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Screening of fungal potentiality in removing heavy metals of industrial waste water

Ahmed Ismail; Mokhtar S. Beheary and Hanan A. Gad
Department of Environmental sciences, Faculty of Science, Port Said University, Egypt.

ABSTRACT

Fungal activities to remove and/or degrade heavy metals in industrial waste water were studied. Water samples were collected from the waste water main drain station in industrial area in Port Said. A total of 55 isolates were grown on 4 types of cultures media. Macroscopically and morphological examination results also showed that among these isolates15are belonging to fungi; 12 isolates are belonging to actinobacteria, whereas 28 isolates are belonging to bacteria. The 15 fungal isolates were screened for their ability to degrade some heavy metals reported in industrial waste water. *Aspergillus niger* and *Aspergillus flavus* were reported as the most active organisms in degrading of heavy metals detected in industrial waste water.

Keywords: *Aspergillus niger*, *Aspergillus flavus*, heavy metals, Industrial waste water, bioremoval

INTRODUCTION

Environmental issues are of increasing concern, and research is now being directed towards applying technology to ameliorate the effects of environmental pollution. This has led to renewed interest in the use of fungi to detoxify environmental pollutants, such as heavy metals, an approach referred to asbioremediation. Most of the heavy metal salts are soluble in water and form aqueous solutions and consequently cannot be separated by ordinary physical means of separation. The conventional treatment procedures used for removal of metals are uneconomical (Say *et al.,* 2003). Therefore, there is a need for a rapid, economical and environmentally benign technology for the removal of metals from polluted environment. The ability of microorganisms to accumulate metal ions from aqueous solutions has been widely reported (Tangaromsuk *et al*., 2002; Al-Garni, 2005; Svecova *et al*., 2006; El-Sherif *et al.*, 2008; Zamil *et al*., 2009).

Biosorption is a low cost and effective biological method which involves the use of dead microorganisms to detoxify and control metal contaminants in the environment (Kapoor, *et al*., 1999; Iskandar *et al*., 2011). Bacteria, fungi, yeast and algae can remove heavy metals from aqueous solutions insubstantial quantities (Brierley, 1990; Gadd 1990; Muraleedharan and Venkobachar, 1991). The uptake of heavy metals by biomass can take place by an active mode (dependent on the metabolic activity) known as bioaccumulation or by a passive mode (sorption and/or complexation) termed as biosorption. (Shumate and Strandberg, 1985) defined biosorption as “a non-directed physico-chemical interaction that may occur between metal/radio nuclide species and the cellular compounds of biological species. Fungi and yeasts accumulate micronutrients, such as Cu, Zn and Mn, and non-nutrient metals, like U, Ni, Cd, Sn, Hg, in amounts higher than the nutritional requirement (Gadd, 1990). The potential of fungal biomass as adsorbent for the removal of heavy metals and radio nuclides from polluted waters was recognized (Jilek *et al.,* 1975; Shumate and Parrott, 1978). Fungal biomass received attention as biosorbent materials for metal-contaminated aqueous solutions, because of its potentiality to grow fast and its availability as an industrial waste product, e.g. *Aspergillus niger* and *Aspergillus fumigates* (Say *et al.,* 2001).

This study was aimed to screen the activity of some fungi to remove heavy metals from industrial waste water.

MATERIALS AND METHODS

Water Samples were collected from the cumulative industrial waste water drain station in industrial free zone area in Port Said. Water samples were collected in clean and sterilized 500 ml glass bottle. Samples were transported to the laboratory in ice bags.

Isolation of microorganisms

Microorganisms that have been isolated from water samples were cultured according to the standard method described by the American Public Health Association (APHA, 2012) on nutrient agar, starch casein agar and potato dextrose agar media . Plates were incubated at 30C˚ for 7 days.

Identification of fungi

Identification of fungi was done at the Laboratory of Environmental Microbiology, Faculty of Science, Port Said University, by macroscopic examination based on cultural characters observed on PDA medium and microscopic examination. Pure cultures of isolated micro-organisms were identified based on characteristic features of colony morphology and reproductive structural characteristics like sporangiophore position, columella and spore shape using keys of **(**Ellis, 1971 and 1976; Pitt, 1979; Domsch *et al*., 1980; Subramanian, 1983; Ellis and Ellis, 1997; Gilman, 2001 and Nagamani *et al*., 2006).

Heavy metals determination

Heavy metals concentrations in waste water samples were determined by acid digestion. 15 ml of HNO_3 were added to 250 ml of each sample, and then evaporated until 25 ml. The solution was transferred into 50 ml volumetric flask and made up to the mark with distilled water. Then, heavy metals (Cu, Zn, Fe, Cd, and Cr) were detected by Atomic Absorption Spectrophotometer (Anton paarmultiwave 3000) using standard protocols as described by Hayat *et al*. (2002).

The percentages of metal ions removed by microbial cultures were calculated according to the equation:

% removal= $(C_i - C_f / C_i) * 100$ where

C_i: initial metal concentration

C_f: final or residual metal concentration

Screening for microbial isolates ability for heavy metals degradation

Fungal isolates were screened for the ability to grow and remediate heavy metals in effluent industrial waste water using nutrient broth medium. The components of this medium were dissolved in industrial waste water then the cultures medium were inoculated by spore suspension of selected microorganisms where 1ml of suspension contains $4x10^6$ spores. The cultures were incubated at 30^{o}C for 15 days. Un-inoculated cultures were used as a control. Microbial growth was expressed as mg dry weight, while the degradation of heavy metals (Cu^{+2}, Zn^{+2}, Fe^{+2}, Cd^{+2} and Cr^{+5}) was estimated using atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

A total of 55 isolates were isolated from industrial waste water on 4 types of cultures media. These isolates were distributed as 12 isolates of them grow on nutrient agar medium, 9 isolates were recorded to grow on starch casein agar medium,16 isolates were grown on Czapek Dox agar medium and 18 isolates were grown on potato dextrose agar medium. Macroscopical and morphological examination results also showed that among these isolates15are belonging to fungi; 12 to actinobacteria and 28 isolates are belonging to bacteria.

Identification of fungal isolates

Identification results of the fifteen fungal isolates revealed that out of 15 species, 4 species were of *Aspergillus*, 3species belonging to the genus *Penicillium* and only 1 species was represented other genera (Table 1)

Table 1: Fungal isolates

Reference number	*Name of isolates*
Iso.1	***Syncephalastrum racemosum***
Iso.2	***Alternaria alternata***
Iso.4	***Betraniella sp.***
Iso.6	***Byssochalamus niveus***
Iso.7	***Emericella nidulans***
Iso.8	***Penicillium oxalicum***
Iso.9	***P. digitatum***
Iso.10	***P. chrysogenum***
Iso.11	***Curvularia lunata***
Iso.12	***Colletotricum sp***
Iso.13	***Cladosporium spherospermum***
Iso.14	***Aspergillus niger***
Iso.15	***A. flavus***
Iso.16	***A. terreus***
Iso.17	***A. tamarii***

Screening of selected isolates for heavy metals removal:

The 15 fungal isolates were screened for their ability to degrade some heavy metals reported in industrial waste water. The heavy metals tested were Cu^{+2}, Fe^{+2}, Cd^{+2}, Zn^{+2} and Cr^{+5} as shown in (Table 2).

Table 2: Screening of microbial isolates to degrade heavy metal.

	Cu^{+2}		Cr^{+5}		Cd^{+2}		Zn^{+2}		Fe^{+2}	
	C_f	%	C_f	%	C_f	%	C_f	%	C_f	%
Control	18.8		32		28.5		20.08		41.6	
Iso. 1	10.23	45.5	30.4	5	16.32	43	18.2	9.3	33.25	20
Iso.2	12.53	33	26.2	18	22.34	21.6	18.09	9.9	39.68	4.6
Iso.4	7.02	62.6	23.7	25.9	19.52	31.5	16.92	15.7	19.4	53.2
Iso.6	6.42	65	27.6	13.7	18.97	33.4	19.82	1	24.27	41.6
Iso.7	10.14	46	24.4	23.7	21.79	23.5	10.72	46.6	37.68	9
Iso.8	9.73	48	24.2	24	18.48	35	12.74	36.5	29.01	30.2
Iso.9	14.48	23	27.2	15	23.25	18.4	19.15	4.6	38.59	7
Iso.10	13.07	30	32	0	17.58	38	20.02	0.2	27.93	32.8
Iso.11	10.21	45.7	32	0	19.05	33	16.27	18.9	29.09	30
Iso.12	15.87	15	32	0	24.01	15.7	18.4	8.36	34.25	17
Iso.13	13.72	27	30.1	5.9	16.53	42	12.58	37.3	12.9	69
Iso.14	6.24	66.8	13.2	27	14.81	48	9.04	54.9	13.7	67
Iso.15	6.73	64	21.2	33.7	13.05	54	8.52	57.5	11.6	72
Iso.16	12.5	33.5	30.4	5.4	21.35	25	7.46	62.8	25.73	38
Iso.17	6.34	66	29	9	15.07	47	10.21	49	38.29	7.9

As cleared from results in Tables (2&3) isolate No.12 (*Aspergillus niger)* and isolate No.13 (*Aspergillus flavus*) were the most active organisms in degrading the majority of tested heavy metals.

Table 3: Growth of fungal isolates (mg/ 50 ml) on industrial waste water

Isolate number	1	2	4	6	7	8	9	10	11	12	13	14	15	16	17
Microbial growth (mg/50 ml)	108	192	270	216	202	198	177	198	194	172	192	350	348	276	265

Shivakumar *et al*. (2014) has isolated 23 fungi from tannery effluent. These included 9 *Aspergillus* sp., *5 Penicillium* sp., *Paecilomyces variotii, Trichoderma viride, Graphium* sp., *Scopulariopsis brevicaulis, Sporothrix schenckii, Fusarium oxysporum* and *Acremonium strictum*. Shazia *et al.* (2013) have isolated 19 fungi from heavy metal contaminated soils. But Kumar *et al*. (2012) found only four metals resistant microbial strains were isolated from soil and sludge and on the basis of morphological, cultural and biochemical characteristics were identified as *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Aspergillus niger*.

Gadd and White (1993) explained that fungi are structurally unique organisms that contribute to significant removal of metal ions from waste water. This is because of their great tolerance towards metals and other adverse conditions, such as low pH and their intracellular metal uptake capacity. Metals and fungi can interact in concert in several ways depending on the type of metal, organism and environment. The impact of heavy metals on the environment has promoted the research to produce alternative, effective and low cost waste water purification scheme. To get rid of the heavy metals from the environment using microbial method, it is necessary to know the tolerance of microbes for particular metal ions. Metal tolerance is defined as the ability of an organism to survive in response to metal. Biological mechanism implicated in fungal survival in metal polluted aqueous solution includes extracellular precipitation, crystallization, transformation of metals, biosorption of cell wall, decreased transport or impermeability, efflux, intracellular compartmentation and sequestration (Gadd, 1993).

The present results recorded two isolates of predominant fungi belonging to genus *A spergillus*. The identification procedure revealed that these species are *A. niger* and *A. flavus.* For *A. niger* growth was recorded as $350mg^{-1}$ and removal percentage of $Cu^{+2}$was 64%,Fe^{+2} (72%), Cd^{+2}(54%), Cr^{+5}(33.7%) and Zn^{+2} (57.5%) where for *A. flavus*; growth was $348mg^{-1}$ with heavy metals removal of (Cu^{+2} $Fe^{+2}$$Cd^{+2}$, Cr^{+5}, Zn^{+2}) was (66.8%, 67%, 48%, 27%, 54.9%) respectively. This is may be due to the fact that *A. niger* and *A. flavus* isolates may assimilate heavy metals inside the cell or may adsorbed them on the cells surface giving them an advantage for treatment of heavy metals in industrial wastewater. Sintuprapa *et al*. (2000) have explained metal accumulation in living *A. niger* and *A. flavus* from paper effluent is higher due to intracellular metal uptake occurring in metabolically active cells in combination with extra cellular adsorption.

Similar to our present study, *Aspergillus* sp. were observed to be the most common genus in the heavy metal contaminated soils (Ahmad *et al.,* 2005; Zafar *et al*., 2007).

CONCLUSION AND RECOMMENDATION

Industrial waste water is a source of water surface pollution by heavy metals which cause negative effects on the environment.

Many microorganisms proved to be able to remove heavy metals from industrial waste water.

Aspergillus species proved to be effective in the removal of heavy metals.

The present study is recommending a further detailed investigation on heavy metals bioremediation by mixing culture of fungi with waste waters, and this technique should go from the Lab scale to large field scale; this will definitely decrease environmental pollution and possible reuse of this waste water.

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ARABIC SUMMARY

دراسة قدرة الفطريات لازاله المعادن الثقيله فى مياه الصرف الصناعى

أحمد اسماعيل عبد القادر, مختار سامي بحيرى,حنان على جاد
قسم علوم البيئه –كليه العلوم- جامعه بورسعيد - مصر

تمت دراسة قدرة الفطريات لإزالةأو تحلل المعادن الثقيلة في مياه الصرف الصناعي؛ حيث تم جمع عينات المياه من محطة الصرف الرئيسية في المنطقة الصناعية ببورسعيد. كان مجموع ما تم عزله من الكائنات 55، وتم نموهمعلى 4 أنواع من الاوساط الغذائيه .
وأظهرت نتائج الفحص الميكروسكوبيه والمورفولوجية أن من بينهذه العزلات (15 ينتمون إلى الفطريات و12ينتمون إلى الاكتينو بكتريا و 28 عزلة ينتمون إلى البكتيريا). تم اختبار العزلات الفطرية على قدرتها على تحلل أو ازالة بعض المعادن الثقيلة التي توجد في مياه الصرف الصناعي ووجد ان *Aspergillus niger* *Aspergillus flavus* من أكفأ الكائنات في معالجه مياه الصرف الصناعى من المعادن الثقيله.