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# Removal of heavy metals from waste water of tanning leather industry by fungal species isolated from polluted soil

**Eman Fathi Sharaf* and Eman Alharbi**

Department of Biology, Faculty of Science, Taibah University, AL-Madinah AL- Munawarah, Kingdom of Saudi Arabia.

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**Seven fungal species (1237 colonies/g dry soil) belonging to three genera were isolated from soil sample (pH 8.7, total soluble salts 0.81 mS/cm, organic carbon 0.25%) located in the industrial city at Al-Madinah Al-Munawarah. Such soil receives a long-term application of untreated industrial effluents. The isolated species were *Aspergillus candidus*, *Aspergillus carneus*, *Aspergillus flavipes*, *Aspergillus flavus* var *columnaris, Aspergillus unguis*, *Cephalosporium curtipes* and *Cylindrophora hoffmannii*. Genus *Aspergillus*, the most dominant, accounted for 95.1% of the total count and was represented by five species. *A. candidus* was the most prevalent species on the isolation plates (37.2% of the total count) followed by *A. flavipes* and *A.unguis*. The isolated fungi were investigated for their potential to remove heavy metals from wastewater effluent of tanning leather industry. Such effluent was alkaline (pH, 8.2) with high content of total soluble salts (30.6 mS/cm) and heavy metals including $Pb^{+2}$, $Cu^{+2}$, $Fe^{+3}$, $Mn^{+2}$, $Cr^{+6}$ and $Sr^{+2}$. The isolated fungi showed significant metal sorption capacity which was species and metal dependent. Almost all the fungi showed more affinity to $Pb^{+2}$ than $Cr^{+6}$ and $Sr^{+2}$. The most dominant *A. candidus* on the isolation plates exhibited the highest activity for biosorption of heavy metals. The results indicate that fungi of contaminated soils have high level of metal biosorption capacities.**

**Key words:** Fungi, industrial wastewater, biosorption, heavy metals.

## INTRODUCTION

Heavy metals are severe pollutants released into the environment and can inhibit biological activity, especially at higher concentrations, with the sensitivity being species dependent (Means and Hinchee, 1994). Some metals can form compounds that can be toxic, carcinogenic or mutagenic, even in very low concentrations (Kazemian and Mallah, 2008; Picardo et al., 2009). Industries such as mining, metals melting, pesticides, tanneries leather industries among others produce waste water loaded with polluted materials (Pahlavanzadeh et al., 2010). The most hazard pollutants are heavy metals such as mercury, lead, cadmium, selenium, copper, chromium and arsenic (Sen and Sharandindra, 2009) which cause great environmental problems (Bunghez et al., 2010). The tanning leather industry, one of the most widespread industries, produce large amount of wastewater containing toxic pollutants including heavy metals. Therefore, the effluent of leather tanning industry must be handled carefully (Lofrano et al., 2006) and should be treated before its reuse or disposal in water bodies

*Corresponding author: Email: emanfsharaf@yahoo.com. Tel: 00966569929160. Fax: 0096648454770.

Abbreviations: **ICP-OES**, Inductively coupled plasma optical emission spectrometry; **TSS**, total soluble salts; **TOC**, total organic carbon.

(Howari and Garmoon, 2003). Such treatments are essential to prevent the contamination of drinking water and the reach of contaminants into food chain (Pang et al., 2010).

In recent years, the search for the removal of toxic metals has focused on new technologies rather than conventional methods like ion exchange, chemical precipitation, ultra filtration and so on (Veit et al., 2005) which are expensive, need energy and can produce waste products that require careful disposal (Ahalya et al., 2003). Therefore, the biological approaches have been considered as an alternative remediation for heavy metals removal from wastewaters (Pena-Castro et al., 2004). One of these methods is the biosorption which can be defined as the ability of biological materials to accumulate heavy metals through metabolism or physico-chemical pathway of uptake (Fourest and Roux, 1992). Although the bioaccumulation of heavy metals is a general character of microbial cells, fungi are superior as they have high ability to accumulate, tolerate and detoxify metals (Zafar et al., 2007) through the metal-binding capacities of their cell walls (Gupta et al., 2000). Accordingly, the present investigation is directed to the removal of some heavy metals from wastewater of tanning leather industry by fungal cells isolated from a polluted soil.

## MATERIALS AND METHODS

### Wastewater sample

Wastewater samples were collected from Tanning leather factory in Al Madinah Al Munawarah, Saudi Arabia.

### Characterization and heavy metals content of wastewater

The pH of the wastewater was measured using pH Meter (Hana, HI 8314). The wastewater was evaluated for their heavy metals content ($Cr^{6}$+, $Cu^{2}$+, $Fe^{3}$+, $Mn^{2}$+, $Pb^{2}$+, $Sr^{2}$+), using inductively coupled plasma optical emission spectrometry (ICP-OES) IRIS Intrepid II XDL/Thermo Electron Corporation, as well as total soluble salts (TSS) at Al-Musa Group for Environmental Laboratories, K.S.A.

### Preparation of wastewater sample for heavy metals detection

A volume of 100 ml of the wastewater was digested with 3 ml concentrated $HNO_3$ (for metal digestion) and 3 ml $H_2O_2$ (for digestion of any residual organic matter) at 80°C and left until the volume reached 15 to 20 ml, then cooled, filtered and completed up to a volume of 100 ml with distilled water (EPA 2007,1987).

### Measurement of heavy metals

The previously prepared sample was injected into the ICP-OES apparatus (EPA 200.7, 1987) through Auto-sampler then transferred to Nubalizer. The nebulized sample was converted to metal atoms and then to the excited atoms through a high heat source (plasma) in the presence of an inert gas. Element-specific emission spectra are produced by radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the emission lines are monitored by photosensitive device. The ICP-OES measured wavelength of the emitted spectra is converted to concentrations using calibration standards specific for each metal.

### Soil sample and soil analysis

Soil sample was collected from nonagricultural soil located in the Industrial City at Al Madinah Al Munawarah, Saudi Arabia in the vicinity of the area where wastes and industrial waste water are disposed. This soil is exposed to different pollutants including heavy metals. Therefore, it is expected that microorganisms isolated from such polluted soil is well adapted and tolerated to such environmental stress. The pH of the soil extract was measured using pH meter (Hana, HI 8314). The total organic carbon (TOC), measured by gravimetric method, and the TSS of the soil extract were estimated at Al-Musa Group for Environmental Lab, K.S.A.

### Mycological analysis

Fungal isolation was carried out using soil dilution plate method (Johnson et al., 1960). A slightly modified Martin's medium was used for such purpose. The medium contained (g/l): dextrose, 10; peptone, 5; $KH_2PO_4$, 1; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4.5H_2O$, 0.01; agar, 15 and Rose Bengal, 3.3 ml. Such components were dissolved in a diluted wastewater (1:15 v/v) instead of water. After sterilization, streptomycin (30 µg/ml) was added. About 20 ml of the cooled medium were added to each Petri-dish (15). 1 ml of soil suspension at the proper dilution was transferred aseptically and distributed equally onto Petri plates which were incubated at 30°C for 10 days. The developing fungal colonies were counted (colonies/g dry soil) and the relative density (R.D. %) of each fungal species was calculated as percentage of the total fungal load. The isolated fungi were identified according to Moubasher (1993), for soil fungi in Qatar and other Arab countries: Raper and Fennell (1965), for genus *Aspergillus* and Gilman (1957), for soil fungi.

### Screening of the isolated fungi for biosorption of heavy metals from wastewater

#### *Biomass preparation*

Two discs (1 cm diameter, 7 days old) of each isolated fungal species were cultured in flasks (Triplicate) containing 50 ml liquid Czapex Dox's medium which contained (g/l): Sucrose, 20; $NaNO_3$, 3; $MgSO_4.7H_2O$, 0.5; $KH_2PO_4$, 1; KCl, 0.5. The flasks were then incubated at 28°C for seven days and then the biomass of each fungus was harvested, washed with distilled water and 1 g of each biomass was used as a fresh adsorbent.

#### *Pre-treatment of wastewater*

The wastewater was pretreated with aluminum sulfate (1% w/v) for 2 h, to precipitate any suspended particles and impurities and then filtered.

#### *Biosorption process*

Sorption process was carried out according to Korenevskii et al. (1999). 1 g from the previously prepared mycelia mats were added to flasks (250 ml capacity) containing 100 ml wastewater. The

**Table 1.** Characterization and heavy metals content of the wastewater.

| Heavy metal | Quantity |
|---|---|
| Strontium ($Sr^{+2}$) (ppm) | 320.3±5.1 |
| Chromium ($Cr^{+6}$) (ppm) | 154.0±2.5 |
| Iron ($Fe^{+3}$) (ppm) | 41.7±1.3 |
| Lead ($Pb^{+2}$) (ppm) | 35.8±1.4 |
| Copper ($Cu^{+2}$) (ppm) | 9.7±0.2 |
| Manganese ($Mn^{+2}$) (ppm) | 3.0±0.01 |
| pH | 8.2 |
| Total soluble salts | 30.6 mS/cm |

flasks were then incubated at 28°C for 90 min in shaking incubator (125 rpm). After that, the wastewater was filtered and the residual heavy metals were measured (as previously mentioned) and the percentage removal of heavy metals was calculated.

### Data analysis

Summary statistics were used to obtain means and standard error (SE). Analysis of variance (ANOVA) to determine the significant differences (P≤0.05) between means, using the Statistical Package for the Social Sciences (SPSS) Statistical Software, was applied.

## RESULTS AND DISCUSSION

The present results reveal that the wastewater of leather industry under investigation was alkaline (pH 8.2) and saline with total soluble salts of 30.6 mS/cm and loaded with heavy metals where the highly quantities were recorded by $Sr^{2+}$ followed by $Cr^{6+}$ (320.3 and 154.0 ppm, respectively). Moderate amounts of $Fe^{3+}$ and $Pb^{2+}$ were detected (41.7 and 35.8 ppm, respectively). However, lower amounts of $Cu^{2+}$ and $Mn^{2+}$ (9.7 and 3.0 ppm, respectively) were observed in the wastewater (Table 1). According to the National Environmental Quality Standards for Industrial effluents (NEQS, 1999), the maximum permissible limit of $Sr^{2+}$ and $Cr^{3+}$ were 1.0 ppm, whereas that for $Fe^{2+}$ and $Pb^{2+}$ were 8.0 and 0.5 ppm, respectively. Meanwhile, the maximum permissible limit for both $Cu^{2+}$ and $Mn^{2+}$ were 1.0 and 1.5 ppm, respectively. Consequently, presence of such amounts of these metals in the present wastewater is considered highly pollutants and represents a hazard for the environment where they are disposed and their removal is urged issue. For comparison, the amount of heavy metals in the present wastewater was notably higher than that recorded in the leather tannery industrial wastewater obtained from leather industry plant in the south of Germany. The metals were Cr, Pb, Cu and Fe with quantities of 33.72, 1.82, 0.19 and 0.21 ppm, respectively (Hussein et al., 1995).

In this study, seven fungal species belonging to three genera were recovered from the soil sample located at the industrial city at Al-Madinah, Al-Munawarah (Table 2). Genus *Aspergillus*, the most dominant, constituted 95.1% of the total count, denoting its highest competitive ability. It comprised five species, of them *Aspergillus candidus* and *Aspergillus flavipes* were of high frequency followed by *Aspergillus unguis*. Both *Cephalosporium curtipes* and *Cylindrophora hoffmannii* were accounted for 4.9% of the total count; an observation indicating the unsuitability of such environment for their survival. In this connection, different soil fungal species can exhibit different degrees of pollution tolerance (Arnold and Kaputska, 1987) and many species could be reduced or completely eliminated in polluted soil (Gildon and Tinker, 1981).

With regard to *A. candidus* it was prevalent on the isolation plates as it accounted for 37.2% of the total load, indicating its highest density. The high dominance of *A. candidus* at such polluted soil denotes its higher degree of tolerance and adaptation to such environmental stress than the other lower dominant ones.

In this regard, weak fungal species sensitive to pollution give a chance to other well adapted fungi, to colonize leading to the increase in their number (Delvel et al., 1999). On the other hand, some fungal species have developed mecha-nisms to protect themselves from the toxic effect of heavy metals and can inhabit polluted environments well; such species can be exploited in removal of heavy metals from polluted wastewater (Glazer and Nikaido, 1995).

It is worthy to mention that, the total count of the isolated fungi was low (1237 colonies/g dry soil). This observation might be attributed to the alkalinity of the present soil (pH 8.7, data not shown), the low content of its organic matter (0.25%, data not shown) and/or the high quantities of the heavy metals, the high salinity (30.6 mS/cm) and alkalinity(pH 8.2) of the wastewater which was used in the isolation medium. The solutions are considered saline if it contain more than 15 mS/Cm of total soluble salts (Landon, 1991).

In this regard, Ezzouhri et al. (2009) reported that, pollution of soil and water by heavy metals may lead to a reduction in microbial community due to the extinction of the sensitive species. Moreover, the long exposure of

**Table 2.** Total count (colony/g dry soil) and relative density (R.D%) of fungi isolated from soil sample located at the industrial city.

| Isolated fungi | Total count (colony/g) | Relative density (%) |
|---|---|---|
| Genus *Apergillus* | 1177 | 95.1 |
| *A. carneus* | 55 | 4.4 |
| *A. flavipes* | 405 | 32.7 |
| *A.flavus* var *columnaris* | 33 | 2.6 |
| *A.candidus* | 460 | 37.2 |
| *A.unguis* | 254 | 20.5 |
| *Cephalosporium Curtipes* | 37 | 2.9 |
| *Cylindrophora hoffmannii* | 23 | 2.0 |
| Total | 1237 | 100 |

**Table 3.** Percentage removal of heavy metals from wastewater by the isolate fungi.

| Fungal species | Sorption of heavy metals (%) | | | | | |
|---|---|---|---|---|---|---|
| | $Pb^{2+}$ | $Cu^{2+}$ | $Fe^{3+}$ | $Mn^{2+}$ | $Cr^{6+}$ | $Sr^{2+}$ |
| *Aspergillus carneus* | 15.0 | 10.3 | 4.3 | 16.6 | 6.0 | 2.2 |
| *Aspergillus flavipes* | 48.0 | 32.9 | 14.8 | 3.3 | 7.3 | 0.6 |
| *Aspergillus flavus* var *columnaris* | 42.4 | 11.3 | 28.5 | 10.0 | 13.2 | 7.0 |
| *Aspergillus candids* | 54.4 | 46.3 | 45.5 | 20.0 | 9.5 | 6.3 |
| *Aspergillus unguis* | 28.2 | 6.1 | 30.4 | 6.6 | 6.4 | 1.2 |
| *Cephalosporium Curtipes* | 36.3 | 21.6 | 33.3 | 0.0 | 1.4 | 6.4 |
| *Cylindrophora hoffmannii* | 10.0 | 39.1 | 5.2 | 6.6 | 10.0 | 4.2 |

LSD between fungi at 5% was 6.8; LSD between metals at 5% was 4.4.

water and soil to heavy metals can produce changes in their microbial populations, reducing their activity and their number (Zafar et al., 2007).

The current isolated fungi were screened for their potential to remove heavy metals from wastewater effluent of leather industry (Table 3). The fungi exhibited significant ($P \leq 0.05$) sorption capacity for all the metal ions simultaneously which was species dependent and differed from metal to metal. However, the most potent biosorbent was *A. candidus* which was also the most dominant on the isolation plates. This observation recalls again and confirms the ability of *A. candidus t*o survive and colonize in the polluted habitat and get resistance for such conditions.

It is noteworthy that, the sorption capacity of all fungi was higher for $Pb^{2+}$ than $Sr^{2+}$ and $Cr^{6+}$. In this connection, biosorbent showed higher affinity towards certain metal in mixed metals solution due to the selectivity of the biosorbent and/or the ambient conditions which may prefer the adsorption of such metal over the others (Sheng et al., 2008).

Regarding this, many filamentous fungi have shown an excellent potential for bioremediation of metals from industrial effluents and wastewaters. Fungi are known for their metal binding abilities and they exhibit not only tolerance to metals (Faryal et al., 2006) but also their detoxification (Zafar et al., 2007). The metal binding capacity of fungi depends on the presence of functional amine, phosphate, sulphate, sulfhydryl, carboxyl and hydroxyl groups on the cell wall materials like chitin and chitosan (An et al., 2001).

Heavy metal resistant fungi were isolated from the soil samples of an electroplating industry where the isolated *Aspergillus niger* could sorb $Cr^{+3}$ and $Ni^{+2}$ from the industrial wastewater (Congeevaram et al., 2007). The heavy metals Mn, Cd, Cr, Cu, Zn and Pb were detected in an industrial wastewater effluent and *A. niger* isolated from sludge, could remove 50% of Cd and 58% of Zn from this effluent (Kumar et al., 2010). On the other hand, *Aspergillus lentulus* could sorb about 99% of Cu from synthetic metal solution (Jha et al., 2011) and *Penicillium citrinum* (Pang et al., 2010) can efficiently remove heavy metals from aqueous solution and effluents.

It could be concluded that soil of industrial places, is favored for isolation of fungal species which can tolerate and adapt to such environmental stress. Such species can be explored in the treatment of industrial wastewater which is loaded with heavy metals that resemble hazard pollutants for the environment. These fungal species are considered cheap, natural and safe green remedies which

which can replace the chemical conventional ones.

Finally, a successful biosorption process needs preparation of good biosorbent and optimization of the ambient conditions; this will be discussed later in a further work.

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