INTRODUCTION and Aim of work

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Environmental pollution caused by heavy metals in industrial effluents is one of the most pressing problems in many densely populated cities worldwide (Boyd et al. 2010). There are different sources for heavy metals in the environment. These sources can be both of natural or anthropogenic origin. Increased industrialization and human activities have impacted on the environment through the disposal of waste containing heavy metals. Mine drainage, domestic effluents, landfill leachate, agricultural runoff, surface finishing industry, energy and fuel production, fertilizer and pesticide industry, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace and atomic energy installation contribute such a kind of waste in the environment (Bradl 2005) mainly in the aquatic systems (Marques et al. 2000).

During evolution, aquatic and terrestrial organisms have developed diverse strategies to maintain an equilibrated relation with heavy metal ions present and available in the surrounding medium. Cells face two tasks, the first is to select those toxic metals essential for growth and exclude those that are not, and the second to keep essential ions at optimal intracellular concentrations (Cobbett and Goldsbrough 2002).

Heavy metals are metals with a density above 5 g/cm^3, thus the transition and post transition metals can be referred to as heavy metals (Hawkes 1997).

Of the 90 naturally occurring elements, 21 are non-metals, 16 are light metals and the remaining 53 (with Arsenic included) are heavy metals.

Heavy metals mentioned in the field of biosorption are usually classified as the following three categories: toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.), whose specific weight is usually more than 5.0 g/cm^3 (Wang and Chen 2006).

According to the IUPAC technical report, the term “heavy metals” is both meaningless and misleading. The term “heavy metal", because it is often used with connotations of pollution and toxicity, is probably the least satisfactory of all the terms quoted as it leads to the greatest confusion. “Heavy” in conventional usage implies high density. “Metal” in conventional usage refers to the pure element or an alloy of metallic elements. Knowledge of density contributes little to prediction of biological effects of metals, especially since the elemental metals or their alloys are, in most cases, not the reactive species with which living organisms have to deal. The term “heavy metals” implies that the pure metal and all its compounds have the same physicochemical, biological, and toxicological properties, which are untrue (Duffus 2002).

Some heavy metals such as Zn, Cu, Ni and Cr are essential or beneficial micronutrients for plants, animals and microorganisms, whereas others, such as Cd, Hg and Pb have no known biological and/or physiological functions. However, all these metals could be toxic at relative low concentrations (Gadd and white 1993). The physiological range for essential heavy metals between deficiency and toxicity is extremely narrow (Clemens 2001).

Heavy metals unlike organic pollutants, they cannot be degraded but accumulate throughout the food chain, producing potential human health risks and ecological disturbances (Lovley 2001; Gadd 2004). Several studies have shown that metals adversely influence microorganisms, affecting their growth, morphology and activities, including symbiotic N_2 fixation (Khan and Scullion 2002; Shi et al 2002; Pereir et al 2006). Many reports have shown that short-term or long-term exposure to toxic metals results in the reduction of microbial diversity and activities in soil (Wang et al 2007).

The toxic characteristics of heavy meals are displayed as follows: (1) the toxicity can last for a long time in nature; (2) some heavy metals even could be transformed from relevant low toxic species into more toxic forms in a certain environment, mercury is such a case; (3) the bioaccumulation and bioaugmentation of heavy metal by food chain could damage normal physiological activity and endanger human life finally; (4) metals can only be transformed and changed in valence and species, but cannot be degraded by any methods including biotreatment; (5) the toxicity of heavy metals occurs even in low concentration of about 1.0–10 mg/L. Some strong toxic metal ions, such as Hg and Cd, are very toxic even in lower concentration of 0.001–0.1 mg/L (Alkorta et al. 2004).

Conventional methods for removing metal ions from aqueous solution have been studied in detail, such as chemical precipitation, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon etc. However, chemical precipitation and electrochemical treatment are ineffective, especially when metal ion concentration in aqueous solution is as low as 1 to 100 mg/L, they also produce large amount of sludge to be treated with great difficulties. Ion exchange, membrane technologies and activated carbon adsorption process are extremely expensive, especially when treating a large amount of water and wastewater containing heavy metal in low concentration, so they cannot be used at large scale (Ahalya et al 2003). These disadvantages of conventional systems together with the need for cost-effective process and safe method for removing heavy metals from discharging effluents has resulted in search for other unconventional materials such as organic or inorganic sorbents (Wang and Chen 2006).

Alternative process is biosorption and/or bioaccumulation. The term biosorption, some times referred to as physical adsorption, describes the ability of inactive, dead or living biomass to bind to heavy metals or contaminants present in dilute solutions. The cell-wall structure is mainly responsible for this property. This method of uptake is independent of the biological metabolic cycle and is known as ‘‘biosorption’’ or ‘‘passive uptake’’. The term bioaccumulation refers to heavy metals passing into the cell across the cell membrane through the cell metabolic cycle. This mode of metal uptake is dependent of the biological metabolic cycle and is known as ‘‘active uptake’’. Most of the studies dealing with microbial metal remediation via growing cells describe the biphasic uptake of metals, i.e., initial rapid phase of biosorption followed by slower, metabolism-dependent active uptake of metals (Naja et al. 2006; Chojnacka 2009).

Biosorption process offers the advantages of low operating costs, minimization of the volume of chemical and/or biological sludge to be disposed of and high efficiency in detoxifying very dilute effluents (Marques et al. 2000). In the concept of biosorption, several physical or chemical processes may be involved such as physical and/or chemical adsorption, ion exchange, coordination, complexation, chelation and microprecipitation. Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids offer many functional groups which can bind metal ions such as carboxylate, hydroxyl, sulphate, phosphate and amino groups. In addition to these functional binding groups, polysaccharides often have ion exchange properties (Beolchini et al. 2006; Naja et al. 2005).

Non-living biomass appears to present specific advantages in comparison to the use of living microorganisms. Killed cells may be stored or used for extended periods at room temperature, they are not subject to metal toxicity and nutrient supply is not necessary. Moreover pretreatment and killing of biomass either by physical or chemical treatments or crosslinking are known to improve the biosorption capacity of biomass. It has also been reported that cell wall soluble proteins, which make complexes with metal ions can be fixed by some denaturation processes such as heat and ethanol treatment. Deactivated yeast cells do not release protein and exhibit higher metal ion removal capacity than live yeast (Goksungur et al. 2005).

Among micro-organisms, fungal biomass offers the advantage of having a high percentage of cell wall material which shows excellent metal-binding properties. Many fungi and yeast have shown an excellent potential of metal biosorption, particularly the genera *Rhizopus, Aspergillus, Streptoverticillum* and *Saccharomyces*. Among bacteria, *Bacillus sp*. has been identified as having a high potential for metal sequestration and has been used in commercial biosorbent preparation1. Besides there are reports on the biosorption of metal(s) using *Pseudomonas sp, Zoogloea ramigera* and *Streptomyces sp*. Among photoautotrophs marine algae became the candidate of interest due to bulk availability of their biomass from water bodies. *Sargassum natans* and *Ascophyllum nodosum* in this group have shown very high biosorptive capacities for various metal(s). Besides marine algae, there are reports on binding of heavy metal(s) to green algae, viz. *Chlorella sp*. and *cyanobacteria* (Wang and Chen 2009; Gupta et al. 2000)

Heavy metal removal by biosorption has been extensively investigated during the last several decades. Some reviews and researches have been published focusing on different aspects of heavy metal biosorption (Davis et al. 2003; Gavrilesca 2004; Malik 2004; Wang and Chen 2006; Yin et al. 2008; Febrianto et al. 2009; Zhang et al. 2009; Tsekova et al. 2010; and Chatterjee et al. 2010). From these reviews, we can see that the research on biosorption is focused on the following three major fields. First, the biosorbents. It is necessary to continue to search for and select the most promising types of biomass from an extremely large pool of readily available and inexpensive biomaterials. Second, the mechanism of biosorption. The mechanism involved in the metal biosorption is only understood to a very limited extent to date. It is necessary to identify the mechanism of metal uptake by biosorbents and understand microbe–metal interactions. Third, large scale experiment. The biosorption process is basically at the stage of laboratory-scale study. It is in great difficulties and almost a failure in attempt of applying biosorption process into practice. Great efforts should be taken to improve biosorption process, including immobilization of biomaterials, improvement of regeneration and re-use, optimization of biosorption process.

The efficiency of metal recovery depends on choice of eluant and elution conditions, as various eluants presenting different desorption mechanisms may be used (Yang et al. 2005). Lowering pH (e.g. with mineral acids) causes metal desorption, resulting from competition between protons and metal ions for binding sites. Mineral acids such as HCl, H_2SO_4 and HNO_3 are efficient desorption agents, although high concentrations may damage biosorbents, limiting their use in subsequent adsorption cycles. Carbonates which form complexes with metal ions are efficient eluants. However, biomass can be damaged due to high equilibrium pH. The eluants used for metal recovery should be: (a) nontoxic, (b) cause no damage to biosorbent to allowreuse and (c) achieve maximum recovery at lowest possible concentrations. This can be expressed by the solid-to-liquid ratio (S/L), the mass of loaded biosorbent per eluant volume, an important parameter to be optimized (Ferraz et al. 2004).

In this study it was aimed to isolate some heavy metal resistant fungal strains from local Egyptian soil, in addition to using baker yeast. The capability of isolated and identified fungal strains to uptake cadmium, copper, Lead and arsenic ions was evaluated.

The effect of increasing concentration of Pb^{+2}, Cu^{+2}, As^{+5} and Cd^{+2} on the efficiency of fungal strains was examined. Optimization of environmental and cultural conditions for the tested strains to ensure maximum growth and metal uptake was achieved. A comparison between the bioaccumulation and biosorption of these metal ions by the selected strains was achieved along the thesis. Finally, the effect of heavy metals on the total DNA, carbohydrates, lipids and protein of the selected fungus were studied.