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Biosorption of uranium and heavy metals using some local fungi isolated from phosphatic fertilizers

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Abstract A number of 26 fungal cultures were isolated from different phosphatic sources, including phosphate fertilizers and rock phosphate. The highest number and percentage of isolates were obtained from tri-phosphate fertilizer and rock phosphate samples (9 isolates with 34.6% for each sample) and then 8 isolates with 30.8% from monophosphate fertilizer. These isolates were tested for heavy metals biosorption on solid medium supplemented with different metal ions at concentration of 50 ppm. Eighteen out of 26 isolates were able to grow on solid medium containing heavy metals. The selected isolates were also screened for removal of heavy metals with different concentrations (0–150 ppm) in liquid medium. Among 18 fungal cultures, 5 isolates were chosen for their high capability to tolerance of high concentration of heavy metals (150 ppm), and it was found that the fungal growth was promoted in the presence of U, Cr^{5+} Cu^{++} for FR1 (0.026, 0.133 and 0.128 g/100 ml), Cr^{5+} for FR7 (0.246 g/100 ml), Zn^{++}, Co^{++} and Pb^{++} for FR13 (0.219, 0.371 and 0.303 g/100 ml), U for FR15 (0.174 g/100 ml) and Zn^{++}, Co^{++} and Pb^{++} for FR16 (0. 203, 0.385 and 0.312 g/100 ml). These isolates FR1 & FR7, FR13, FR15 & FR16 belong to genus *Aspergillus* as identified by their morphological properties, respectively.

Introduction

Heavy metals are considered to be chemical elements which include 69 elements, of which 16 are synthetic. Some of these elements are extremely toxic to human beings, even at very low concentrations (Roane and Pepper, 2000; Wang and Chen, 2006 and Sahni, 2011). The main heavy metals associated with environmental contamination, and which offer potential danger to the ecosystem, are copper (Cu), zinc (Zn), silver (Ag), lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), strontium (Sr), cesium (Cs), cobalt (Co), nickel (Ni), thallium (Tl), tin (Sn) and vanadium (V) (Wang and Chen, 2006). In general, metal ions can be classified as follows: (1) Essential and important for metabolism (Na, K, Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn and Mo); (2) toxic heavy metals (Hg, Cr, Pb, Cd, As, Sr, Ag, Si, Al, Tl), which have no

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biological function (in ecotoxicology terms, hexavalent forms of Hg, Cr, Pb and Cd ions are the most dangerous); (3) radionuclides (U, Rn, Th, Ra, Am, Tc), which are radioactive isotopes and, although toxic to cells, they are nonetheless important in nuclear medicine procedures; 4) semi-metals or metalloids (B, Si, Ge, As, Sb, Te, Po, At, Se), which exert distinct biological effects on metals. However, metals are predominantly present in the environment in cationic and anionic forms in semimetals, and As is often classified as heavy metal (Ahluwalia and Goyal, 2007).

To date, numerous physicochemical methods have been used to remove heavy metals from the environment. Chemical precipitation and ion exchange are the most used ones, but oxidation/reduction, filtration, electrochemical treatment, reverse osmosis, membrane technology and evaporation recovery are also common (Ahluwalia and Goyal, 2007). However, if the concentration is too low, these techniques have an excessive cost and are not efficient (Vullo et al., 2008). To solve this problem, biological processes have been carried out. Biosorption is the process in which some microorganisms, such as bacteria, fungi or algae, are used to remove environmental contaminants. Some microorganisms are able to remove heavy metals and others pollutants from the environment. This means that microorganisms may be used as a cheap and efficient bioremediation tool (Wang and Chen, 2009). The processes implicated in heavy-metal detoxification include absorption, adsorption, ion exchange, surface complexation and precipitation (Muñoz et al., 2012).

Microorganisms can survive in all environments because of their innate ability to take up the pollutants as nutrients such as heavy metals due to absorptive/accumulative capability. Soil microorganisms are known to play a key role in the mobilization and immobilization of metal cations, thereby changing their availability to plants (Birch and Bachofen, 1990). Species such as *Penicillium*, *Aspergillus*, *Pseudomonas*, *Sporophyticus*, *Bacillus* and *Phanerochaete* are found to be very useful for the removal of heavy metals such as chromium and nickel (Congeevaram et al., 2006; Nilanjana et al., 2008; Abdelaty et al., 2011; Kumar et al., 2011 and Nanda et al., 2011).

The present work aims to evaluate the ability of the isolated fungal species from some phosphatic fertilizers to remove uranium and some heavy toxic metals.

Materials and methods

Sampling

Three representative samples (monophosphate fertilizer, triphosphate fertilizer and rock phosphate) were collected from different ecological sources in sterilized plastic bags. Both monophosphate and tri-phosphate fertilizer samples were collected from Abu Zaabl Fertilizers & Chemical Industries Co., and rock phosphate was obtained from Plateau Abu Tartur Western Sahara.

Media used

Medium (1): Potato-dextrose agar (PDA) (Difco Manual, 1984) was used for isolation maintenance and preservation of the fungal isolates. Its composition was as follows ($g\,l^{-1}$): potatoes, 200; dextrose, 20; agar, 20 and adjusted to pH 5.0.

Medium (2): Czapek Dox agar medium (Thom and Church, 1926) was used to quantitative and qualitative estimation of heavy metals removing fungi. Its composition was as follows ($g\,l^{-1}$): Sucrose, 30; $NaNO_3$, 3; K_2HPO_4, 1; KCl, 0.5; $FeSO_4$, 0.1; $MgSO_4{\cdot}7H_2O$, 0.5; agar, 20 and adjusted to pH 5.0. *The above medium composition was modified by addition of different metal ions to heavy metals biosorption. **Czapek Dox broth medium was the same as Czapek Dox agar medium without adding agar.

Preparation of metal solution

Stock metal solution contained 1000 mg/l concentration each of Ni^{++} ($NiCl_2{\cdot}7H_2O$), Zn^{++} ($ZnSO_4{\cdot}6H_2O$), Cd^{++} ($CdSO_4{\cdot}7H_2O$), Pb^{++} ($Pb(NO_3)$), Cr^{++++++} ($K_2Cr_2O_7$), U (UO_2) or/and Cu^{++} ($CuSO_4.5H_2O$) was prepared by dissolving heavy metals salt in distilled water. The working metal solution of 25, 50 75, 100 and 150 mg/l concentration was prepared from the stock solution. Metal solutions were sterilized by 0.2 μm pore-size Millipore sterile filters.

Isolation of heavy metals removing fungi

Ten gram of representative samples in phosphate fertilizer was suspended in 90 ml of sterile tap water and shaken thoroughly for 10 min. Heavy metals removing fungi were isolated from collected samples by spread plate techniques using medium 1. The plates were incubated at 28–30 °C for 24–48 h. Developed colonies were picked, purified and preserved at 5 °C on agar slant for further studies.

Maintenance of cultures

Stocks culture slants were maintained at 5 °C on preservation medium (medium 1) after incubation at 28–30 °C for 24–48 h.

Screenings of the most efficient heavy metals removing fungi

Screening the most efficient isolates was carried out using two steps. In primary screening step (qualitative estimation), fungal isolates were inoculated on plate agar medium (medium 2) supplemented with different heavy metal salts and the concentration of the metal salts was maintained at 50 ppm of the medium. The plates were incubated at 28 °C for 48 h. The same method was carried out with control plates (plates without metal). Each sample was made in triplicate. Finally, the plates were incubated at 28 °C for 48 h to observe the growth of fungi on solid medium.

In the secondary screening step, the quantitative estimation was carried out in plugged Erlenmeyer flasks (250 ml) containing 100 ml of Czapek Dox broth medium (medium 2) supplemented with a range of heavy metals concentrations (25–150 ppm). So, 7 sources of heavy metals such as, $NiCl_2{\cdot}7H_2O$, $ZnSO_4{\cdot}6H_2O$, $CdSO_4{\cdot}7H_2O$, $Pb(NO_3)$, $K_2Cr_2O_7$, UO_2 and/or $CuSO_4{\cdot}5H_2O$ were applied separately to give different concentrations of Ni^{++}, Zn^{++}, Cd^{++}, Pb^{++}, Cr^{++++++}, U or/and Cu^{++} ions, respectively. Theses flasks were inoculated with 2% of tested isolates and incubated at 28–30 °C 7 days. At the end of incubation period, Samples of fungal culture were filtrated using filter paper no. 1 washed twice and dried at 70 °C to constant weight.

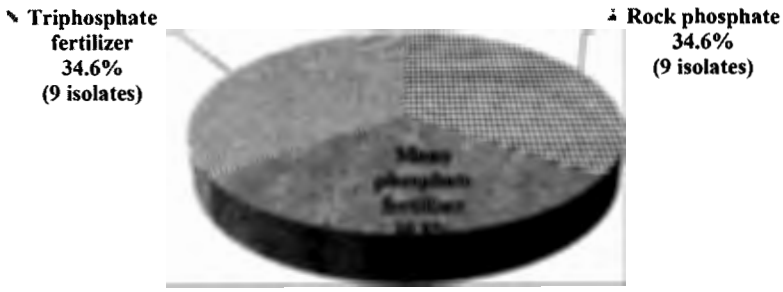

Fig. 1 The percentage distribution of microbial isolates obtained from different phosphate fertilizer samples.

Table 1 Growth of fungal isolates on solid medium supplemented with different metal ions at 50 ppm.

Isolates code	Sources of isolation	Growth of fungi at different heavy metal						
		Co^{++}	Cd^{++}	Cu^{++}	Cr^{+5}	Zn^{++}	Pb^{++}	U
FR 1	RP	+	+	+	+	+	+	+
FR 2	RP	+	+	+	+	+	+	+
FR 3	RP	+	+	+	+	+	+	+
FR 4	RP	–	–	–	–	+	–	–
FR 5	RP	+	+	+	+	+	+	+
FR 6	RP	+	+	+	+	+	+	+
FR 7	M	+	+	+	+	+	+	+
FR 8	M	–	–	–	–	+	–	–
FR 9	M	–	–	–	–	+	–	–
FR10	M	+	+	+	+	+	+	+
FR11	T	+	+	+	+	+	+	+
FR12	T	+	+	+	+	+	+	+
FR13	T	+	+	+	+	+	+	+
FR14	T	–	–	–	–	+	–	–
FR15	T	–	–	–	–	+	–	–
FR16	T	–	–	–	–	+	–	–
FR17	T	–	–	–	–	+	–	–
FR18	T	+	+	+	+	+	+	+
FR19	T	+	+	+	+	+	+	+
FR20	M	+	+	+	+	+	+	+
FR21	M	+	+	+	+	+	+	+
FR22	M	–	–	–	–	+	–	–
FR23	M	+	+	+	+	+	+	+
FR24	RP	+	+	+	+	+	+	+
FR25	RP	+	+	+	+	+	+	+
FR26	RP	+	+	+	+	+	+	+

FR = Fungal isolates, RP = Rock phosphate, M = Mono phosphate fertilizer, T = Tri-phosphate fertilizer.
+ = Growth; – = Non Growth.

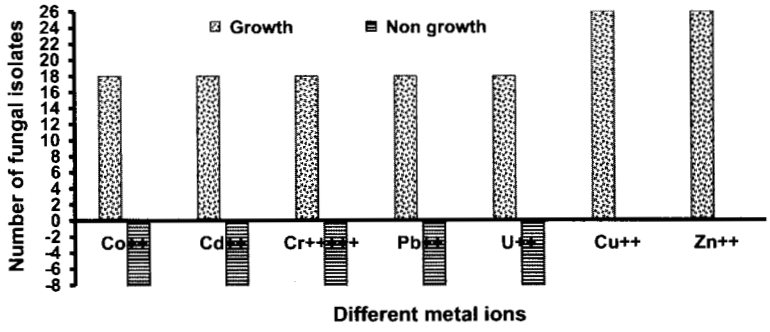

Fig. 2 The distribution number of heavy metal removing fungal isolates in the presence of different metal ions at 50 ppm.

Identification of the most efficient isolates

The most efficient fungal isolates were identified based on the microscopic shape and color of conidia according to Barnett and Hunter (1998).

Statistical analysis

The correlation coefficient between samples was analyzed with Microsoft Office Excel (2013).

Result and discussion

Isolation of heavy metal removing fungi

In the present study, 26 fungal cultures were isolated from different phosphate fertilizer and phosphatic ore samples on medium 2. The highest number of isolates was obtained from rock phosphate (9 isolates) and tri-phosphate fertilizer (9 isolates) and followed by mono phosphate fertilizer (8 fungal isolates). The percentage distribution of microbial isolates was illustrated in Fig. 1. The highest figure of isolates percentage was shown in samples collected from rock phosphate and tri-phosphate fertilizer, being 34.6% followed by isolates obtained from mono phosphate fertilizer isolates (30.8%).

Screening the most efficient heavy metals removing isolates

Qualitative estimation

Preliminary selection of removing fungal isolates was based on their ability to grow on solid medium 2 supplemented with different metal ions at 50 ppm. After 48 h of incubation period, growth of fungal isolates in the presence of metal ions such as Co^{++}, Cd^{++}, Cu^{++}, Cr^{5+} Zn^{++}, Pb^{++} and U was detected and recorded in Table 1. Data showed that among

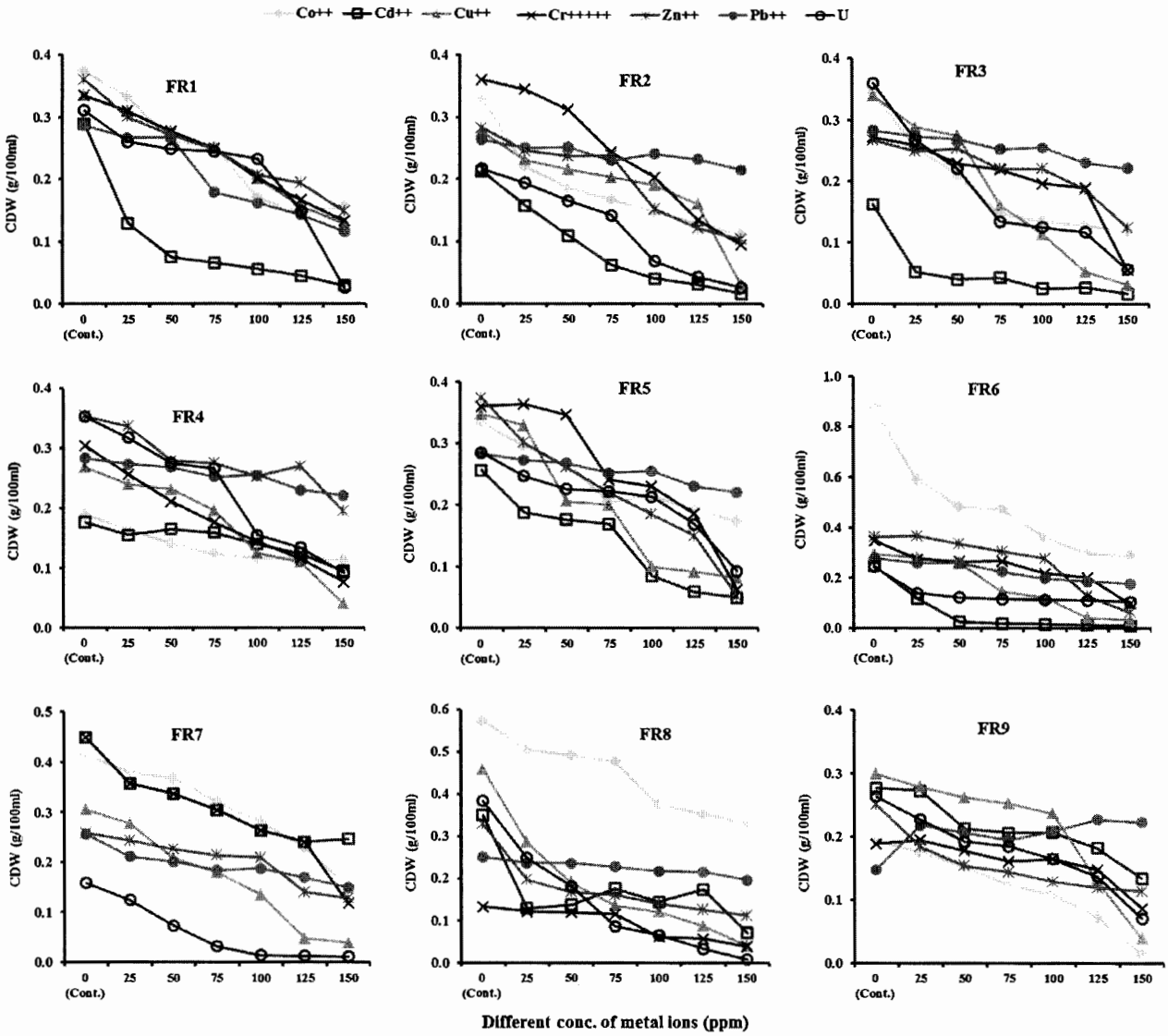

Fig. 3 Cell dry weight of fungal isolates as influenced by different heavy metals concentrations during 8 day at 28°C.

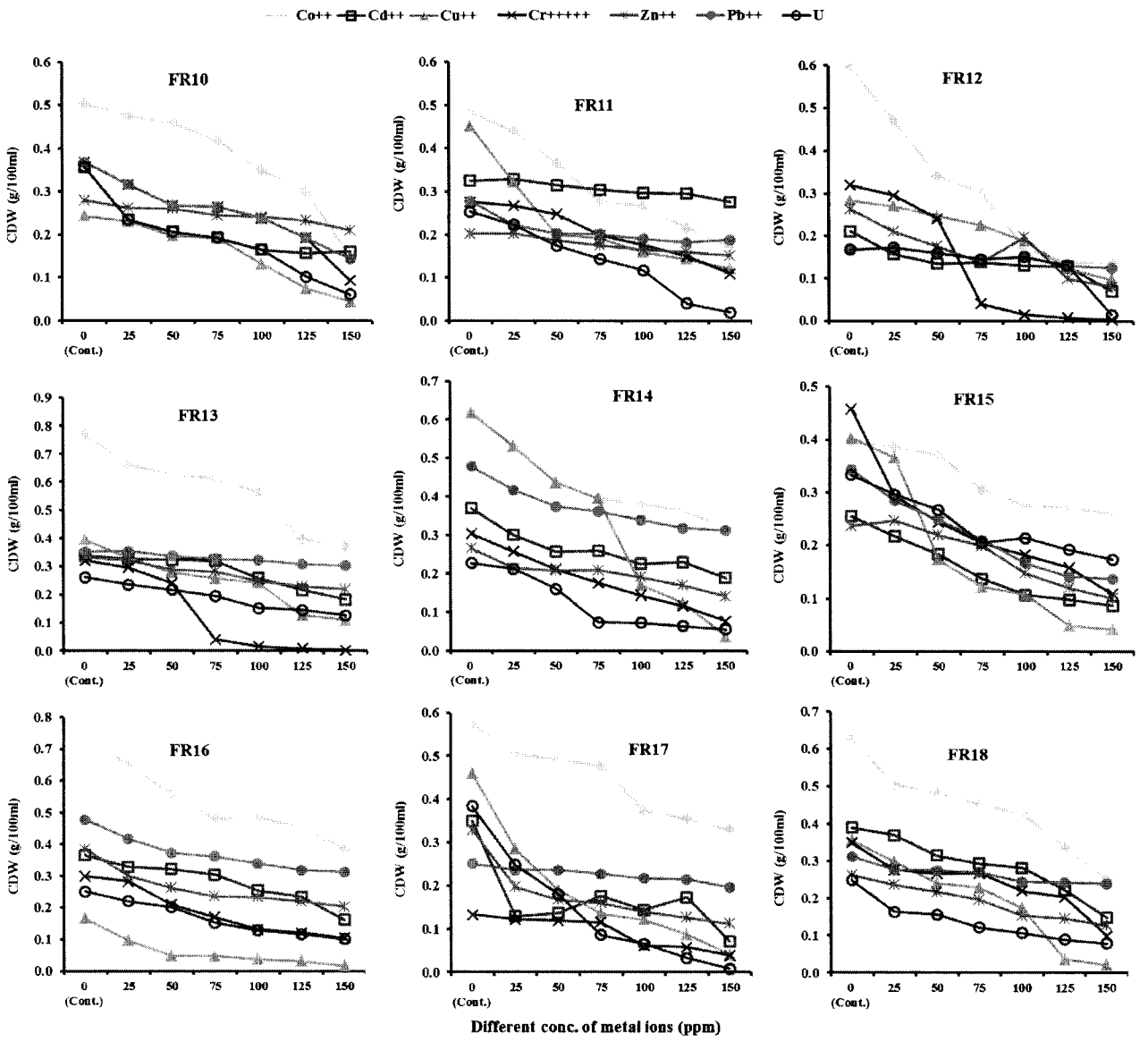

Fig. 3 (*continued*)

these 26 fungal isolates, 18 isolates gave signs of growth on agar plats supplemented with metal ions and demonstrated positive results (+). Data illustrated in Fig. 2 showed the distribution number of metal ions removing isolates. It's clearly showed the ability of all 26 fungal isolates to grow in the presence of Zn^{++} and Cu^{++} while only 18 out of 26 fungal isolates gave the positive result (growth) in the presence of other metal ions (Co^{++}, Cd^{++}, Cr^{5+}, Pb^{++} and U).

Similar results were obtained by Valix and Loon (2003). They found that the hyphae growth of *Aspergillus flavus* and *A. niger* exposed to heavy metals was mapped using the tolerance index. Species of the genus *Fusarium* and other have been isolated from contaminated soils, and their ability to tolerate the presence of different heavy metals has been investigated by Zafar et al. (2007). Moreover, Dwivedi et al. (2012) isolated heavy metal tolerant fungi from samples of sewage, sludge and industrial effluent contaminated with heavy metals (Pb, Cr and Ni) which found many strain tolerant to Pb, some tolerant to Cr and some tolerant to Ni at 25 ppm. All fungal strains exhibited growth at lower concentration of metals but it became reduced in the presence of higher concentration due to the increase in length of the lag phase as compared to control sample (Iram et al., 2012). Also, Dwivedi et al. (2012) observed that inhibition of some of the fungal isolates at higher concentration of heavy metals is mainly related to the various biological factors. This toxic effect of higher concentration of heavy metals on growth of fungi has been recognized by Malik (2004).

Quantitative estimation of heavy metals removing isolates

Eighteen fungal isolates were cultivated in liquid medium supplemented with different metal ions (Co^{++}, Cd^{++}, Cu^{++}, Cr^{+5}, Zn^{++}, Pb^{++} and U) at different concentrations (0–150 ppm). Data illustrated by Fig. 3 indicated a decrease

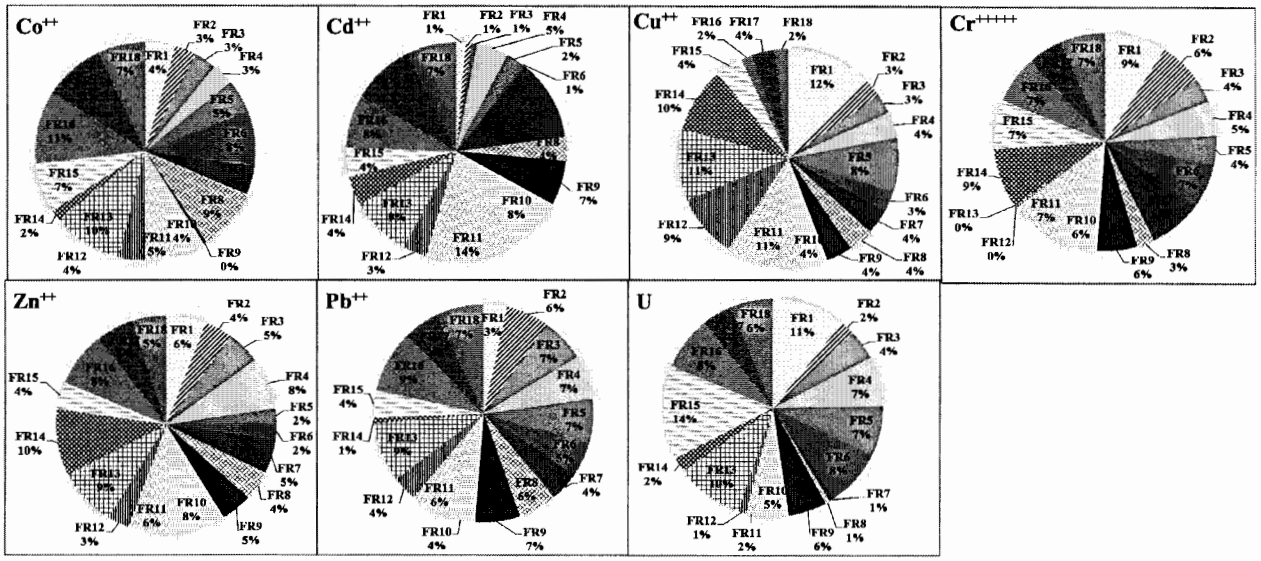

Fig. 4 The percentage distribution of heavy metal biosorption by fungal isolates at 150 ppm.

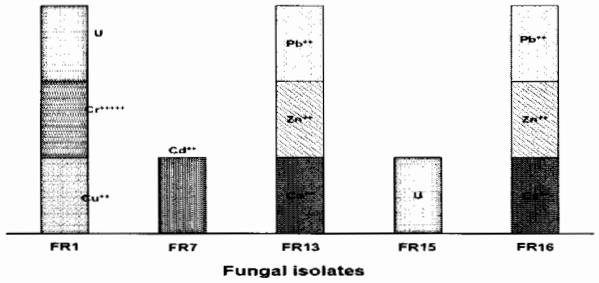

Fig. 5 The numbers and types of heavy metals biosorption (at 150 ppm) by the most efficient fungal isolates.

in the growth of tested fungal isolates by increasing the different metal ions concentration up to 150 ppm. Similar studies have been reported by Congeevaram et al. (2006). Ozer and Ozer (2003) found that high metal biosorption was obtained at initial metal concentration ranging from 50 to 100 mg l^{-1} at 30 °C temperature and pH 5.0. Ahmed et al. (2006) noticed that the biosorption of heavy metals was affected by initial metal concentration and type of the tested fungi.

The percentage distribution of metal ions removing fungal isolates at 150 ppm was illustrated in Fig. 4. The highest percentage of fungal growth in medium supplemented with different metal ions at 150 ppm was detected in percent of Co^{++} represent 10% and 11% by FR13 & FR16, Cu^{++} & Cr^{+5} represent 12% and 9% by FR1, Cd^{++} with 11% and 12% by FR11 & FR7, Zn^{++} with 9%, 10% and 8% by FR13, FR14 & FR16, Pb^{++} with 9% by FR13 & FR16 and U represent 11% and 15% by FR1 & FR15, respectively.

Among 18 fungal cultures, 5 isolates were chosen for their high capability to tolerance of high concentration of heavy metals (150 ppm), and it was found that the fungal growth was promoted in the presence of U, Cr^{5+}, Cu^{++} for FR1 (0.026, 0.133 and 0.128 g/100 ml), Cr^{5+} for FR7 (0.246 g/100 ml), Zn^{++}, Co^{++} and Pb^{++} for FR13 (0.219, 0.371 and 0.303 g/100 ml), U for FR15 (0.174 g/100 ml) and Zn^{++}, Co^{++} and Pb^{++} for FR16 (0. 203, 0.385 and 0.312 g/100 ml) respectively as shown in Fig. 5.

Statistical analysis revealed a high negative correlation coefficient (r) between the growth of fungi and metal ions concentrations for all fungal isolates ranged from −0.70 to −0.99, except FR4 in the presence of $Cr^{+5}$and FFR6 in the presence of Zn^{++} (a high positive correlation coefficient $r = 1$). The lowest negative correlation coefficient was recorded in medium supplemented with Cd^{++} for FR8 ($r = -0.30$), whereas, in the presence of Pb^{++} for FR9, the correlation coefficient was low positive ($r = -0.45$).

From all the previous data, it could be summarized that FR1, FR7, FR13, FR15 and FR16 were the best fungal isolates for growth on medium supplemented with metal ions at concentration (150 ppm). So, these isolates were selected for subsequent studies.

Identification of the most efficient isolates

According to the morphological properties (microscopic shape and color of conidia) of fungal isolates FR1, FR7, FR13, FR15 and FR16 were subjected to the preliminary classification to be the genus *Aspergillus* according to Barnett and Hunter, (1998).

In this respect, different species of *Aspergillus* have been reported as efficient heavy metals reducers (Congeevaram et al., 2006). Moreover, Dwivedi et al. (2012) found that many heavy metal tolerant isolates were identified as *Aspergillus niger* (Pb2, Cr10, Ni19, Ni27, Ni33), and *Aspergillus flavus* (Pb7, Pb8, Ni35, Ni36), respectively by the laboratory culture.

Conclusion

From the above study it is concluded that, several fungal cultures were isolated from phosphate fertilizer and rock phosphate. Five fungal isolates were found to be more efficient isolates for growth in liquid medium supplemented with different heavy metals at high concentration (up to 150 ppm). These

isolates FR1, FR7, FR13, FR15 and FR16 were identified as *Aspergillus* according to morphological properties.

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