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Fungal Biosorption for Cadmium and Mercury Heavy Metal Ions Isolated from Some Polluted Localities in KSA

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A B S T R A C T

Persistent heavy metal pollution poses a major threat to all life forms in the environment due to its toxic effects. These metals are very reactive at low concentrations and can accumulate in the food web, causing severe public health concerns. The use of microbial biosorbents is eco-friendly and cost effective; hence, it is an efficient alternative for the remediation of heavy metal contaminated environments. Microbes have various mechanisms of metal sequestration that hold greater metal biosorption capacities. The goal of microbial biosorption is to remove and/or recover metals and metalloids from solutions, using living or dead biomass and their components. This paper aims to biosorption of cadmium and mercury heavy metal ions by using some heavy metal ions resistance local fungal isolates with some agricultural wastes for removing it from industrial and municipal wastewater collected from some KSA localities using enrichment culture technique. Eighteen fungal isolates were identified according to key for fungal identification as the following: *Acremonium sp.*, *Alternaria alternata*, *Alternaria chlamydosporum*, *Aspergillus fumigatus*, *Aspergillus ochraceus*, *Aspergillus wentii*, *Cladosporium cladosporioides*, *Cunninghamella elegans*, *Curvularia lunata*, *Fusarium chlamydosporum*, *Mucor racemosus*, *Penicillium aurantiogriseum*, *Penicillium chrysogenum*, *Penicillium expansum*, *Penicillium oxalicum*, *Rhizopus stolonifer* and *Trichoderma viride*. Two most potent fungal strains viz. *Alternaria alternata* and *Penicillium aurantiogriseum* were selected as the most potent fungal strains with tolerant up to 1000 ppm concentration for both $HgCl_2$ and $CdCl_2$ heavy metals. Optimum contact time for *Alternaria alternata* and *Penicillium aurantiogriseum* with both heavy metals under investigation (Cadmium and mercury) is five days. The optimum pH in both cases was 6. The optimum temperature was 30°C. The growth of both fungi *Alternaria alternata* and *Penicillium aurantiogriseum* on cadmium and mercury ions decreased with increasing of ions concentrations. This indicated the potential of these identified fungi as biosorbent for removal of high concentration metals from wastewater and industrial effluents.

Introduction

It is well recognized that the presence of heavy metals in the environment can be detrimental to a variety of living species, including man. Industrial wastewaters are considered the most important sources of heavy metal pollution. Heavy metal pollution

has become a serious environ- mental issue in the last few decades. There is a need to develop potential technology that can remove toxic heavy metals ions found in polluted environments. One of the most serious environmental problems is heavy metal pollution in water and soil. The presence of heavy metals even in traces is toxic and detrimental to both flora and fauna. Wastes containing metals are directly or indirectly being discharged into the environment, which is a serious threat to human life (Ayangbenro, and Babalola, 2017).

Discharge from industry contains various organic and inorganic pollutants. Among these pollutants are heavy metals which can be toxic and / or carcinogenic and which are harmful to humans and other living species (Renge *et al.,* 2012). The heavy metals of most concern from various industries include lead (Pb), zinc (Zn), copper (Cu), arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni) and mercury (Hg) (Mehdipour *et al.*, 2015). They originate from sources such as metal complex dyes, pesticides, fertilizers, fixing agents (which are added to dyes to improve dye adsorption onto the fibers), mordents, pigments and bleaching agents (Rao *et al.,* 2010).

In developed countries, legislation is becoming increasingly stringent for heavy metal limits in wastewater. Various treatment techniques employed for the removal of heavy metals include chemical precipitation, ion exchange, chemical oxidation, reduction (Electrochemical treatment), reverse osmosis (Membrane technologies), ultra filtration, electrodialysis and adsorption (FU and Wang, 2011). However, some disadvantages, such as high cost, incomplete removal, high-energy consumption, and / or generation of toxic wastes accompany these technologies. Therefore, a cost-effective treatment that efficiently removes heavy metals from industrial effluents is needed.

Among these methods, adsorption is the most efficient as the other technique. Ion exchange, membrane technologies are extremely expensive. An advanced and cost effective technique for the removal of heavy metals from the waste waters has been directed towards biosorption. Some of the promising natural biosorbents like algae, fungi, bacteria and yeast have proved to be potential due to their metal sequestering properties and the tendency for decreasing the concentration of heavy metal ions in the solution (Volesky, 1986).

Microorganisms including fungi and bacteria have been reported to extract heavy metals from wastewater through bioaccumulation and biosorption. Microorganisms can uptake heavy metal ions either actively (bioaccumulation) and /or passively (biosorption). Biosorption refers to the passive heavy metal ions uptake by different forms of biomass, which may be dead or alive. The advantages of biosorption are low cost, high efficiency of heavy metal ions removal from dilute solutions, regeneration and possible metal ions recovery. An attempt was therefore, made to isolate fungi from sites contaminated with heavy metals for higher tolerance and removal from wastewater.

Using microorganisms (*i.e.* fungi, bacteria, algae and yeasts) as biosorbents to remove metal ions from wastewater offers a potential alternative to existing methods. The adsorption method is a relatively new process and is emerging as a potentially preferred alternative for the removal of heavy metals because it provides flexibility in design, high-quality treated effluent and is reversible and the adsorbent can be regenerated (FU and Wang, 2011).

The major sources of cadmium include metal refineries, smelting, mining and the photographic industry and it is listed as a Category-I carcinogen by the International Agency for Research on Cancer (IARC) and a group B-1 carcinogen by the USEPA (Friberg *et al.*, 1992).

The toxicity of cadmium to microorganisms damage nucleic acid, denature protein, inhibit cell division and transcription, inhibits carbon and nitrogen mineralization (Ayangbenro, and Babalola, 2017), while the toxicity of mercury decrease population size, denature protein, disrupt cell membrane, inhibits enzyme function.

Mercury is also harmful and it is a neurotoxin that can affect the central nervous system. If it is exceeded in concentration, it can cause pulmonary, chest pain and dyspnea (Namasivayam and Kadirvelu, 1999).

In this paper, it has been aimed at portraying the biosorption process, various methods followed for the heavy metal removal from wastewater, and we attempted to optimize the performance of the laboratory scale bioremoval experiments.

The effect of operational conditions (concentrations of cadmium and mercury, contact time, pH, and temperature) were also investigated in this study.

In addition, this paper surveys the various fungal isolates as natural bioadsorpents used as adsorbents and natural biosorbents for the removal of cadmium and mercury from wastewater.

This process obtained from biological material and is comparatively cheap. However, cost analysis is an important criterion for selection of an adsorbent for heavy metal removal from wastewater.

Materials and Methods

Collection of samples

Samples of soils, sewage, sludge and industrial effluents were collected in sterilized containers from sewage treatment plants at Taif, KSA. These samples were brought to laboratory and kept in refrigerator at 4°C for further processing.

Preparation of heavy metal solutions

The 1000-ppm stock solutions of Cd and Hg ions were made in double distilled water using $CdCl_2$, and $HgCl_2$. The 25, 50, 100,250,500 and 1000 ppm solutions of these heavy metals were prepared from 1000 ppm stock solution by dilution with double distilled water. The stock solution of heavy metals was sterilized separately through bacteriological filters and added to sterilized potato dextrose and nutrient broth to make its concentration 25, 50,100,250 and 500 ppm.

Isolation of heavy metal resistant fungi

Fungal isolates were isolated from samples of sewage, sludge and industrial effluents by serial dilution method using potato dextrose agar. Heavy metals polluted soil samples were serially diluted up to 10^9dilutions using sterile saline and the diluted samples are plated on the sterile potato dextrose agar (PDA) plates amended with Mercuric chloride (25, 50, 100, 250, 500 and 1000 ppm) and Cadmium chloride (25, 50, 100, 250, 500 and 1000 ppm) using spread plate method.

The plates incubated at 27°C for 4 to 7 days. Plates examined and different isolates further purified by repeated single colony isolation. The fungal isolates identified using cultural morphology, cellular morphology and biochemical tests. Cultural morphology to determine the colony color, shape and texture

studied on PDA medium. All fungal isolates were maintained on glucose peptone medium containing 20 g/l glucose, 20 g/l peptone, 5 g/l yeast extract, and 15 g/l agar, at pH 7 and maintained on GPYA (Glucose Peptone Yeast Extract Agar) medium [composition (g/L): glucose-40; peptone-5; yeast extract-5; agar-30; pH-5.6] incubated at room temperature for 48hrs.

Purification

The purification procedure of the fungal isolates was carried out by the agar streak plate method. All fungal colonies of different forms and colour showing separate growth on both Czapeck-Dox's agar and PDA media were picked up and restreaked following the zig-zag method onto the agar surface of plates containing the same isolation media. At the end of incubation period, only the growth, which appeared as a single separate colony of distinct shape and color, was picked up and restreaked again for several consecutive times onto the surface of agar plate of isolation media to ensure its purity. Purity was checked up microscopically and morphologically. Pure isolates only were subcultured on slants of its specific isolation medium and kept for further investigation. The purified colonies were prepared to be used for a complete identification process and other studies. The pure cultures of were maintained on Potato dextrose agar (PDA) slants at 4°C.

Identification of heavy metals resistance fungal isolates

The cultures were identified based on macroscopic (colonial morphology, colour, texture, shape, diameter and appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia and presence of sterile mycelium). Pure cultures of fungi isolates were identified with the help of literature (Domsch *et al.*, 1980; Barnett and Hunter, 1999).

Parameters controlling the resistance of two most potent fungal strain to cadmium and mercury

To produced mycelium pellets, 6 agar plugs (5 mm) originating from actively growing seven days old PDA solid cultures (log phase) (Anahid *et al.*,2011),were collected and inoculated in 250 ml conical flasks containing (100 ml) autoclaved (121°C,15 min and 15 psi) potato dextrose broth (PDM) medium. Flasks were incubated in incubator at 28°C for 7 days in dark conditions. A 7 days old mycelium was used as the inoculum in the bioaccumulation experiments (Prigione *et al.*, 2009; Kacprzak and Malina, 2005).

Mycelial pellets obtained after incubation periods were harvested through Whatman filter paper No.42 and washed three times with deionized water to remove any residual growth media from biomass. Pellets were heat inactivated by autoclaving and dead biomass was used immediately thereafter (Slaba and Dlugonski, 2011).

An appropriate amount of washed live biomass was dried in oven at 80ºC overnight. The dried mycelia were grinded using a mortar to obtain powder in the smallest particle size and subsequently used as a biosorbent. The smaller particles resulted in a larger surface area (Zhou, 1999). Biomass has been crushed to prevent particle aggregation for enhancing the biosorption capacity. The dry biomass was stored at room temperature in polyethylene tubes in a vacuum desiccator until use (Ezzouhri *et al.*, 2010).

Effect of contact time

Time course experiments were conducted in 250 mL Erlenmeyer flasks with a working

PDB volume of 100 mL contaminated with 1000 ppm cadmium and mercury concentrations for two most potent fungal isolates at pH 6 for 1 and 7 days (Kacprzak and Malina, 2005).

Effect of pH

The bioaccumulation of cadmium and mercury ions by the two most potent fungal isolates was carried out at different pH ranging from 4-7.5. Fungal inoculated culture medium containing heavy metals was incubated at pH of 4, 4.5, 5, 5.5, 6, 6.5, 7 and 7.5.The initial pH of solutions was adjusted by adding 0.1 M solutions was adjusted by adding 0.1 M HCL and 0.1 M NaOH. After incubation periods, the culture medium was filtered and the mycelium was weighted.

Effect of temperatures

Bioaccumulation of cadmium and mercury by the two most potent fungal isolates was carried out at different temperature ranging from 20 to 45°C. Fungal inoculated culture medium containing cadmium and mercury was at temperature of 20, 25, 30, 35, 40 and 45°C. After incubation under all optimal conditions, the fungal mycelia were weighted.

The parameters (initial metal concentration, contact time, pH and temperature), which were considered in a cadmium and mercury biosorption assay by dried mycelia, were the same as those for biosorption by dead mycelia except that 0>2 g of dried biomass powder was placed in each Erlenmeyer flask.

The effects of initial metal ion, initial pH and contact time on were examined using one way ANOVA followed by post-Hov multiple comparisons by Duncan's method. The difference was considered significant when $P<0.05$.

Results and Discussion

Identification of cadmium and mercury ions resistance fungal isolates

Eighteen heavy metals fungal isolates were identified of based on macroscopic (colonial morphology, colour, texture, shape, diameter and appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia and presence of sterile mycelium). Pure cultures of fungi isolates were identified with the help of literature. The heavy metals resistant fungal isolates were identified as *Acremonium sp.*, *Alternaria alternata*, *Alternaria chlamydosporum*, *Aspergillus fumigatus*, *Aspergillus ochraceus*, *Aspergillus wentii*, *Cladosporium cladosporioides*, *Cunninghamella elegans*, *Curvularia lunata*, *Fusarium chlamydosporum*, *Mucor racemosus*, *Penicillium aurantiogriseum*, *Penicillium chrysogenum*, *Penicillium expansum*, *Penicillium oxalicum*, *Rhizopus stolonifer* and *Trichoderma viride* (Table 1).

Resistance of eighteen heavy metals resistance fungal strains

Eighteen well identified heavy metals fungal strains were applied against both cadmium and mercuric chloride at different ppm viz. 25, 50,100,250, 500 and 1000 ppm respectively. Three fungal strains *Acremonium* sp., *Fusarium chlamydosporum* and *Trichoderma viride* exhibited high sensitivity to all different cadmium concentrations. Out of eighty fungal strains fifteen, fourteen strains tolerated and resistance to Cadmium at 25 and 50 ppm respectively.

Eleven fungal strains were exhibited resistance to cadmium concentrations at 100 ppm. Six fungal strains were exhibited

resistance to cadmium concentrations at 250 ppm. Only three fungal strains were exhibited tolerance to cadmium concentrations at 500ppm viz. *Alternaria alternata, Penicillium aurantiogriseum* and *Penicillium expansum*.

Only two fungal strains were exhibited to high resistance to cadmium concentration 1000 ppm viz. *Alternaria alternata* and *Penicillium aurantiogriseum*. These two most potent high resistance fungal strains to heavy metals (Cadmium chloride at 1000 ppm) were used to completed study.

These results indicated inhibition of growth of fungal strains at higher concentration of heavy metals. Similar observations about toxic effect of higher concentrations of heavy metals on growth of fungi have been reported by many authors (Table 2).

Data recorded in table 3 reveled that only two fungal strains viz. *Acremonium sp.* and *Rhizopus stolonifer* were sensitive to all mercuric concentration (25,50,100,250,500 and 1000 ppm), while all another tested sixteen strains exhibited resistance to mercuric chloride 25 ppm.

Twelve fungal strains were exhibited resistance to mercuric chloride 50 ppm while nine fungal strains exhibited resistance to mercuric chloride 100 ppm. Out of eighty heavy metals, resistance fungal strains only six fungi exhibited resistance to 250 ppm mercuric chloride viz. *Alternaria alternata*, *Aspergillus niger*, *Aspergillus ochraceus*, *Penicillium aurantiogriseum*, *Penicillium expansum* and *Penicillium oxalicum*.

Only three fungal strains exhibited resistance to mercuric chloride (500 ppm) while only two fungal strains exhibited resistance to mercuric chloride (1000 ppm) viz. *Alternaria alternata* and *Penicillium aurantiogriseum*.

Parameters affecting the growth of the potent two fungal strains *Alternaria alternata* and *Penicillium aurantiogriseum* on cadmium and mercury respectively

Contact time

An increase in percentage of biosorption for cadmium and mercury by *Alternaria alternata* and *Penicillium aurantiogriseum* was observed time increased and later decreased after a longer time as shown in table 4.

pH

The effect of pH on percentage biosorption of heavy metals is depicted in table 5 for both cadmium and mercury by using *Alternaria alternata* and *Penicillium aurantiogriseum*, the sorption increased at pH 6. This implies that an optimum percentage of biosorption was achieved at pH between 5 and 6.

Temperature

The sorption percentage increased with temperature for the heavy metals and experienced a significant reduction after the optimum temperature was reached. The maximum biosorption capacity of the biosorbent for cadmium and mercury by *Alternaria alternata* and *Penicillium aurantiogriseum* was achieved at temperature of 30°C. Further increase in temperature gave low effect or no on sorption percentage (Fungal growth). Therefore, the optimum temperature needed for the effective biosorption of the heavy metals in this experiment for the cadmium and mercury metals range from 25°C to 35 °C (Table 6).

Heavy metals concentrations

The growth of *Alternaria alternata* and *Penicillium aurantiogriseum* at different concentrations of two tested heavy metals

cadmium and mercury were decreased with increasing concentrations from 25 – 1000 ppm. If the toxicity of cadmium and mercury increased the growth of two most potent fungi decreased (Table 7).

Once toxic metals are present in the environment, they are cycled between its abiotic and biotic elements, posing toxicity in the latter group. The most dangerous metals the so-called "toxic trio" *i.e.* cadmium (Cd), lead and mercury for which no biological function has been found (Chojnacka, 2010).

Biosorption, bioaccumulation, biotransformation, and bio mineralization are the techniques employed by microorganisms for their continued existence in metal polluted environment. These strategies have been exploited for remediation procedures (Gadd, 2010; Lin and Lin, 2005). Heavy metal removal can be carried out by living organisms or dead biological materials. Large scale feasibility applications of biosorptive processes have shown that dead biomass is more applicable than the bioaccumulation approach, which involves the use of living organisms and thus requires nutrient supply and a complicated bioreactor system. In addition, the toxicity of pollutants, as well as other unfavorable environmental conditions, can contribute to the inability to maintain a healthy microbial population.

The cellular structure of a microorganism can trap heavy metal ions and subsequently adsorb them onto the binding sites of the cell wall (Malik, 2004).

This process is called biosorption or passive uptake, and is independent of the metabolic cycle. The amount of metal sorbed depends on the kinetic equilibrium and composition of the metal at the cellular surface. The mechanism involves several processes, including electrostatic interaction, ion exchange, precipitation, the redox process, and surface complexation (Yang *et al.,* 2015).

However, many characteristic attributes of living microorganisms have not been exploited in large-scale applications (Park *et al.,* 2010). The choice organism must develop resistance towards metal ions as it comes into contact with the heavy metal pollutant to achieve the goal of remediation. The organism of choice may be native to the polluted environment or isolated from another environment and brought to the contaminated site (Sharma *et al.,* 2000).

Biotic methods exploit natural biological processes that allow certain plants and microorganisms to help in the remediation of metals in soil and water (Hashim *et al.,* 2011). Bioremediation is gaining importance in recent times as an alternate technology for the removal of elemental pollutants in soil and water, which require effective methods of decontamination (Srivastava and Majumder, 2008).

Biosorption and bioaccumulation are two processes involved in biotreatment studies. Heavy metal bioaccumulation is as active process including metabolic activity within living organisms (Lesmana *et al.,* 2009). Biosorption is a term that usually describes the removal of heavy metals from an aqueous solution through their passive binding to a biomass (Pacheco *et al.,* 2011). In bioaccumulation, the first stage is biosorption and then, subsequent stages, related to the transport of pollutant (mainly via energy-consuming active transport systems) into the inside of cells occur (Chojnacka, 2010). Apart from using living biomass, dead and dried biomasses have been introduced as anew field of bio treatment technology. Many studies have revealed that inactive/dead microbial biomass can passively bind metal ions via various physicochemical mechanisms (Wang

and Chen, 2009)). It has been suggested that the pretreatment modifies the surface characteristics/ groups or by exposing more metal- binding sites (Dhankhar and Hooda, 2011).

Eighteen fungal isolates tolerant to heavy metals were isolated from samples of soil, sewage, sludge and industrial effluent contaminated with heavy metals using standard methods (Solarsk *et al.,* 2009). Out of eighteen three fungal strains *Acremonium* sp., *Fusarium chlamydosporum* and *Trichoderma viride* exhibited high sensitivity to all cadmium contraptions. Only three fungal strains were exhibited tolerance to cadmium concentrations at 500 ppm viz. *Alternaria alternata*, *Penicillium aurantiogriseum* and *Penicillium expansum*. Only two fungal strains were exhibited to high resistance to cadmium concentration 1000 ppm viz. *Alternaria alternata* and *Penicillium aurantiogriseum*.

Twelve fungal strains were exhibited resistance to mercuric chloride 50 ppm while nine fungal strains exhibited resistance to mercuric chloride 100 ppm. Out of eighty heavy metals, resistance fungal strains only six fungi exhibited resistance to 250 ppm mercuric chloride viz. *Alternaria alternata*, *Aspergillus niger*, *Aspergillus ochraceus*, *Penicillium aurantiogriseum*, *Penicillium expansum* and *Penicillium oxalicum.* Only three fungal strains exhibited resistance to mercuric chloride (500 ppm) while only two fungal strains exhibited resistance to mercuric chloride (1000 ppm) viz. *Alternaria alternata* and *Penicillium aurantiogriseum*.

This indicated inhibition of growth of the fungal isolates at higher concentration of two heavy metals. Similar observations about toxic effect of higher concentration of heavy metals on growth of fungi and bacteria have been reported (Malik, 2004; Rama *et al.*, 1997).

The maximum uptake of 1000 ppm of cadmium was observed *Alternaria alternata* and *Penicillium aurantiogriseum*. Also maximum uptake of mercury 1000 ppm found in *Alternaria alternata* and *Penicillium aurantiogriseum*.

The minimum uptake of 1000ppm of mercury was observed with *Alternaria alternata* and *Penicillium aurantiogriseum*. Wherever there was less growth, there was higher uptake of cadmium and vice versa.

Table.1 Identification of eighteen isolates cadmium and mercury ions resistance fungal isolates

No.	Heavy metals resistance fungi	No.	Heavy metals resistance fungi
1	*Acremonium sp.*	10	*Curvularia lunata*
2	*Alternaria alternata*	11	*Fusarium chlamydosporum*
3	*Alternaria chlamydosporum*	12	*Mucor racemosus*
4	*Aspergillus fumigatus*	13	*Penicillium aurantiogriseum*
5	*Aspergillus niger*	14	*Penicillium chrysogenum*
6	*Aspergillus ochraceus*	15	*Penicillium expansum*
7	*Aspergillus wentii*	16	*Penicillium oxalicum*
8	*Cladosporium cladosporioides*	17	*Rhizopus stolonifer*
9	*Cunninghamella elegans*	18	*Trichoderma viride*

Table.2 Effect of Cadmium ions concentration (ppm) on the growth of Eighteen identified fungal strains

No.	Heavy Metal Conc. / Organism	Control (Without $CdCl_2$)	Cadmium concentrations (ppm)					
			25	50	100	250	500	1000
1	*Acremonium* sp.	9.8±0.6	-ve	-ve	-ve	-ve	-ve	-ve
2	*Alternaria alternata*	13.5±2.4	12.6±1.8	10.8±2.1	9.7±1.1	6.1±0.7	3.8±0.9	1.9±0.4
3	*Alternaria chlamydosporum*	15.8±1.3	10.9±1.2	7.6±0.8	3.8±0.4	1.3±0.4	-ve	-ve
4	*Aspergillus fumigatus*	13.9±1.7	10.5±1.2	8.1±0.7	1.9±0.6	-ve	-ve	-ve
5	*Aspergillus niger*	17.9 ± 3.2	12.3± 1.7	8.8± 0.9	3.6± 0.7	-ve	-ve	-ve
6	*Aspergillus ochraceus*	14.6±1.8	9.7±1.1	6.3±0.5	2.4±0.6	-ve	-ve	-ve
7	*Aspergillus wentii*	12.3±0.5	8.6±0.4	5.9±0.5	1.4±0.3	-ve	-ve	-ve
8	*Cladosporium cladosporioides*	13.5±0.7	8.7±0.9	4.8±0.6	1.7±0.4	-ve	-ve	-ve
9	*Cunninghamella elegans*	11.3±0.9	6.1±0.3	3.9±0.5	-ve	-ve	-ve	-ve
10	*Curvularia lunata*	12.4±0.9	6.5±1.7	2.6±0.5	-ve	-ve	-ve	-ve
11	*Fusarium chlamydosporum*	10.2±0.6	-ve	-ve	-ve	-ve	-ve	-ve
12	*Mucor racemosus*	9.4±0.6	5.7±0.9	4.2±0.6	-ve	-ve	-ve	-ve
13	*Penicillium aurantiogriseum*	11.3±2.1	10.7±1.8	10.2±2.1	8.9±0.8	4.6±0.6	1.1±0.3	0.4±0.1
14	*Penicillium chrysogenum*	10.8±0.6	6.3±1.2	4.9±0.7	1.7±0.9	0.8±0.3	-ve	-ve
15	*Penicillium expansum*	12.7±1.2	10.3±1.4	9.0±0.8	7.9±1.2	3.1±0.5	0.8±0.3	-ve
16	*Penicillium oxalicum*	10.9±0.8	8.6±0.4	8.2±0.9	6.5±0.9	3.8±0.6	-ve	-ve
17	*Rhizopus stolonifer*	8.7±0.9	3.6±0.8	-ve	-ve	-ve	-ve	-ve
18	*Trichoderma viride*	11.2±1.4	-ve	-ve	-ve	-ve	-ve	-ve

The data are expressed as fresh weight (in grams) ± standard deviation of three independent experiments.

Table.3 Effect of Mercury (Hg) ions concentration (ppm) on the growth of certain fungal species

No.	Heavy Metal Conc. / Organism	Control (Without $HgCl_2$)	Mercuric Concentrations (ppm)					
			25	50	100	250	500	1000
1	*Acremonium sp.*	9.8±0.6	-ve	-ve	-ve	-ve	-ve	-ve
2	*Alternaria alternata*	12.6±1.7	9.7±1.9	9.2±2.3	7.3±1.2	5.2±0.9	2.3±0.6	1.1±0.3
3	*Alternaria chlamydosporum*	15.8±1.3	8.3±0.7	1.4±0.5	-ve	-ve	-ve	-ve
4	*Aspergillus fumigatus*	13.9±1.7	8.9±0.8	5.2±0.6	2.1±0.3	-ve	-ve	-ve
5	*Aspergillus niger*	18.7± 2.4	17.4± 1.6	15.6± 2.3	14.2± 0.9	8.6±0.4	0.8±0.1	-ve
6	*Aspergillus ochraceus*	14.6±1.8	10.6±0.8	7.9±0.8	3.5±1.3	1.1±0.4	-ve	-ve
7	*Aspergillus wentii*	12.3±0.5	4.3±0.8	-ve	-ve	-ve	-ve	-ve
8	*Cladosporium cladosporioides*	13.5±0.7	6.3±0.8	3.7±0.5	-ve	-ve	-ve	-ve
9	*Cunninghamella elegans*	11.3±0.9	5.8±0.4	1.9±0.7	-ve	-ve	-ve	-ve
10	*Curvularia lunata*	12.4±0.9	4.9±0.8	-ve	-ve	-ve	-ve	-ve
11	*Fusarium chlamydosporum*	10.2±0.6	7.4±0.7	3.8±0.6	1.2±0.4	-ve	-ve	-ve
12	*Mucor racemosus*	9.4±0.6	2.4±0.7	-ve	-ve	-ve	-ve	-ve
13	*Penicillium aurantiogriseum*	10.8±1.5	9.1±0.8	8.5±1.7	7.9±0.6	4.8±0.7	1.6±0.3	0.7±0.1
14	*Penicillium chrysogenum*	10.8±0.6	8.1±0.7	5.7±0.8	2.9±0.4	-ve	-ve	-ve
15	*Penicillium expansum*	12.7±1.2	9.6±0.3	6.8±0.6	3.8±0.4	1.5±0.6	-ve	-ve
16	*Penicillium oxalicum*	10.9±0.8	8.9±0.8	7.4±0.6	5.3±0.5	1.9±0.5	-ve	-ve
17	*Rhizopus stolonifer*	8.7±0.9	-ve	-ve	-ve	-ve	-ve	-ve
18	*Trichoderma viride*	11.2±1.4	4.1±0.6	-ve	-ve	-ve	-ve	-ve

The data expressed as fresh weight in grams ± standard deviation of three independent experiments.

Table.4 Effect of contact time into growth of two most potent fungal strains that incubated for 48, 72, 96, 120,144,168 and 192 hours with cadmium chloride and mercuric chloride (1000ppm)

Cadmium chloride uptake			Mercuric chloride uptake		
Contact time (h)	*Alternaria alternata* dry weight(g/100ml)	*Penicillium aurantiogriseum* dry weight (g/100ml	Contact time (h)	*Alternaria alternata* dry weight (g/100ml)	*Penicillium aurantiogriseum* dry weight (g/100ml
48	1.99±0.5	0.41±1.3	48	1.12±0.8	0.71±0.4
72	2.21±0.3	0.52±0.9	72	1.32±1.2	0.82±0.6
96	2.42±0.4	0.67±0.4	96	1.45±0.7	0.9l±0.1
144	2.45±0.3	0.82±0.6	144	1.50±0.3	0.99±0.5
168	2.23±0.3	0.81±0.1	168	1.44±1.7	0.90±1.7
192	2.22±0.1	0.81±1.4	192	1.43±0.2	0.90±1.6

Table.5 Effect of different pH values on the growth of two fungal strains *Alternaria alternata* and *Penicillium aurantiogriseum* on cadmium and mercury

Cadmium chloride uptake			Mercuric chloride uptake		
pH	*Alternaria alternata* dry weight (g/100ml)	*Penicillium aurantiogriseum* dry weight (g/100ml)	pH	*Alternaria alternata* dry weight (g/100ml)	*Penicillium aurantiogriseum* dry weight (g/100ml)
4	2.12±0.1	1.60±0.2	4	1.30±0.2	0.90±0.1
4.5	2.23±0.2	1.62±0.3	4.5	1.33±0.1	0.95±0.2
5	2.34±0.1	1.70±0.2	5	1.49±0.4	0.96±0.1
5.5	2.41±0.1	1.81±0.1	5.5	1.51±0.5	0.98±0.1
6	2.48±0.2	1.82±0.1	6	1.50± 0.1	0.99 ±0.2
6.5	2.45±0.4	1.81±0.4	6.5	1.49±0.1	0.97±0.1
7	2.44±0.1	1.80±0.1	7	1.44±0.3	0.96±0.1
7.5	2.10±0.3	1.70±0.1	7.5	1.22±0.3	0.96±0.2

Table.6 Effect of temperature on the growth of two fungal strains *Alternaria alternata* and *Penicillium aurantiogriseum* on cadmium and mercury

Cadmium chloride uptake			Mercuric chloride uptake		
Temperature (°C)	*Alternaria alternata* dry weight (g/100ml)	*Penicillium aurantiogriseum* dry weight (g/100ml)	Temperature (°C)	*Alternaria alternata* dry weight (g/100ml)	*Penicillium aurantiogriseum* dry weight (g/100ml)
20	2.39±0.5	1.50±0.2	20	1.75±0.1	0.85±0.1
25	2.48±0.12	1.51±0.1	25	1.81±0.4	0.99±0.1
30	2.54±0.1	1.50±0.1	30	1.82±0.1	0.99±0.0
35	2.11±0.1	1.44±0.2	35	1.80±0.2	0.85±0.0
40	1.51±0.2	1.43±0.2	40	1.80±0.1	0.80±0.1
45	1.22±0.5	1.42±0.4	45	1.71±0.2	0.77±0.0

Table.7 Effect of different contraptions (ppm) of cadmium and mercury on the growth of two fungal strains *Alternaria alternata* and *Penicillium aurantiogriseum* on cadmium and mercury

Cadmium chloride uptake			Mercuric chloride uptake		
Concent-rations (ppm)	*Alternaria alternata* dry weight (g/100ml)	*Penicillium aurantiogriseum* dry weight (g/100ml)	Concent-rations (ppm)	*Alternaria alternata* dry weight (g/100ml)	*Penicillium aurantiogriseum* dry weight (g/100ml)
25	13.4±0.1	11.2±0.3	25	10.7±0.2	10.3±0.2
50	12.5±0.5	10.5±0.1	50	9.5±0.1	9.1±0.1
100	10.2±0.2	9.2±0.2	100	8.2±0.2	8.5±0.1
250	7.5±0.1	5.1±0.4	250	5.9±0.1	5.6±0.2
500	4.3±0.1	2.4±0.1	500	2.9±0.5	2.1±0.1
1000	2.5±0.2	0.8±0.1	1000	2.5±0.2	1.8±0.1

The highest uptake of cadmium and mercury by *Alternaria alternata* and *Penicillium aurantiogriseum* isolates indicated more binding sites on cell wall of these fungal strains and their potential as biosorbent to remove cadmium and mercury from soil, sewage, wastewater and industrial wastewater containing higher concentration of cadmium and mercury.

These results showed that both *Alternaria alternata* and *Penicillium aurantiogriseum* are suitable for using as cadmium and mercury accumulators in wastewater. Similar results with respect to biosorption of cadmium and other heavy metals by fungi and bacteria have been reported earlier (Chang *et al.,* 1997; Puranik and Paknikar, 1999; Costa *et al.,* 2001; Pardo *et al.,* 2003; Kefala *et al.,* 1999; Ghoslan *et al.,* 1999; Say *et al.,* 2001; Watanabe *et al.,* 2003; Ozdemir *et al.,* 2004; Ayangbenro, and Babalola, 2017).

Kumar *et al.,* (2014) isolated five fungi that tolerate Pb, Cd and Cr. *Penicillium chrysogenum*, *Aspergillus nidulans*, *Aspergillus flavus*, *Rhizopus arrhizus*, *Trichoderma viride*. Fungi *Aspergillus nidulans*, *Rhizopus arrhizus* and *Trichoderma viride* showed maximum uptake capacity of 25.67 mg/g for Pb, 13.15 mg/g for Cd and 2.55 mg/g of Cr respectively. Fungal biomass has been explored by several researchers for its potential to remove copper from wastewater. The use of fungal biomass for such purposes has been hindered due to problems such as small particle size, poor mechanical strength, low density and rigidity (Ayangbenro and Babalola, 2017).

However, the use of a suitable matrix can potentially overcome these problems. Thus, Iqbal and Edyvean (2004) used a low cost, physically strong and highly porous matrix, namely" Loofah sponge" for the immobilized biomass of *Phanerochaete chrysosporium*, and a maximum adsorption capacity of 50.9 mg/g at pH 6 with 98% removal reported.

The fungi has highly porous, their mesh structure provides ready access and a large surface area for the biosorption.

Numerous studies have demonstrated that microorganisms have ability to remove heavy metals from wastewater with better performance and lower cost compared with conventional technologies (Congeevaram *et al.,* 2007).

Various researchers have shown that *Aspergillus niger* can effectively remove

uranium, lead, cadmium and copper ions (Kapoor *et al.,* 1999). Huang and Huang (1996) and Huang *et al.,* (1988) investigated the use of *Aspergillus oryzae* to remove cadmium and copper ions from aqueous solution.

Small particle size with low density, poor mechanical strength and rigidity are some of the physical problems encountered when applying biomass as a biosorbent (Han *et al.,* 2005). Immobilization of the biomass within a suitable matrix can overcome these problems by offering ideal size, mechanical strength, rigidity and porous characteristics to the biological material (Trujillo *et al.,* 1995).

Several immobilized biomass systems have been successfully used as adsorbing agents to remove heavy metals (Pan *et al.,* 2005). Loofa sponge is a natural, environmentally friendly biomaterial. It is abundant, cheap, rigid, non-toxic, chemically inert and highly porous. The use of loofa sponge material for the immobilization of algae, fungal hyphae and yeast cells has been successfully demonstrated (Akhtar *et al.,* 2008). However, the use of loofa sponge- immobilized *Aspergillus terreus* for metal biosorption has not been investigated. Ho *et al.,* (2006) reported that free *Aspergillus terreus* has high capacity for adsorbing metal ions from aqueous solutions. Sun *et al.,* (2010) studied that lead, mercury and cadmium biosorption from solutions by loofa sponge immobilized *Aspergillus terreus* and evaluate the applicability of the immobilized *Aspergillus terreus* for the removal of lead from industrial wastewaters.

Various factors influence the microbial remediation of metals. They include the bioavailability of the metal to the microbe, concentration of pollutants, electron acceptors, moisture content, nutrients, osmotic pressure, oxygen, pH, redox potential, soil structure, temperature, and water activity. The bioavailability of each metal in soil is influenced by factors such as the buffering capacity, cation exchange capacity, clay minerals content, metal oxide, and organic matter (Tak *et al.*,2013; Mani and Kumar,2014; Brar *et al.*,2006).

Although some heavy metals play important roles in the physiological, biochemical, and metabolic processes of living organisms, functioning as co-factors for some enzymes, micronutrients, regulators of osmotic pressure, and stabilization of molecules, the majority of them have no known biological function in living organisms and are toxic when generated in excess (Fachola *et al.,* 2016). The toxicity of metals is the ability of a metal to cause undesirable effects on organisms. This depends on the heavy metal bioavailability and the absorbed dose (Rasmussen *et al.,* 2000). The threat posed by heavy metals to the health of living organisms is worsened by their continuously persistent nature in the environment. Toxicity increases when the medium becomes acidic and nutrient-deficient, and when the soil structure is poor, especially in mining environments (Mukhopadhyay and Maiti, 2010).

At acidic pH levels, heavy metals tend to form free ionic species, with more protons available to saturate metal binding sites. This means that at higher hydrogen ion concentrations, the adsorbent surface is further positively charged, thus reducing the attraction between adsorbent and metal cations.

Therefore, heavy metal becomes more bioavailable, thereby increasing its toxicity to microorganisms and plants. At basic conditions, metal ions replace protons to form other species, such as hydroxo-metalcomplexes. These complexes are soluble in some cases (Cd, Ni, and Zn), while those of

Cr and Fe are insoluble. The solubility and bioavailability of heavy metals can be influenced by a small change in the pH level. Temperature also plays an important role in the adsorption of heavy metals. It has two major effects on the adsorption process. Increasing the temperature will also, increase the rate of adsorbate diffusion across the external boundary layer and in the internal pores of the adsorbate particles, because liquid viscosity decreases as temperature increases. It also affects the equilibrium capacity of the adsorbate, depending on whether the process is exothermic or endothermic. Temperature changes affect the stability of the metal ion species initially placed in solution; stability of the microorganism–metal complex depends on the biosorption sites, microbial cell wall configuration, and ionization of chemical moieties on the cell wall. An increase in the sorption capacity of lead, from 0.596 to 0.728 mg/g, was obtained when the temperature was raised from 25 to 40° (Arjoon *et al.*, 2013).

Heavy metal toxicity affects microbial population size, diversity, and activity, as well as their genetic structure. It affects the morphology, metabolism, and growth of microorganisms by altering the nucleic acid structure, disrupting the cell membranes, causing functional disturbance, inhibiting enzyme activity and oxidative phosphorylation, and causing lipid peroxidation, osmotic balance alteration, and protein denaturation (Fashola *et al.*, 2016).

At lower initial solute concentrations, the ration of the initial molecules of solute to the available surface area is low; subsequently, the sorption becomes independent of the initial concentration. However, at higher concentrations, the sites available for sorption become fewer compared with the molecules of solute present. Hence, the removal of solute is strongly dependent upon the initial solute concentration (Dhankharand Hooda, 2011). An increased metal uptake by increasing the initial metal ion concentration is a result of the increased driving force of the concentration gradient, rather than the increased initial metal ion concentration (Ghorbani *et al.*, 2008). Several researches have also found similar results as this study (Soleimani *et al.*, 2016).

The two most potent fungal isolates *Alternaria alternata* and *Penicillium aurantiogriseum* showing maximum tolerance up to 1000 ppm of both cadmium and mercury metals are tested for potential microbes to remove these heavy metals from soil, sewage and industrial wastewater and the most efficient microbes for removal of heavy metals from liquid media are identified. Further studies to realize their potential for removal of heavy metals by these fungal strains that mixed with some agricultural wastes as biosorbent agents from industrial effluents are in progress.

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