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# Fungal diversity in different three Egyptian soils under heavy metals stress

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**Abstract**— The objective of this study is to evaluate the diversity of fungi in heavily contaminated soils with heavy metals and characterize the most resistant isolates. Three soil samples were collected from different soil types such as clay alluvial (FFS), calcareous (WNS) and sewage farm (ARS) soils. Four heavy metals (Cd, Zn, Pb and Ni) under various concentrations and their effect on soil properties were investigated. The data showed that Alluvial and Wadi-Natrun soils had the highest values of maximum adsorption and fixation capacity of Cd, Zn, Pb and Ni metals. The highest counts of fungi were recorded in the calcareous soil and alluvial one treated with Ni. Twenty isolates were obtained from counted plates in the highest heavy metals concentrations. These isolates were screened on solid and liquid media enriched with increasing of tested heavy metals concentrations. Isolates No. FM01 and FM14 were the most tolerant for the highest concentrations of the applied Zn, Pb and Cd. Using of nano zero valent iron (nZVI) to enhance growth of fangal isolates was investigated. Over 90 % of 1700 ppm of Zn was removed by nZVI and fungal isolates could be able to grow well. As a result of morphology characteristics; isolates FM01 which tolerate 1600 ppm of Zn was classified as *Aspergillus nidulans*, while isolate FM04 which tolerate 1600 ppm of Pb and 20 ppm of Cd was recognized as *Aspergillus fumigates.* The isolate FM14 was tolerated 20 ppm of Cd and classified as *Aspergillus flavus.* Isolates FM10 and FM15 were tolerated 1700 ppm of Zn and characterized as *Aspergillus niger* and *Aspergillus terreus* respectively. The isolate FM20 which tolerate 1500 ppm of Pb and 20 ppm of Cd was classified as *Penicillium* sp.

**Key words** — Fungal resistance, Heavy metals, *Aspergillus* sp., Contaminated soil, nano zero valent iron (nZVI).

## 1 INTRODUCTION

HEAVY metals contamination of soil is widespread due to metal processing industries, tannery, combustion of wood, coal and mineral oil, traffic, and plant protection materials. Metals can exist in the soil solution as free cations (e.g. $Cu^{+2}$, $Cd^{+2}$, $Zn^{+2}$) or organic ligands (e.g. $ZnCl^{+}$, $CdCl^{-3}$, metal citrates) and associated with colloidal material (Hissler and Probst, 2006). Heavy metals occur naturally in the soil environment from the pedogenetic processes of weathering of parent materials at levels that are regarded as trace and rarely toxic (Pierzynski *et al.*, 2000 and Kabata-Pendias and Pendias, 2001). Due to the disturbance and acceleration of nature's slowly occurring geochemical cycle of metals by man, most soils of rural and urban environments may accumulate one or more of heavy metals to cause risks to human health, plants, animals, ecosystems, or other media (Khan *et al.*, 2008 and Zhang *et al.*, 2010). Ling-Yu *et al.* (2010) stated that the accumulation of heavy metals, such as Cr, Ni, Cu, As, Cd, and Zn in soils was significantly affected by land use patterns.

Some heavy metals (cobalt, chromium, nickel, iron, manganese and zinc) are essential and required by organisms as micro nutrients (Bruins *et al.*, 2000). They are involved in redox processes, in order to stabilize molecules through electrostatic interactions, as catalysts in enzymatic reactions, and regulating the osmotic balance (Hussein *et al.*, 2005). On the other hand, many other metals such as Ag, Al, Cd, Au, Pb, and Hg have not biological role (nonessential) and potentially toxic to living organisms, especially microorganisms (Hussein *et al.*, 2005 and Xincai *et al.*, 2008). However, at high levels, both of the essential and non-essential metals become toxic to the organisms. These heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity (Roane and Pepper, 2000). Heavy metals can damage microbial cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of DNA. Toxicity of these heavy metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions (Nies, 1999). Microbial population responses to heavy metal contamination provide a relevant model for ecological studies to assess the influence of environmental characteristics (Guo *et al.*, 2009). Several studies have demonstrated that metals influence microorganisms by affecting their growth, morphology and biochemical activity (Sandaa *et al.*, 2001; Tsai *et al.*, 2005 and Pérez-de-Mora *et al.*, 2006) and diversity (Dell Amico *et al.*, 2008). Microbial survival in heavy metal polluted soils depends on intrinsic biochemical properties, physiological and/or genetic adaptation including morphological, as well as environmental modifications of metal speciation (Abou-Shanab *et al.*, 2007). Soil microorganisms play critical roles in the environment such as cycling mineral compounds and decomposition of organic material. Environmental stress caused by heavy metals generally decreases the diversity and activity of soil microbial populations, and upsets the ecological balance of population interactions within the soil community.

The specific aims of presented study are determining the amounts of pollutants prevailing in the soil used in three types of technological activities (intensive agriculture, industrial activities, sewage water disposal), and identifying the numbers of Fungi in the examined soils, studying the effect of increasing concentration of soil heavy metals or pesticides on the microbial structure in the studied soils. As well as determining the minimum inhibitory concentration from pollutants for Fungal isolated from artificially polluted soils.

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# 2 MATERIALS AND METHODS

## 2.1 Soil samples

Three topsoil (0-20 cm depth) samples were collected from different sites and types in Egypt. These samples represented to alluvial soil (experimental farm of the faculty of agriculture, Cairo university, Giza), calcareous soil (Wadi-Natrun) and sewage farm sandy soil (Abu-Rawash) which were symbolized as (FFS), (WNS) and (ARS), respectively. The collected soil samples were air-dried, crushed and stored as fine soil particles (< 2 mm).

## 2.2 Characteristics of collected soil samples

1. Particle size distribution was carried out by Pipette method as described by Gee and Bauder (1986).
2. Organic matter was determined by wet oxidation method (Sparks, 1996).
3. Total CaCO3 was determined using callin's calcimeter (Sparks, 1996).
4. Soil pH and EC were measured in a standing 1: 2.5 soil water suspension using combined electrode pH meter and conductivity meter, respectively (Jackson, 1973).
5. Soluble cations Na and K were measured by flame photometer, while Ca, Mg, and soluble anions (Cl, SO4, CO3, and HCO3) were determined titrimetrically according to Page et al (1982).
6. The concentration of heavy metals (Cd, Zn, Pb, and Ni) in the Aqua-regia, DTPA extracts of adsorption isotherm supernatants solution were determined using atomic absorption spectrophotometer (Perkin Elmer A Analyst 100 U.S.A).

## 2.3 Effect of heavy-metal pollution on soil fungal populations

Portions of 100 g soil were weighted in 150 ml plastic cups and artificially contaminated with increasing concentrations of Cd (0,3,5 and 8 ppm), both Pb and Zn (70,250 and 400 ppm) and Ni (50,100 and 400 ppm) as solutions of sulphate salts. These concentrations were selected according to the guidelines of the critical limits of soil heavy metal content. Tap water was added to soil to bring the soil moisture up to field capacity, then incubated for 7 days at constant temperature (25˚C) through which the soil moisture was maintained at field capacity (F.C.). At the end of the soil incubation period, portions of spiked soils were subjected to the enumeration of fungi as well as Aqua-Regia and DTPA extractable Cd, Zn, Pb and Ni.

## 2.4 Enumeration and isolation of fungal populations

Enumeration and isolation of the dominant fungal populations was carried out the soils contaminated with heavy metals (Zn, Pb, Ni and Cd). Soil samples were serially diluted (Kapoor and Paroda, 2007) and plated onto potatoes dextrose agar medium as described by Atlas (2000). Colony forming units (CFU/g soil) were counted after incubating plates at 28 ˚C for 7 days.

A total of 20 fungal isolates which tolerated the highest levels of the tested heavy metals was collected. Fungal isolates were purified by repeated streaking on potatoes dextrose agar medium plates.

## 2.5 Screening of heavy metal resistant Fungi

Fungal isolates were screened for their resistance to heavy metals and pesticides in solid and liquid medium according to Bisht *et al.* (2012). A loopful growth of stock culture was inoculated into 5 ml sterile distilled water, vortexed and 0.1 ml of the culture suspension was inoculated onto agar medium supplemented with increasing concentrations of the examined heavy metals at the following concentrations (Table 1).

**Table 1. Heavy metal concentrations (ppm) in growth media used for screening the isolated fungi.**

| level | Cd | Zn | Pb | Ni |
|---|---|---|---|---|
| **1** | 2 | 25 | 100 | 25 |
| **2** | 5 | 50 | 300 | 50 |
| **3** | 8 | 75 | 500 | 75 |
| **4** | 11 | 100 | 700 | 100 |

Growth of the microorganisms was further challenged in the specific liquid media of fungi containing different concentrations of the examined heavy metals. Erlenmeyer flasks (250 ml) containing 100 ml of the specific medium supplemented with the examined concentration of heavy metal were inoculated with 1ml mother culture and incubated at 28 ˚C on a rotary shaker at 100 rpm. Growth of fungi in their liquid media was recorded by measuring their dry biomass weight after 15 days incubation at 30 ˚C on a rotary shaker at 175 rpm rotary shaker (Darwesh, 2008).

## 2.6 Synthesis of Nanoscaled Zero-Valent Iron (nZVI)

Synthesis of nZVI is based on borohydride reduction of Fe(III) (Wang *et al.* 2006). For the synthesis of 1.5 g of nZVI; 5.34 g $FeCl_3$ was dissolved in a 4/1 (v/v) ethanol/water mixture and stirred on a magnetic stirrer. On the other hand, 1 M sodium borohydride solution was prepared. The final $BH^{4-}$/ $Fe^{3+}$ ratio is adjusted to 3, since excess borohydride is needed for better growth of nanoparticles.

To separate the black iron nanoparticles from the liquid phase, magnet technique was used. At this point, solid particles were washed at least three times with 25 ml portions of absolute ethanol to remove all of the water. This washing process is probably the key step of synthesis since it prevents the rapid oxidation of zero-valent iron nanoparticles. Synthesized nanoparticles were finally dried in oven at 50 $^{o}$C overnight.

## 2.7 Phenotypic identification technique of fungal isolates

Fungi isolates were morphologically identified using light microscope (kyowa optical model microlux 73) equipped with a camera by-which the micro-photography of the isolates was taken.

# 3 RESULTS AND DISCUSSION

## 3.1 Soil characteristics

Three soil samples were collected from different soil type and location. The selected soils represent normal clay textured

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alluvial in Faculty's Farm (FFS) soil and calcareous in Wadi El-Natrun (WNS) soil containing 14.4 % $CaCO_3$ as well as sewage farm soil in Abu-Rawash region (ARS) containing 6.1 % Organic Matter (OM) as presented in Table (2). The results showed that the WNS soil is alkaline and had pH level reached to 8. The data showed that Zn metal recorded the highest concentrations in all the investigated soils followed by Pb as in the order: Zn> Pb> Ni> Cd. In addition, it was found that sewage-farm soil (ARS) contained the highest concentrations of aqua-regia and DTPA extracted heavy metals especially for Pb and Zn but still in the range of marginal polluted soils as indicated by Angelova *et al.* (2010) and Erika-Andrea *et al.* (2010).

**Table 2. Physical and chemical characteristics of the investigated soil samples.**

| Soil Analysis | | | Faculty's Farm (FFS) | Abo Rawash (ARS) | Wadi El-Natrun (WNS) |
|---|---|---|---|---|---|
| pH (1:2.5) | | | 7.76 | 7.32 | 8.06 |
| EC dS/ m (1:2.5) | | | 1.43 | 1 | 1.26 |
| Soluble anions (me/l) (1:2.5) | | $HCO_3$ | 3.3 | 4.1 | 2.9 |
| | | $Cl^-$ | 4 | 2.6 | 3.8 |
| | | $SO_4^{--}$ | 7.5 | 3.6 | 6.6 |
| Soluble cations (me/l) (1:2.5) | | $Na^+$ | 7 | 3.1 | 5.5 |
| | | $K^+$ | 0.3 | 0.8 | 0.2 |
| | | $Ca^{++}$ | 5.4 | 4.3 | 6.4 |
| | | $Mg^{++}$ | 2.2 | 2.1 | 1.2 |
| Organic Matter (OM) | | (g/kg) | 13 | 61 | 8 |
| $CaCO_3$ | | (g/kg) | 11 | 26 | 144 |
| Total N | | (g/kg) | 1.4 | 24 | 0.8 |
| Heavy metals (mg/kg soil) | Pb | AQ.R* | 2.75 | 133.15 | 10.2 |
| | | DTPA | 0.76 | 6.63 | 1.2 |
| | Cd | AQ.R | 2.75 | 0.9 | 0.06 |
| | | DTPA | 0.03 | 0.14 | 0.02 |
| | Zn | AQ.R | 77.8 | 446.6 | 55.1 |
| | | DTPA | 5.5 | 23.06 | 7.6 |
| | Ni | AQ.R | 4.03 | 2.23 | 0.48 |
| | | DTPA | 0.55 | 0.1 | 0.05 |

**AQ.R*= Aqua-Regia extract**

### 3.2 Fungal diversity in heavy metals artificially polluted soils

The total fungal counts (TFC) of the investigated soils under the stress of increasing concentrations of heavy metals (Cd, Zn, Pb and Ni) were presented in Table (3). The data showed that counts of fungi decreased with increasing the concentration of all the tested heavy metals in all the investigated soils. The highest values were noticed in the calcareous soil (WNS) and alluvial one (FFS) treated with Ni and reached to 21 and 30 * $10^4$ cfu/g soil, respectively. In case of Cd and Pb, The soil of sewage farm (ARS) showed the highest of TFC. With respect to Zn, it is interesting to notice that the highest of TFC values was recorded in the calcareous soil (WNS) which could be rendered to the antagonism of Zn-Ca which inhibited the toxicity of Zn ion. Elekes and Busuioc (2011) stated that heavy metal absorption by higher fungi is influenced primarily by the species, but also by soil pH and concentration of other metals in the soil and this interaction of chemical elements can be synergistic and/or antagonistic.

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**Table 3. Total Fungal counts in FFS, ARS and WNS Soils polluted with Heavy metals**

| Heavy metal concentrations (ppm) | | TFC*$10^4$ | | |
|---|---|---|---|---|
| | | ARS | FFS | WNS |
| Control | | 30 | 100 | 10 |
| Cd | 3 | 9 | 3.2 | 8 |
| | 5 | 1 | 0.4 | 0.4 |
| | 8 | 0.1 | 0.15 | 0.37 |
| Zn | 70 | 1.6 | 2.5 | 24 |
| | 250 | 1 | 0.07 | 2.4 |
| | 400 | 0.1 | 0.03 | 0.13 |
| pb | 100 | 15 | 0.34 | 1.8 |
| | 250 | 1.2 | 0.13 | 0.4 |
| | 400 | 0.4 | 0.09 | 0.08 |
| Ni | 50 | 2.2 | 30 | 21 |
| | 100 | 0.2 | 10 | 2.5 |
| | 400 | 0.02 | 4 | 0.3 |
| Mix (Zn,Cd,Ni,pb) | 1* | 0.2 | 0.80 | 0.4 |
| | 2* | 0.1 | 0.9 | 0.06 |
| | 3* | 0.01 | 0.5 | 0.053 |

**TFC = Total Fangal Counts; 1= (3 Cd, 70 Zn, 100 Pb, 50 Ni); 2 = (5 Cd, 250 Zn, 250 Pb, 100 Ni); 3 = (8 Cd, 400 Zn, 400 Pb, 400 Ni).**



### 3.3 Isolation and Screening of heavy metal tolerant fungi on solid media

Twenty fungal isolates were obtained from counted plates in the highest heavy metal concentrations in all the soils and subjected for screening test on solid nutritional media enriched with increasing concentrations of heavy metals. The screening results of heavy metals resistant fungi over 1000 ppm of Zn, Ni and Pb and 12 ppm of Cd evaluated on solid medium was reported (data not found in this paper). All isolates had good growth on solid medium containing tested heavy metals concentrations until 1000 ppm of Zn, Ni and Pb and 12 ppm of Cd (the data not represented in this paper). The results showed that isolates No. FM01 and FM14 were the most tolerant for the highest concentrations of the applied Zn, Pb and Cd (1500, 1500 and 20 ppm). Generally, all fungi isolates were tolerant to 1100 ppm Pb concentration.

The biomass of fungal growth under heavy metals effect were measured as (mg dry weight/100 ml) on nutritional liquid media containing 1500 ppm of Zn and Pb or 20 ppm of Cd. The growth curves presented in Table (4) showed that the maximum biomass of fungi isolates was recorded at 10 days then decreased according to the normal growth curves. It was found that isolate No. FM10 had the highest biomass growth at 1500 mg Zn/kg. Isolate no. FM04 showed the highest resistances to Pb stresses followed by isolate FM14 in the following order: FM04> FM14> FM16> FM20. With respect to Cd stresses (20 ppm), isolate FM14 was the most resistance (the highest biomass growth) as shown in the order: FM14> FM20> FM04.

The fungal isolate FM04 was the most resistant to Pb, while isolates FM10 and FM15 were the most tolerant to Zn concentrations up to 1700 ppm in the liquid media (Table 4). Four isolates namely; FM04, FM14, FM16 and FM20 were tolerated 20 ppm of Cd in liquid medium. However, the isolate FM14 was the most tolerant to Cd (Figure 1).

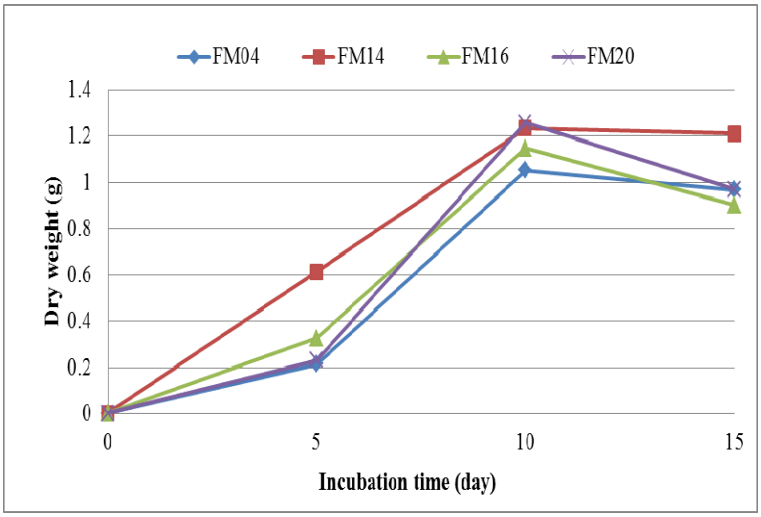


**Fig. 1. Growth of four isolates in liquid medium containing 20 ppm of Cd.**

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**Table 4. Screening of heavy metals resistant fungal isolation in liquid medium.**

| Isolate No. | Heavy metal | Concentrations (ppm) | Incubation time (day) | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 5 | 10 | 15 |
| FM01 | Control | | 0.0 | 0.50 | 1.12 | 0.834 |
| | Zn | 1500 | 0.0 | 0.31 | 0.67 | 0.661 |
| | | 1600 | 0.0 | 0.026 | 0.025 | 0.014 |
| | | 1700 | 0.0 | 0.0 | 0.0 | 0.0 |
| FM04 | Control | | 0.0 | 1.28 | 2.35 | 1.985 |
| | Pb | 1500 | 0.0 | 1.14 | 2.14 | 1.884 |
| | | 1600 | 0.0 | 0.236 | 0.842 | 0.624 |
| | | 1700 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Cd | 20 | 0 | 0.214 | 1.052 | 0.968 |
| | Zn | 1500 | 0.0 | 0.16 | 0.94 | 0.807 |
| FM09 | Control | | 0.0 | 0.75 | 1.50 | 1.21 |
| | Zn | 1500 | 0.0 | 0.10 | 1.22 | 1.128 |
| FM10 | Control | | 0.0 | 1.19 | 1.98 | 1.73 |
| | Zn | 1500 | 0.0 | 0.65 | 1.70 | 1.66 |
| | | 1600 | 0.0 | 0.852 | 1.369 | 0.741 |
| | | 1700 | 0.0 | 0.258 | 0.951 | 0.687 |
| FM14 | Control | | 0.0 | 0.97 | 1.75 | 1.85 |
| | Cd | 20 | 0 | 0.613 | 1.235 | 1.212 |
| | Pb | 1500 | 0.0 | 0.80 | 1.53 | 1.755 |
| | Zn | 1500 | 0.0 | 0.30 | 0.85 | 0.735 |
| FM15 | Control | | 0.0 | 0.66 | 1.21 | 0.802 |
| | Zn | 1500 | 0.0 | 0.40 | 1.00 | 0.959 |
| | | 1600 | 0.0 | 0.547 | 0.951 | 0.654 |
| | | 1700 | 0.0 | 0.159 | 0.753 | 0.357 |
| FM16 | Control | | 0.0 | 0.73 | 1.29 | 0.961 |
| | Cd | 20 | 0 | 0.325 | 1.147 | 0.902 |
| | Pb | 1500 | 0.0 | 0.61 | 0.93 | 0.485 |
| FM20 | Control | | 0.0 | 1.10 | 2.02 | 1.80 |
| | Pb | 1500 | 0.0 | 0.31 | 0.53 | 0.4 |
| | Cd | 20 | 0.0 | 0.231 | 1.258 | 0.97 |

### 3.4 Enhancement of fungal isolates growth by using nano zero valent iron (nZVI)

The aim of this study is to enhance the growth of fungal isolates which reverse affected by increase of Zn concentrations. The growth of three fungal isolates (FM01, FM10 and FM15) in liquid media containing 1700 ppm Zn before and after treated by nano zero valent iron (nZVI) is presented in Figure (2). The results show that the growth of three fungal isolates in medium after treated by nZVI was very enhanced nearly to control which growing in medium without heavy metals (Fig. 2). This likely due to remove Zn from liquid media. This result was agreed with Liang *et al.* (2014) who reported that with the solids concentration of 1 g/L nZVI, more than 85% of $Zn^{2+}$ could be removed within 2 h.

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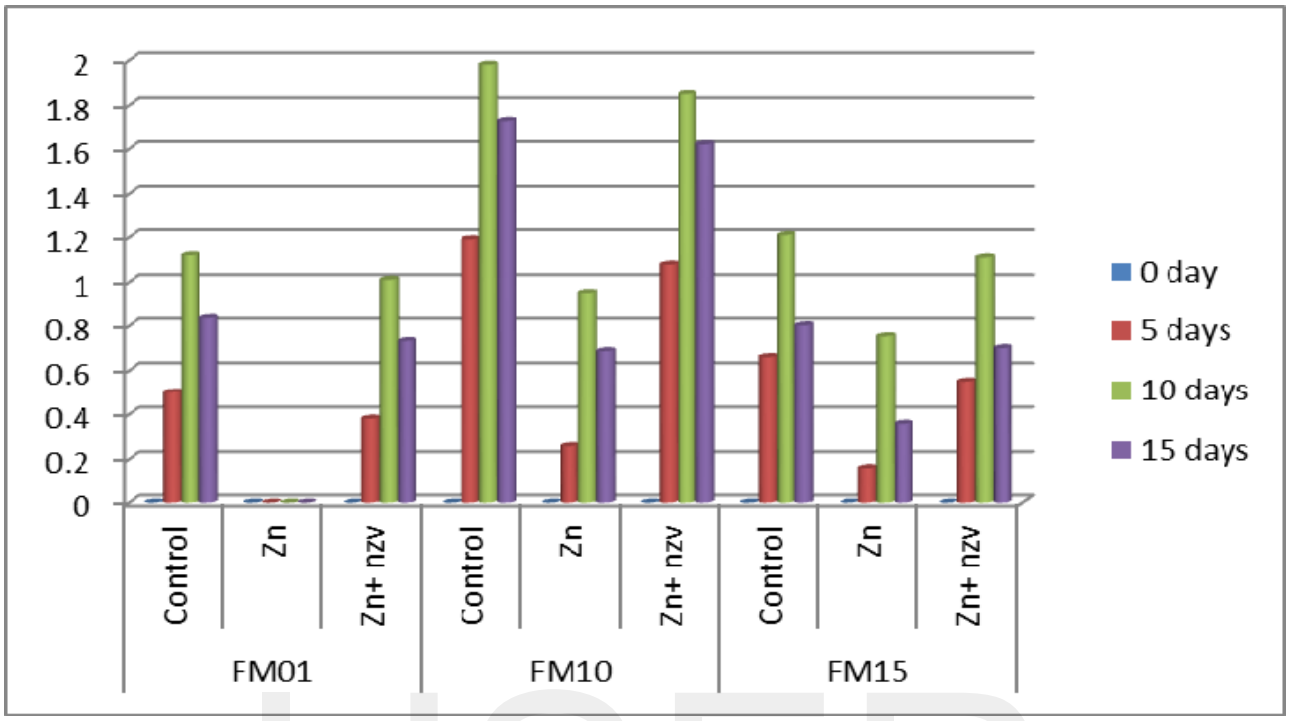


**Fig. 2. Efficiency of using nano zero valent iron to remove Zn toxicity on three fungal isolates.**

### 3.5 Morphology and identification of the heavy metals tolerant fungi colonies

The screened fungi colonies which tolerated the maximum concentrations of each heavy metal were purified and subjected to microscopic investigation. The morphology of fungi colony was described including its color and the approximate taxonomy according to Iram *et al.* (2012) and the results was presented in Table (5). The obtained morphological description showed the isolates were classified to two defferent genera namely *Aspergillus* and *Penicillium*. Isolates FM01 which tolerate 1600 ppm of Zn was classified as *Aspergillus nidulans*, while isolate FM04 which tolerate 1600 ppm of Pb and 20 ppm of Cd was recognized as *Aspergillus fumigates*. The isolate FM14 was tolerated 20 ppm of Cd and classified as *Aspergillus flavus*. Isolates FM10 and FM15 were tolerated 1700 ppm of Zn and characterized as *Aspergillus niger* and *Aspergillus terreus* respectively. The isolate FM20 which tolerate 1500 ppm of Pb and 20 ppm of Cd was classified as *Penicillium* sp. (Table 5).

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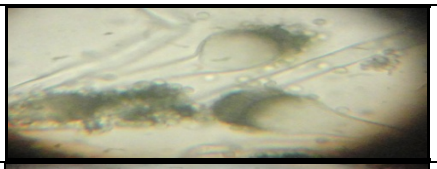
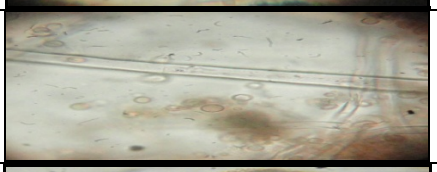
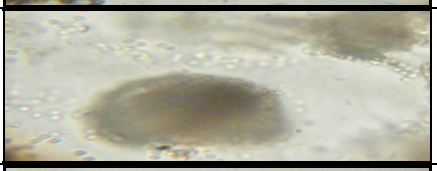
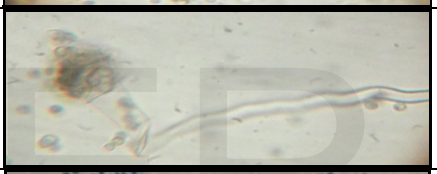
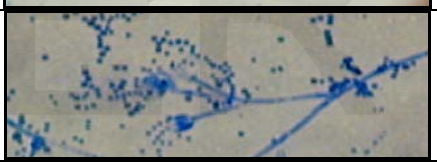
**Table 5. Morphology of the screened heavy metals tolerant colonies of soil fungi isolates**

| Isolates No. | Visual Identification | Photo | Soil location |
|---|---|---|---|
| FM01 | *Aspergillus nidulans* |  | (ARS) |
| FM04 | *Aspergillus fumigates* |  | (ARS) |
| FM10 | *Aspergillus niger* |  | (FFS) |
| FM14 | *Aspergillus flavus* |  | (WNS) |
| FM15 | *Aspergillus terreus* |  | (WNS) |
| FM20 | *Penicillium* sp. |  | (FFS) |

**Abu-Rawash (ARS); Alluvial soil (FFS); Wadi-el-Natrun (WNS)**

## 4 REFERENCES

**Abou-Shanab, R.A.I.; Van Berkum, P. and Angle, J.S. (2007).** Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphre of *Alyssum murale*. Chemosphere, 68: 360-367.

**Angelova1, V.R.; Ivanova, R.V.; Todorov, J. M. and Ivanov, K. (2010).** Lead, Cadmium, Zinc, and Copper Bioavailability in the Soil-Plant-Animal System in a Polluted Area. The Scientific World Journal, 10: 273-285.

**Ashraf, M.S.; Ranjha, A.M.; Yaseen, M.; Ahmad, N. and Hannan, A. (2008).** Zinc adsorption behavior of different textured calcareo soils using Freundlich and Langmu models, Pak. J. Agri. Sci., 45(1):1-7.

**Atlas, R. M. (2000).** Handbook of Microbiological Media. CRC Press, 2ed, New York. 2005 pp.

**Bisht, S.S.; Praveen, B.; Rukmini, M.; and Dhillon, H. (2012).** Isolation and Screening of Heavy Metal Absorbing Bacteria from the Industry Effluent Sites of the River Nagavali. Inter. J Pharm. Sci., Res., 3(5): 1448-1451.

**Bruins Mark, R.; Sanjay, K. and Frederik, O.W., (2000).** Microbial Resistance to Metals in the Enviroinment. Ecotox. Environ. Saf., 45(3): 198-207.

**Darwesh, O.M. (2008).** Protection of Agricultural Soil near textile industry plants by bioremediation of textile dyes residues. M.Sc. Thesis, Faculty of Agriculture, Cairo University, Giza, Egypt. 163p.

**Dell'Amico, E.; Mazzocchi, M.; Cavalca, L.; Allievi, L. and Andreoni, V. (2008).** Assessment of bacterial community structure in a long-term copper-polluted ex-vineyard soil. Microbiol. Res., 163: 671-683.

**Elekes, C.C. and Busuioc, G. (2011)**. The effect of metallic content of soil on the absorption and accumulation for some species of fungi used in soil's Bioremediation. Seria Agronomie, 54 (2):127-132.

**Erika-Andrea, L.; Mirela, M.; Marin, Ş.; Oana, C.; Cecilia, R. and Valer, M. (2010).** Assessment of Pb, Cd, Cu and Zn Availability for Plants in Baia Mare Mining Region. Journal of Plant Development, 17: 139-144.

**Gee, G.W. and Bauder, J.W. (1986).** Particle-size analysis, in: Klute, A. (Ed.), Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods. S Sci. Soc. Amer., Madison, WI, pp. 383-411.

**Guo, Z.; Megharaj, M.; Beer, M.; Ming, H.; Rahman, M.M.;**

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ISSN 2229-5518

**Wu, W. and Naidu, R. (2009).** Heavy metal impact on bacterial biomass based on DNA analysis and uptake by wild plants in the abandoned copper mine soils. Biores. Techn., 100: 3831-3836.

**Hissler, C. and Probst, J.L. (2006).** Impact of mercury atmospheric deposition on soils and streams in a mountainous catchment (Vosges, France) polluted by chlor-alkali industrial activity: the important trapping role of the organic matter. Sci. Total Environ., 361: 163–178.

**Hussein, H.; Sfarag, K. and Kandil, H.M. (2005).** Tolerance and uptake of heavy metal by *pseudomonads*. Proc. biochem., 40: 955-961.

**Iram, S.; Arooj, A. and Parveen, K. (2012).** Tolerance potential of fungi isolated from polluted soil of Multan, Pakistan. Journal of Biodiversity and Environmental Sciences, 10: 27-34.

**ISO 11466. (1995).** Soil quality, Extraction of Trace Elements Soluble in Aqua Regia. ISO, International Organization for Standardisation.

**ISO 14870. (2001).** Soil quality -- Extraction of trace elements by buffered DTPA solution. ISO, International Organization for Standardisation.

**Jackson, M.L. (1973).** Soil Chemical Analysis, Prentice Hall of India (Pvt) Ltd.,New Delhi. 187 p.

**Kabata-Pendias, A. and Pendias, H. (2001).** Trace Elements in Soils and Plants, CRC Press, Boca Raton, Fla, USA, Third edition. 432 P.

**Kapoor, k.k. and Paroda, S.H. (2007).** Experimental Soil Microbiology, First Edition. 85p.

**Khan, S.; Cao, Q.; Zheng, Y. M.; Huang, Y. Z. and Zhu, Y. G. (2008).** Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China, Environmental Pollution, 152 (3): 686–692.

**Liang, W.; Dai, C.; Zhou, X. and Zhang, Y. (2014)**. Application of Zero-Valent Iron Nanoparticles for the Removal of Aqueous Zinc Ions under Various Experimental Conditions. PLoS ONE 9(1): e85686.

**Lindsay, W.L. and Norvell, W.A. (1978).** Development of a DTPA soil test for zinc, iron, manganese and copper. S Sci. Soc. Amer. J., 42: 421-428.

**Ling-yu, B.A.I.; Zeng Xi-bai, L.; Lian-fang, L.I.; Pen C. and Shu-hui, L.I. (2010).** Effects of land use on heavy metal accumulation in soils. Agricultural Sciences in China. 9 (11): 1650-1658.

**Nies, D.H. (1999).** Microbial heavy metal resistance. Appl. Microbiol. biotechnol., 51: 730.

**Orhue, E.R. and Frank, U.O. (2011).** Fate of some heavy metals in soils: a review. Journal of Applied and Natural Science, 3(1): 131-138.

**Page, A.L.; Miller R.H. and Keeney, D.R. (1982).** Methods of Soil Analysis, part 2, 2nd., Agronomy monograph No 9. ASA, SSSA, Madison. 1159 p.

**Pérez-de-Mora, A.; Burgos, P.; Madejón, E.; Cabrera, F.; Jaeckel, P. and Schloter, M. (2006).** Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. Soil Biol. Biochem., 38: 327-341.

**Pierzynski, G. M.; Sims, J. T. and Vance, G. F. (2000).** Soils and Environmental Quality, CRC Press, 2nd edition, Inc. Boca Raton,Florida. pp 155-207.

**Roane, T.M. and Pepper, I.L. (2000).** Microbial responses to environmentally toxic cadmium. Microb. Ecol., 38: 358-364.

**Rowel, D.L. (1994).** Soil Science. Methods and Applications. Longman Scientific and Technical, Harlow, Essex, UK. 350 p.

**Sandaa, R.A.; Torsvik, V. and Enger, Q. (2001).** Influence of long-term heavy-metal contamination on microbial communities in soil. Soil Biol. Biochem., 33: 287-295.

**Sparks, D.L. (1996).** Methods of Soil Analysis. Part 3, Chemical Methods. Soil Sci. Soc. Amer. B. ser., no. 5, Madison, Wis., 5p.

**Tsai, Y.P.; You, S.J.; Pai, T.Y. and Chen, K.W. (2005).** Effect of cadmium on composition and diversity of bacterial communities in activated sludges. Intern. Biodet. Biodegr., 55: 285-291.

**Wang, W.; Jin, Z.; Li, T.; Zhang, H. and Gao, S. (2006).** Preparation of spherical iron nanoclusters in ethanol–water solution for nitrate removal. *Chemosphere* 65:1396- 1404.

**Xincai, C.; LtaoChen, J.S.; WeiXiang, W. and YingXU, C. (2008).** Immobilization of heavy metals by *Pseudomonas putida* CZ1/geothie composites from solution. Colloids and Sursaces B: bio interface., 61:170-175.

**Zhang, C.; Liu, X.; Dong, F.; Xu, J.; Zheng, Y. and Li, J. (2010).** Soil Microbial Communities Response to Herbicide 2,4-dichlorophenoxyacetic Acid Butyl Ester. Eur. J. Soil Biol., 46: 175-180.

**Zovko, M. and Romic, M. (2011).** Soil Contamination by Trace Metals: Geochemical Behaviour as an Element of Risk Assessment, Earth and Environmental Sciences, Dr. Imran Ahmad Dar (Ed.), ISBN: 978-953- 307-468-9, InTech, Available from: http://www.intechopen.com/books/earthandenvironmentalsciences/soil contamination by trace metals geochemical behavior as an element of risk assessment.

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