In this study, the myco-reduction potential of fungi isolated from soil was ascertained by Norkrans shake flask experiment contaminated with chromium(VI). Fungal tolerance assay and induced tolerance training of the fungi were also carried out. Aspergillus niger, Penicillium, and Saccharomyces strains were isolated from the soil samples using culture based technique. Norkrans samples were collected and analyzed for Cr(VI) concentration using diphenylcarbazide spectrophotometric method. Penicillium strain was observed to be most effect at Cr(VI) concentrations of 16.1 and 8.1 mg L⁻¹ since it was able to reduce Cr(VI) more than Saccharomyces strain and A. niger on day 20. Bio-sorption kinetics for this study was better described by pseudo second order model while Langmuir isotherm model fitted better to the equilibrium data. There was virtually steady increase in fungal growth for all the treatments through-out the experimental period. Significant negative correlation (p < 0.05) was observed between fungal growth and Cr(VI) reduction rate. The results from the induced tolerance training showed that Penicillium had the highest tolerance index (TI) values at 18, 20, and 25 mg L⁻¹ concentrations of Cr(VI) compared to A. niger and Saccharomyces strain. These results demonstrated that these fungi have the potential to bio-absorb Cr(VI) and if properly harnessed, could be used in place of conventional remediation technology to clean-up the Cr(VI) contaminant in the field.

**KEYWORDS**

bio-sorption, chromium (VI), contamination, fungal tolerance assay, kinetics, Penicillium

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**1 | INTRODUCTION**

Contamination of environmental receptors such as soil, groundwater, surface water, and air with poisonous chemicals is among the problems facing industrialized countries [1–3]. Most of these contaminants (which could be heavy metal) emanate from industries [4–6] and elimination of heavy metal such as Cr(VI) from contaminated water is important since they are toxic to aquatic organisms and humans. The use of Cr(VI) in electroplating, leather tanning, chromate preparation and in metal finishing has led to its existence as a common contaminant in the soil.

The clean-up of soil contaminated with toxic elements requires the use of conventional and biological remediation technologies. Soil remediation is the removal of poisonous compounds from the soil by physicochemical and biological means [7–9]. Conventional methods of soil clean-up include excavation, washing, thermal adsorption,
and vitrification. The removal of chromium from contaminated environments by conventional technologies has a couple of drawbacks which include the fact that these techniques are money-consuming, require a lot of energy, cause physicochemical and biotic disturbance of soil, and do not thoroughly clean-up or remove contaminants from contaminated sites [1,10,11]. Plants and microorganisms are currently used to eliminate contaminants from the environment in a process called biological remediation. Biological remediation of chromium contaminants by fungi and bacteria is more effective than the conventional methods since it is ecofriendly, cheap, natural, and well embraced by the public [12].

Cr(VI) is among the most toxic and common metals affecting human health upon bioaccumulation. As a result of proliferation of industries, high amount of Cr(VI) waste is currently an environmental challenge. Among the different chromium valence states, Cr(VI) and Cr(III) are of environmental significance owing to their ability to reside firmly in the environment. Cr(III) has been proven to be involved in protein, cholesterol, and fatty acid metabolism while Cr(VI) is an oxidizing agent that can cause kidney problem, cardiovascular shock [13], and cancer [14]. A number of bacterial isolates have been highlighted to eliminate Cr(VI) from the environment; however there are limited information on the use of fungi and yeast for the removal of chromium from the ecosystem [15].

Therefore, the aim of this work was to isolate fungi from the soil and assess their potential to bio-absorb Cr(VI) in the laboratory. In addition to this, comprehensive bio-sorption kinetics and equilibrium studies were done using different isotherm models. Considering the practice of bioremediation, this investigation could have beneficial future use in eliminating Cr(VI) from the environment and total elimination of this metal from the ecosystem will guarantee chromium-free water and soil for future use.

2 | MATERIALS AND METHODS

2.1 | Soil samples collection

Soil samples were collected in polyethylene bag from refuse dumpsite, automobile, and furniture workshop in Benin City, Nigeria. Samples were kept at 4 °C before isolation.

2.2 | Preparation of fungal medium

Potato dextrose agar (PDA) medium was prepared following manufacturer's instructions and sterilized by autoclaving at 121 °C for 15 min [16].

2.3 | Fungal isolation

Stock solutions of soil samples were prepared by weighing 1 g of soil samples on a weighing balance (Radag Wagi Elektronizne, Poland) and transferred into sterile test tubes containing 9 ml of autoclaved reverse osmosis water. One milliliter of the stock solution was serially diluted [17,18] and inoculated by pour plate technique and culture plates were incubated upside down at ambient temperature (28 °C ± 2) for 5 days.

2.4 | Cultural characterization and identification of fungal strains

Cultural features of fungal strains were done according to the method described by Imarhiagbe et al. [16] and the major remarkable macroscopic features in strain identification were the colony diameter and color. According to the method described by Riddell [19], microscopic identification of fungal isolates was done by examining under the microscope using lactophenol cotton blue [20]. Microscopic features for the strain identification were phialide shape, branching pattern, stipe ornamentation, stipe width, stipe length, spore length, spore width, spore ornamentation, and spore shape. Isolates from the mixed culture plates were aseptically streaked on freshly prepared PDA medium, sealed with paraffin paper and incubated upside down at ambient temperature (28 °C ± 2) for 5 days. Then, isolates were preserved in agar slants for future use.

2.5 | Preparation of Norkrans medium

One liter of the medium was prepared as described by Igiehon [1] with little modification. Briefly, the medium ingredients (glucose 20.0 g; potassium phosphate (KH2PO4) 0.6 g; potassium hydrogen orthophosphate (K2HPO4) 0.4 g; 5H2O) 0.005 g; manganese (II) sulphate tetrahydrate (MnSO4.4H2O) 0.005 g; zinc sulphate ferric citrate (C6H5O7Fe. heptahydrate (ZnSO4.7H2O) 0.0044 g; cobalt (II) chloride hexahydrate (COC12.6H2O) 0.001 g; calcium chloride dihydrate (CaCl2.2H2O) 0.132 g; thiamine hydrochloride (C12H17CIN4OS.HCl) 0.0001 g; magnesium sulphate heptahydrate (MgSO4.7H2O) 0.5 g; asparagine (C4H8N2O3) 1.2 g) were weighed and transferred into a conical flask containing 1 L of reverse osmosis water and stirred properly using magnetic stirrer. Thirty milliliter (30 ml) of the medium was dispensed into flasks, covered with bugs, sealed with aluminium foil and autoclaved at 121 °C for 15 min.

2.6 | Myco-reduction experiment in Norkrans medium

The autoclaved Norkrans medium was inoculated with two balls of fungal biomass from the pure culture plates.
and incubated in a shaker incubator in a randomized manner at 60 rpm for 5 days. Analytical grade Cr(VI) was then added to the flasks at concentrations of 16.1, 8.1, 4.0, and 2.0 mg L\(^{-1}\) including the control (without the fungi). Control without Cr(VI) but with fungi and that with only the Norkrans medium but without the fungi and Cr(VI) were also set up. Each treatment contains five replicates.

### 2.6.1 Cr(VI) and Cr(III) quantification

Fungal reduction potential of Cr(VI) was determined by quantifying Cr(VI) in Norkrans samples. Samples were collected from the Norkrans flasks on days 0, 5, 10, and 20. 5 ml of the samples were collected in McCartney bottles to determine the concentration of Cr(VI) using diphenylcarbazide spectrophotometric method [21,22]. Briefly, Norkrans samples were transferred to flasks and the pH of the samples was adjusted to 1.0 ± 0.3 with 0.3 N H\(_2\)SO\(_4\) and diluted with distilled water. Diphenylcarbazide was added, mixed, and allowed to stand for color development. Samples were then measured at 540 nm using spectrophotometer. In addition, the concentrations of trivalent chromium [Cr(III)] at the end of the experimental period (day 20) were detected from the Norkrans flask culture as described by Ackerley et al. [23]. Norkrans culture solutions were filtered through Whatman filter paper before measuring Cr (III) concentrations.

### 2.6.2 Cr(VI) bio-sorption test

Fungal bio-sorption test was done with little modification according to the method employed by Bennett et al. [24]. Briefly, 2 g of fungal biomass from Cr(VI) flask experiment was taken on the last day (day 20) of the experiment, dried at room temperature for 2 days and ground into fine powder. Thirty milliliter of H\(_2\)SO\(_4\)/HNO\(_3\) solution was added to the fungal mycelial powder and exposed to heating. The digested samples were subjected to spectrophotometric analyses and results were recorded. This was also done for the control experiments and the differences in spectrophotometric readings were taken as the amount of Cr(VI) absorbed.

### 2.6.3 Bio-sorption kinetics

Many kinetic models have been used to explain the mechanism and reaction order of absorption systems. In this study, the bio-sorption of hexavalent chromium by the fungal strains was tested with the following equations of kinetic models: zero-order and pseudo-second order model (Table 1) which are used for studying the rate controlling step and bio-sorption mechanism [25,26].

### 2.6.4 Equilibrium studies using adsorption isotherms

Adsorption isotherm is an indispensable model which explains the phenomenon governing the movement of a material (e.g., metal) from a liquid phase to a solid phase at a constant pH and temperature. The relationship between bio-adsorbed metal ion and the remaining metal ion in the aqueous solution is defined when adsorbate (metal) is in contact with the adsorbent (fungal strain) for a sufficient contact time which is expressed with mathematical correlation using adsorption isotherms [27]. In this study, two parameters isotherm models (Langmuir and Freundlich) were used for fitting bio-sorption data of fungal biosorbent [28,29].

### 2.7 Chromium tolerance assay

This was done following the method of Ortiz et al. [30]. Briefly, liquid samples were taken from the experimental flasks on days 0, 5, 10, and 20 inoculated in PDA and incubated at ambient temperature (28 °C ± 2) for 5 days. Thereafter, viable cells were counted and results were recorded in log\(_{10}\) cfu ml\(^{-1}\). Five replicates were done for each treatment.

### 2.7.1 Induced–tolerance training

Fungal isolates were inoculated on 2% malt extract agar (MEA) modified with 16.1 mg L\(^{-1}\) of Cr(VI). MEA fungal plates without chromium were used as control. Fungal isolates from the culture plates amended with 16.1 mg L\(^{-1}\) were subsequently sub-cultured in freshly prepared MEA amended with increasing Cr(VI) concentrations of 18, 20, and 25 mg L\(^{-1}\) and culture plates were incubated at room temperature (28 °C ±2) for 5 days. Induced tolerance training was determined by calculating the tolerance index (TI) which is the total number of fungal isolates in MEA amended with increasing Cr(VI) concentrations divided by the total number of fungal isolates in the control MEA plates.

### 2.8 Statistical analysis

Data were cleaned in Microsoft excel and exported to SPSS. Graphs were plotted in Microsoft excel. Normality test was done for data, data were transformed, outliers were removed, mean values and standard errors were determined using Microsoft excel. Analysis of variance (ANOVA) and

### Table 1

| Kinetic model          | Linearized form                  | Reference |
|------------------------|----------------------------------|-----------|
| Zero order             | \((q_t - q_e) = q_e - k_0 t\)    | [26]      |
| Pseudo second order    | \(t/q_t = 1/K^2q_e^2 + 1/q_e t\) | [25]      |
correlation were done in SPSS. Duncan multiple test was used to ascertain differences in mean and p-values less than 0.05 were considered significant.

3 | RESULTS

3.1 | Culturable fungal detection

The culture based technique revealed the presence of *Penicillium* strain, *Aspergillus niger*, and *Saccharomyces* strain in the soil samples. *Penicillium* strain and *A. niger* were observed to have greenish to black spores and brown to black spores respectively while *Saccharomyces* strain had creamy spores (Table 2). In addition, microscopic examination unveiled the presence of stipe and phialide in *Penicillium* and *A. niger* which were absent in the yeast cell *Saccharomyces* strain (Table 3). Conversely, asci-containing spores were present in the yeast cells. Upon characterization and identification, these fungi were subsequently used for the chromium reduction assay.

3.2 | Myco-remediation by fungal strains: quantification of Cr(VI) and Cr(III)

Figure 1a–d show the myco-reduction potential of *Saccharomyces* strain, *A. niger*, and *Penicillium* strain. Cr(VI) reduction at a concentration of 16.1 mg L\(^{-1}\) was observed for all the treatments amended with these fungi except for control. Similar outcome was observed for treatments amended with 8.1, 4.0, and 2.0 mg L\(^{-1}\). *Penicillium* strain was observed to be the most effective at concentrations of 16.1 and 8.1 mg L\(^{-1}\) since it was able to reduce the contaminant more than *Saccharomyces* strain and *A. niger* on day 20 (Fig. 1a and b). On the contrary, *A. niger* was most effective at concentrations of 4.0 and 2.0 mg L\(^{-1}\) (Fig. 1c and d). The reduction of Cr(VI) observed could be related to the conversion of Cr(VI) to Cr(III) and intracellular accumulation of chromium (bio-sorption) by the fungi.

Thus, Norkrans samples analysis revealed the presence of Cr(III) in all the treatments except for the control experiment suggesting that the fungi may have triggered the conversion of Cr(VI) to Cr(III). In the medium amended with *Penicillium* strain, 1.00 ± 0.10, 2.00 ± 0.11, 0.10 ± 0.00, and 0.20 ± 0.00 mg L\(^{-1}\) levels of Cr(III) were detected in the 16.1, 8.1, 4.0, and 2.0 mg L\(^{-1}\) of Cr(VI) treatments, respectively, while *Saccharomyces* inoculated medium had 1.50 ± 0.10, 2.00 ± 0.00, 1.00 ± 0.00, 0.10 ± 0.30 concentrations of Cr(III) in 16.1, 8.1, 4.0, and 2.0 mg L\(^{-1}\) of Cr(VI) treatments, respectively (Table 4).

3.2.1 | Cr(VI) bio-sorption potential of fungal strains

For the bio-sorption assay, *Penicillium* strain absorbed 3.00, 3.00, 2.00, and 0.10 mg L\(^{-1}\) of Cr(VI) for treatments amended with 16.1, 8.1, 4.0, and 2.0 mg L\(^{-1}\) of Cr(VI), respectively (Table 5). At a concentration of 16.1 mg L\(^{-1}\) of Cr(VI), *Saccharomyces* strain was found to absorb the least amount of Cr (VI) (1 mg L\(^{-1}\)) even though this strain was able to reduce Cr(VI) better than *A. niger* at this concentration (Fig. 1a).

3.2.2 | Bio-sorption kinetics

The kinetic analyses for the Cr(VI) bio-sorption test was done by using the pseudo second order and zero order kinetic constant. The pseudo second order analyses showed significant regression coefficient (\(R^2\)) of 0.99 for all the Cr(VI) treatments inoculated with *Penicillium* strain, *A. niger*, and *Saccharomyces* strain while the zero order indicated no significant \(R^2\) for all the treatments (Table 6).

3.2.3 | Equilibrium studies using adsorption isotherms

Similarly, two isotherm models were used for the equilibrium study namely the Langmuir and Freundlich isotherm models. The outcome of the study showed that the Langmuir isotherm has a significant \(R^2\) of 0.99 while Freundlich model did not show significant relationship with the data as relatively low \(R^2\) values (<0.99) were obtained (Table 7).

3.3 | Fungal tolerance to Cr(VI)

Fungal viable cell counts from the tolerance experiments showed that Cr(VI) was not toxic and had no effect on the fungi (Fig. 2a–d). At a concentration of 16.1 mg L\(^{-1}\), *A. niger* had maximum growth on day 5 (Fig. 2a) and day 20 at a concentration of 8.1 mg L\(^{-1}\) (Fig. 2b). At a concentration of

### Table 2: Macroscopic characterizations of the of the isolated fungal strains

| Fungal strain     | Color of spore | Colony color     | Colony diameter (mm) × ±SE |
|-------------------|----------------|-----------------|-----------------------------|
| *Penicillium* strain | Greenish black | Greenish black  | 41 ± 1.00                   |
| *A. niger*        | Brown to black | Black           | 44 ± 1.10                   |
| *Saccharomyces* strain | Creamy        | White to cream  | 15 ± 0.10                   |

\(× ±SE\), mean ± standard error.
4.0 mg L\(^{-1}\) of Cr(VI), *Saccharomyces* strain biomass depleted to 4.1 log\(_{10}\) cfu ml\(^{-1}\) on day 20 after reaching its peak on day 10 with a count of 4.2 log\(_{10}\) cfu ml\(^{-1}\) (Fig. 2c). *Penicillium* and *Saccharomyces* strains had the least biomass on day 20 for treatments amended with 2.0 mg L\(^{-1}\) (Fig. 2d).

### 3.3.1 Induced tolerance training

The induced-tolerance training assay outcome showed that *Penicillium* strain, *A. niger*, and *Saccharomyces* strain were able to tolerate higher Cr(VI) concentrations of 18, 20, and 25 mg L\(^{-1}\) (Table 8). Also, the TI values for the trained fungal strains (subjected to increasing Cr(VI) concentrations) were relatively higher than the TI values for the untrained fungal strains [cultivated in medium that was not amended with Cr(VI) or the control].

### 4 DISCUSSION

Microorganisms play a significant role in the elimination of Cr(VI) from polluted soil. They develop tolerance to chromium by different mechanisms, such as intracellular accumulation, adhesion of chromium to cell surfaces, intracellular precipitation, and complexation by exopolysaccharides [31]. This always motivates the isolation of fungal from a variety of soil environments that can be exploited for bioremediation or mycoremediation purposes [32]. In this study, a similar phenomenon was employed to isolate fungal strains from refuse dumpsite, automobile and furniture workshop.

Hence, isolated *Penicillium*, *A. niger*, and *Saccharomyces* were characterized macroscopically based on color of colonies, color of spores, and colony diameter (Table 2). *Penicillium* strain had greenish black spores and greenish black colonies with an average diameter of 41 ± 1.00 while *A. niger* had brown to black spores and black colonies with a mean diameter of 44 ± 1.10. *Saccharomyces* strain was however characterized with white to creamy colonies with an average diameter of 15 ± 0.10 (Table 2).

Microscopic characterizations of fungal strains were done by considering fungal spore length, spore width, spore shape, spore ornamentation, stipe length, stipe width, stipe ornamentation, phialide shape, and branching pattern. It was observed that *Penicillium* strain possessed spores that were globose and coarse with a length of 3.5 µm and width of 2.7 µm while *A. niger* had smooth to rough conidia in chains with a length of 4.5 µm and width of 2.3 µm. But smooth to rough ovoid spores present in asci were observed for *Saccharomyces* strain (Table 3). Microscopic observation also revealed the presence of phialide and stipe in *Penicillium* and *A. niger* which is in agreement with the
results of Kamili et al. [33] (Table 3). Furthermore, the mycoremediation results from this study have shown that the fungal strains isolated namely: *Saccharomyces* strain, *A. niger*, and *Penicillium* strain were effective in reducing Cr(VI) in the Norkrans liquid medium. However, *Penicillium* strain was the most effective at Cr(VI) concentrations of 16.1 and 8.1 mg L\(^{-1}\) since it was able to reduce the contaminant to 8.0 and 3.0 mg L\(^{-1}\), respectively, on day 20 (Fig. 1a and b). The reason for such effectiveness could be that the fungus produced more mycelia [1] thereby allowing more Cr(VI) to penetrate into the microbial cell biomass. On the contrary, *A. niger* had the greatest absorption capacity at Cr(VI) concentrations of 4.0 and 2.0 mg L\(^{-1}\) as the fungus was able to eliminate Cr(VI) from the liquid medium to concentrations of 1 and 0.2 mg L\(^{-1}\), respectively, on day 20. At concentrations of 4.0 and 2.0 mg L\(^{-1}\), *Saccharomyces* strain was least effective in reducing Cr(VI). However, the control for all the treatments had constant Cr(VI) concentrations through-out the experimental period. Control experiments with Norkrans medium and fungi only but without Cr(VI) and those with only the Norkrans medium but without the fungi and Cr(VI) gave zero (0) readings indicating that the substrate that was read in the spectrophotometer was strictly Cr(VI) contaminant and not fungal cells or the Norkrans medium. These fungi were able to remove Cr(VI) from the medium by possibly transforming or reducing Cr(VI) to less toxic Cr(III) by enzymatic process or by potentially bio-accumulating this metal within their cell biomass [3,34].

Table 4 shows the concentrations of Cr(III) that were detected at different concentrations of Cr(VI) treatments inoculated with various fungal strains. Cr(III) was found at the highest concentration of 2 mg L\(^{-1}\) in the 8.1 mg L\(^{-1}\) of Cr(VI) treatment inoculated with *Penicillium* and *Saccharomyces* strains. However, relatively lower concentrations of

| TABLE 4 | Cr(III) concentrations in Norkrans culture solutions |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cr(III) (mg L\(^{-1}\)) | 16.10 × ±SE | 8.10 × ±SE | 4.00 × ±SE | 2.00 × ±SE |
| Isolate | | | | |
| Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Penicillium* strain | 1.00 ± 0.10 | 2.00 ± 0.11 | 0.10 ± 0.00 | 0.20 ± 0.00 |
| *A. niger* | 1.00 ± 0.30 | 1.67 ± 0.01 | 0.10 ± 0.10 | 0.10 ± 0.30 |
| *Saccharomyces* strain | 1.50 ± 0.10 | 2.00 ± 0.00 | 1.00 ± 0.00 | 0.10 ± 0.30 |

\(\times ±SE = \text{Mean} ± \text{standard error.}\)
Cr(III) were detected in other treatments inoculated with the same fungi and *A. niger*. The presence of Cr(III) in the Norkrans medium suggests that these fungal strains may have the enzymatic capabilities needed to convert Cr(VI) to Cr(III), therefore demonstrating their ability for adaptation [35]. The decrease in Cr(VI) concentration (Fig. 1) in culture media of *Penicillium*, *Saccharomyces*, and *A. niger* may be due to a metabolic reduction of Cr(VI) to Cr(III), fungal uptake of Cr(VI) without reduction and passive uptake of Cr(VI) without reduction to Cr(III). Metabolic uptake with reduction, passive uptake as well as active uptake without reduction (bioaccumulation) of metal ions including Cr(VI) are known processes of microbial bioremediation [23,36–40].

The differences in intracellular Cr(VI) concentration among the fungal strains (Table 5) might therefore be related to variations in the rate of Cr(VI) uptake or reduction to

| TABLE 5  | Cr(VI) bio-sorption profiles of fungal isolates |
|----------------|-----------------------------------------------|
| Isolate       | Mean intracellular Cr(VI) (mg L\(^{-1}\))       |
|               | 16.10 × SE | 8.10 × ± SE | 4.00 × ± SE | 2.00 × ± SE |
| *Penicillium*  |            |            |            |            |
| strain        | 3.0 ± 0.100 | 3.0 ± 0.001 | 2.0 ± 0.000 | 0.1 ± 0.001 |
| *A. niger*     | 2.0 ± 0.000 | 2.5 ± 0.002 | 2.0 ± 0.000 | 1.0 ± 0.001 |
| *Saccharomyces*| 1.0 ± 0.000 | 2.0 ± 0.010 | 1.0 ± 0.001 | 0.1 ± 0.001 |

× ± SE, mean ± standard error.

| TABLE 6  | Bio-sorption kinetics parameters for the bio-sorption of Cr(VI) by MSR1 strain |
|----------------|-----------------------------------------------|
| Fungal isolate | Cr(VI) concentrations (mg L\(^{-1}\)) |
|               | 16.10 | 8.10 | 4.00 | 2.00 |
| *Penicillium*  | Experimental \(q_e\) | 5.000 | 2.500 | 1.500 | 1.000 |
|                 | Pseudo second order |            |            |            |            |
|                 | \(K_{2p}\) | 1.580 | 1.800 | 1.000 | 0.100 |
|                 | \(q_e\) | 4.500 | 2.230 | 1.200 | 1.000 |
|                 | \(R^2\) | 0.999 | 0.990 | 0.992 | 0.991 |
|                 | Zero order |            |            |            |            |
|                 | \(K_0\) | 0.251 | 0.256 | 0.200 | 0.100 |
|                 | \(q_e\) | 3.110 | 1.120 | 1.000 | 0.100 |
|                 | \(R^2\) | 0.856 | 0.850 | 0.855 | 0.755 |
| *A. niger*      | Experimental \(q_e\) | 5.000 | 2.000 | 1.400 | 1.100 |
|                 | Pseudo second order |            |            |            |            |
|                 | \(K_{2p}\) | 1.500 | 1.100 | 1.000 | 0.100 |
|                 | \(q_e\) | 4.460 | 1.210 | 1.210 | 0.100 |
|                 | \(R^2\) | 0.992 | 0.991 | 0.990 | 0.990 |
|                 | Zero order |            |            |            |            |
|                 | \(K_0\) | 0.201 | 0.206 | 0.201 | 0.101 |
|                 | \(q_e\) | 4.010 | 1.220 | 1.000 | 1.000 |
|                 | \(R^2\) | 0.806 | 0.700 | 0.755 | 0.755 |
| *Saccharomyces* | Experimental \(q_e\) | 4.000 | 2.000 | 1.300 | 1.000 |
|                 | Pseudo-second order |            |            |            |            |
|                 | \(K_{2p}\) | 1.300 | 1.000 | 1.000 | 0.100 |
|                 | \(q_e\) | 3.360 | 1.100 | 1.110 | 0.100 |
|                 | \(R^2\) | 0.991 | 0.990 | 0.991 | 0.990 |
|                 | Zero order |            |            |            |            |
|                 | \(K_0\) | 0.200 | 0.106 | 0.101 | 0.101 |
|                 | \(q_e\) | 3.010 | 1.220 | 1.000 | 0.100 |
|                 | \(R^2\) | 0.706 | 0.700 | 0.705 | 0.705 |
Cr(III) among the fungi. It is likely that the relatively low concentrations of Cr(III) detected for 16.1 mg L\(^{-1}\) treatments inoculated with \textit{A. niger} and \textit{Penicillium} strain (Table 4) may be due to the high concentrations of Cr(VI) absorbed by the fungal strains (Table 5) since intracellular accumulation of hexavalent chromium might affect the normal functioning of microorganisms [38]. As regards chromium bioaccumulation and toxicity, Ksheminska et al. [41] highlighted different mechanisms of chromium remediation by diverse strains of the yeast \textit{Pichia guilliermondii}. The authors found that all the tested yeast strains responded to hexavalent chromium with great alterations in protein profile patterns, which included suppression and induction of specific polypeptides. In particular, they detected the suppression of a 59 kDa and induction of a 62 kDa proteins in treatments amended with Cr(VI) and concluded that these proteins function in bioremediation of Cr(VI) by \textit{P. guilliermondii}. The biosorption profile (Table 5) of fungal isolates indicated the accumulation of Cr(VI) in fungal mycelia and this was deduced from the relatively higher values obtained for fungi amended with Cr(VI) compared to the control [without Cr(VI)]. For the treatment with 2.0 mg L\(^{-1}\) of Cr(VI), \textit{A. niger} was found to absorb the highest amount (1 mg L\(^{-1}\)) of Cr(VI). There was also significant negative correlation (\(p < 0.05\)) between the \textit{A. niger} growth (Fig. 1c and d) and reduction of Cr(VI) (Fig. 2c and d) for treatments amended with 2.0 and 4.0 mg L\(^{-1}\) of Cr(VI). This showed that increase in fungal biomass led to a decrease in Cr(VI). This finding is in line with the result of Bennett et al. [24] who reported absorption of Cr(VI) by \textit{Aspergillus flavus} and \textit{A. niger}.

Table 6 shows the kinetic constants of pseudo second order and zero order estimated for the bio-sorption of Cr(VI) by fungal strains. The results for zero order which has low \(R^2\) (< 0.98) did not show significant correlation with the kinetic data which shows that this model is not valid for this study. However, the pseudo second order equation with relatively higher \(R^2\) (0.99) showed a better fit than the zero order models. Moreover, the equilibrium \(q_e\) agrees with experimental \(q_e\) value (Table 6). The suitability of the pseudo
second order kinetic model shows that the bio-sorption of Cr(VI) by *Penicillium*, *A. niger*, and *Saccharomyces* biomass followed chemisorption process and also play a significant role as the rate limiting step.

Equilibrium data analysis is necessary to establish an equation to design and optimize an operating system. In this study, two parameter isotherm model namely, Langmuir and Freundlich isotherm were used to investigate the correlation between the Cr(VI) adsorbed onto *Penicillium*, *A. niger*, and *Saccharomyces* biomass at their respective concentrations. The $R^2$ and isotherm constant for the models are present in Table 7.

Langmuir isotherm was observed to show a better fit than the other isotherm model indicating a monolayer mode of absorption with high $R^2$ of 0.99 (Table 7). "The Langmuir isotherm is based on the assumption of surface homogeneity such as equal availability of absorption sites and monolayer surface coverage [31]." It is similarly based on the idea that no interaction occurs between the absorbed Cr(VI) species [28]. The equilibrium parameter ($R_L$) was utilized in projecting whether absorption platform is "promising" or "unpromising." $R_L$ value indicates the shape of isotherm to either be promising (i.e., when $R_L > 1$ or $R_L = 1$) or unpromising (i.e., when $R_L < 0$ or $R_L < 1$) or irreversible when $R_L$ is equal to 1.

**TABLE 8** Tolerance index of the fungal isolates with and without “induced tolerance training” at different concentrations of Cr(VI)

| Isolate          | Cr(VI) concentration (mg L$^{-1}$) | Tolerance index (TI) |
|------------------|------------------------------------|----------------------|
|                  |                                    | With training $\times \pm$ SE | Without training (16.1 mg/l) $\times \pm$ SE |
| *Penicillium* strain | 18                                 | 0.880 ± 0.01          | 0.5000 ± 0.001       |
| *A. niger*        | 18                                 | 0.810 ± 0.001         | 0.5000 ± 0.000       |
| *Saccharomyces* strain | 18                                | 0.610 ± 0.001         | 0.4000 ± 0.000       |
| *Penicillium* strain | 20                                 | 0.700 ± 0.001         | 0.5100 ± 0.000       |
| *A. niger*        | 20                                 | 0.670 ± 0.000         | 0.5100 ± 0.001       |
| *Saccharomyces* strain | 20                                | 0.554 ± 0.000         | 0.5300 ± 0.001       |
| *Penicillium* strain | 25                                 | 0.500 ± 0.000         | 0.5200 ± 0.002       |
| *A. niger*        | 25                                 | 0.470 ± 0.000         | 0.5500 ± 0.000       |
| *Saccharomyces* strain | 25                                | 0.454 ± 0.001         | 0.4300 ± 0.001       |

$x \pm SE$, mean ± standard error.
zero (0). The $R_L$ values for Cr(VI) bio-sorption on *Penicillium*, *A. niger*, and *Saccharomyces* biomass were found to be promising with $R_L$ values greater than zero (0) but less than one (1) (Table 7) and this is in agreement with the results obtained by Samuel et al. [31] who reported $R_L$ value of 0.441 for Cr(VI) absorbed on *Penicillium griseofulvum* MSR1 biomass.

The results of this study also showed that the Freundlich isotherm is not applicable to these data as low $R^2$ values were obtained (Table 7). This suggests that these data are not in conformity with non-ideal adsorption on heterogeneous surfaces and multilayer sorption mode [29]. Therefore, the results indicate that the Langmuir isotherm model exhibited a better fit than the Freundlich isotherm model tested, demonstrating that the surfaces of the fungal sorbents are homogenous. These results show that each fungal binding site accepts only one chromium (IV) molecule and the sorbed Cr(VI) molecules are arranged as a monolayer, all binding sites are energetically equal and that the sorbed Cr(VI) species do not interact between each other. In an attempt to know if Cr(VI) had effect on or was toxic to the fungal strains, tolerance assay was carried out. The idea behind this assay is that if increase in fungal viable cell counts is directly proportional to increase in the experimental days, it is an indication that these fungi are resistant to Cr(VI) and this was the observation in this study (Fig. 2a–d). For the treatment containing 16.1 mg L$^{-1}$ of Cr(VI), *Saccharomyces* strain, *A. niger*, and *Penicillium* strain had the highest viable cell counts on day 5 after which fungal cells declined (Fig. 2a). The reason for this decline could be that the fungal cells were producing toxic metabolites that affected their viability. On the other hand, there was virtually steady growth increase in the treatments containing 8.1 and 2.0 mg L$^{-1}$ of Cr(VI) through-out the experimental period (Fig. 2b–d). This showed that the fungi were able to grow at these concentrations and this is similar to what was observed by Bennett et al. [24] who reported increase in fungal growth in Cr(VI) contaminated environment at different pH and temperatures. Correlation analyses showed significant negative correlation ($p < 0.05$) between fungal growth and chromium reduction. In the treatments containing 2.0 and 8.0 mg L$^{-1}$ of Cr(VI), *A. niger* had the highest count of $4.5 \log_{10} \text{cfu ml}^{-1}$ on day 20.

The induced-tolerance training assay results showed that *Penicillium* strain, *A. niger*, and *Saccharomyces* strain were able to tolerate the increasing concentrations of Cr(VI). *Penicillium* strain had the highest TI values for all the concentrations of Cr(VI) compared to *A. niger* and *Saccharomyces* strain (Table 8). The TI values of *Penicillium* strain subjected to training at Cr(VI) concentrations of 18, 20, and 25 mg L$^{-1}$ were 0.880, 0.700 and 0.500 mg L$^{-1}$, respectively. Also, the TI values of *Saccharomyces* strain subjected to training were 0.610, 0.554, and 0.454, respectively. However, the TI values of the untrained fungal strain (fungal strain cultivated in medium that was not amended with Cr(VI)) were lower than the TI values of their respective trained fungal counterparts. Thus, the relatively high TI values observed indicates that chromium amended medium was able to enhance fungal tolerance to Cr(VI) and therefore the fungal strain may have the potential to tolerate higher concentrations of chromium with the consequential effect of bio-absorbing and getting rid of the heavy metal from the environment [42,43].

Chromium tolerance and reduction potential of *Saccharomyces* strain, *A. niger*, and *Penicillium* strain demonstrated that these fungi could be explored biotechnologically to bio-absorb and consequently remove chromium especially the more toxic Cr(VI) from the environments so as to make them suitable for agricultural [44,45] recreational and possibly industrial purposes. However, studies on bacterial remediation of Cr(VI) have also been reported. In particular, Ndedy Aka and Babalola [3] reported multiple heavy metal resistance of bacteria isolated from chromium, nickel, and cadmium contaminated soil where it was highlighted that bacterial tolerance to heavy metal was due to several processes such as diffusion of metal into the mycelia, efflux, and oxidation processes.

In conclusion, results from this study have shown that *Penicillium* strain, *A. niger*, and *Saccharomyces* strain have the potential to remove Cr(VI) from the environment by absorbing the metal without the metal having effects on their growth as depicted by the chromium tolerance assay. Moreover, the pseudo second order kinetics explain the chemisorption process while the Langmuir isotherm indicates a monolayer bio-sorption for all the fungal strains at the different Cr(VI) concentrations and therefore, these fungi can be more effective in the treatment of hexavalent chromium from contaminated soil. Also, there is the possibility of these fungi thriving well in an environment with relatively higher concentrations of Cr(VI) if subjected to induced-tolerance training. The reduction of Cr(VI) to less toxic Cr(III) and/or bio-sorption potential of these fungi can therefore be harnessed as an alternative and sustainable strategy to the expensive and less effective conventional remediation technologies.

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CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

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