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# Identification of the fungi absorbing heavy metals isolated from waste deposits of zinc factories, Zanjan province, Iran

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**Abstract:** The disposed wastes of zinc industries contain large amounts of heavy metals such as $Pb^{2+}$ and $Cd^{2+}$. These elements are considered as hazardous elements to human beings and other organisms. This study aims at introducing the fungal isolates that evolved to be compatible with waste deposits during the time and can absorb $Pb^{2+}$ and $Cd^{2+}$ions. During the spring and autumn in 2011, eight samples of sediment contaminated by waste were collected from Zanjan zinc industrial zone and fungal isolates were isolated. The degree of tolerance against 0–2500 mg/L $Pb^{2+}$ and $Cd^{2}$ was measured using minimum inhibitory concentration (MIC) experiment. Results revealed that two *Aspergillus* sp. isolates namely A.BZ1 isolated in spring and A.PZ1 isolated in the autumn showed the highest resistance and the maximum growth rate. The result of sorption capacity by live and dead biomasses of two isolates against $Pb^{2+}$ and $Cd^{2+}$ions with different metal concentrations showed the highest uptake by living biomass of A.PZ1 with 53.75 mg/g and 7.02 mg/g and minimum adsorption in dead biomass was 3.65 and 0.19 mg/g. The results of contact time on isolates sorption revealed that A.PZ1 with 1.715 mg/g sorption has 72.97 % removal efficiency in the first 30 minutes. After the identification of these two isolates and the combination of morphological criteria and sequencing of the ITS-rDNA region, the *A. fumigatus* was identified. The prevalence of isolate population, metal tolerance and the genome information in fungi are three traits that can be used as biomarkers for monitoring contaminants in the environment.

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**Key words:** *Aspergillus fumigatus*, biomarker, biosorption, heavy metals, MIC

## INTRODUCTION

Zanjan zinc industrial zone is located at five kilometers to the south of Zanjan city which hosts more than 46 industrial units being active in the production of zinc ingot and its derivatives. The deposited wastes of this group of industries contain elements such as lead, zinc, cadmium, cobalt, nickel and arsenic which are categorized as hazardous and toxic wastes to the nature. Unchecked excretion of these elements to the environment can through the soil and lower lands over time and therefore threaten human health and the environment through entering the food chains (Khamesy & Asadi 2009).

Elimination of heavy metals in low concentrations from aquatic solutions and environments using physicochemical methods is exorbitant and requires sophisticated technology. Replacement of the new purification methods of water contaminated with heavy metals using absorption of heavy metals by microorganisms (bio-adsorption) can be effective in the reduction of such contaminants in the environment. The previous studies showed that some of these microorganisms such as bacteria, algae, yeasts and fungi were well established and well recognized to able to absorb a large amount of metal ions (Ahalya et al. 2003; Mashitah et al. 1999). Fungi may be more efficient for the elimination of heavy metals from industrial wastes than microbes since they have high heavy metal tolerance and some other factors such as, high capacity for cell wall bands, high intracellular capacity for metal sorption and low pH (Gadd 1986).

The sorption capacity of microorganisms such as fungi, algae and plants for removal of heavy metal ions, radio–nuclides and, in some cases, lowering the toxicity of such substances have interested microbio–logists, biotechnologists and environmental engineers. As a result, several concepts of biological metal purification and also biological treatment of environmental contamination have been proposed, some of which having been employed in pilot or industrial scale (Bargar et al. 2008; Macek et al. 2008).

Metals, such as Fe, Zn, Cu and Co scanty concentrations are essential elements for metabolism and growth of fungi (Gadd 1986). Fungi and yeasts can also store innutritious elements such as cadmium,

mercury, uranium, silver and gold in themselves up to a constant level. Both live and dead cells of fungi can increase their capacity for the sorption of toxic elements and valuable metals (Arıca et al. 2001). The absorption of metal elements by fungi and yeast has rendered them as a desirable method for eliminating heavy metals from industrial wastes and recycling valuable metals (such as silver and gold) in aquatic processes. The fungal mass potential in the elimination of metals from industrial wastes has been presented by different scholars (Shumate et al. 1985, Zajic et al. 1972).

Fungi and yeasts have been used in various fermentative processes (Volesky 1991). Some of which can continually provide biomass sources for the biological absorption of metal elements (Brown et al. 2001). The present study aims at isolation and identification of heavy-metal-absorbing fungi species which are compatible with the waste deposits of zinc factories and introduction of the mentioned fungal species as biomarkers for monitoring environmental contaminants.

## MATERIALS AND METHODS

### Sampling

Eight sediment samples were obtained from the stream bed form at the depth of about 0-10 cm, in two periods of time, namely, spring (rainfall season) and autumn (dry season). The samples were collected by sterile spatula and placed in sterile plates which were kept at the temperature of 4 ºC (Prabhat et al. 2016) and were analyzed in the laboratory of the Environmental Department of Zanjan province, Iran.

### Isolation and preservation of fungal isolates

Samples from the collected sediments, which were soluble, were inoculated on the agar media (Harley et al., 1993).The YGC agar medium was prepared using Yeast Extract Glucose Chloramphenicol (37 g/l, pH 6) and distilled water. It was sterilized in an autoclaving at 121°C for 15 min and 124 k Pa and cooled down to room temperature under a microbiology hood. Sediment samples were diluted using sterile water with 200 mg/L $Pb(NO_3)_2$ and 200 mg/L $Cd(NO_3)_2.xH_2O$ and transferred on YGC agar surface and incubated at 28 ± 1 °C for 5 days. For isolation the grown fungi, a colony was obtained from each growing fungal species and placed on plates containing WA (water agar) and PDA (Potato Dextrose Agar). Incubation conducted at 28 ± 1 °C for 5 days. Single spore isolates were obtained for each growing colony (Choi et al. 1999). After the growth of fungi and the formation of colonies the fungal isolates were preserved on filter paper.

### Determination of Minimum Inhibitory Concentration (MIC)

To select metal–resistant isolates, the Minimum Inhibitory Concentration (MIC) (that is, a metallic concentration inhibiting fungal isolate growth) test was utilized (Zafar et al. 2007). First, different concentrations of $Pb(NO_3)_2$ and $Cd(NO_3)_2$ (0.00, 250, 1000, 2000, 2500 mg/L) in YGC medium were prepared. Then, it was sterilized at 121 °C for 15 minutes in the autoclave then cooling down to room temperature and distribute into sterile plates. Later, using mono – spray method, one 2–milimeter piece was separated from each newly grown mycelium and was then placed in plates containing YGC with $Pb(NO_3)_2$ and stored at the temperature of 28 °C inside an incubator for 5 days. An experiment was conducted by three replicates of each concentration. After incubation, the lowest concentration of metal ions which inhibited the growth rate of fungi was defined as the minimum inhibitory concentration of the heavy metal (MIC) which inhibited visible growth of fungi (Price et al. 2001). The isolates showed better growth rate after incubation were considered concerning tolerance to metals.

### Sorption capacity of live fungal biomass

The removal of lead and cadmium by live biomasses of two fungal strains which had the maximum growth was examined in two 250 mL Erlenmeyer flasks containing 150 mL culture medium (SDB) (Qayyum, et al. 2016). The medium was contaminated using lead and cadmium ions solution and maintained to have 100, 200 and 300 mg/L concentrations. The pH level of all flasks were adjusted to the pH 5 and then sterilized, 5-mL sample of each flask was collected using a test tube and one drop of $HNO_3$ normal was added to each in order to analyze ions (initial metal concentration $C_i$). Subsequently, they were cooled down to about the room temperature and inoculated by spore suspensions of the isolates. Then all flasks were placed on an orbital shaker (at 150 rpm) at 28 ± 1 ºC for 5 days. All samples were filtrated and supernatant fraction was analyzed for the remaining ion concentrations ($C_f$). Then, all the biomasses were washed and dried in the oven at 50 ºC and weighed to measure the mass of biomass (w). The initial ($C_i$) and final ($C_f$) metal concentrations were determined before and after the process. All of the experiments were replicated twice. The absorption capacity of live biomass was estimated according to equation (1) (Zhang et al. 1998).

$$q_e = ( C_i - C_f )\times v/m \qquad (1)$$

Where:

$q_e$ Amount of metal ion adsorbed per amount of adsorbent (milligram per gram)

$C_i$ Initial metal ion concentration (milligram per liter) before adding the fungus

$C_f$ Residual metal ion concentration (milligram per liter) after adding the fungus

w Weight of dry biomass (gram)

v Final volume of solution (liter)

The removal efficiency of the elimination of heavy metals was calculated using the equation (2), (Anbia et al. 2012).

$$RE = \frac{(C_i - C_f)\times 100}{C_i} \qquad (2)$$

**Sorption capacity of non-alive biomasses**

Live biomasses of two isolates were prepared by SDB culture media and after the growth period, the biomasses were harvested from the medium, washed twice in distilled water, and inactivated using autoclave at 121 ºC. Samples were dried at 60 ºC for 24 h, and were broken into pieces in porcelain pounder and dried again in the oven for 2 hours and cooled in a desiccator. Initial batch adsorption experiments of the isolates were carried out with 0.04 mg of dried biomass in a 10mL solution at pH 5 (lead and cadmium 10 mg/L) (Azizi et al. 2012).

A 2 mL of each sample in the test tube was obtained and kept in the refrigerator 4 ºC to analyze $Pb^{2+}$ and $Cd^{2+}$. All of the experiments were replicated twice. Samples were placed on an orbital shaker (150 rpm) at 28±1 ºC for 280 minutes and then were filtered and the supernatant fraction was analyzed to determine the $C_f$ of $Pb^{2+}$and $Cd^{2+}$.

**Preparing the solution**

Stock solution (1000 mg/L) of Pb was prepared using salts of $Pb(NO_3)_2$ and $Cd(NO_3)_2$. The concentration ranges varied between 5, 10, 15, 20 mg/L for a single metal solution. All pH levels were adjusted with 0.1 N HCl or 0.1 N NaOH. In this experiment, all different temperatures, volume of samples, pH and the liquidity of the solution were considered stable at each concentration.

**Method of calculation**

The standard solutions for atomic absorption device were prepared from the basic 1000 mg/L solutions. After calibration of the device measurement of the reference material, the samples' blank were considered. All the prepared samples were measured by VARIAN AA110 Atomic absorption device.

**Identification of the fungal isolates**

**Morphological characterization**: The slide culture technique was employed for the initial identification of the selected isolations. First, the sterile microscopic slides were prepared and a 10-mm piece of semisolid sterile medium was placed in each of these slides. A single spore of each isolate was placed on it, and the slide was placed in a Petri dish containing 2 drops of sterile distilled water and was preserved in the incubator at the temperature of 28 ºC for 5 days. After the growth, the fungal samples were stained using lactophenol cotton blue and examined using a microscope under magnification of 100x in order to identify the fungal taxon (Hong et al. 2010). Colony characteristics and microscopic studies were recorded from cultures grown on CYA (Czapek Yeast Extract Agar) and MEA (Malt Extract Agar) plates after seven days incubation in MEA at 25 ºC and in CYA at 37 ºC (Hong et al. 2006).

**Molecular characterization:** Total genomic DNA was extracted from 7-days colonies according to the procedure described by Liu et al. (2000) with some modifications. In order to examine the accuracy of DNA extraction, electrophoresis was conducted in 0.8 % agarose gel. Nucleic acid stain for visualization of double-stranded DNA was performed by DNA Safe Stain (Sinaclon, Iran). The ITS–rDNA region was amplified by polymerase chain reaction using ITS-1 F (5'–CTT GGT CAT TTA GAG GAA GTA A–3') (Gardes & Bruns 1993) and ITS–4 (5'–TCC TCC GCT TAT TGA TAT GC–3') (White et al. 1990) primers.

In the present study, the ready PCR mixture made by the Denmark Ampliqon Company containing dNTPs, Taq-DNA polymerase, $MgCl_2$ and PCR buffer was used. The amplification was performed in a volume of 25 µL using 10 pm of each primer and 10 ng of genomic DNA in the Thermocycler MJ-PTC 200 model (Peltier thermal cycler).

The PCR cycles included an initial denaturation at 95 °C for 90 seconds, followed by 35 cycles consisting denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 30 s, and a final extension for 6 min at 72 °C.

PCR products of five isolates were sequenced by Macrogen Company (South Korea) in forward direction. Sequencing results were first edited by the CHROMAS version 1.7.6 (http://www.technelysium.com.au) and Editseq ver. 5.01. The Basic Local Alignment Search Tool (BLAST) was utilized to compare the similarities of nucleotide sequences with GenBank database (http://www.ncbi.nlm.nih.gov/blast) (Altschul et al. 1997).

## RESULTS

After cultivating the spring and autumn sediment samples, six isolates forming the grown dominant population were selected. According to the appearance features of the grown colonies, *Aspergillus* isolates formed the highest population. In a similar study, Akhtar et al. (2013) separated twelve *Aspergillus* isolates from the soils contaminated using heavy metals. In several studies an *Aspergillus* isolate has used for the absorption of heavy metals such as bio-decolorization of textile dye effluent by *A. fumigatuus* (Rana & Samir 2014; Tehrani et al. 2015). The use of *A. terreus* live biomass for elimination of Chromium (Sugasini et al. 2014). The utilizing *A. foetidus* for lead elimination (Chakraborty et al. 2013) and mercury elimination by *A. flavus* (Kurniati et al. 2014).

**Minimum Inhibitory Concentration (MIC)**

Result of measuring the radial growth diameters of fungal colonies on the medium contaminated with lead nitrate and cadmium ions are shown in the Table 1 and (Fig. 1). Four isolates of autumn samples, including A.PZ1, A. PZ2, A.PZ3, and A.PZ4 and two isolates of the spring samples A.BZ1 and A.BZ2 which had the growth potential were selected.

According to the results, the selected isolates revealed a good tolerance for lead and cadmium elements and their MIC were estimated to be more than

2000 mg/L. Among the isolates, A.BZ1 spring isolate with the maximum growth diameter 0.5 cm and A.PZ1 autumn isolate with the maximum growth diameter 0.4 cm were selected as the appropriate isolates for the continuation of the study. The capacities of different fungi for elimination of copper and lead ions were assessed by Price et al. (2001). The previous study has shown that *Aspergillus niger* is the best choice for heavy metal eliminations in comparison with other fungi (Hong et al. 2010).

**Absorption capacity of lead and cadmium by live biomass**

When a single ion was used, A.PZI isolate showed 53.7 mg/g sorption, which is the highest absorption rate in comparison with A.BZI isolate. Also, when cadmium ion was used as the single ion, A.PZI isolate with the absorption of 7.020 mg/g, showed more absorption rate than A.BZI isolate with the absorption of 5.719 mg/g. The results of sorption by the two isolates are presented in (Fig. 2).

The absorption results of $Pb^{2+}$ and $Cd^{2+}$ions in the form of the binary ions showed that spring isolate (A.BZI) with the lead ion adsorption of 21.579 mg/g had the most adsorption rate in comparison with autumn isolate (A.PZI) with the absorption of 10.951 mg/g. In comparison with cadmium ion, A.BZI isolate with the absorption of 6.286 had more uptake rate than A.PZI isolate with the absorption of 4.094 mg/g. The results of sorption by the two isolates are presented in (Fig. 3).

**Adsorption capacity of lead and cadmium by non-alive biomass**

The results of adsorption capacity using the non-alive biomass of two isolates for the concentration of 10 mg/L from $Pb^{2+}$and $Cd^{2+}$ ions, respectively demonstrated the autumn isolate possess the most adsorption capacity of 3.651 mg/g for lead and also 0.83 mg/g for cadmium ion (Fig. 4).

**Table 1.** Minimum Inhibitory Concentration (MIC) of $Pb^{2+}$ and $Cd^{2+}$ ions by autumn isolates (A.PZ1, A.PZ2, A.PZ3, A.PZ4) and spring isolates (A.BZ1, A.BZ2) of *Aspergillus fumigatus*.

| Metal concentration (mg/L) | Autumn isolates | | | | Spring isolates | |
|---|---|---|---|---|---|---|
| | A.PZ1 | A.PZ2 | A.PZ3 | A.PZ4 | A.BZ1 | A. BZ2 |
| 0 | 5.6 | 6 | 5.7 | 5.2 | 7 | 7 |
| 250 | 5.2 | 5.4 | 4.2 | 5.1 | 5.8 | 6.2 |
| 500 | 5 | 4.8 | 4 | 4 | 5.6 | 6 |
| 1000 | 3.8 | 3.4 | 2.8 | 2.9 | 3.5 | 2.2 |
| 1500 | 1.4 | 1.3 | 2 | 1.2 | 1.5 | 1.2 |
| 2000 | 0.4 | 0.2 | 0.2 | 0 | 0.5 | 0.2 |
| 2500 | 0 | 0 | 0 | 0 | 0 | 0 |

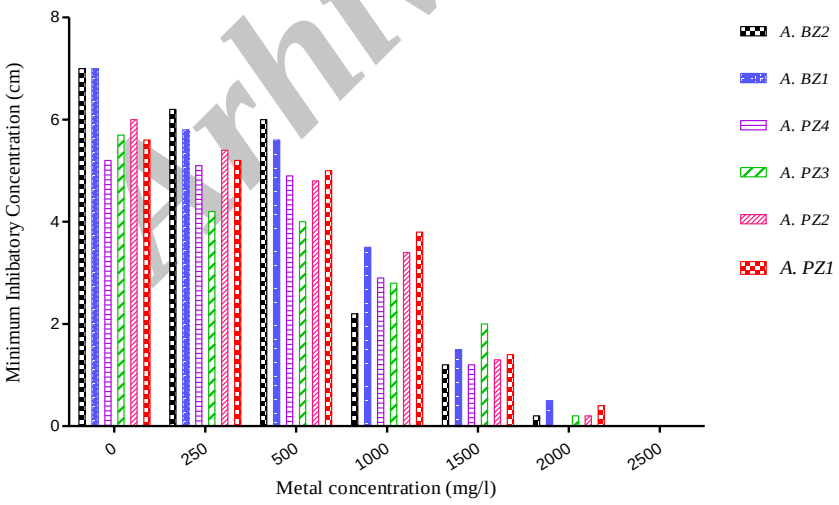



**Fig. 1**. Metal concentrations and radial growth of autumn isolates (A.PZ1, A.PZ2, A.PZ3, A.PZ4) and spring isolates (A.BZ1, A.BZ2) of *Aspergillus fumigatus.*

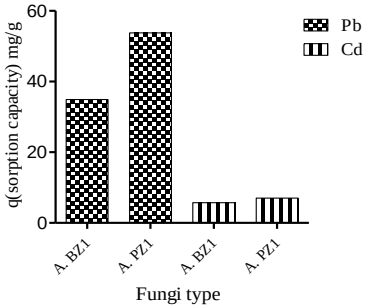



**Fig. 2.** Diagram of single absorption of $Pb^{2+}$ and $Cd^{2+}$ ions by *Aspergillus fumigatus* isolates (A.BZ1 and A. PZ1).

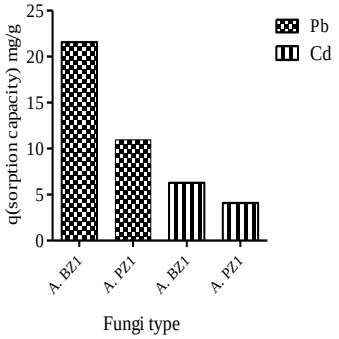



**Fig. 3.** Diagram of binary absorption of $Pb^{2+}$ and $Cd^{2+}$ ions by *Aspergillus fumigatus* isolates (A.BZ1 and A.PZ1) at 28°C and pH 5.

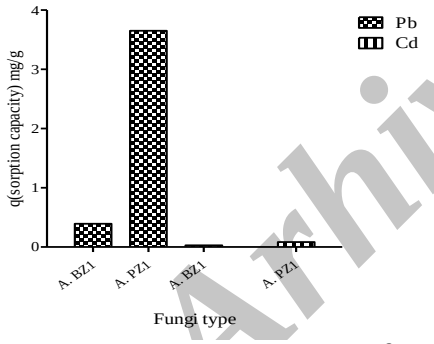



**Fig. 4.** Diagram of single adsorption of $Pb^{2+}$ and $Cd^{2+}$ ions by *Aspergillus fumigatus* isolates (A.BZ1 and A.PZ1).

Absorption rate means against joint ion of lean and cadmium revealed that autumn isolate (*A.* PZ1) was determined to be maximum 1.645 mg/gr for lead ion and spring isolate (*A.* BZ1) was determined to be maximum 0.196 mg/gr for cadmium ion (Fig. 5).

### Effects of contact time on the adsorption capacity

### Single ion experiment

To determine the effects of contact time for $Pb^{2+}$ and $Cd^{2+}$ions uptake by non-alive biomass of A. BZI and A. PZI isolates, experiments were conducted against 10 mg/L concentration of $Pb^{2+}$ and $Cd^{2+}$ions in the form of single ion for 480 minutes and in pH 5. The results are presented in Table 2.

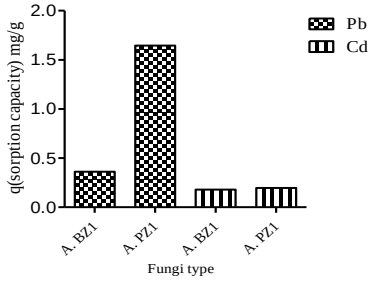



**Fig. 5**. Diagram of binary absorption of $Pb^{2+}$ and $Cd^{2+}$ions by *Aspergillus fumigatus* isolates (A.BZ1 and A.PZ1).

According to Fig. 6, the sorption of metal was rapid in the first stage of contact time and then decreased and after 120 minutes it reached an equilibrium level. The absorption of lead by biomasses of A.PZ1showed 1.715 mg/g in the first 30 minutes with maximum removal efficiency of 72.97 % and in contrast, the spring isolation with the 0.285 mg/g had the least lead ion absorption with the removal efficiency of 12.12 %in the first 10 minutes. For cadmium ion uptake, the results of this experiment showed maximum uptake of 0.103 mg/g by A.PZ1 isolate with the removal efficiency of 13.89%. It had the highest absorption efficiency in the first 30 minutes in comparison with the spring isolates.

The sorption competition between separate directions showed that the reaction between live biomass of isolates against the elements had an important role in the biological absorption, the physicochemical properties. The biomass type and the reaction of the fungal isolate against metal ions are important factors in biosorption (Hong et al. 2010).

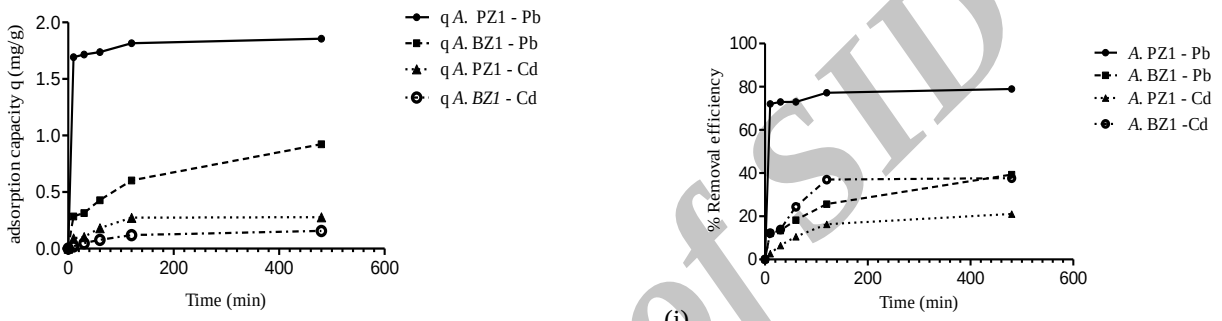
Gadd (1993) reported that the difference in metal absorption by live organisms depended on chemical factors of the cell wall. The results of a study on *A. fumigatus* showed that this fungus has a high ability for lead absorption and the lead absorption rate depended on the growth rate. The result of study by Al-Garniet al. (2009) on *A. fumigatus* in aquatic solution showed that dry biomass of this fungus was a suitable choice for cadmium absorption. On the other hand, the metal ion absorption depended on fungus growth rate and the isolates. The rate of metal ion absorption by fugal isolates under the effect of time expressed the absorption capacity of the absorbent. This feature is used for designing the purification systems and increasing purification efficiency. In a similar study, the absorption of heavy metals by *Rhizopus* showed that *Rhizopus* isolates could absorb 4.33 mg/L chromium ion and 2.72 mg/L of cadmium ion (Ahmad et al. 2005).

### Identification of Fungal Species

Micro morphological observations were carried out using MEA colonies and for colony criteria description using CYA colonies (Hong et al. 2006).

**Table 2**. Effects of contact time on biosorption of $Pb^{2+}$ and $Cd^{2+}$ ions by *Aspergillus fumigatus* isolates.

| Time (min) | Pb | | | | Cd | | | |
|---|---|---|---|---|---|---|---|---|
| | A.BZ1 | | A.PZ1 | | A.BZ1 | | A.PZ1 | |
| | q | Re | q | Re | q | Re | q | Re |
| | mg/g | % | mg/g | % | mg/g | % | mg/g | % |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0.285 | 12.12 | 1.693 | 72.02 | 0.020 | 2.71 | 0.090 | 12.20 |
| 30 | 0.315 | 13.40 | 1.715 | 72.97 | 0.048 | 6.44 | 0.103 | 13.89 |
| 60 | 0.428 | 18.19 | 1.738 | 73.93 | 0.078 | 10.50 | 0.180 | 24.40 |
| 120 | 0.603 | 25.63 | 1.815 | 77.23 | 0.120 | 16.27 | 0.273 | 36.94 |
| 480 | 0.923 | 39.25 | 1.855 | 78.93 | 0.155 | 21.01 | 0.278 | 37.62 |




(i)

**Fig. 6**. Absorption and the removal efficiency of $Pb^{2+}$ and $Cd^{2+}$ ions by non alive biomass of *Aspergillus fumigatus* isolates (A.BZ and A.PZ).

The ITS–rDNA region was amplified using the primers ITS1 and ITS4 and deposited under GenBank accession No. KY563660 = A.PZ1 and KY563661 = A.BZ1. A sequence similarity survey was performed using BLASTn (Altschul et al. 1990) algorithm available via GenBank. After the combination of morphological criteria and sequencing of the ITS-rDNA region, *A. fumigatus* Fresenius, Beitr. Mykol. 81: 18. 1863. was identified which is illustrated and briefly described here:

The growth rate of colony after 7 days of incubation in dark conditions at 25 ºC on MEA (Malt Extract Agar) reached 59–70 mm and at 37 ºC on CYA (Czapek Yeast Extract Agar) reached 38–74 mm in different isolates. The colony greyish turquoise or dark turquoise to dark green to dull green, velutinous. Conidial head columnar. The size of stipes are 32.5–392 × 2.5–7 µm. Vesicles pyriform to subclavate, sometimes subglobose, but rarely globose, 6–25 µm. The conidia globose to ellipsoidal, smooth to finely rough, 2–5 × 2–5 µm. Phialides 6–13×2–4 µm (Fig. 7).

*Aspergillus fumigatus* has a worldwide distribution and is a cosmopolitan fungus with several habitats, such as soil and human (Samson et al. 2007; Raper and Fennell 1965).

## DISCUSSION

Zinc industry wastes contain a high percentage of heavy metals, such as lead and cadmium with acidic base. Results of isolation experiment of sediment zinc waste industries during two seasons showed that *A. fumigatus* isolates had the most dominated population. The MIC experiment results on the isolates against $Pb^{2+}$ and $Cd^{2+}$represented that the *A. fumigatus* A.BZ1 isolate from the spring samples with 5 mm growth and the A.PZ1 isolate from the autumn samples with 4 mm growth against 2000 mg/L demonstrated the most resistance against heavy metals. All resistant isolates can be ranked as A.BZ1 > A.PZ1 > A.PZ2, A.PZ3, A.BZ2 > A.PZ4. The comparison of the absorption rate of elements by live biomass of spring and autumn isolates in return for $Pb^{2+}$ and $Cd^{2+}$with the concentration of 100 to 300 mg/L and pH 5 displayed that the autumn live biomass of A.PZI against lead ion had 53.7 mg/g and against cadmium ion had7.07 mg/g with maximum sorption. The effects of contact time on adsorption with non-alive biomass against $Pb^{2+}$ and $Cd^{2+}$ions indicated, when all the sites were occupied, the absorption rates of ions became equal to the elimination rate of ions and as contact time increased, the rate of adsorption had no growth.

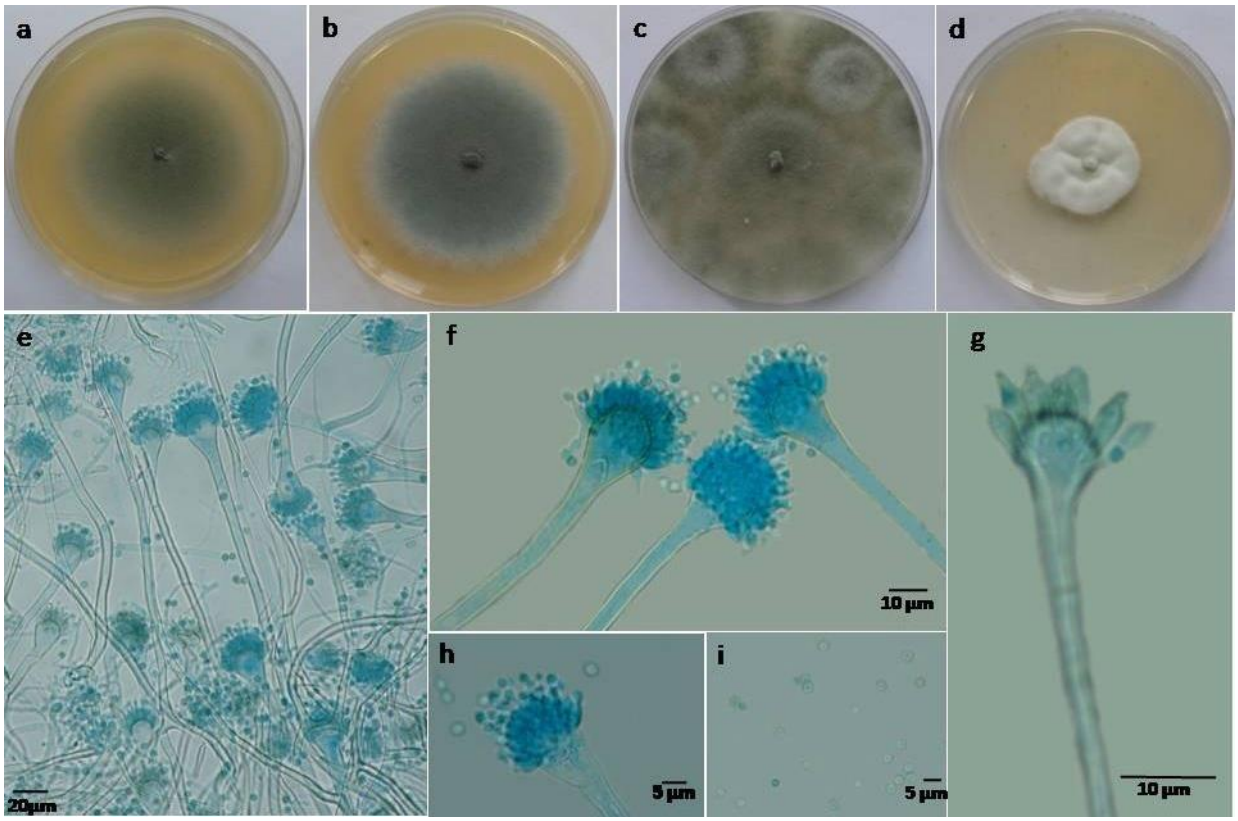



**Fig. 7.** *Aspergillus fumigatus*. a, b. Seven days colonies of autumn (A.PZ1) and spring (A.BZ1) isolates on MEA; c, d. Seven days colonies of autumn (A.PZ1) and spring (A.BZ1) isolates on CYA; e, f. conidiophores; g. vesicle and phialides; h, i. conidiophores with conidia.

Furthermore, the removal efficiency will be increased with the absorption rate. The change of sorption rate showed the limitation of sites for the sorbent for the adsorption confronted with ion competitions and gradually these sites were occupied and the continuation of absorption had a decreasing trend with the completion of absorption capacity. The knowledge of contact time and the absorption rate are two important factors in designing the biological treatment systems using biological absorbents. The results of the identification of the isolates reveal that two isolates A.PZ1 and A.BZ1 belong to the *A. fumigatus*. The presence of the dominant population of isolates this fungus in contaminated sites during the time, the sorption rate, and the ability of live biomasses in the affinity of heavy metals are the main parameters and they can be used in the live organism introduction into environmental contaminant detection as a biomarker.

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# شناسایی قارچ‌های جاذب عناصر سنگین جداسازی شده از رسوبات پسماند کارخانجات روی زنجان، استان زنجان

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**چکیده:** پسماندهای دفعی صنعت روی محتوی مقادیر بالایی از عناصر سنگین از جمله سرب و کادمیوم هستند. این عناصر از نظر خطر برای سلامت انسان و سایر موجودات، از عناصر پر خطر محسوب می‌گردند. هدف از این مطالعه معرفی جدایه‌های قارچی می باشد که در طول زمان با رسوبات پساب روی سازگار شده و توان جذب یون سرب وکادمیوم داشته باشند. طی دو فصل بهار و پائیز در سال ۱۳۹۰، هشت نمونه از رسوبات آلوده پساب شهرک تخصصی روی زنجان جمع آوری گردید و جدایه های قارچ .*Aspergillus* sp جدا سازی شدند. با آزمایش MIC (حداقل غلظت مهاری) تحمل جدایه ها نسبت به عناصر سرب و کادمیوم درمقابل غلظت ۰ تا ۲۵۰۰ میلی گرم در لیتر تعیین گردید. جدایه‌ی بهار A.BZ1 و از جدایه‌ی پائیز A.PZ1 با حدکثر رشد به عنوان مقاومترین جدایه‌ها در مقابل دو عنصر انتخاب گردیدند. نتایج ظرفیت جذب توسط بیومس زنده و مرده این دو جدایه قارچ در مقابل غلظت‌های متفاوت یون سرب و کادمیوم نشان داد بیشترین جذب توسط بیومس زنده A.PZ1 به ترتیب ۵۳/۷۵ mg/g و ۷/۰۲ mg/g، کمترین جذب برای بیومس مرده آن ۳/۶۵ mg/g و ۰/۱۹ mg/g بدست آمد. نتایج اثر زمان در جذب جدایه ها نشان داد که جدایه A.PZ1 در ۳۰ دقیقه اول با جذب ۱/۷۱۵ میلی گرم بر گرم از راندمان جذب ۷۲/۹۷ ٪ برخوردار می‌باشد. پس از شناسایی ریخت شناختی این دو جدایه و تلفیق صفات ریخت شناختی و تعیین توالی ناحیه ژنومی ITS-rDNA، گونه *Aspergillus fumigatus* شناسایی گردید. غالب بودن جمعیت، مقاومت در مقابل فلزات و اطلاعات ژنوم در قارچها سه ویژگی است که از آن‌ها می‌توان به عنوان نشانگرهای زیستی برای ردیابی آلاینده‌ها در محیط استفاده نمود.

**کلمات کلیدی:** *Aspergillus fumigatus*، نشانگر زیستی، جذب زیستی، عناصر سنگین، ظرفیت جذب

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