb as well as Cu were analyzed using SEM analyzer. *Aspergillus niger* biosorbents were mounted on aluminum slabs with gold coating for better visualization of micrographs. To identify molecular compound present before and after adsorption, the sample was subjected to FTIR analysis to measure the absorbance of infrared radiation by the Lake Gerio water sample. Resultant spectrum was then used to identify the functional groups present in the compound.

2.4. Batch Sorption Studies

A batch equilibration method employed in Sarma *et al.* (2015) was used to realize the sorption experiments. Using a suitable pH neutralizer, the Lake Gerio water sample pH was adjusted before experimentation to approximately 6, in accordance with optimum obtained in Pansuphaphol *et al.* (2014). Separate mixtures of cultured 5, 10 and 15g *Aspergillus niger* and the water sample, making a total of 0.02 L of mixture, were shaken on a horizontal shaker for 20, 100 and 180 mins. Minitab 17.0 based on the CCD-RSM was initiated using 5-15g dosage, 20-180 min contact time and 25-50℃ temperature as Factors A, B and C to give the number of experimental runs and factor value combinations to be used. After every run, samples were taken out and the Cu and Pb metals present after sorption were filtered out. Before then, the final concentrations of the metals were determined using AAS or EDX analyzers, depending on their ability to detect the concentration levels in the final water sample. Factors A, B and C were tied against 2 responses (R2 and R1), named percent removal or removal efficiency (RE) and the respective metals uptake, q_e (mg/g). Both output parameters are given by Equations 1 and 2 (Gutti *et al.*, 2015; Musumba *et al.*, 2020; Waweru *et al.*, 2016), respectively:

$$RE(\%) = \frac{C_i - C_f}{C_i} \times 100 \qquad\qquad (1)$$

$$q_e = \frac{V(c_i - c_f)}{M} \tag{2}$$

where, C_i = initial metal ion concentration (mg/L), C_f = final metal ion concentration (mg/L), M = mass of the biosorbent (g) and V = volume of the reaction mixture or metal solution (L).

2.5. Biosorption Models and Optimization

In Minitab V21.1, 'Stat' menu was entered and 'Create Response Surface Design' was selected within which 3 continuous factors were defined. Face centered alpha value (1.682) with single or 1 block, 20 runs and default number of center points was allowed. Low and high cube points levels were then defined for the respective factors to create a randomize number of runs. Suggested experimental values for each run was recorded and then plugged into Equations 1 and 2 to calculate R1 and R2 to complete the worksheet table. R1 and R2 response surface designs were then analyzed where the confidence interval is two-sided, box-cox transformation is null and confidence level for all intervals is 95.

Values of RE and q_e were calculated using Equations 1 and 2 and inputted into the RSM DOE for each run in the Minitab tool. Expected outcome were statistical performance variables estimates and 4 suitable model for both q_e and RE based on Cu and Pb sorption. These models were used to calculate the R1 and R2 predictions. Under the optimization sub-menu, maximum responses for given combinations of input or factors were determined and showcased using 3D contour plots. In addition, the desirability under which such occurs was noted.

3. Results and Discussion

3.1. SEM Micrograph of Aspergillus niger

Morphology of Pb and Cu explained earlier and determined with the use of SEM machine, resulted in a microporous structure observed at 2000× and 1000× resolution for the biosorption, as shown in Figure 1. SEM micrographs of treated and untreated biosorbent were compared to understand the morphological structures that occurred in the surface of the biosorbent (Yusuf, 2019) against the biosorption of Pb ions. Visible changes between the treated and untreated biosorbent in terms of surface pores, morphological disintegration and cylindrical structures were observed, as discussed in Al Shammari *et al.* (2023). Presence of Pb crystals over the surface of the *Aspergillus niger* biosorbent evidenced the biosorption potential of dead fungal biomass. SEM of *Utricularia aurea* (Yoonaiwong *et al.*, 2011) and *Aspergillus niger* (Santhi & Jagadeeswari, 2015) sorption of Pb and *Aspergillus awamori* sorption of Cd (El-Sayed, 2015) also revealed noticeable changes in structural patterns after adsorption.

3.2. Concentration of Metallic Elements

Elemental composition information based on X-rays emitted when metal ions sample solution before and after adsorption are bombarded with electrons reveals no presence of both Cu and Pb. Before sorption, C, Ca and K seemed to be more concentrated in the Lake Gerio water, as evidenced in Figure 2, while C, N and K forms most part of the mixture after adsorption.
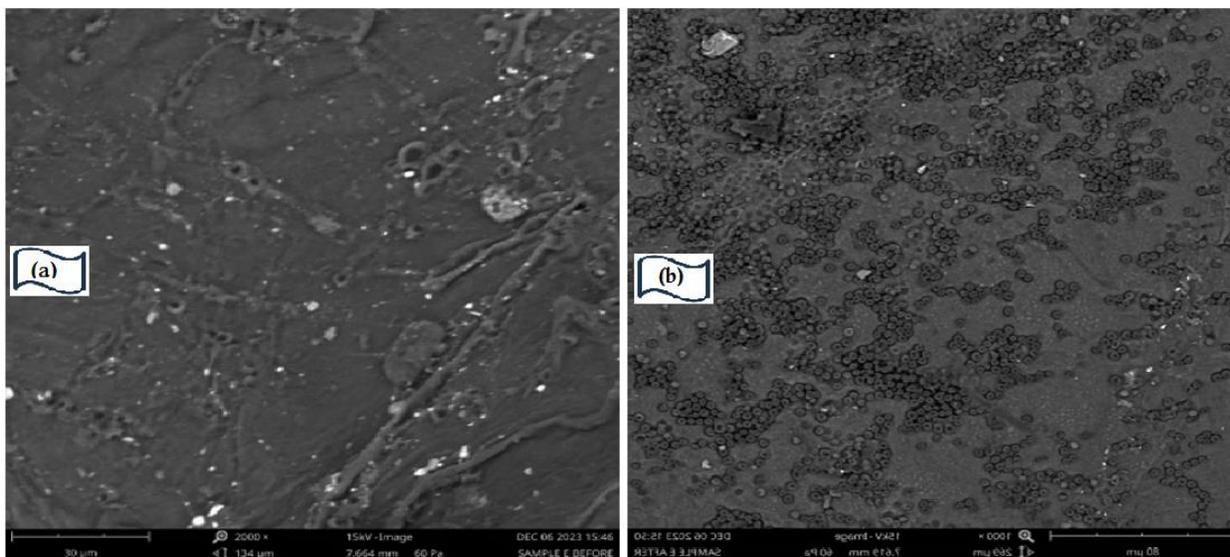
Figure 1: SEM (a) Before and (b) After Biosorption
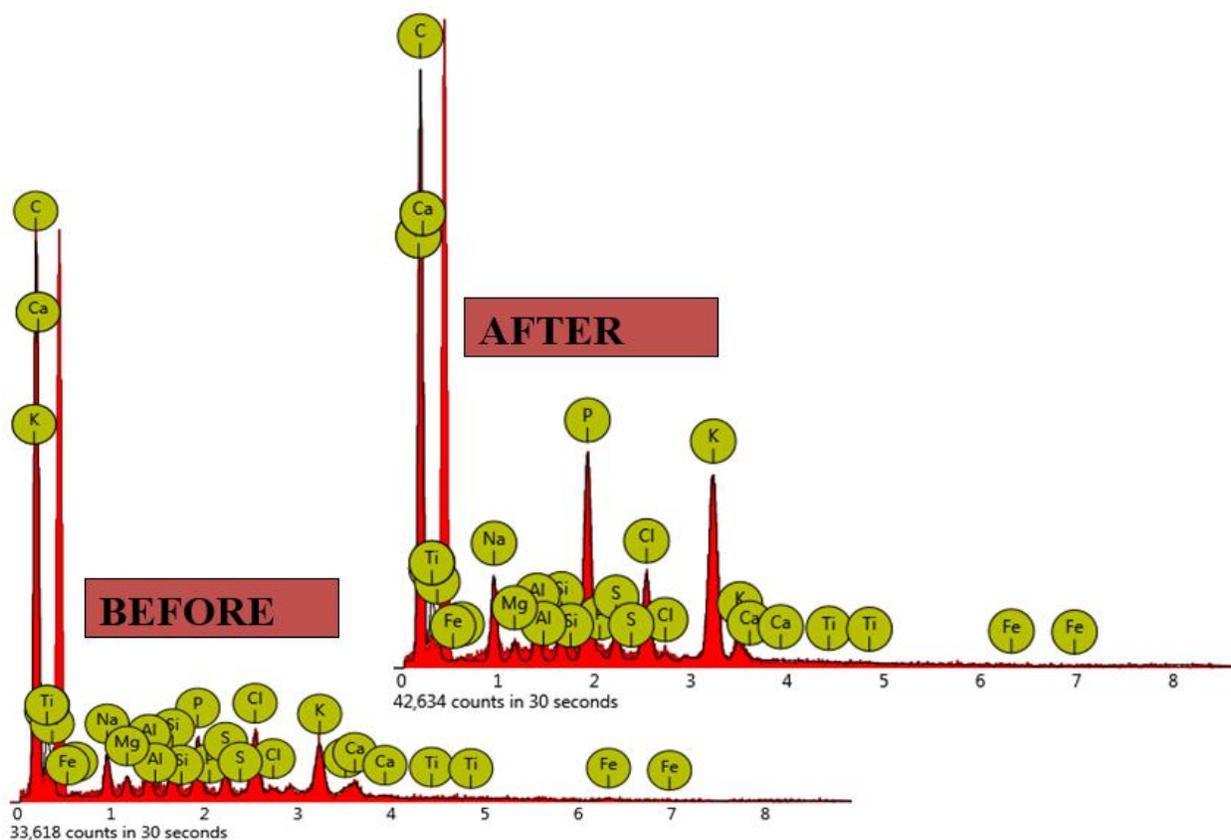
Figure 2: EDX Images of *Aspergillus niger* Before and After Sorption

EDX in both Figure 2 and Table 1 show zero Pb and Cu in the fungi-water mixture before and after adsorption. It is in consonance with findings of Luka *et al.* (2021) where EDX analysis also revealed no Pb^{2+} concentration in the aqueous solution examined. There are however clear evidences of reduction in concentrations of C (from 61.01-58.54 ppm), N (24.17-17.90 ppm), S (0.92-0.77 ppm),

Si (1.11-0.9 ppm), Al (1.03-0.79 ppm), Ca (0.89-0.49 ppm), Mg (0.66-0.18 ppm), Fe (0.6-0.06 ppm) and Ti (0.12-0.00 ppm) after sorption, whose REs are respectively 4.2%, 25.94%, 16.3%, 18.92%, 21.36%, 44.94%, 72.73%, 90% and 100%. It further demonstrate the capability of the microorganism to remove several other minerals from the lake water. Perhaps, 0 ppm of Cu^{2+} and Pb^{2+} in the sample can be attributed to the EDX machine's poor ability to detect very low-concentration elemental compostion in the Lake Gerio water mixture. Hence, the employment of additional metallic mineral detection technique such as the AAS can be used in this case.

Table 1: Atomic and Weight Concentrations of Metallic Ions Present Before and After Adsorption

S/No.	Element	Before Adsorption		After Biosorption	
		Atomic Conc.	Weight Conc.	Atomic Conc.	Weight Conc.
1.	Carbon (C)	69.90	61.01	70.91	58.54
2.	Nitrogen (N)	23.74	24.17	18.60	17.90
3.	Potassium (K)	1.08	3.06	3.40	9.13
4.	Chlorine (Cl)	1.08	2.77	2.61	5.55
5.	Phosphorus (P)	0.89	2.00	1.30	3.16
6.	Sodium (Na)	1.00	1.67	1.60	2.53
7.	Silicon (Si)	0.54	1.11	0.41	0.90
8.	Aluminium (Al)	0.53	1.03	0.43	0.79
9.	Sulphur (S)	0.39	0.92	0.40	0.77
10.	Calcium (Ca)	0.31	0.89	0.29	0.49
11.	Magnesium (Mg)	0.37	0.66	0.05	0.18
12.	Iron (Fe)	0.15	0.60	0.02	0.06
13.	Titanium (Ti)	0.04	0.12	0.00	0.00

After adsorption, concentration of many of the metallic ions in the Lake Gerio water sample portrays the water as drinkable, especially Al, Mg and Ca whose concentrations are below WHO standard (Mahmud et al., 2016). Titanium is not typically found in high concentrations in natural lake water, as also found by Linnik & Zhezherya (2015) on Ukraine water bodies. It is more commonly associated with minerals and rocks in the earth's crust. However, trace amounts of Ti can be present in natural water bodies, such as Lake Gerio having 0.12 ppm (within WHO guideline value of 0.1 mg/L) as a result of weathering and erosion of Ti-containing minerals in the surrounding geological formations. Additionally, human activities such as industrial discharges or mining operations can introduce Ti into water bodies which also affects seabirds, as researched by Hauser-Davis et al. (2020). It is now obvious that the sensitivity of EDX is limited, and it may not be able to detect elements present in very low concentrations. In the case of Cu and Pb, their concentrations in the Lake Gerio water sample may have been below the detection limit of the EDX instrument used in the study. Moreover, EDX is a surface analysis technique, which means that it only detects elements present on the surface of the sample. If Cu and Pb were present in the interior of the *Aspergillus niger* biomass, they may not have been detected by EDX. And whether Cu and Pb can be reduced/removed from the sample collected is dependent on the adsorbent performance. Spanelova et al. (2003) and Palanivel et al. (2023) mentioned that the biosorption process depends on the composition of the fungal cell wall.

3.3. Modelled Experiment and Ions Composition from AAS

Initial Pb concentration (0.19 mg/L) and Cu concentration (0.145 mg/L) was detected by AAS, as showcased in Table 2. Ogwu & Kwabuge (2019) discloses lower composition of Pb (i.e., 0.0007 ppm) and Cu (i.e., 0.005 ppm) in Lake Gerio 4 years ago, revealing a significant rise compared to this study findings. Collin *et al.* (2022) assert that 42% reduction in plant's root growth is observed if Pb is in excess in soil. So, a Pb^{2+} concentration of 0.19 mg/L in Lake Gerio water would be equivalent to 190 mg/kg in soil, assuming a density of 1 g/cm³ or 1,000 g/L for the soil. Consequently, Pb intoxication effect on planted crops on fertile soil around the lake using the lake water, will be observed – as the amount falls above 116.352 mg/Kg allowed for agricultural plants, as calculated using Castro-Bedriñana *et al.* (2021) report. Cu ranged from 90-120 mg Cu/kg in surface soils according to Park & Cho (2011), less than 145 mg/Kg (in soil equivalence of 0.145 mg/L in Lake Gerio water) and favorable if it were to be maintained within acceptable limit of 270 mg/Kg in soil for plant growth.. At each level of the DOE runs, AAS analysis also gives the composition of the metal ions after sorption. The fact that these C_fs are lower than the constant C_i for the respective ions signifies serious adsorption effect of the *Aspergillus niger* on the Lake Gerio water sample after application.

Table 2: Runs for Varied Empirical Conditions and Final Concentrations of Metal After Sorption

Run	Factors			Pb		Cu	
	A: Time (min)	B: Temp. (°C)	C: Dosage (g)	C_i (mg/L)	C_f (mg/L)	C_i (mg/L)	C_f (mg/L)
1.	100	37.5	5	0.19	0.1	0.145	0.023
2.	100	37.5	10	0.19	0.02	0.145	0.024
3.	100	37.5	10	0.19	0.19	0.145	0.013
4.	100	37.5	10	0.19	0.08	0.145	0.048
5.	180	50	15	0.19	0.1	0.145	0.03
6.	20	50	15	0.19	0.12	0.145	0.084
7.	100	50	10	0.19	0.07	0.145	0.14
8.	20	25	15	0.19	0.05	0.145	0.061
9.	180	25	5	0.19	0.13	0.145	0
10.	100	37.5	15	0.19	0.18	0.145	0.057
11.	180	37.5	10	0.19	0.188	0.145	0
12.	100	37.5	10	0.19	0.01	0.145	0.102
13.	20	37.5	10	0.19	0.11	0.145	0.066
14.	100	25	10	0.19	0.04	0.145	0.104
15.	180	25	15	0.19	0.024	0.145	0
16.	100	37.5	10	0.19	0	0.145	0
17.	20	50	5	0.19	0	0.145	0.093
18.	100	37.5	10	0.19	0	0.145	0.104
19.	180	50	5	0.19	0.16	0.145	0.023
20.	20	25	5	0.19	0	0.145	0.024

C_f declined to as low as 0 ppm in run 16-18 and 20 for Pb sorption and run 9, 11, 15 and 16 for Cu sorption. Though, the temperatures, contact time and dosage amounts at which they occur are distinct, points to the existence of optimal factor values within the range 100% removal was achieved. Under the CCD Minitab DOE, these optimal conditions under the highest desirability value can be found. Based on the differences in C_i and C_f of the metals after sorption, it can be inferred that the metal which experienced a greater reduction in concentration and thus was best adsorbed by the fungi is Cu. Cu^{2+} concentration discovered in the sample before and after adsorption is below 3mg/L WHO standard in drinking water (Liu *et al.*, 2023). But using a completely different experimental conditions, Sravani *et al.* (2012) reported Pb above Cu. It can be observed that Run 10 performs very poorly in terms of the adsorption of both Cu and Pb in their study. Final concentration of Cu after sorption was 0.000117 mg/L, which is only a slight reduction from $C_i = 0.145$ mg/L. Similarly, C_f of Pb after sorption was 0.000179 mg/L, which is also a relatively small reduction from the initial value of 0.19 mg/L. While EDX is valuable for surface analysis and elemental identification in solid samples, it is not the appropriate technique for detecting and quantifying metal concentrations in solution at the levels mentioned. AAS capability is also rivalled by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique (Lindholm-Lehto, 2019; Voica *et al.*, 2012).

3.4. FTIR of Lake Gerio Water Sample

FTIR analysis was carried out on the *Aspergillus niger* biosorption produced in order to identify the functional group responsible for the sorption. Figure 3 depicts the infrared spectroscopy image of the *Aspergillus niger* before adsorption of the Pb and Cu ions in Lake Gerio water sample. It clearly showed 8 peaks of various functional groups on the samples that correspond to their vibrational frequencies in a molecule.
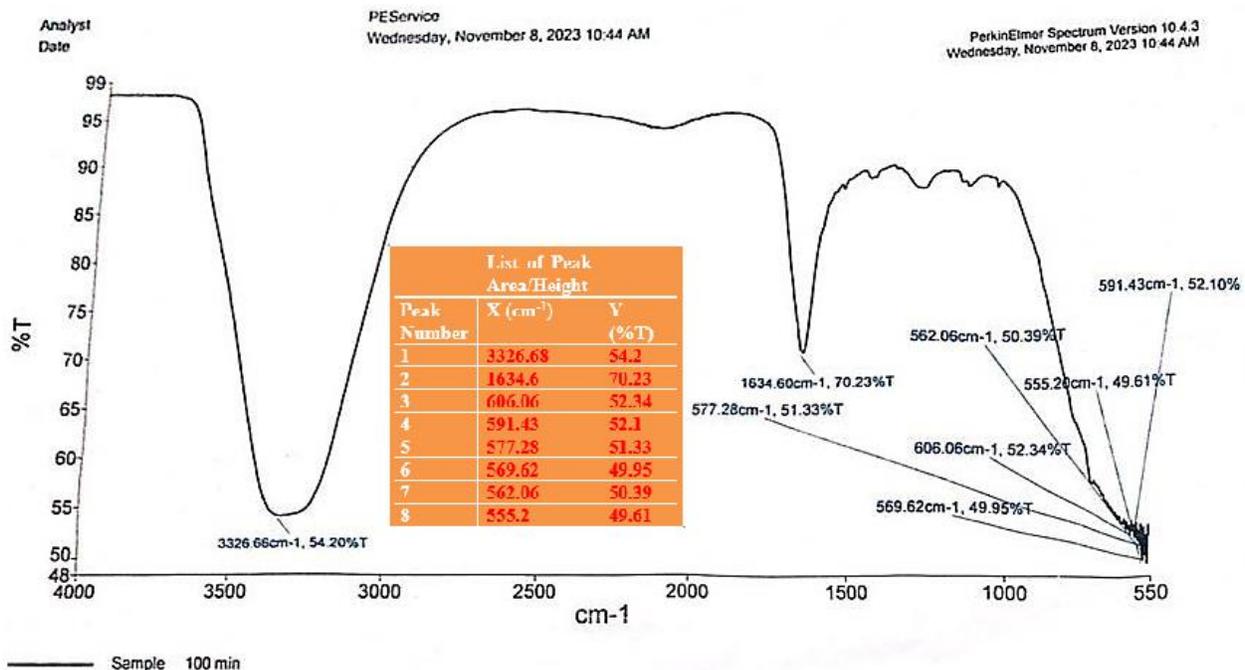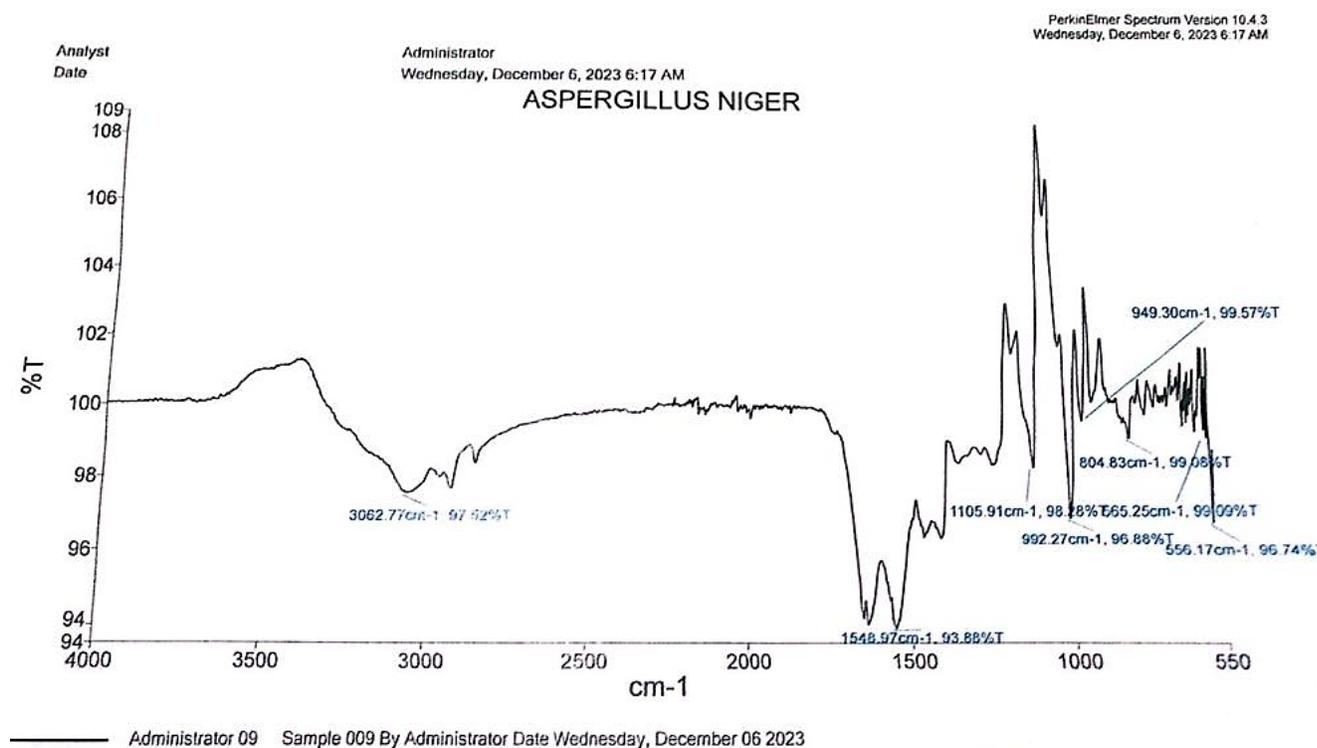

Figure 3: FTIR of *Aspergillus niger* Before Adsorption

Wavelength of 3062.77 cm^{-1} typically corresponds to C-H stretching vibrations in aliphatic non-aromatic hydrocarbons, such as methyl or methylene groups, nearly out of the range reported by Aranda *et al.* (2022) for tannins during Cu adsorption; that of 1548.97 cm^{-1} indicates C=C stretching vibrations, often found in aromatic compounds like benzene rings; 1105.91 cm^{-1} value is suggestive of C-O stretching vibrations, common in alcohols, ethers or esters; wave number equal to 992.27 cm^{-1} may be associated with C-N stretching vibrations, often found in amines (Flouty *et al.*, 2019); an 804.83 cm^{-1} value indicates C-H bending vibrations, commonly observed in various organic compounds; region of 556.17 cm^{-1} is associated with C-Cl stretching vibrations, often found in chlorinated compounds (Waweru *et al.*, 2016) and; lastly, 565.25 cm^{-1} typically corresponds to out-of-plane bending vibrations, often observed in substituted aromatic compounds. Figure 4 FTIR depicts the infrared spectroscopy image of the *Aspergillus niger* after biosorption of Pb and Cu from Lake Gerio, showing peaks of 8 various functional groups on the samples. These peaks correspond to vibrational frequencies of the functional groups in a molecule – correspondingly, as demonstrated in Figure 3.

Figure 4: FTIR of *Aspergillus niger* After Adsorption

Intensities of these peaks differ as shown in Figure 4, indicating potential changes in the abundance or interactions of these functional groups as a result of the adsorption process (Gaplovska *et al.*, 2018; Simonovicova *et al.*, 2021). For example, if the intensity of a peak at a specific wave number decreases after adsorption, it may suggest that the corresponding functional group is involved in the binding of the metal ions. Conversely, if the intensity of a peak increases after adsorption, it may

suggest that the corresponding functional group is no longer involved in the binding of the metal ions (Cu^{2+} & Pb^{2+}).

3.5. Biosorption Model, Fitting and Statistics

After entering calculated q_e (R1) from the experimental investigation for all the 20 runs and choosing the CCD scheme in the RSM Design Expert software (Minitab 21.1), models in line with the 3 chosen factors were generated. All inputs and declarations suggested a quadratic model for the Pb q_e and RE observations, as given by Equations 3 and 4, respectively.

$$R1_{Pb} = \begin{matrix} 0.00208 - 2.39519 \times 10^{-6}A - 5.34127 \times 10^{-5}B - \\ 8.71396 \times 10^{-5}C - 1.12436 \times 10^{-6}AB + 3.81469 \times 10^{-7}AC \\ -1.494 \times 10^{-7}BC - 1.19595 \times 10^{-8}A^2 + 7.13338 \times 10^{-7}B^2 \\ + 1.12436 \times 10^{-6}C^2 \end{matrix} \tag{3}$$

$$R2_{Pb} = \begin{matrix} 306 - 0.070A - 13.9B + 9.9C - 0.00303\,A^2 + 0.193B^2 \\ -0.586C^2 - 0.0024AB + 0.0553AC - 0.122BC \end{matrix} \tag{4}$$

In terms of uncoded units, quadratic modelled equations for Cu q_e and RE values, are given by Equations 5 and 6, respectively.

$$R1_{Cu} = \begin{matrix} -2.88546 \times 10^{-4} + 1.32303 \times 10^{-6}A + 5.73632 \times 10^{-5}B - \\ 1.14751 \times 10^{-4}C - 1.16667 \times 10^{-9}AB - 1.84583 \times 10^{-7}AC \\ -1.41333 \times 10^{-7}BC + 9.2661 \times 10^{-9}A^2 - 7.59661 \times 10^{-7}B^2 \\ +5.51879 \times 10^{-6}C^2 \end{matrix} \tag{5}$$

$$R2_{Cu} = \begin{matrix} -217 - 0.256A + 18.27B - 11.3C + 0.00374\,A^2 - 0.2395B^2 \\ +0.765C^2 - 0.0006AB - 0.0153AC - 0.073BC \end{matrix} \tag{6}$$

These equations were used to compute the predicted responses observed in Table 3; while the percent difference between the predicted and observed values can be used to evaluate the accuracy of the models.

Runs 1, 4, 5, 6, 9, 15, 17, 19 and 20 for Pb sorption have % differences of their observed vs. predicted q_es ranging from 1.357-18.542%, indicating that the predicted values are very close to the experimental values. On the other hand, runs 2, 3, 7, 8, 10, 11, 12, 13, 14, 16, and 18 have larger % differences of $\geq 26\%$, suggesting that the model (Equation 3) may not be as accurate in predicting the q_e for those runs. By implications, the model may need further refinement or improvement to better capture the underlying relationships for the experimental conditions associated with the runs exhibiting larger % differences. Also in Table 3, small q_e % differences for Cu ranging from 0-18.95% occur in runs 1, 4-6, 8-9, 11, 13-15, 17, 19-20 while the remainder are $\geq 24.793\%$. Therefore, Equation 5 have similar attribute with Equation 3 in predicting q_e, but is far better given that larger number of the observations have q_e values that's approximately equal to the calculated. Whether these predictions best fit the responses from empirical runs is best demonstrated using predicted versus actual data points plot in Figure 5 as well as the 10 selected statistical metrics in Table 4.

Table 3: R1 and R2 Predictions Based on the Biosorption Quadratic Models

Run	**Pb** q_e (Expt.)	q_e (Prdct.)	RE (%)	**Cu** q_e (Expt.)	q_e (Prdct.)	RE (%)
1.	0.00036	0.00042	47.36842	0.000488	0.00046	84.13793
2.	0.00034	0.000231	89.47368	0.000242	0.000182	83.44828
3.	0	0.000231	0	0.000264	0.000182	91.03448
4.	0.00022	0.000231	57.89474	0.000194	0.000182	66.89655
5.	0.00012	0.000098	47.36842	0.000153	0.000124	79.31034
6.	9.33E-05	0.000076	36.84211	8.13E-05	6.85E-05	42.06897
7.	0.00024	0.000305	63.15789	1E-05	4.87E-05	3.448276
8.	0.000187	0.000138	73.68421	0.000112	0.000112	57.93103
9.	0.00024	0.000221	31.57895	0.00058	0.000584	100
10.	1.33E-05	0.000099	5.263158	0.000117	0.000179	60.68966
11.	0.000004	0.000029	1.052632	0.00029	0.000344	100
12.	0.00036	0.000231	94.73684	0.000086	0.000182	29.65517
13.	0.00016	0.00028	42.10526	0.000158	0.000138	54.48276
14.	0.0003	0.00038	78.94737	0.000082	7.72E-05	28.27586
15.	0.000221	0.000224	87.36842	0.000193	0.000173	100
16.	0.00038	0.000231	100	0.00029	0.000182	100
17.	0.00076	0.000721	100	0.000208	0.00022	35.86207
18.	0.00038	0.000231	100	0.000082	0.000182	28.27586
19.	0.00012	0.000133	15.78947	0.00058	0.000571	100
20.	0.00076	0.000745	100	0.000208	0.000228	35.86207

Observed RE of Pb and Cu in Table 3 and the predicted values in Figure 5 (b & d) together gives predicted vs. actual RE plots. Only 4-5 data points fall on the linear lines in their respective scatter graphs with very low R^2 values of 0.4925 and 0.6757, signifying a poor fit with significantly higher % differences. Additional statistical measurements including adjusted and predicted R^2 can be used to describe the model performance. Based on these metrics (also shown in Table 4), the Cu biosorption model appears to be the better model, as it has higher R^2, adjusted R^2, and predicted R^2 values compared to the Pb biosorption model. These higher values indicate that the Cu biosorption model provides a better fit to the data and has stronger predictive performance compared to the Pb biosorption model. Mean in Table 4 represents the average value of the responses, Coefficient of Variation (C.V.%) provides a measure of the variability of the data relative to the mean and Adequate Precision (adq. prec.) is a measure of the signal-to-noise ratio in the model. According to Milad & Hossein (2017), adequate precision > 4 is desirable which is in agreement with 7.801 and 10.418 equal values (for Pb^{2+} and Cu^{2+} uptakes) obtained herein. Here a 3.465 adequate precision for Pb^{2+} % removal is undesirable, but Cu^{2+} % removal with 5.458 > 4 is desirable. A lower C.V.%, higher adq. prec., and consistent mean value indicate a more reliable and accurate model with better predictive performance. Hence, q_e model (Equation 3) for Pb sorption with 49.36% C.V. < 59.22 C.V.% for RE model (Equation 4) is the most suitable for the metal sorption from Lake Gerio water sample, while Equation 6 is above 5 for Cu, taking C.V.% only into consideration. It is observed that

a lower C.V.% does not correspond to a high R^2, and vice versa. Ranking from best to worst based on the considerations of C.V.%, adq. prec., and mean values, picks Equation 5 as the most favorable followed by 3, 6 and 4. It again puts Cu biosorption efficiency ahead of Pb in this study.
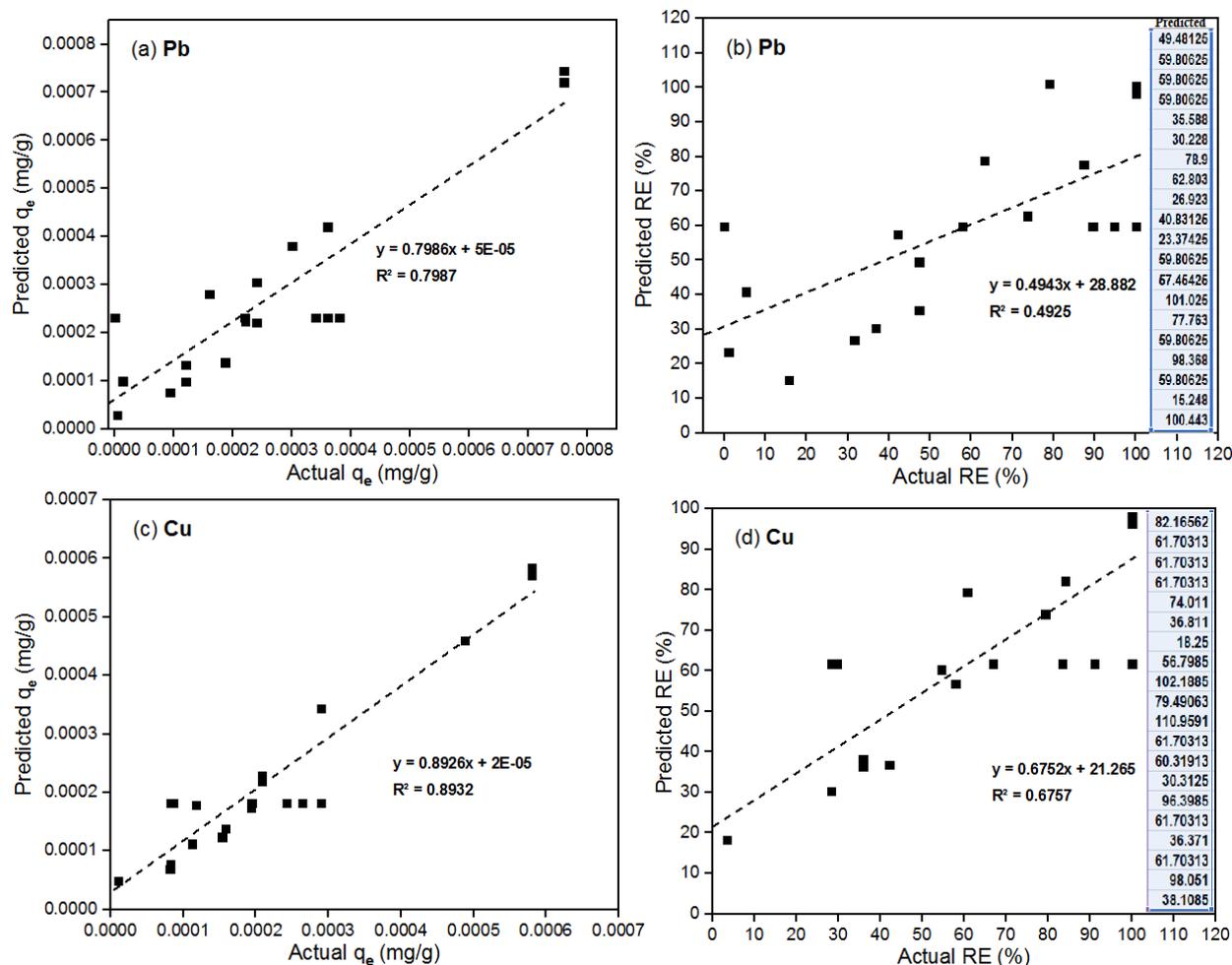

Figure 5: Predicted Versus Actual Output Responses

Table 4: Model Summary Statistics

Parameter	Pb		Cu	
	R1 (q_e)	R2 (RE)	R1 (q_e)	R2 (RE)
Std. Dev.	0.0001298	34.7217	0.0000727	24.0521
R^2	79.87%	49.25%	89.32%	67.57%
Adj. R^2	61.76%	3.58%	79.71%	38.38%
PRESS	0.00000043	30982.04	1.379E-7	11587.42
R^2 (Pred.)	48.32%	0.00%	72.13%	35.04%
AICc	–260.09	-	-	-
BIC	–282.14	-	-	-
C.V.%	49.36	59.22	32.91	37.54
Adq. Prec.	7.801	3.465	10.418	5.458
Mean	0.0002629	58.63	0.0002209	64.07

3.6. Factor Effect on Responses

3D surface plots for Pb and Cu uptake (R1) and % removal (R2) responses in Figure 6 and 7, respectively demonstrate the relationship between the independent variables (A = contact time, B = temperature, and C = dosage) and the dependent variables. Different types of curvature showcased by the 3D surface shapes signals sensitivity of the responses with changes in 2 factors variations. For the R1 plots in Figure 6 (a-c), the curved surfaces illustrate the combined effects of A, B and C on Pb^{2+} uptake. An upward surface curve in a certain direction (Abubakar *et al.*, 2024), suggests that increasing the values of the corresponding independent variables leads to an increase in Pb uptake. Conversely, downwards curvature of the surface, indicates a decrease in Pb uptake with increasing values of the independent variables. Similarly, for the R2 plots, Figure 6 (d-f), the curved surfaces depict the combined effects of factors A, B and C on the RE of Pb.
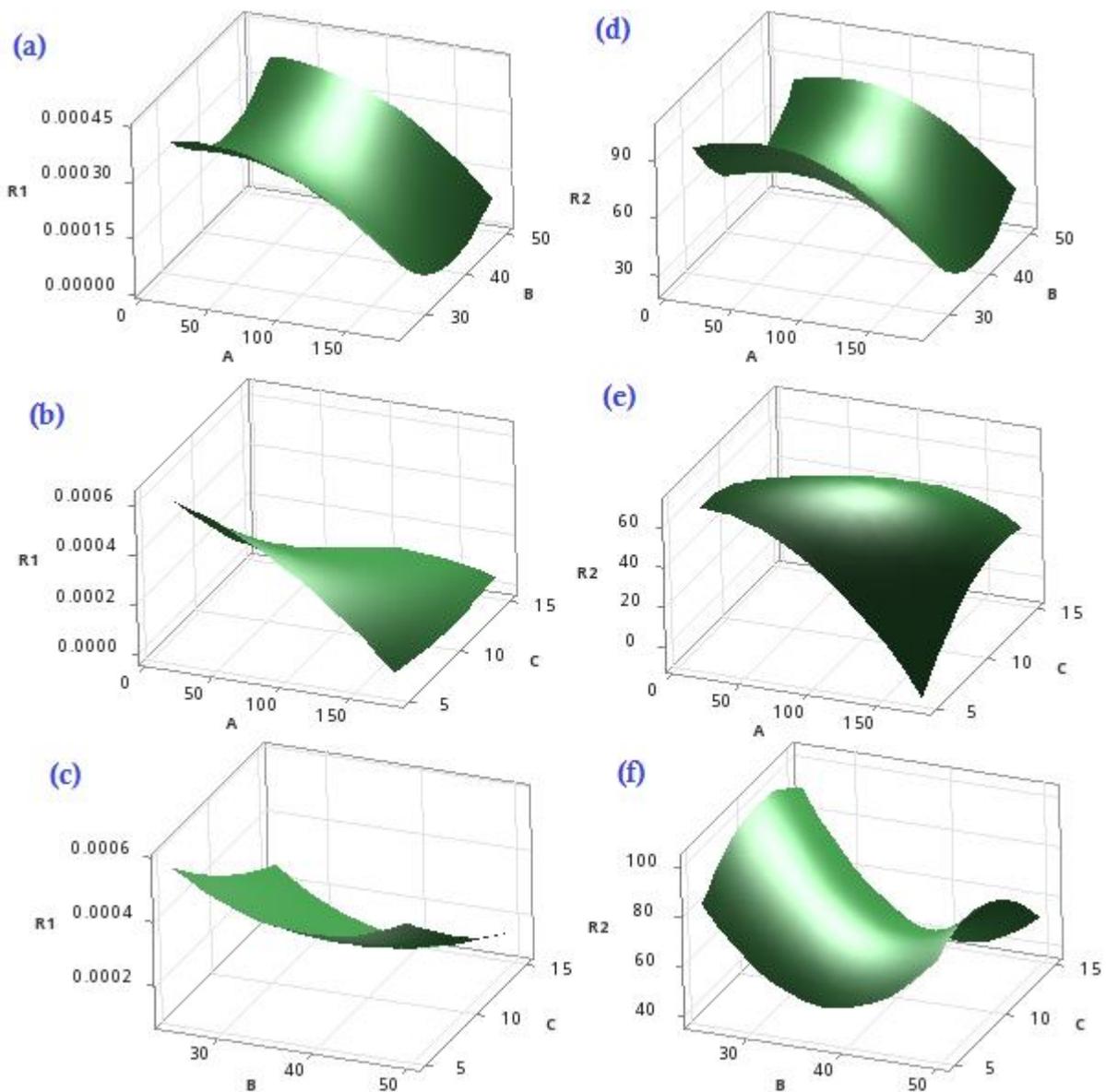
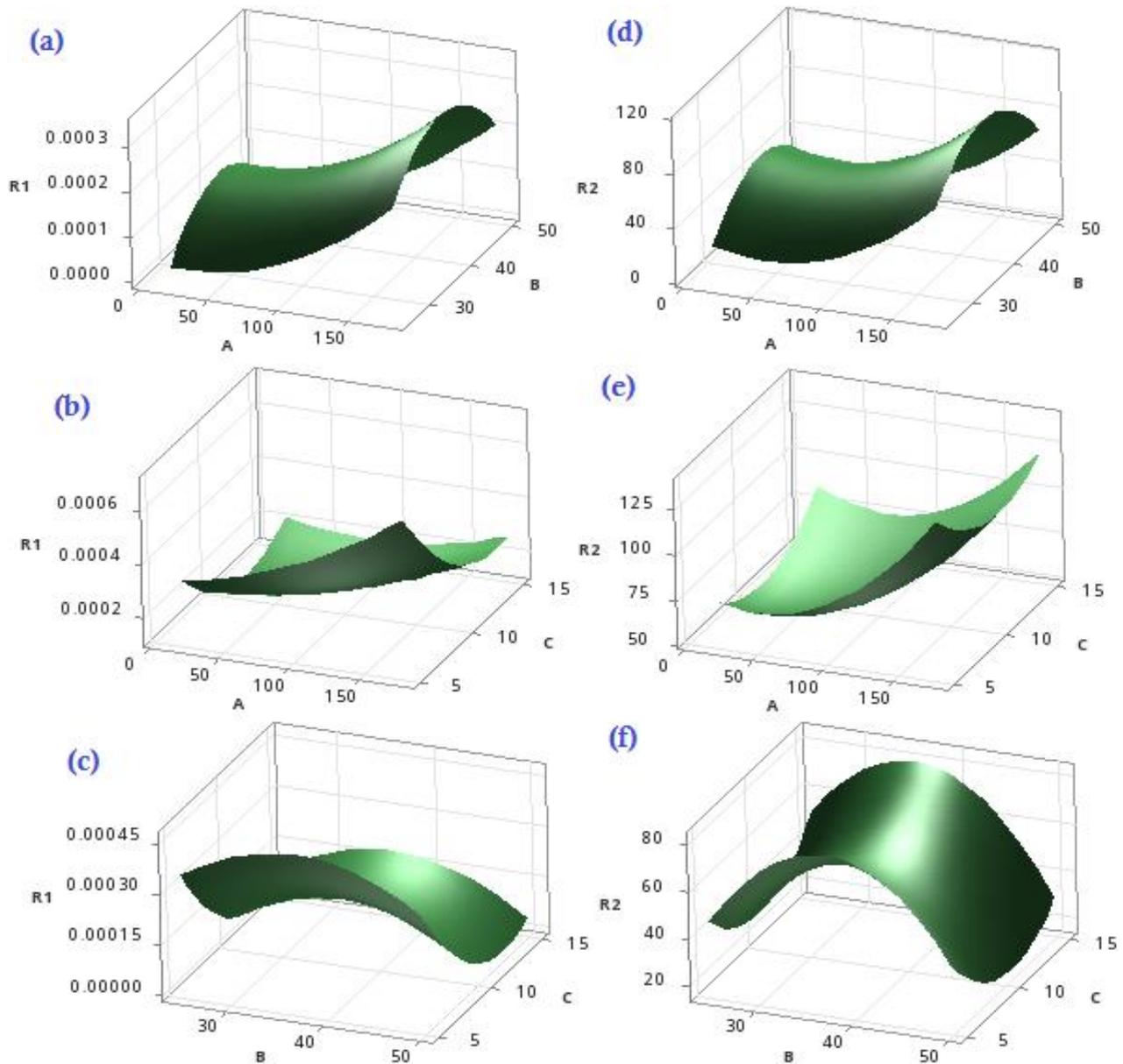**Figure 6:** 3D Surface Plot for Pb^{2+} Uptake (R1) and Percent Removal (R2)

Optimal combinations can be determined by finding the peak or highest point on the surface plot. In Figure 6 (a & d), the highest point on the surface plot corresponds to a contact time of approximately 120 min, a temperature of 40℃, and a dosage of 10 g. This combination of factors maximizes Pb uptake and its RE. Combination of factors that maximizes Pb uptake in Figure 6b is 120 min, 40℃ and 5g *Aspergillus niger* dosage, while achievable optimum RE derived from Figure 6e is at 120 min, 40℃ and 15g. In Figure 6 (c & f), the highest point on the surface plot corresponds to 120 min, 40℃ and 10g and 120 min, 40℃ and 15g for R1 and R2, respectively. The values of (R1, R2) corresponding to the optimal combinations of A, B, and C in Figures 6a, b, c, d, e, and f are therefore (–0.00513, 53.208), (–0.004977, 38.878), (–0.00513, 53.208), (–0.00513, 53.208), (–0.005226, 38.238) and (–0.005226, 38.238). Unexpectedly, all q_e for Pb are negative based on optimal predictive estimates given by Equation 3 in terms of actual factors, as against its positive experimental values in terms of coded factors in Table 3. It points to error in the Pb data and thus impossible to pick an optimum. Same idea is applied in finding the optimal response and factor combinations for Cu^{2+} sorption using *Aspergillus niger* from Figure 7 generated by Minitab.

Maximum Cu sorption from Lake Gerio is achieved at 120 min, 40℃ and varying adsorbent dosage. Optimum dosage from Figure 7 (a & c) is 10g, Figure 7 (b & d) is 5g and Figure 7 (e & f) is 15g. From Figure 7 (a-f), these optimal factors correspond to (q_e, RE) of (0.0002035, 66.796), (0.0005023, 89.701), (0.0002035, 66.796), (0.0005023, 89.701), (0.0001805, 82.141) and (0.0001805, 82.141), respectively. Optimal factor is 120 min, 40℃ and 5g, corresponding to 0.0005023 mg/g and 89.701% in Figure 7 (b & d). It is worthy of note that the 3D surface plots in Figures 6 and 7 are based on the model equations developed using the experimental findings, rather than the experimental findings themselves. Based on the experimental findings, they correspond to keeping A = 100 min, B = 37.5℃ and C = 10g as constant. In addition, the 3D surfaces do not sufficiently give the optimal responses since in each plot either one of the three factors (A, B & C) is not participating. In essence, the foregone optimal combinations and responses are for just 2-factor variations only and not for 3-factor situation.

3.7. Optimal Operating Conditions

Optimal combinations of 3 factors and individual responses are showcased in Table 5 in accordance with the highest desirability obtained in the study. Pb uptake of 0.0007452 mg/g and Cu uptake of 0.0006965 mg/g represent the amount of Pb^{2+} and Cu^{2+} ions, respectively, that can be adsorbed by *Aspergillus niger* biomass per gram of the biosorbent. These values indicate the effectiveness of *Aspergillus niger* in removing Pb and Cu from the aqueous solution. Relatively lower Cu uptake value ($q_{e\,Pb} > q_{e\,Cu}$) indicates that while *Aspergillus niger* is effective in adsorbing Cu, its affinity for Pb is comparatively higher. Also considering the optimal factor conditions, it's clear that the maximum uptake capacity for Pb is achieved in a significantly shorter time (20 min) compared to Cu (180 min). A % removal of Pb and Cu of 105.047 and 137.368%, respectively implies that more Pb^{2+} and Cu^{2+} ions were removed from the solution than were originally present. Deviations from the maximum achievable RE (i.e., 100%) is attributed to possible experimental error, variability in measurements, or limitations in the analytical techniques used to quantify the initial and final concentrations of Pb and Cu. However, substituting A = 20 min, B = 25℃ and C = 5g in Equation 3

and 4 gives $q_{ePb} = 0.000191$ mg/g and RE $\cong 100\%$. This new finding attests to the fact that, under the given conditions (equal dosage = 5g), *Aspergillus niger* is more efficient in removing Cu, while A, B, C for Pb RE gives impractical q_{ePb} despite a desirability of unity.


Figure 7: 3D Surface Plot for Cu^{2+} Uptake (R1) and Percent Removal (R2)

Table 5: Optimal Combination Based on Highest Desirability

Solution	A (min)	B (°C)	C (g)	Fit	Composite Desirability
Pb: Figure 6(a-c)	20	25	5	R1 = 0.0007452 mg/g	0.980590
Pb: Figure 6(e-f)	53.9394	25	8.33333	R2 = 105.047%	1.000000
Cu: Figure 7(a-c)	180	37.1212	5	R1 = 0.0006965 mg/g	1.000000
Cu: Figure 7(e-f)	180	37.1212	5	R2 = 137.368%	1.000000

Choosing q_{eCu} whose RE is maximum and falls below 100% is advised for this study. As such, q_{eCu} 0.000571 mg/g consistent with 98.051% RE at 50 min, 50℃ and 5g dosage is an alternative optimal combination predictable using Equation 5. Afolabi *et al.* (2022) also obtained 86.27 and 98.85% RE for Cu and Pb, respectively using orange peels as adsorbent, in close consonance with this result. Using *Aspergillus niger* as adsorbent, Xu *et al.* (2021) achieved 96.21-100% Pb removal from aqueous solution. In an anaerobic sewage sludge system, Ndiritu *et al.* (2017) effectively extracted 98.8% Pb and 98.2% Cu using hydrogen peroxide. Combined used of PBD in Minitab and CCD in Design-Expert resulted in 73.9% Pb removal by lactic acid bacteria (Goudarzi *et al.*, 2020). Peaks of the optimum R1 and R2 for Pb and Cu sorption are both represented in Figure 8.
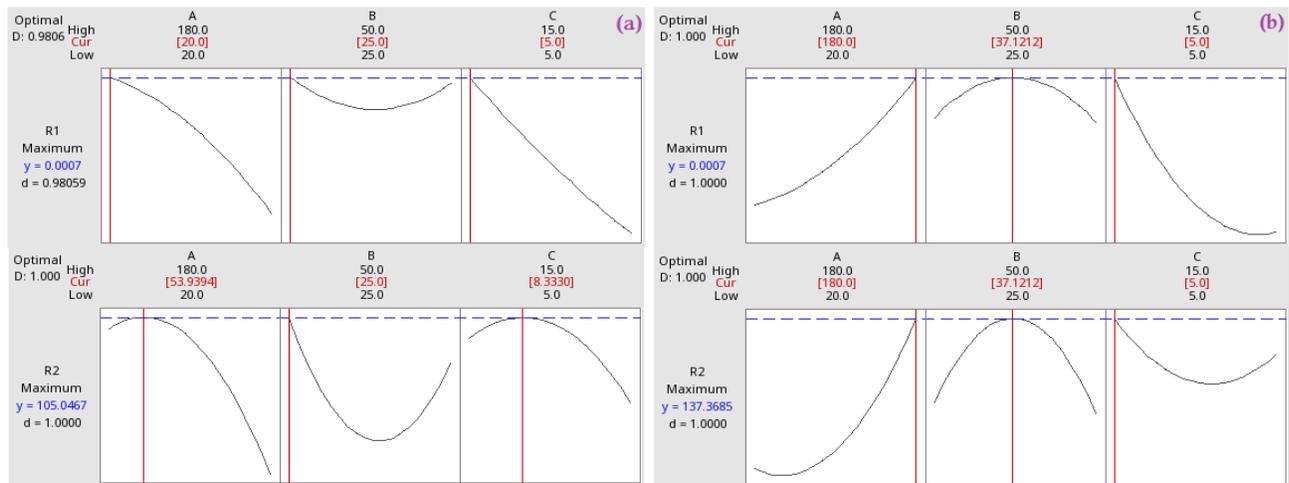
Figure 8: Desirability for (a) Pb and (b) Cu Sorption CCD Optimization Using Minitab

Aspergillus niger appears to be more efficient in terms of lower adsorbent dosage and contact time for Pb removal compared to Cu. The lower dosage and shorter contact time for Pb make *Aspergillus niger* a promising candidate for Pb remediation applications. Overall, Cu^{2+} is best removed by the biosorbent at 37.1212℃ moderate temperature. If model performance is to be put aside, Pb sorption by *Aspergillus niger* is more rapid and efficient given its low contact time of 20 min against 180 min for Cu and 250 min optimal realized in Khoshraftar *et al.* (2023) using dolomite. While CCD is effective for exploring the response surface, optimization within the design space can be challenging. The optimal conditions may lie outside the experimentally investigated region, requiring additional experimentation, as observed in Figure 8 for R2 > 100%. CCD may not be as efficient when higher-order models (quadratic or cubic) are needed. This is evidenced in Equation 3 which gives negative predicted q_e. In such cases, other designs like BBD or more advanced designs may be more appropriate.

Conclusions

Lake Gerio is paramount to crop production in Northeastern Nigeria and the study of its water quality is important for various purposes, including for consumption. Pb concentration of 0.19 mg/L is suitable for drinking and detrimental for crop production while 0.145 mg/L Cu concentration collectively revealed by AAS analysis of the Lake Gerio water sample will support plant growth.

Sorption of Cu and Pb and other heavy metals were achieved in the laboratory in batch mode at varying time (20-180 min), *Aspergillus niger* adsorbent dosage (5-15g) and temperature (25-50℃) as evidenced in their reduced final concentrations after sorption. CCD run in Minitab produces 4 model equations for q_e and RE for both Pb and Cu that is partly suitable for predicting those responses due to $R^2 < 0.9000$ obtained; even though SEM and FTIR analysis showcased considerable adsorptive property of the fungal biomass. Predicted optimal parameter combinations for Pb^{2+} sorption is 20 min, 25℃ and 5g sorbent dose, corresponding to an uptake of 0.000191 mg/g whereas maximum Cu^{2+} uptake of 0.0006965 mg/g occur at 180 min, 37.1212℃ and 5g *Aspergillus niger*. RE > 100% estimated by Equations 4 and 6 is not practical, but is the highest, implying promising performance of *Aspergillus niger* in the bioremediation of heavy metal contamination of Lake Gerio. Understanding optimal conditions such as contact time, temperature, and adsorbent dosage can help in designing more efficient and cost-effective metal removal processes using *Aspergillus niger*. Researchers should carefully consider the specific characteristics of their experimental system and the goals of their study when choosing an experimental design so as to avoid inadequacies witnessed using CCD in this study.

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