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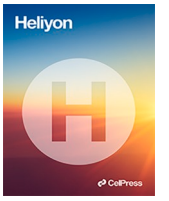

Research article

Biosorption performance of the multi-metal tolerant fungus *Aspergillus* sp. for removal of some metallic nanoparticles from aqueous solutions

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ABSTRACT

The wide spread of nanotechnology applications currently carries with it the possibility of polluting the environment with the residues of these nanomaterials, especially those in the metallic form. Therefore, it is necessary to study the possibility of treating and removing various nanoscale metal pollutants in environmentally friendly ways. The present study focused on the isolation of multi-metal tolerant fungi to be applied in the bioremoval of Zn, Fe, Se, and Ag nanoparticles as potential nanoscale metal pollutants. *Aspergillus* sp. has been isolated as multi-metal tolerant fingus and investigated in the bioremoval of targeted nanometals from their aquoues solutions. The effect of biomass age, pH, and contact time was studied to determine the optimal biosorption conditions for fungal pellets towards metal NPs. The results showed a high percentage of fungal biosorption on the of two-day-old cells, which amounted to 39.3, 52.2, 91.7, and 76.8% of zinc, iron, selenium, and silver, respectively. The pH 7 was recorded the highest percentage of NPs removal for the four studied metals i.e. 38.8, 68.1, 80.4, and 82.0% of Zn-, Fe-, Se- and Ag-NPs, respectively. The contact time required between *Aspergillus* sp. and the metal nanoparticles to obtain the best adsorption was only 10 min in the case of Zn and Ag, but it was 40 min for both Fe and Se NPs. The efficiency of living fungal pellets in removing the four metallic NPs exceeded that of dead biomass by 1.8, 5.7, 2.5, and 2.5 folds for Zn, Fe, Se and Ag, respectively. However, utilization of dead fungal biomass for metallic NPs removal could be considered more applicable to the actual environmental applications.

1. Introduction

The extensive of nanotechnology across industrial, agricultural and medical applications leads to increase their emissions into the environment, especially water [1–5]. Engineered nanoparticles can discharge into wastewater during their use and disposal. NPs will reach drinking water sources and natural aquatic ecosystems if water and wastewater treatment plants are ineffective or unable to remove them from the water. This will lead to increased exposure of plants, microbes, animals and humans to dangerous heavy metals, which threatens bad health and environmental effects [6]. However, it is still unclear how NPs move through the environment and

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what their fate is [7]. In contrast to the analogous bulk materials, NPs' huge surface area to volume ratio creates novel materials with altered properties [8]. However, these altered properties, together with NPs' extremely small size, may also increase the material's bioavailability and toxicity [6]. The toxicity may be absorbed by the skin, lungs, or gastrointestinal tract and may result in secondary toxicity by way of oxidative stress, inflammation, DNA damage, apoptosis, or translocation inside organs and tissues [9]. Therefore, it is important to create a quick and efficient way to eliminate NPs before releasing them into the environment [10].

According to reports, coagulation, flocculation, filtering, biotransformation, and aggregation are all effective methods for removing metallic NPs from aqueous solutions. In contrast to conventional chemical and physical methods, biological removal approaches of heavy metals using plants, microbes, or their metabolites are more efficient and environmentally friendly. Metal absorption uptake can take place either passive or active processes. The "passive uptake" referred to the surface entrapment and non-metabolic sorption of metallic ions onto the binding sites of the biosorbent. These passive processes could occur in case of either living or dead cells. Whereas "active absorption" processes involves the metabolic passage of heavy metals into the living cell through the cell membrane [11–13].

For the elimination of heavy metal ions, both live and dead fungi have been identified as a potential class of excellent and low-cost adsorbents for heavy metals. Numerous commercial fermentation processes employ fungi, which could provide a cost-effective and reliable source of biomass for the elimination of metal ions. Fungi can also be readily produced in large quantities using basic fermentation methods and low-cost growing substrates. Fungi can eliminate heavy metals and other contaminants by complexing, exchanging ions, or physically adsorbing the metal cations with functional fungal cell wall groups [9,14]. It should be noted that various fermentation industries produce large amounts of fungal biomass as waste, which can be used as heavy metal biological adsorbents. Fungi can also be readily produced in large quantities using basic fermentation methods and affordable growing media [15,16].

Although several studies have demonstrated the efficacy of various fungi in removing heavy metals, few have dealt with the removal of nanoparticles from aqueous solutions. Hence, this study was conducted to isolate metal-tolerant fungi to be used for the bio-removal of metallic nanoparticles from their aqueous solutions. Some factors that influence the bioremoval of the targeted metallic nanoparticles also have been investigated.

2. Materials and methods

2.1. Isolation of heavy metals tolerant fungi

Forty-eight rhizosphere soil samples were collected from some garden trees in the cities of Shebeen El-Kom (Monofia governorate; 30.558033°N, 31.015295°E) and El-Qanatir El-Khairia (Qalyubia governorate; 30.188652°N, 31.135415°E). In addition, six samples of spoilage household food samples were collected from El-Qanater El Khayreya city. All samples were collected in sterilized bags then brought to laboratory under aseptic conditions and kept in refrigerator at 4 °C for further processing.

Isolation of heavy metals-tolerant fungi was performed using "consecutive enrichment culturing technique" [1]. In brief, 10 g of soil/spoilage household food sample was (in triples) enriched in 250-mL conical flasks containing 100-mL potato dextrose broth medium (PDB) amended with a mixture of Zn, Fe, Se, and Ag metals (as common heavy metals). The concentrations of applied metals were 1000 ppm of Zn^{2+} and Fe^{2+} (from $ZnSO_4.7H_2O$, and $FeSO_4.7H_2O$, respectively), 500 ppm of Se^{2+} (from Na_2SeO_4) and 100 ppm of Ag^{2+} (from $AgNO_3$). The lower concentrations of selenium and silver were used due to the highly toxicity of both elements. Flasks were incubated under orbital shaking conditions (125 rpm) at room temperature for 72 h. The enrichment cultures were successively repeated three times by transferring 10 mL of the previous culture to fresh enrichment medium and incubated under the same conditions [17]. One-milliliter aliquot from each flask was then transferred to a 1%-saline water tube for progressive dilution to 10^{-5}. Then, a 100-μL portion of the suspension was spread on the surface of potato dextrose agar (PDA) plates supplemented with 50 μg/mL ampicillin and multi metal mixture for isolation. The concentrations of multi heavy metal in the isolation PDA plates were 100 ppm for Zn^{2+} and Fe^{2+}, 50 ppm for Se^{2+} and 10 ppm for Ag^{2+}. Isolation plates were incubated at 28 °C for 7 days, and then representatives of fungal colonies with different morphological features were selected, and subcultured on agar plates of the same isolation medium for purification. Selected fungal isolates were maintained on slants of the same isolation medium for further investigations [1].

2.2. Screening for heavy metals tolerance

Fungal isolates were screened for their tolerance to different concentrations of Zn^{2+}, Fe^{2+}, Se^{2+} and Ag^{2+} individually. The tested concentrations were 500, 1000 and 1500 ppm each for Zn^{2+} or Fe^{2+}. The concentrations of Se^{2+} were 500, 750 and 1000 ppm; while the concentrations of Ag^{2+} were 100, 300 and 500 ppm. The screening was performed following the agar well diffusion method with minor modifications [1]. In brief, fungal isolates were activated on PDA-heavy metals plates and incubated for 4 days at 28 °C until sporulation. The spores were collected, suspended in sterile water, and diluted to give a number of spores 10^6 spores/ml (depending on haemocytometer slide counting). Wells (6 mm diameter) on the middle of PDA-heavy metal plates were made using sterile cork borer. A volume of 60 μL of fungal spore suspension was placed into the wells and the plates were incubated at 28 °C for 72 h. The growth diameter of fungal isolates on PDA-Heavy metals was measured as indication for heavy metals tolerance with comparing to the growth on normal PDA medium as control.

2.3. Morphological characterization of the selected fungi

The most potent fungal isolate that tolerate high heavy metal concentrations was identified up to genus level using macroscopic and

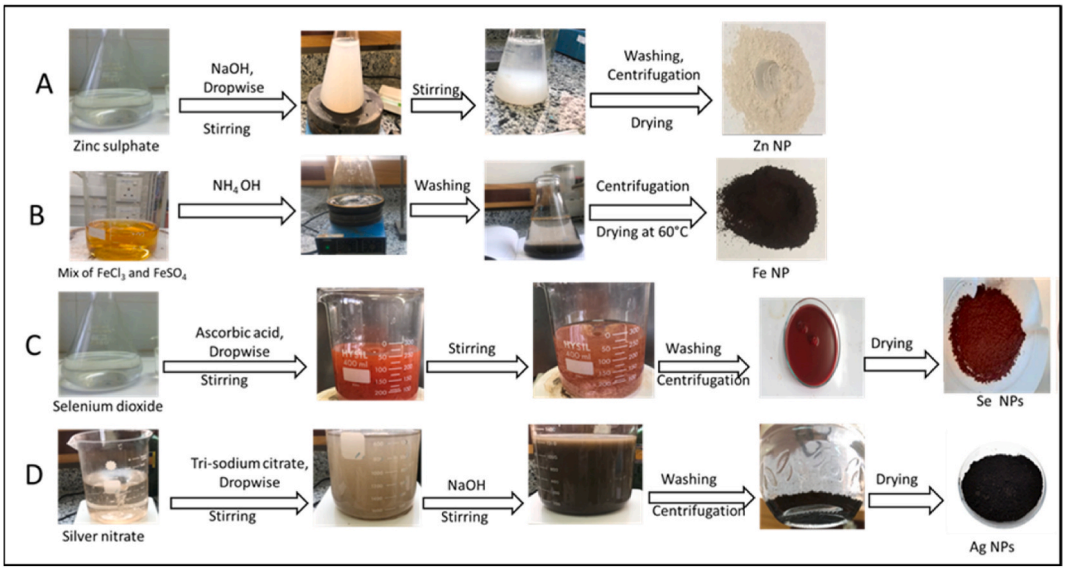

Fig. 1. Flowchart for preparation of metal nanostructures, zinc (A), Iron (B), selenium (C) and silver (D).

microscopic characterizations. The macroscopic observation was determined through the growth on different media (Czapek-Dox, Sabouraud's dextrose, Yeast Extract–Peptone–Glycerol (YPG) and PDA). Colony shape, color and measurement the colony diameters were noted [18,19]. Microscopic observation through the stages of mycelial growth at different incubation periods on PDA medium was determined by light microscope (Priorlux Ac 240V with magnification of 40x).

2.4. Chemical synthesis of metals nanostructures

In this study, chemical reduction (bottom-up method) was used to convert metal ions into their nanoforms according to the established methods in the literatures (Fig. 1 (A - D) [20,21]. In brief, zinc nanoparticles was synthesized by dropping 1% of sodium hydroxide (as reducing agent) into 2% zinc sulfate with stirring until the white precipitates was observed. Iron nanoparticles were synthesized by using ammonium hydroxide as reducing agent for $FeCl_3$ and $FeSO_4$ (2:1 w/w) in a final ratio of 1:5 (v/v) and mixing until the black sediment formed. Synthesis of selenium nanoparticles were performed by reducing selenium dioxide (1%) using ascorbic acid (0.1g to 30 mL) that dropped with stirring until observation of red sediment. Silver nanoparticles was prepared by wise dropping tri-sodium citrate solution (0.1g in 10 mL) in to $AgNO_3$ solution (0.1g in 100 mL) with stirring at 60 °C, then NaOH (3%) was gradually dropped until the color changed to dark brown (precipitation).

The precipitates of Zn-, Fe-, Se-, Ag-NPs were collected by centrifugation (10,000×*g* for 15 min) and washed with distilled water several times until the pH became neutral. Then the precipitated particles were washed with absolute alcohol to remove any impurities and prevent the components from re-oxygenized, centrifuged at 10,000×*g* for 15 min and dried at 60 °C to a constant weight.

2.5. Characterization of metal nanoparticles

The average particle size, size distribution and morphology of the prepared metal nanostructures were studied using high resolution transmission electron microscope (JEOL, JEM-2100 HRTEM). In order to load the sample into the TEM, a drop of well-dispersed nanoparticle dispersion was applied on an amorphous carbon-coated 200 mesh carbon grid and dried at ambient temperature [22].

2.6. Fungal-removal of metal nanoparticles

The most successful isolate of the chosen fungi tolerant to heavy metals was chosen for bio-removal studies of metal nanoparticles from synthetic wastewater. Biosorption experiments were carried out using fungal pellets to evaluate their ability to biologically remove metal nanoparticles and the effect of the age of the cultured biomass, contact time, and pH on its efficiency. All bioremoval experiments were performed as batch experiments in 250-mL Erlenmeyer flasks individually containing 300 ppm of Zn, Fe, Se, or Ag in their nanoforms plus 5 g of fungus pellets (as a bioremoval agent). Preparation of fungal pellets was performed by inoculating fungal spores into 250-mL Erlenmeyer flask containing 50 mL of PDB-Heavy metals and incubation at 28 °C on a rotary shaker for eight days [1].

The pellets were harvested by filtration on filter paper (whatman No.4) at 2, 4, 6 and 8 days to study the influence of fungal age on the biosorption of metal nanoparticles. After washing pellets by sterilized DW, it were placed in conical flasks contains 50 mL of synthetic wastewater containing individual nanometals as contaminant. The pH values of wastewater/nanoparticals solutions were

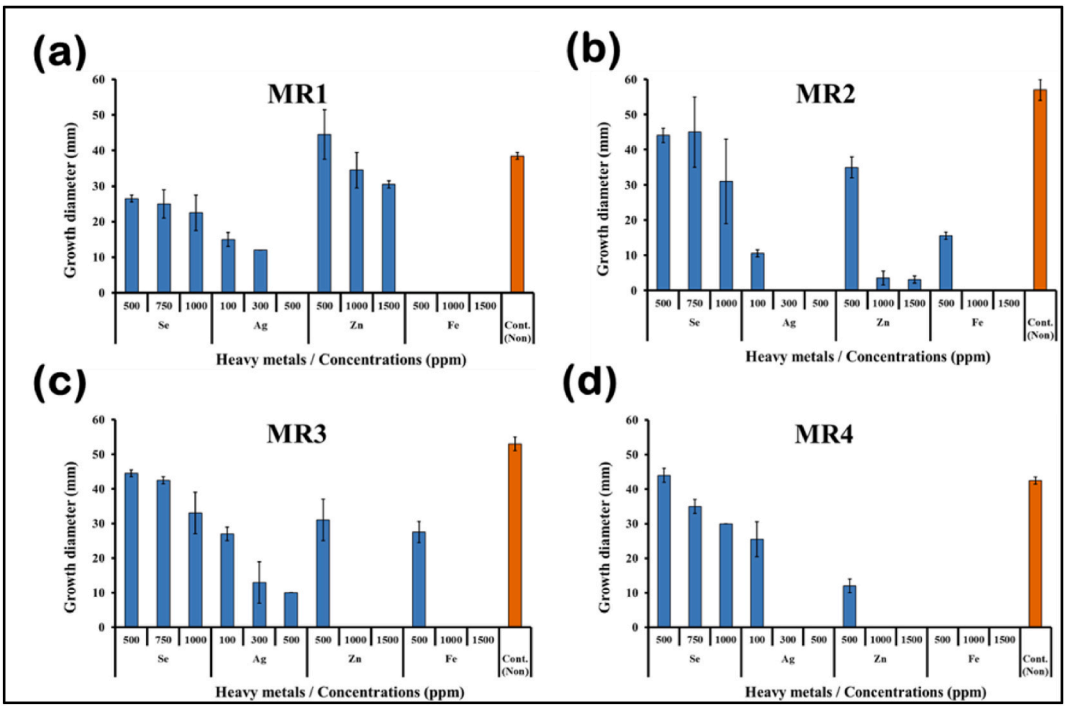

Fig. 2. Growth diameter of fungal isolates No. MR1, MR2, MR3, and MR4 on PDA plates containing different metal ions (Zn^{2+}, Fe^{2+}, Se^{2+} and Ag^{2+}) with different concentrations.

adjusted at different values i.e. 2, 5, 7, 9 and 11 to study the effect of pH on metal removal by the fungus. Contact time between fungal pellets and metallic nanoparticles was investigated by incubating the mixtures under shaking at 120 rpm for 10, 20, 30 and 40 min at ambient temperature.

The efficiency of metallic nanoparticle removal was estimated by measuring the concentration of the targeted metal(s) in the mycelial filtrate (after removing pellets by nylon sieve of 1 mm mesh). Metallic-NPs were measured by Agilent 5100 Inductively Coupled Plasma–Optical Emission Spectrometer (ICP-OES) according to the procedures of the manufacture.

2.7. Dry fungal biomass for biosorption of metal nanoparticles

As it is more viable and safer in terms of biological contamination, the possibility of using dead dried fungal biomass was studied to remove the studied metal nanoparticles from their solutions. The dead fungal biomass used in this experiment was prepared by drying the fungal pellets of selected fungus at 70 °C for 24 h, and then gently grinding them in a home kitchen electrical grinder. Individually, 50 mL of 300 ppm of Zn-, Fe-, SE-, or Ag-NPs suspensions (with pH 7) were mixed with 3 g of dead fungal biomass for 10 min. The amount of metallic NPs removed per dry biomass was estimated after subtracting the residual metal amount in the aqueous filtrate (after sieving with 1 mm mesh nylon sieve). For comparison, fresh fungal pellets were used to remove metallic NPs with similar conditions and measured by ICP-OESs. The amounts of NPs removed per gram dry biomass or its equivalent wet biomass were used as the base of comparison.

Moreover, the dead fungal biomass was investigated as bioremoval agent for a mixture of the four mentioned metallic NPs in a synthetic wastewater. A mixture of 300 ppm from each Zn, Fe, Se and Ag NPs (at pH 7) was mixed with three g of dried fungal biomass at ambient temperature. After 10 min contacting time (with 125 rpm obituary shaking), the solution was filtrated with gauze fabric to allow passing only metallic NPs (MNPs). The residual metal concentration in filtrate was analyzed and a mixture without any treatment was served as a control.

2.8. Statistical analysis

All experiments were performed in triplicates and results were presented as average ± standard error (SE).

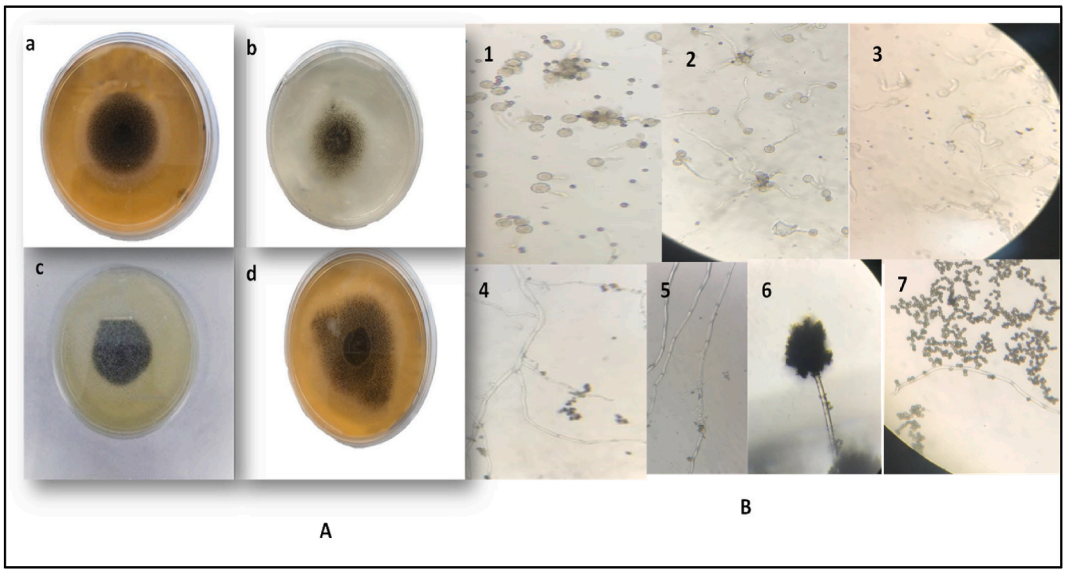

Fig. 3. Morphological characteristics of fungal isolate MR3; (A) macroscopic using different media, YPG (a), DOX (b), PDA (c) and Sabouraud's dextrose (d). (B) The microscopic observations at different incubation periods: (1, 2) after incubation for 24 h (conidia formed germ tube), (3, 4, 5, and 6) after 48h of incubation (germ tube grow into hyphal mycelium and forming aerial hyphae), (7) after 72 h (aerial hyphae forming conidiophores forming the vesicle has sterigmata carrying conidia).

3. Results and discussion

3.1. Isolation of heavy metals tolerant fungi

Isolation of heavy metals tolerant fungi was targeted in order to be investigated for the bioremoval of metallic nanoparticles from wastewater. Samples were successively three times enriched in the presence of Zn^{2+}, Fe^{2+}, Se^{2+} and Ag^{2+} salts, then plated on PDA-heavy metals for isolation. Four fungal isolates namely MR1, MR2, MR3 and MR4 with different morphological characteristics were obtained as heavy metals-tolerant fungi. The fungal isolates MR1 and MR3 were isolated from soil samples, while MR2 and MR4 were isolated from spoilage sample of bread and pomegranate fruit, respectively. The concentration of heavy metals in enrichment cultures were 1000 ppm of Zn^{2+} and Fe^{2+}, 500 ppm of Se^{2+} and 100 ppm of Ag^{2+}. The low numbers of fungal isolates may be returned to toxicity of heavy metals used in enrichment culture [23]. On the other hand, the ability to grow on such high concentrations of toxic metals indicates that the obtained fungal isolates are considered heavy metal tolerant fungi.

3.2. Screening for highly heavy metals tolerance

The obtained four heavy metals-tolerating fungal isolates were screened for their highly tolerance against different concentrations of individual Zn^{2+}, Fe^{2+}, Se^{2+} and Ag^{2+} salts. The diameter of fungal growth on the PDA plate was used as an indicator of the tolerance of the heavy metal present [15,24]. Data illustrated in figure (2 (a - d)) indicates that the four fungal isolates were able to grow in all investigated concentrations of Se ions i.e. 500, 750, and 1000 ppm. However, growth diameter of fungi decreased with the increase in metal concentration. Isolates MR1 and MR4 didn't grow on all tested Fe concentrates i.e. 500, 1000, and 1500 ppm (Fig. 2 a, and d). Although isolate MR1 showed good growth on zinc in all concentrations used (i.e. 500, 100, and 1500 ppm), isolate MR3 outperformed it in growth on both selenium and silver (Fig. 2 a, and c). The concentration of and 500 ppm of Ag prevented the growth of all fungal isolates except MR3 isolate (Fig. 2 c).

Based on tolerance and growth diameters on heavy metals containing agar plates, the isolate MR3 has been selected as the most tolerant isolate.

3.3. Morphological identification of heavy metal-tolerant fungus

The macroscopic and microscopic characteristics of MR3 isolate were used its identification.

The macroscopic characterization of MR3 isolate colonies was examined through observing the fungal growth on potato dextrose and Czapek's dox agar at 28 °C. The fungus was initially white, and quickly became black with conidial production. In the comparison with different medium such as yeast peptone glucose agar and sabouraud's dextrose agar, the colonies produced black conidia at the center and white mycelia towards the edge as shown in Fig. (3 A). Colony diameter after 3 days of incubation at 28 °C was 50.3, 30.4, 40.6, and 50.8 mm on PDA, Dox, YPG and sabouraud's dextrose agar plates.

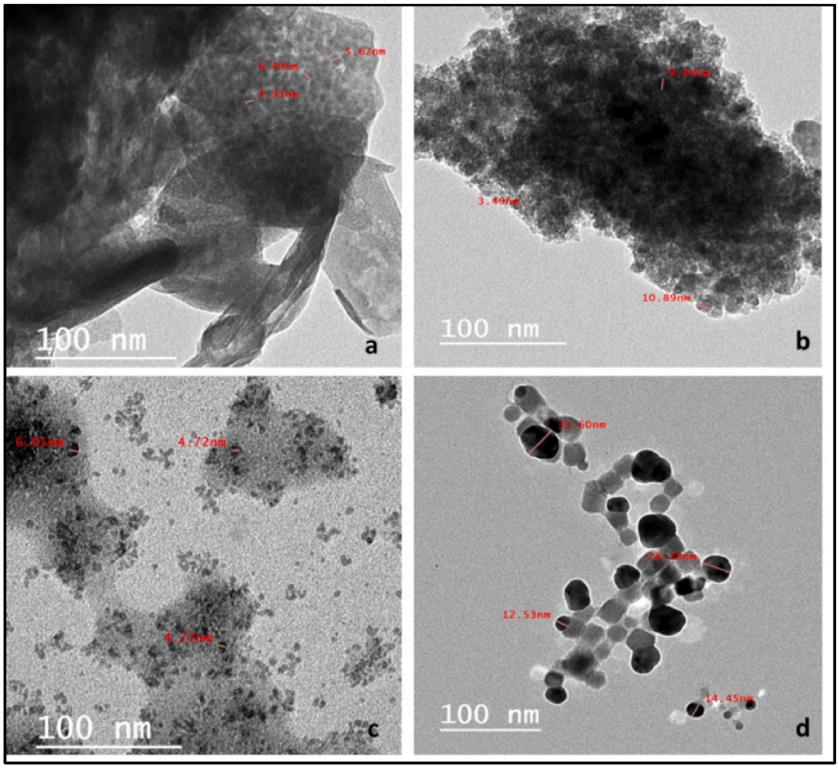

Fig. 4. HRTEM characterization of Zn (a), Fe (b), Se (c) and Ag (d) nanoparticles.

The microscopic observations were examined by light microscope (Priorlux Ac 240V with magnification of 40X) at different incubation periods of fungal growth on PDA plates as shown in Fig. (3 B). After 24 h, conidia were germinated forming vegetative cells which formed germ tubes. After 48 h, germ tubes developed into hyphal mycelium which branches dichotomously forming aerial hyphae. Then grow to form conidiophores which swell at the apex forming the vesicle part of the conidiophore. After 72 h, the vesicles form the primary sterigmata known as the phialides. The sterigmata form the secondary sterigmata that start to produce the conidial spores [18].

From macroscopic and microscopic observations, the isolate MR3 was identified as *Aspergillus* sp. according to Silva et al. [18].

3.4. Preparation and characterization of metallic nanoparticles

The main aim of this research is to establish an ecofriendly method for the bioremoval of metallic nanoparticles from wastewater. Hence, the study targeted some common metals that usually used in nanoforms either in industry or ever research. Zn-, Fe-, Se- and Ag-NPs were synthesized by chemical reduction as a common rout for their synthesis in the large scale application (and expected to be found in many wastewater resources). The size and shape of nanoparticles are critical features that influence their properties and also influence their interactions with surrounded flora and microflora [25]. Therefore, the prepared nanometals were subjected to characterization using High Resolution Transmission Electron Microscopy (HRTEM). TEM analysis of the chemically synthesized Zn, Fe, Se and Ag particles confirms that they are nano-sized with spherical shape (as illustrated in Fig. 4 (a - d)). However, the size of prepared nanoparticles was varied from one to another. ZnNPs and SeNPs had a diameter of less than 10 nm, Fe-NPs varied from 3 to 15 nm, and AgNPs were between 10 and 35 nm. The average size and shape of the obtained nanoparticles were consistent with those reported in the relevant literature [1,20,26,27].

3.5. Application of heavy metal-tolerant Aspergillus sp. in NPs-bioremoval

According to previous experiments, the isolate *Aspergillus* sp. (MR3) was selected as a highly heavy metal-tolerant fungus. There is a correlation between fungi's capacity to absorb or adsorb heavy metals and their capacity to tolerate them [28,29]. Due to the ability to tolerate different heavy metals with relatively high concentrations, the fungus *Aspergillus* sp. (MR3) was investigated for the bio-removal of some metallic nanoparticles from synthetic wastewater. The investigation included the utilization of the pellets of *Aspergillus* sp. (MR3) to individually remove Zn-, Fe-, Se- and Ag-NPs from synthetic waste water containing metals. Many factors can affect the bio-removal capacity of heavy metals using the live pellets of a particular fungus [30]. Some of these factors were considered in this research, such as the effect of fungal age, contact time between fungal biomass and heavy metals, and the pH of metallic

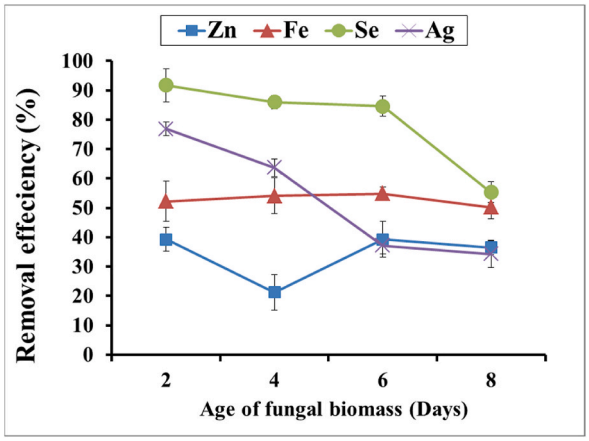

Fig. 5. Efficiency capacity (%) of *Aspergillus* sp. biomass for removing of Zn, Fe, Se and Ag NPs at different biomass age.

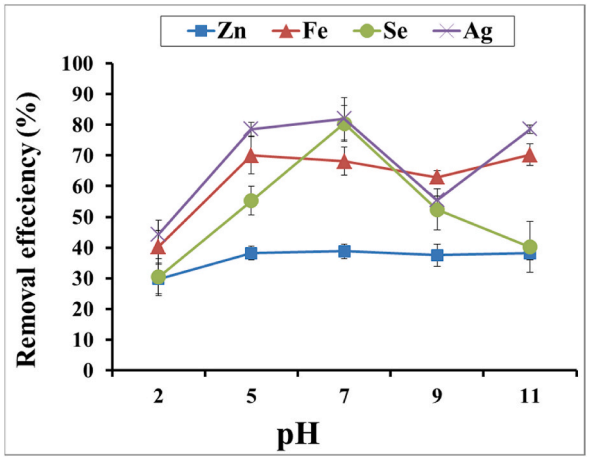

Fig. 6. Removal efficiency (%) of Zn, Fe, Se and Ag NPs by *Aspergillus* sp. biomass under various pH values.

nanoparticle solution.

3.5.1. *Impact of fungal biomass age*

The uptake of heavy metals and pollutants by fungi depends primarily on "the bio-sorption to cell wall" as well as the cellular uptakes of the "essential metals" into the cells via "specific transporters" [31]. The age of fungus is a main parameter that affects either cell wall composition (functional groups including amine, carbonyl, thiol, hydroxyl groups, phosphate and sulfate) as well as the ability of uptake metals from surrounding environments [32].

Thus, the impact of *Aspergillus* sp. age on the biosorption efficiency of MNPs via its pellets was investigated. After harvesting of *Aspergillus* sp. biomass at different incubation periods (different ages), the obtained pellets was mixed with Zn, Fe, Se and Ag NPs suspensions (at pH 7) for 3 h. The results indicated that the biomass of the two-day-old *Aspergillus* sp. fungus gave the best removal percentage of the four nanoparticles under study, as shown in Figure (6). It recorded 39.3, 52.2, 91.7 and 76.8% removal percentages for Zn, Fe, Se and Ag NPs, respectively. The percentage of metal NPs removed generally decreased as the fungal biomass aged (the lowest percentages of removal occurred by the eight-day-old pellet) (Fig. 5). In a similar study, Delgado et al. [33], studied the bio-sorption of Cu^{+2}, Cd^{+2} and Ni^{+2} ions by *Fusarium flocciferum* and reported that the older cultures showed a decrease in metal bio-sorption capacity comparing with the fresh biomass. These findings, as well as those from Kapoor and Viraraghavan [34], indicate that cells in the early stages of development have a higher capacity to absorb metal ions than those in the later stages of life.

3.5.2. *Effect of pH*

pH is one of the most important environmental factors that affecting the absorption of minerals from their aqueous solutions. It could alter the biosorbent surface charge, degree of ionization and availability of the functional groups such as hydroxyl (R–OH), carboxyl (R–COOH), amino ($–NH_2$) and sulfhydryl (–SH) groups [35]. A wide range of pH values (that is, 2, 5, 7, 9, and 11) were applied to examine their effect on the biosorption of Aspergillus sp. pellets towards Zn, Fe, Se and Ag Nps.

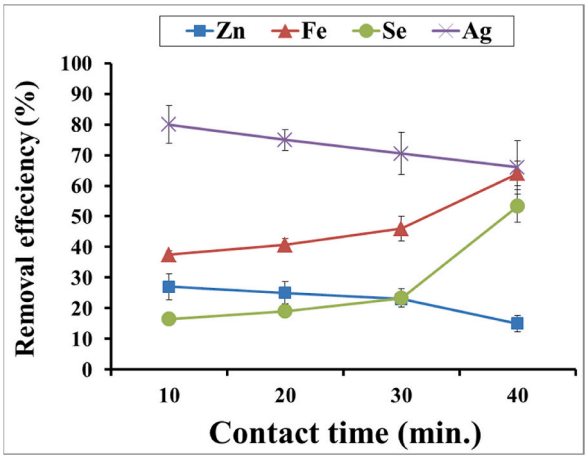

Fig. 7. Biosorption capacity (%) of *Aspergillus* sp. towards (Zn, Fe, Se and Ag) nanoparticles at different contact time.

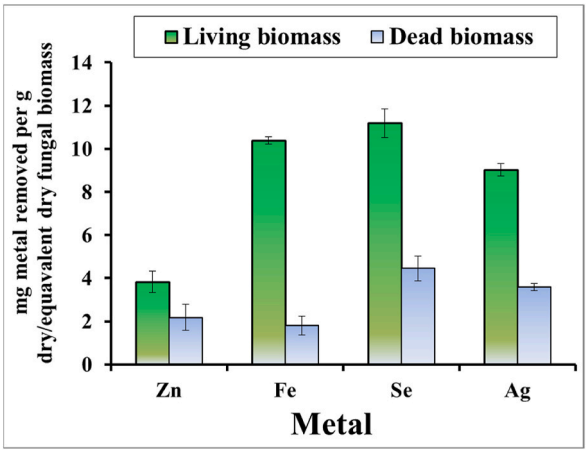

Fig. 8. The amount of adsorbed Zn, Fe, Se and Ag-nanoparticles per gram of the dry mass or its equivalent of the wet mass of the fungus *Aspergillus* sp.

The results (Fig. 6) showed that the biosorption of metallic NPs at neutral pH was noted as the best one toward all tested MNPs. The pellets of *Aspergillus* sp. were able to remove 38.8, 68.1, 80.4, and 82.0% of Zn, Fe, Se and Ag-NPs, respectively when the pH value was seven. The lowest removals of NPs were recorded at pH 2, in such an acidic environment H+ ions occupy the functional groups in the cell wall presenting a limitation for carrying metal ions in these groups [36]. While with the increase of pH, more functional groups such as carboxyl and phosphate with negative charges were produced and the ability of the biomass to absorb metal ions increases [37].

3.5.3. *Effect of contact time*

Successful biosorption removal of pollutants from an environment depends on the length of time that biomass is in contact with contaminants [38]. Hence, the pellets of *Aspergillus* sp. were mixed with Zn, Fe, Se and Ag NPs for different periods to verify the optimal contact time for bioremoval of these nanometals. The experiment was conducted on a rotary shaker at 125 rpm, pH 7.0 and 28 °C ±2 for 10, 20, 30 and 40 min as incubation time. The results in Figure (7) show that the best removal percentage of both Zn and Ag by *Aspergillus* sp. was within 10 min, which was 27.0 and 80.0% for Zn and Ag, respectively. While, 40 min was needed for *Aspergillus* sp. pellets to cause 64.0, 53.4% removal for both Fe- and Se-NPs, respectively. Notably, longer incubation with Zn and Ag-NPs was coupled with decreases of the binding of such NPs and the fungal biomass. This could be attributed to the saturation of "the free binding active sites in the outer surface" with time leading to the occurrence of the biosorption in "the outer surface instead of the inner surface" [39].

3.5.4. *Living vs dead fungal biomass*

To comparison between the activity of both living and dead biomass in removing of Zn, Fe, Se, and Ag NPs, MNPs solutions were inoculated with 3g of living or dead biomass individually. The amount of adsorbed MNPs per gram of the dry mass or its equivalent of

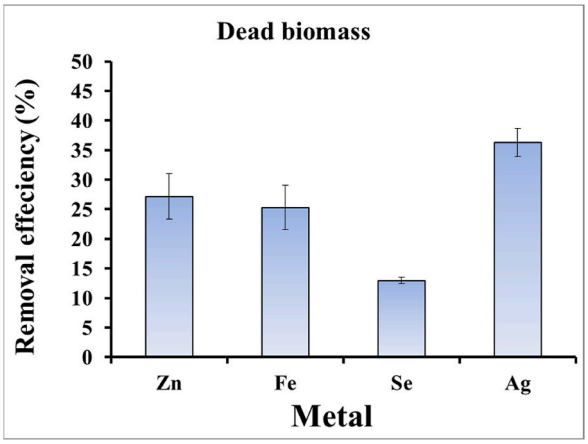

Fig. 9. Effect of the dead biomass of *Aspergillus* sp. on removal of mixture of (Zn, Fe, Se and Ag) nanoparticles.

the wet mass of the fungus *Aspergillus* sp. was calculated and illustrated in Fig. (8). Illustrated data revealed that the activity of living fungal pellets in removing the four MNPs exceeded that of dead biomass by 1.8, 5.7, 2.5, and 2.5 folds for Zn, Fe, Se and Ag, respectively. However, for dry fungal biomass, the removal of Se and Ag was higher than that of Fe and Zn. While, in wet fungal biomass, the removal of Se and Fe was slightly higher than that of Ag and much more than that of Zn nanoparticles.

The fact that the biosorption process increased when dealing with live biomass suggests that fungal biomass's uptake of metal NPs was more reliant on cellular activity than just a passive adsorption process. Similar results have been reported by Saad et al. [16] who found that bio-uptake of Cu^{2+} by live mass of *Aspergillus* sp. was higher than dead biomass treated with either alkali or boiling.

Notwithstanding, dead biomass has an application advantage over live biomass in heavy metal removal especially in industrial applications due to health concerns of live cells. Also, systems containing live cells are likely to be more sensitive to metal ion concentration (toxic effects) and adverse operating conditions (pH and temperature). Also, a continuous supply of nutrients is required for systems using live cells, and mineral recovery and regeneration of biosorbents are more complex for living cells. Furthermore, dead biomass can be obtained cheaply commercially as a waste product from industrial fermentation processes [40].

3.6. Application of dead Aspergillus sp. biomass for multi-metal removal from synthetic wastewater

From the practical point of view, metallic nano-pollutants do not exist in a single form in the wastewater. Therefore, it is necessary to study the ability of the isolated multi-metal tolerant fungus *Aspergillus* sp. to remove multi-nanometals in wastewater. The dead biomass of *Aspergillus* sp. was used as the biosorpant instead of fungal pellets due to its applicability in industrial and environmental applications. Synthetic wastewater containing a mixture of Zn, Fe, Se and Ag NPs was prepared and the dried dead fungal biomass was applied. The biosorption efficiencies (shown in Fig. 9) were recorded for each metal in the nanometal mixture. From the results, it is clear that it is relatively less than the removal results for each nano-element individually present in the aqueous solution (as in Fig. 8). Such results could be attributed to the completion between different metals on the active sites on the outer surface of fungal biomass. However, despite the low efficiency of removing metal NPs via dead fungal biomass, it can be considered as a promising initial step for further investigation towards environmentally friendly bioremediation of toxic NPs present in aquatic environments.

4. Conclusion

Isolation and screening of heavy metal tolerant fungi is an important step to obtain isolates that are likely to be able to remove nanoparticles from their aqueous solutions. The multi-metal tolerant fungus *Aspergillus* sp. is a good candidate for Zn, Se, Fe, and Ag NPs removal either in single or mixture state. The adsorption capacity of heavy metals by wet fungal biomass was higher than the equivalent dry biomass, which can be attributed to the abundant charges on their surfaces as well as the active transport of metal ions. The biosorption process is affected by several factors including the pH of the NPs solution, contact time, fungal age, as well as the dry, dead and alive status of the fungal biomass. However, despite the low efficiency of removing metal NPs via dead fungal biomass, it can be considered as a promising initial step for further investigation towards environmentally friendly bioremediation of toxic NPs present in aquatic environments.

Author contribution statement

Marwa A. Shalaby: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Osama M. Darwesh: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ibrahim A. Matter: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohamed M. Gharieb: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] O.M. Darwesh, H. Li, I.A. Matter, Nano-bioremediation of textile industry wastewater using immobilized CuO-NPs myco-synthesized by a novel Cu-resistant *Fusarium oxysporum* OSF18, Environ. Sci. Pollut. Res. (2022) 1–13, https://doi.org/10.1007/s11356-022-23360-7.

[2] C.H.M. Hofman-Caris, P.S. Bäuerlein, W.G. Siegers, S.M. Mintenig, R. Messina, S.C. Dekker, Ch Bertelkamp, E.R. Cornelissen, A.P. van Wezel, Removal of nanoparticles (both inorganic nanoparticles and nanoplastics) in drinking water treatment - coagulation/flocculation/sedimentation, and sand/granular activated carbon filtration, Environ. Sci.: Water Res. Technol. 8 (2022) 1675–1686, https://doi.org/10.1039/d2ew00226d.

[3] H.A. Saleh, I.A. Matter, M.T.A. Abdel-Wareth, O.M. Darwesh, Molluscicidal, histopathological and genotoxic effects of *Scenedesmus obliquus* and *Spirulina platensis* extracts and their biosynthesized zinc oxide nanoparticles on *Biomphalaria alexandrina* snails, Aquacult. Res. 53 (10) (2022) 3680–3695, https://doi.org/10.1111/are.15872.

[4] O. Olkhovych, N. Svietlova, Y. Konotop, O. Karaushu, S. Hrechishkina, Removal of metal nanoparticles colloidal solutions by water plants, Nanoscale Res. Lett. 11 (518) (2016), https://doi.org/10.1186/s11671-016-1742-9.

[5] O.M. Darwesh, S.S. Ali, I.A. Matter, T. Elsamahy, Nanotextiles waste management: controlling of release and remediation of wastes, in: E. Andrea, T.A. Nguyen, P. Nguyen-Tri (Eds.), Nanosensors and Nanodevices for Smart Multifunctional Textiles, Elsevier, 2021, pp. 267–286, https://doi.org/10.1016/b978-0-12-820777-2.00016-9.

[6] A. Popowich, Q. Zhang, X.C. Le, Removal of nanoparticles by coagulation, J. Environ. Sci. 38 (2015) 168–171, https://doi.org/10.1016/j.jes.2015.10.001.

[7] H.H.M. Salih, A.M. El Badawy, T.M. Tolaymat, C.L. Patterson, Removal of stabilized silver nanoparticles from surface water by conventional treatment processes, Adv. Nanoparticles 8 (2) (2019) 21–35, https://doi.org/10.4236/anp.2019.82002.

[8] A. Lateef, O.M. Darwesh, I.A. Matter, Microbial nanobiotechnology: the melting pot of microbiology, microbial technology and nanotechnology, in: A. Lateef, E. B. Gueguim-Kana, N. Dasgupta, S. Ranjan (Eds.), Microbial Nanobiotechnology, 2021, pp. 1–19, https://doi.org/10.1007/978-981-33-4777-9_1.

[9] O.M. Gomaa, A.Y. Jassim, A. Chanda, Bioremoval of PVP-coated silver nanoparticles using *Aspergillus niger*: the role of exopolysaccharides, Environ. Sci. Pollut. Res. 29 (21) (2022) 31501–31510, https://doi.org/10.1007/s11356-021-18018-9.

[10] L. Zhang, J. Mao, Q. Zhao, S. He, J. Ma, Effect of $AlCl_3$ concentration on nanoparticle removal by coagulation, J. Environ. Sci. 38 (2015) 103–109, https://doi.org/10.1016/j.jes.2015.04.014.

[11] A. Kapoor, T. Viraraghavan, D.R. Cullimore, Removal of heavy metals using the fungus *Aspergillus niger*, Bioresour. Technol. 70 (1) (1999) 95–104, https://doi.org/10.1016/S0960-8524(98)00192-8.

[12] R. Dhankhar, A. Hooda, Fungal biosorption–an alternative to meet the challenges of heavy metal pollution in aqueous solutions, Environ. Technol. 32 (5) (2011) 467–491, https://doi.org/10.1080/09593330.2011.572922.

[13] S.A. Gouda, A. Taha, Biosorption of heavy metals as a new alternative method for wastewater treatment: a review. Egypt, J. Aquat. Biol. Fish. 27 (2) (2023) 135–153, https://doi.org/10.21608/ejabf.2023.291671.

[14] A. Ayele, S. Haile, D. Alemu, M. Kamaraj, Comparative utilization of dead and live fungal biomass for the removal of heavy metal: a concise review, Sci. World J. 2021 (2021), 5588111, https://doi.org/10.1155/2021/5588111.

[15] P.K. Rose, R. Devi, Heavy metal tolerance and adaptability assessment of indigenous filamentous fungi isolated from industrial wastewater and sludge samples, Beni-Suef Univ. J. Basic Appl. Sci. 7 (4) (2018) 688–694, https://doi.org/10.1016/j.bjbas.2018.08.001.

[16] A.M. Saad, M.M. Saad, N.A. Ibrahim, D. El-Hadedy, E.I. Ibrahim, A.Z.K. El-Din, H.M. Hassan, Evaluation of *Aspergillus tamarii* NRC 3 biomass as a biosorbent for removal and recovery of heavy metals from contaminated aqueous solutions, Bull. Natl. Res. Cent. 43 (1) (2019), https://doi.org/10.1186/s42269-019-0046-5.

[17] M.S. Hasanin, O.M. Darwesh, I.A. Matter, H. El-Saied, Isolation and characterization of non-cellulolytic *Aspergillus flavus* EGYPTA5 exhibiting selective ligninolytic potential, Biocatal. Agric. Biotechnol. 17 (2019) 160–167, https://doi.org/10.1016/j.bcab.2018.11.012.

[18] D.M. Silva, L.R. Batista, E.F. Rezende, M.H.P. Fungaro, D. Sartori, E. Alves, Identification of fungi of the genus *Aspergillus* section *nigri* using polyphasic taxonomy, Braz. J. Microbiol. 42 (2) (2011) 761–773, https://doi.org/10.1590/s1517-83822011000200044.

[19] S.A. Alsohaili, B.M. Bani-Hasan, Morphological and molecular identification of fungi isolated from different environmental sources in the Northern Eastern desert of Jordan, Jordan J. Biol. Sci. 11 (3) (2018) 329–337.

[20] N.A.A. Aboud, W.M. Alkayat, D.H. Hussain, Simple chemical synthesis of zinc oxide and copper oxide nanoparticles for biological protection, Sys. Rev. Pharm. 11 (6) (2020) 1188–1195.

[21] K. Hachem, M.J. Ansari, R.O. Saleh, H.H. Kzar, M.E. Al-Gazally, U.S. Altimari, S.A. Hussein, H.T. Mohammed, A.T. Hammid, E. Kianfar, Methods of chemical synthesis in the synthesis of nanomaterial and nanoparticles by the chemical deposition method: a review, J. Bionanoscience 12 (3) (2022) 1032–1057, https://doi.org/10.1007/s12668-022-00996-w.

[22] Y. Wang, Z. Zhu, F. Xu, X. Wei, One-pot reaction to synthesize superparamagnetic iron oxide nanoparticles by adding phenol as reducing agent and stabilizer, J. Nano Res. 14 (4) (2012) 755, https://doi.org/10.1007/s11051-012-0755-2.

[23] K.E. Giller, E. Witter, S.P. Mcgrath, Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review, Soil Biol. Biochem. 30 (10–11) (1998) 1389–1414, https://doi.org/10.1016/s0038-0717(97)00270-8.
[24] R.P. Calvillo-Medina, Determination of fungal tolerance index to heavy metals and heavy metal resistance tests, Bio-protocol 11 (21) (2021) 4218, https://doi.org/10.21769/bioprotoc.4218, 4218.
[25] J. Jeevanandam, A. Barhoum, Y.S. Chan, A. Dufresne, M.K. Danquah, Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations, Beilstein J. Nanotechnol. 9 (2018) 1050–1074, https://doi.org/10.3762/bjnano.9.98.
[26] O. Darwesh, I. Matter, M. Eida, H. Moawad, Y.-K. Oh, Influence of nitrogen source and growth phase on extracellular biosynthesis of silver nanoparticles using cultural filtrates of *Scenedesmus obliquus*, Appl. Sci. 9 (7) (2019) 1465, https://doi.org/10.3390/app9071465.
[27] A.K. Sidhu, N. Verma, P. Kaushal, Role of biogenic capping agents in the synthesis of metallic nanoparticles and evaluation of their therapeutic potential, Front. Nanotechnol. 3 (2022), 801620, https://doi.org/10.3389/fnano.2021.801620.
[28] E. Mohammadian, A. Babai Ahari, M. Arzanlou, S. Oustan, S.H. Khazaei, Tolerance to heavy metals in filamentous fungi isolated from contaminated mining soils in the Zanjan Province, Iran, Chemosphere 185 (2017) 290–296, https://doi.org/10.1016/j.chemosphere.2017.07.022.
[29] C.L. Văcar, E. Covaci, S. Chakraborty, B. Li, D.C. Weindorf, T. Frențiu, M. Pârvu, D. Podar, Heavy metal-resistant filamentous fungi as potential mercury bioremediators, J. Fungi 7 (5) (2021) 386, https://doi.org/10.3390/jof7050386.
[30] I. Acosta-Rodríguez, J.F. Cárdenas-González, A.S. Rodríguez Pérez, J.T. Oviedo, V.M. Martínez-Juárez, Bioremoval of different heavy metals by the resistant fungal strain *Aspergillus niger*, Bioinorgan. Chem. Appl. (2018) 1–7, https://doi.org/10.1155/2018/3457196, 2018.
[31] S. Siddiquee, K. Rovina, S.A. Azad, Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: a review, J. Microb. Biochem. Technol. 7 (6) (2015) 384–393, https://doi.org/10.4172/1948-5948.1000243.
[32] H. Zhang, X. Yuan, T. Xiong, H. Wang, L. Jiang, Bioremediation of co-contaminated soil with heavy metals and pesticides: influence factors, mechanisms and evaluation methods, Chem. Eng. J. 398 (2020), 125657, https://doi.org/10.1016/j.cej.2020.125657.
[33] A. Delgado, A.M. Anselmo, J.M. Novais, Heavy metal biosorption by dried powdered mycelium of *Fusarium flocciferum*, Water Environ. Res. 70 (3) (1998) 370–375, https://doi.org/10.2175/106143098x125019.
[34] A. Kapoor, T. Viraraghavan, Fungi as biosorbents, in: J. Wase, C. Forster (Eds.), Biosorbents for Metal Ions, Taylor &Francis, London, 1997, pp. 67–85.
[35] G. Chen, G. Zeng, L. Tang, C. Du, X. Jiang, G. Huang, H. Liu, G. Shen, Cadmium removal from simulated wastewater to biomass byproduct of *Lentinus edodes*, Bioresour. Technol. 99 (15) (2008) 7034–7040, https://doi.org/10.1016/j.biortech.2008.01.020.
[36] E. Guibal, C. Roulph, P. Le Cloirec, Uranium biosorption by a filamentous fungus *Mucor miehei* pH effect on mechanisms and performances of uptake, Water Res. 26 (8) (1992) 1139–1145, https://doi.org/10.1016/0043-1354(92)90151-s.
[37] R. Sudha Bai, T.E. Abraham, Studies on chromium(VI) adsorption-desorption using immobilized fungal biomass, Bioresour. Technol. 87 (1) (2003) 17–26, https://doi.org/10.1016/s0960-8524(02)00222-5.
[38] D. Teclu, G. Tivchev, M. Laing, M. Wallis, Bioremoval of arsenic species from contaminated waters by sulphate-reducing bacteria, Water Res. 42 (19) (2008) 4885–4893, https://doi.org/10.1016/j.watres.2008.09.010.
[39] M.M. Zareh, A.S. El-Sayed, D.M. El-Hady, Biosorption removal of iron from water by Aspergillus Niger, NPJ Clean Water 5 (1) (2022), https://doi.org/10.1038/s41545-022-00201-1.
[40] R.B. Narsi, N. Garima, Fungus - an alternative for bioremediation of heavy metal containing wastewater: a review, J. Sci. Ind. Res. 64 (2005) 93–100.