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Removal Efficiency of the Heavy Metals Zn(II), Pb(II) and Cd(II) by *Saprolegnia delica* and *Trichoderma viride* at Different pH Values and Temperature Degrees

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The removal efficiency of the heavy metals Zn, Pb and Cd by the zoosporic fungal species *Saprolegnia delica* and the terrestrial fungus *Trichoderma viride*, isolated from polluted water drainages in the Delta of Nile in Egypt, as affected by various ranges of pH values and different temperature degrees, was extensively investigated. The maximum removal efficiency of *S. delica* for Zn(II) and Cd(II) was obtained at pH 8 and for Pb(II) was at pH 6 whilst the removal efficiency of *T. viride* was found to be optimum at pH 6 for the three applied heavy metals. Regardless the median lethal doses of the three heavy metals, Zn recorded the highest bioaccumulation potency by *S. delica* at all pH values except at pH 4, followed by Pb whereas Cd showed the lowest removal potency by the fungal species and vice versa in case of *T. viride*. The optimum biomass dry weight production by *S. delica* was found when the fungus was grown in the medium treated with the heavy metal Pb at pH 6, followed by Zn at pH 8 and Cd at pH 8. The optimum biomass dry weight yield by *T. viride* amended with Zn, Pb and Cd was obtained at pH 6 for the three heavy metals with the maximum value at Zn. The highest yield of biomass dry weight was found when *T. viride* treated with Cd at all different pH values followed by Pb whilst Zn output was the lowest and this result was reversed in case of *S. delica*. The maximum removal efficiency and the biomass dry weight production for the three tested heavy metals was obtained at the incubation temperature 20°C in case of *S. delica* while it was 25°C for *T. viride*. Incubation of *T. viride* at higher temperatures (30°C and 35°C) enhanced the removal efficiency of Pb and Cd than low temperatures (15°C and 20°C) and vice versa in case of Zn removal. At all tested incubation temperatures, the maximum yield of biomass dry weight was attained at Zn treatment by the two tested fungal species. The bioaccumulation potency of *S. delica* for Zn was higher than that for Pb at all temperature degrees of incubation and Cd bioaccumulation was the lowest whereas *T. viride* showed the highest removal efficiency for Pb followed by Cd and Zn was the minor of the heavy metals.

KEYWORDS: Bio-removal, Efficiency, Fungi, Heavy metals, Hydrogen ions, Incubation temperature

Heavy metal releases to the environment have been increasing continuously as a result of industrial activities since the industrial revolution and technological development, posing a significant threat to the environment and public health because of their toxicity, accumulation in the food chain and persistence in nature. Heavy metals are present in nature and industrial wastewater. Due to their mobility in natural water ecosystems and their toxicity, the presence of heavy metals in surface water and groundwater poses a major inorganic contamination problem.

Conventional techniques commonly applied in removing heavy metals from wastewater include chemical (precipitation/neutralization) or physical (ion exchange, membrane separation, electrodialysis and activated carbon adsorption) methods (Atkinson *et al.*, 1998; Matheickal *et al.*, 1987). Generally, these processes are efficient in removing the bulk of metals from solution at high or moderate concentrations. However, chemical processes produce a large amount of metallic sludge, making metal recovery difficult and increase the pollution load on the environment (Guibal *et al.*, 1992). When applied to dilute metal waste or lower concentrations of metal ions, these processes are either ineffective or not cost-effective (Huang and Huang 1996; Matheickal *et al.*, 1987).

Biological methods of metal removal, defined as biosorption, have been recommended as cheaper and more effective techniques to solve the water pollution problem (Gomes *et al.*, 1998, Khoo and Ting, 2001; Knorr, 1991). In biosorption, either live or dead microorganisms or their derivatives are used, which complex metal ions through the action of ligands or functional groups located on the outer surface of the cell (Bolton and Gorby, 1995). Biosorption regarded as physicochemical interactions of metal ions with the cellular compounds of biological species (Kapoor and Viraraghavan, 1998). The mechanism of uptake can be due to ion exchange, chelation, chemical complexation with microbial cell surface groups, adsorption, and diffusion through cell walls and membranes (Churchill *et al.*, 1995; Kuyucak and Volesky, 1988; Muraleedharan *et al.*, 1991), which differ depending on the species used, the origin and processing of the biomass and solution chemistry.

Microorganisms including bacteria, algae, fungi and yeast are found to be capable of efficiently accumulating

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heavy-metal ions and used as biosorbents (Chen and Yiacaumi, 1997; Gadd, 1992, 1993; Galun *et al.*, 1987; Kratchovil and Volesky, 1998; Mullen *et al.*, 1989; Veglio and Beolchini, 1997; Volesky, 1990; Yetis *et al.*, 2000). The use of fungi as biosorbents have been proven more efficient and economical for removal of toxic metals from dilute aqueous solutions and treating effluents charged with toxic metallic ions by biosorption because of its filamentous morphology and high percentage of cell walls (Addour *et al.*, 1999; Volesky and Holan, 1995). Moreover, fungi can also be easily grown in substantial amounts using inexpensive growth media to obtain large quantity of biomass (Kapoor *et al.*, 1999). In the case of fungal biomass, removal of metal ions from aqueous solutions has been studied with strains of *Penicillium* (Galun *et al.*, 1983a, b), *Rhizopus arrhizus* (Tsezos and Velosky, 1982a, b; Tobin *et al.*, 1984), *Rhizopus oryzae* and *Aspergillus oryzae* (Huang and Huang, 1996), *Aspergillus niger* (Kapoor *et al.*, 1999) and *Mucor rouxii* (Gardea-Torresdey *et al.*, 1996; Mullen *et al.*, 1992).

The pH value of the metal solutions affects the biosorption efficiency and the removal process because it determines the availability of the metal in a soluble form for adsorption, and dictates the overall surface charge of the adsorbent (Tobin *et al.*, 1994). It also affects the surface charge of the biosorbents and the degree of ionization (Galli *et al.*, 2003). The temperature of incubation was also have an effecting role on removing process efficiency of the heavy metals from aqueous solutions (Chen and Yiacaumi, 1997; Gadd, 1990; Veglio and Beolchini, 1997). This paper aimed for studying the potency of the zoosporic fungal species *Saprolegnia delica* (using cyst cells) and the terrestrial fungus *Trichoderma viride*, isolated from polluted water drainages in the Delta of Nile in Lower Egypt, as biosorbents for the removal efficiency of the heavy metals zinc Zn(II), lead Pb(II) and cadmium Cd(II) from aqueous solutions. The three heavy metals were found as the most pollutants in the polluted water drainages in Lower Egypt and *S. delica* was tested for the first time. The removal efficiency of these heavy metals by the two fungal species were followed and compared at different pH values and temperature degrees.

Materials and Methods

Tested fungal species. Two fungal species of which one belonging to zoosporic fungi; *S. delica* and *T. viride* which, related to terrestrial fungi, were tested for their bio-removal efficiency of the heavy metals at different ranges of pH values and temperatures. The two tested fungi were repeatedly isolated in high occurrence and were the commonest fungal species in the polluted water drainages (Plate 1) in the Nile's Delta region, Lower Egypt. The polluted waters in these drainage often used by farmers for irrigation of cultivated plants (Plate 2) because there is no alternative clean unpolluted source. Pure cultures of the zoosporic fungus *S. delica* LAF34 were deposited in the Laboratory of Aquatic Fungi, Botany Department, Faculty of Science, Assiut University, Assiut, Egypt. A strain of *T. viride* (TV MLD42; isolated from polluted water drainages) was preserved on slants of Czapek yeast extract agar medium which consisted of in g/*l*; K_2HPO_4 1, $NaNO_3$ 3.0, $MgSO_4 \cdot 7H_2O$ 0.5, KCl 0.5, $FeSO_4 \cdot 7H_2O$ 0.01, glucose 10.0, yeast extract 2.0, peptone 3.0, agar 15.0 and chloromphenicol antibiotic 1. The strain was then allowed to grow at temperatures between $28 \pm 2^{\circ}C$ in an incubator.

Plate 1. One polluted water drainage from which the tested two fungal species were isolated in high occurrence.

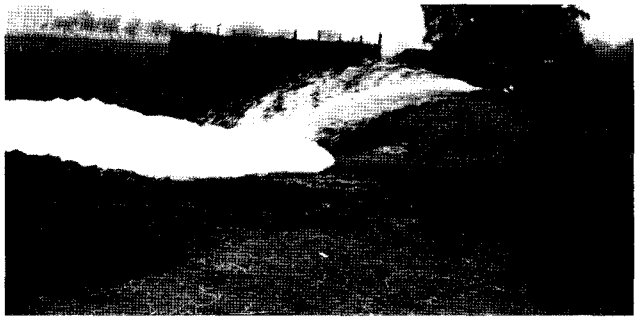

Plate 2. Cultivated plants in Nile's Delta, sometimes, irrigated by farmers with the heavy metals polluted waters because there is no alternative clean water source.

Applied heavy metals. Stock and separate solutions of the heavy metals Zn^{+2}, Pb^{+2} and Cd^{+2} were prepared in deionized water to give 30 $\mu g/ml$ of Zn^{+2}, 8 $\mu g/ml$ of Pb^{+2} and 2 $\mu g/ml$ of Cd^{+2}. Zn was used in the chemical formulation as the metal salt; zinc sulphate ($ZnSO_4$), lead in the form of lead acetate $(CH_3COO)_2Pb$ and Cd as cadmium chloride ($CdCl_2$). These concentrations were the median lethal doses (LD_{50}) of the tested zoosporic fungus; *S. delica* as determined by previous experiment (unpublished data). Also, stock solutions of the same chemical formulation of the heavy metals were prepared by the same way but to give the median lethal doses of the terrestrial fun-

gus *T. viride*. The median lethal dose in this case was 150 *μg/ml* and it was equal for the three individual heavy metals as it was also determined by previous experiment. The median lethal doses of the three heavy metals for the tested fungal species were highly greater than the concentration of these heavy metals at all investigated hyper-polluted water drainages in the Delta of Nile, Lower Egypt (Ali, 2007).

Bio-removal parameters. The two selected fungal species were tested for their efficiency in the removal of the heavy metals Zn, Pb and Cd under two effective parameters, pH value and temperature. The pH values tested were 2, 4, 6, 8, 10 and 12. The temperatures tested were 15, 20, 25, 30 and 35°C.

The media. For testing the bio-removal activity of the selected zoosporic fungal species; *S. delica* for the three applied heavy metals, glucose peptone broth (GPB) medium was used (Willoughby and Pickering 1977) during this study. This medium consists of in g/*l*; glucose 3, Peptone 1, $MgSo_4 \cdot 7H_2O$ 0.128, KH_2PO_4 0.0136 in addition to trace micro-nutrient elements, mg/*l*; $CaCl_2$ 8, $FeCl_2$ 0.5, $MnCl_2$ 0.5, $CuSO_4$ 0.1 and $ZnSO_4$ 0.1. Sodium benzylpenicillin and streptomycin sulphate at concentrations of 0.5 mg/*l* were added to the medium after autoclaving while the conical flasks were still hot. For determination of the metal tolerance of *T. viride*, Sabouraud liquid medium (Scharlau), which consisted of 1% peptone and 2% dextrose, pH 5.8 as described by Errasquin and Vazquez (2003) was used. Peptone, rather than other nitrogen-containing organic substrates, was used because of its comparatively low metal binding (Garcia-Toledo *et al.*, 1985).

Inoculation, treatments and incubation. Zoospores suspension of *S. delica* was prepared by growing the fungus in sterilized cultures of water sesame seeds at 20°C for a period of 8 days. Zoospores discharge starting from the second day of incubation period and extended till the eighth day of vegetative growth. Freshly homogenous spore suspension was also prepared as an inoculum in case of *T. viride*. Glucose peptone and Sabouraud liquid media were poured into 250 *ml* conical flasks (50 *ml* medium each) and conical flasks were plugged and sterilized. The pH values of the growth media were adjusted with 0.1 N HCl and NaOH to give various initial hydrogen ion concentrations (pH values); 2, 4, 6, 8, 10 and 12. Sterilized conical flasks containing the specific nutritive media of the tested fungal species were cooled until 40°C and they were adjusted to give different concentrations of the tested heavy metals at their median lethal doses as indicated previously. Three milliliters suspension of zoospores cysts (approximate number is 4×10^{12} zoospores) of *S. delica* were withdrawn under aseptic conditions with a sterilized pipette and used as inocula for the conical flasks containing glucose peptone broth medium. Also, conical flasks containing Sabouraud liquid medium were inoculated each with three ml of the freshly prepared homogenous spore suspension of *T. viride*. The previous experiment was repeated thrice. The prepared conical flasks for testing the bio-removal efficiency of *S. delica* for the applied heavy metals at the different pH values were incubated at 20°C in case of *S. delica* and at 28 ± 2°C in case of *T. viride*, each for eight days in a rotating incubator at an agitation rate of 150 round per minute (rpm). Another group of conical flasks was prepared for studying the impact of temperature degree on bioaccumulation potency of the heavy metals by *S. delica* and *T. viride*. These conical flasks were prepared by the same way but their pH value of the growth medium was adjusted at 6 in case of the heavy metal Pb and at pH 8 for Zn and Cd in case of *S. delica* whereas for *T. viride* the pH value was adjusted at 6 for the three heavy metals. These conical flasks were incubated for eight days at temperature ranges of 15°C, 20°C, 25°C, 30°C and 35°C in a rotating incubator at an agitation rate of 150 round per minute (rpm). At the end of the bio-removal tests, the biomass pellets were harvested using filter papers (Whatman No. 1) and washed three times with deionized water to remove the residual growth medium, dried in oven at 75°C until constant weight and the dry weight of biomass pellets was calculated. Culture filtrates were used for estimation of the bio-removal efficiency of *S. delica* and *T. viride* for the three applied heavy metals Zn, Pb and Cd.

Analysis of the heavy metal ions. The concentrations of un-adsorbed heavy metals (zinc, lead and cadmium) in the sorption media were analysed and determined using Atomic Absorption Spectrometer (AAS, Varian). The bio-removal potency of the heavy metal by the two tested fungi was calculated from the difference between the initial and final (unabsorbed) heavy metal concentrations. The data at each heavy metal was expressed as *μg/ml*. The measurement results were expressed as three significant digits.

Results

The data presented in Table 1 and Fig. 1 indicate that the highest bio-removal potency of *S. delica* for Zn was generally obtained in the alkaline side (around neutral medium). The bio-removal efficiency of *S. delica* for Zn was of lowest value at pH 4 (highly acidic medium) matching 2.7% of total added Zn concentration and then it was increased at pH 6 (37.1% of initial Zn was sequestered). However, the maximum bioaccumulation potency of *S. delica* for Zn was attained at pH 8 (alkaline side)

Table 1. Efficacy of pH and temperature on the percentage (%) of the bio-removal of the heavy metals Zn, Pb and Cd (treated as median lethal doses) by *S. delica* and *T. viride* from their aqueous media

Fungal species	Heavy metals	Parameters										
		pH values						Temperature degrees				
		2	4	6	8	10	12	15°C	20°C	25°C	30°C	35°C
S. delica	Zn	–	2.7	37.1	44.0	37.7	32.7	35.3	44.0	43.0	42.7	35.3
	Pb	–	62.5	90.0	71.3	67.5	27.5	18.8	90.0	82.5	68.8	24.6
	Cd	–	35.0	45.0	80.0	70.0	65.0	25.0	80.0	70.0	70.0	70.0
T. viride	Zn	17.28	18.3	61.7	18.3	19.0	–	7.0	12.7	54.3	27.3	20.7
	Pb	36.42	38.3	66.5	42.1	38.4	–	45.7	47.5	68.3	42.7	34.5
	Cd	50.31	73.9	74.2	54.7	37.1	–	10.5	18.0	61.0	28.7	34.0

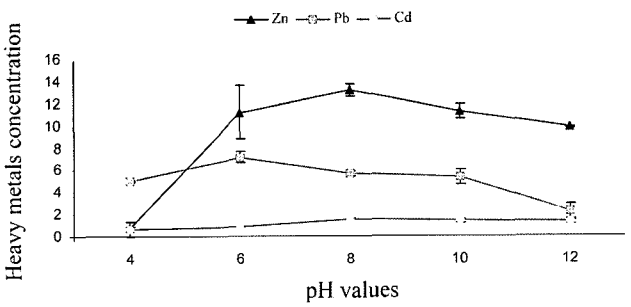

Fig. 1. Effect of pH on bio-removal efficiency ($\mu g/50\ ml$ culture medium) of the heavy metals Zn, Pb and Cd by the fresh biomass of *S. delica*.

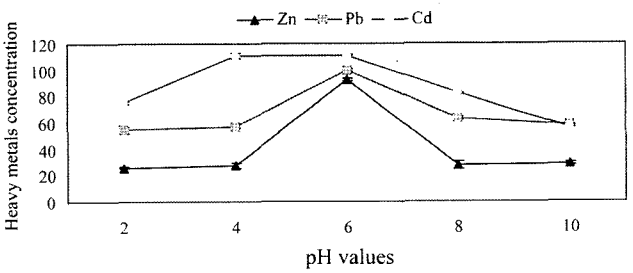

Fig. 2. Effect of pH on bio-removal efficiency ($\mu g/50\ ml$ culture medium) of the heavy metals Zn, Pb and Cd by the fresh biomass of *T. viride*.

where 44.0% of total added Zn was removed. The bio-removal activity of *S. delica* for Zn at pH values 10 and 12 was slightly lower (37.7 and 32.7% of total Zn was removed) than the maximum record at pH 8.

As regards bio-removal potency of *S. delica* for the heavy metal Pb, the maximum value of bio-removal was found at pH value of 6 (around neutral medium; 90% of the total added Pb was sequestered) followed by that at pH 8 (71.3% of total Pb initial concentration was removed, Table 1). Bioaccumulation activity of *S. delica* for Pb was found to be sensitive for alkaline medium where it was inhibited at pH values 10 and sharply declined at pH 12 (67.5 and 27.5%, respectively of the initial Pb concentration were eliminated the medium). Cadmium bio-removal efficiency by *S. delica* was improved in the alkaline medium compared in case of the acidic medium which showed the lowest biosorption rate for Cd. The maximum bioaccumulation potency was detected in the alkaline radical at pH 8 (80% of Cd were removed, Table 1) and it was slightly decreased at pH values 10 and 12 (70 and 65%, respectively of initial Cd concentration were sequestered, Table 1). Regardless, the concentration of the median lethal doses of the three heavy metals, Zn showed the highest bio-removal potency by *S. delica* at all pH values, except at pH 4, followed by Pb whilst Cd showed the lowest removal efficiency by the fungal species. It is worth to mention that *S. delica* did not absolutely grow at pH 2.

As regards *T. viride*, the optimum bio-removal efficiency for the three individual heavy metals was found at pH 6 (Fig. 2). The removal potency of *T. viride* for the heavy metal Zn increased with rising the pH value in the acidic radical until reaching the maximum at pH 6. The amounts of the bio-removed Zn by *T. viride* at alkaline pH values (pH 8 and 10; 18.31 and 19.03%, respectively of initial Zn were removed, Table 1) were nearly alike to that at pH 2 and 4 (17.28 and 18.33% of total Zn were eliminated). Similar results were also obtained for the bio-removal efficiency of *T. viride* to Pb where it was increased with climbing the pH in acidic aqueous medium till 6 (optimum amount; 66.53% of initial Pb was sequestered). The amount of removed Pb reduced gradually with rising the pH value in the alkaline medium. The bio-removal potency of *T. viride* for Cd increased with increasing pH value in the acidic medium until reaching the optimum amount at pH 6 (74.19% of initial Cd concentration removed) and then dropped with rising the pH value in the alkaline side. However, the potency of *T. viride* to remove Cd was approximately equal at pH values 4 and 6 (Fig. 2). Regardless the initial concentrations of the three applied heavy metals, *T. viride* was able to remove high amounts of Cd from the medium at all pH values followed by Pb and Zn removal was the lowest. *T. viride* did not grow absolutely at pH 12.

As shown in Fig. 3, the highest amount of the mycelial

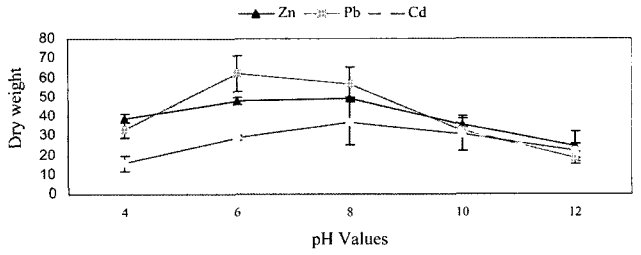

Fig. 3. Effect of pH on mycelia production (mg/50 *ml* culture medium) by *S. delica* treated with different concentrations of the heavy metals Zn, Pb and Cd.

dry weight of *S. delica* was found when the zoosporic fungal species was treated with 8 *μg/ml* of Pb (LD_{50}) at pH values 6 and 8. At 30 *μg/ml* application of Zn, the mycelial dry weight of *S. delica* was low at pH 4, then increased ascendingly at pH 6 until reach the maximum value at pH 8 followed by a decline at pH 10 and it was recorded the lowest level at pH 12. At 8 *μg/ml* supplements of Pb, the biomass dry mycelia of *S. delica* was also low at pH value 4 (33.0 mg) and nearly equal to that at pH 10 (32.5 mg). The optimum dry weight of mycelia was obtained at pH 6 and the mycelial dry weight slightly lowered at pH 8. However, the dry mycelia of *S. delica* was pronouncedly dropped at pH 12. Cd treatments of *S. delica* at 2 *μg/ml* suppressed the biomass dry weight production at pH 4. Then, the dry mycelia raised at pH 6 and climbed to the maximum level at pH 8. Thereafter, the dry mycelia of *S. delica* descindingly declined at pH 10 and 12. It was found that the dry weight of mycelia were approximately equals at pH 6 (29.0 mg) and 10 (30.5 mg).

It is noteworthy that Pb treatment produced the highest amount of biomass dry weight at the two pH values 6 and 8, which were also activated the bio-removal efficiency of the heavy metal by *S. delica.* At these pH values, in comparison, Zn application came next in the quantity of biomass dry weight production by *S. delica* while Cd treatment markedly dropped the biomasses.

The data presented in Fig. 4, show that the maximum yield of biomass dry weight by *T. viride* treated with Zn,

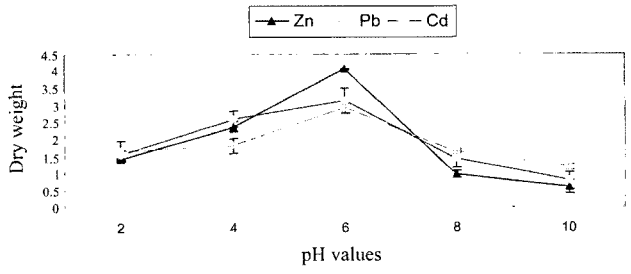

Fig. 4. Effect of pH on mycelia production (g/50 *ml* culture medium) by *T. viride* treated with different concentrations of the heavy metals Zn, Pb and Cd.

Pb and Cd at their median lethal dose was obtained at pH 6 for the three heavy metals. The optimum production of biomass dry weight at this pH (6) was recorded in case of Zn supplement followed by Cd and Pb output was the lowest. Generally, *T. viride* treated with Cd produced the highest biomass of dry weight at all pH values and Pb came second while Zn was the lowest. The production of biomass dry mycelia by *T. viride* supplemented with Zn was elevated with rising pH value in acidic medium with the optimum amount at pH 6. In the alkaline medium at pH 8 and 10, the biomass yield of dry weight was inhibited with rising the pH value and the amounts of biomass were lower than at pH 2 and 4. A similar results were also found when *T. viride* treated with the heavy metals Pb and Zn and the amount of biomass dry weight production was higher in case of acidic compared with the alkaline medium.

Our data indicated in Table 1 and Fig. 5 reveal that, the bio-removal efficiency of *S. delica* for the three tested heavy metals at their median lethal doses was at the lowest value at 15°C compared with the other degrees used for incubation. At the median lethal dose of Zn (30 *μg/ml*), the maximum bioaccumulation potency of *S. delica* was obtained at 20°C where 44% of added Zn was eliminated. Good results were also found at temperatures 25°C and 30°C (43 and 42.7% of initial Zn concentration were removed) but the bio-removal efficiency still lower than that at 20°C. The removal potency of *S. delica* for Zn was equal at 15°C and 35°C (35.5% of initial Zn concentration were sequestered). The bio-removal of Pb by *S. delica* was also affected by the degree of the temperature and it was optimum at 20°C (90% of initial Pb concentration was eliminated). With increasing the temperature, the bio-removal efficiency of *S. delica* for Pb was in the following order: 25°C > 30°C > 35°C (82.5, 68.8, 24.6%, respectively of total Pb concentration were removed). The lowest amount of Pb bio-removal by *S. delica* was recoded at 15°C (18.8% of initial Pb was sequestered). The potency of *S. delica* to remove Cd was also attained

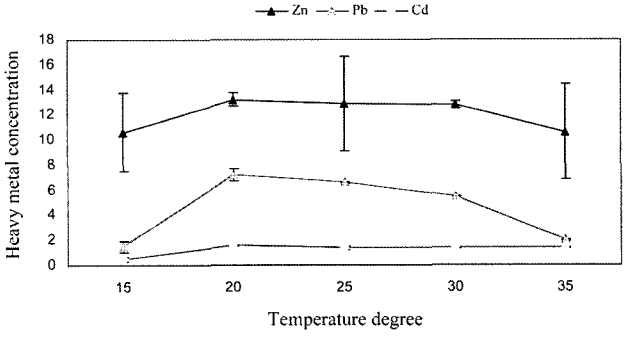

Fig. 5. Effect of temperature on bio-removal efficiency (*μg*/50 *ml* culture medium) of the heavy metals Zn, Pb and Cd by *S. delica.*

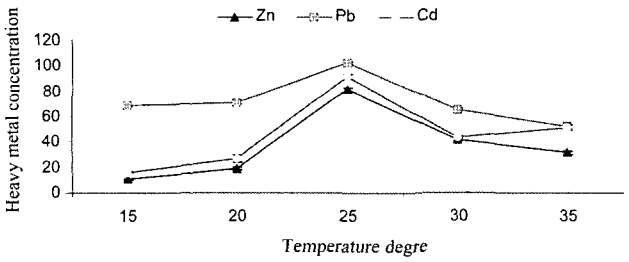

Fig. 6. Effect of temperature on bio-removal efficiency (μg/ 50 *ml* culture medium) of the heavy metals Zn, Pb and Cd by *T. viride*.

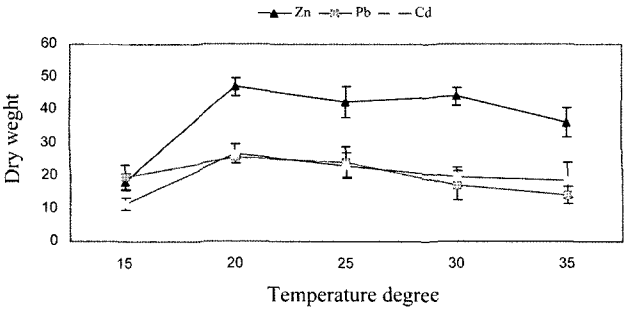

Fig. 7. Effect of temperature on biomass production (mg/ 50 *ml* culture medium) by *S. delica* treated with different concentrations of the heavy metals, Zn, Pb and Cd.

at 20°C (80% of Cd initial concentration was eliminated). However, with rising the temperature for 30°C and 35°C the bioaccumulation activity of *S. delica* for Cd was not affected and remained stable equal to that at 25°C (70% of total added Cd concentration were removed). The removal efficiency of Cd by *S. delica* still at its lowest value at 15°C (only 25% of total Cd concentration was sequestered) compared with other incubated temperatures.

Regardless the degree of temperature, it is obvious that the bio-removal potency of *S. delica* for Zn was highest than that for Pb at all temperature degrees and Cd bioaccumulation was the lowest.

As presented in Table 1 and Fig. 6, the bio-removal potency of *T. viride* for the heavy metal Zn was increased with rising the incubation temperature from 15°C to 20°C (7.00 and 12.67%, respectively of total Zn were removed) and reached the optimum value at 25°C (54.33% of initial Zn was sequestered). Elevated temperatures at 30°C and 35°C reduced the removal efficiency (27.33 and 20.67%, respectively of total Zn was sequestered) of the terrestrial fungus for the heavy metal Zn but their removal potency were greatly higher than at low temperatures (15°C and 20°C). The efficiency of *T. viride* to remove Pb was also optimum at 25°C incubation temperature (68.33% of initial Pb concentration was eliminated) and the potency for the heavy metal removal was activated at the low temperatures (15°C and 20°C) compared with elevated temperatures (30°C and 35°C). Similarly, Cd bio-removal by *T. viride* from the aqueous medium was at the maximum value at 25°C (61.00% of initial Zn concentration was sequestered) and high temperatures (30°C and 35°C; 28.67 and 34.00%, respectively of initial Cd concentration were removed) were more efficient in Cd removal compared with low incubation temperatures (15°C and 20°C; 10.50 and 18.00%, respectively of total Cd concentration were eliminated). Apart from the initial concentrations of the heavy metals, the amounts of Pb removed by *T. viride* from the aqueous medium were higher than that obtained in case of Cd at all incubation temperatures and Zn was the lowest.

The results demonstrated in Fig. 7 show that, the maximum yield of the biomass of *S. delica* amended with the heavy metals Zn, Pb and Cd at their median lethal doses was obtained at 20.0°C. The dry mycelia production was also doing well at 25.0°C but still of lower value than that at 20°C. The highest biomass production of *S. delica* treated with 30 $\mu g/ml$ of Zn was found at 20°C followed by that at 30°C. Then, the yield of mycelia decreased at 25°C and 35°C. The lowest biomass dry weight production was obtained at 15°C. Treatment of *S. delica* with 8 of Pb $\mu g/ml$ suppressed the biomass production at 15°C. Thereafter, the biomass dry weight production climbed at the optimum amount at 20°C before it was gradually dropped at elevated temperatures 25°C to 30°C to 35°C. The different incubation temperatures affected the biomass of *S. delica* supplemented with 2.0 $\mu g/ml$ of Cd. The lowest amount of biomass dry weight was obtained at 15°C whereas the maximum yield of dry mycelia was attained at 20°C. The biomass dropped at 25°C and showed a subsequent decline at 30°C and 35°C. General note show that the biomass production by *S. delica* supplemented with Zn was the highest at all incubated temperatures, except at 15°C, than in case of Pb and Cd. However, the biomass yield of dry weight seemed to be more or less similar in case treatment of *S. delica* with Pb and Cd at the individual incubation temperatures.

The data shown in Fig. 8 reveal that the biomass production dry weight by *T. viride* treated with the heavy metals Zn, Pb and Cd was affected by the heavy metal and incubation temperature. The maximum yield of biomass dry weight by *T. viride* was attained at 25°C for three applied heavy metals Zn, Pb and Cd. The biomass dry weight production by *T. viride* was ascendingly increased from 15~20~25°C. Then, it decreased recording the lowest values at elevated incubation temperatures (30 and 35°C). The biomass dry weight of *T. viride* treated with the heavy metal Pb was also climbed with uprising the incubation temperature until 25°C before it declined at the hihgh temperatures (30 and 35°C). The production of dry weight mycelia by *T. viride* amended with the heavy

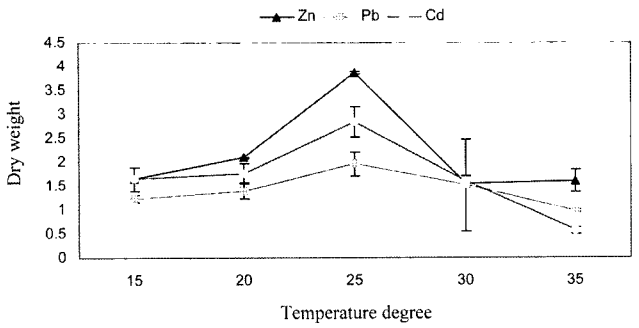

Fig. 8. Effect of temperature on biomass production (g/ 50 *ml* culture medium) by *T. viride* treated with different concentrations of the heavy metals, Zn, Pb and Cd.

metal Cd lie intermediate in the amount between Zn (the highest) and Pb (the lowest).

Discussion

The results showed a great difference between the removal efficiency of the two tested fungi. The optimum bio-removal potency of the zoosporic fungal species *S. delica* for the three applied heavy metals, at their median lethal doses, was around neutral medium. The removal activity of *S. delica* for Zn(II) and Cd(II) was in the maximum level at pH 8 and for Pb(II) was at pH 6. Apart from the median lethal doses of the three heavy metals, the highest bio-removal efficiency by *S. delica* was recoded for Zn at all pH values except pH 4, Pb came next and Cd gave the lowest bioaccumulation efficiency by the fungal species. In case of the terrestrial fungal species *T. viride*, the removal efficiency was optimum at pH 6 for the three applied heavy metals and the fungus was able to remove high amounts of Cd from the medium at the different pH values followed by Pb and Zn removal was the lowest.

Our results clearly indicated that the bio-removal efficiency of the tested heavy metals by the two selected fungal species was strongly dependent upon the biosorbent fungal species, the pH value of the aqueous media and the properties of the metal ions. This is in agreement with similar study carried out by Babich and Stotzky (1979) who found that the toxicity of Pb to some fungal species was potentiated under acidic conditions (pH 5 and 6). Also, in accordance with this result, Babich and Stotzky (1982) found that increasing the pH value from acidic to alkaline levels completely eliminated the toxicity of Ni to *Achlya* sp. and *Saprolegnia* sp. Fourest *et al.* (1994) reported that the maximum sorption capacity of *R. arrhizus* for lead was observed at pH 7.0. In addition, several investigators (Akar *et al.*, 2006; Arica *et al.*, 2004; Bayramoglu *et al.*, 2003; Brady *et al.*, 1994; Lo *et al.*, 1999; Podgorskii *et al.*, 2004; Yan and Viraraghavan, 2003; Yang *et al.*, 2005) reported that the biosorption of lead, cadmium and zinc from aqueous solutions by the live and waste biomasses of several biosorbent fungal species varied and it was observed between pH 4.0 and 6.0 depending upon the heavy metal and the fungal tested. Thus, different metals have different pH optima, due to the different solution chemistry of the metals (Macaskie and Dean, 1989).

The sharp decline or switch off the removal efficiency of the three heavy metals by the two tested fungi at low pH values was also reported by Ross and Townley (1986) who noted that the removal of copper by *P. spinulosum* decreased at lower pH. Similarly, the uptake and binding of the heavy metals; Zn, Pb and Cd were severely inhibited when pH was below 3 by the fungi; *Penicillium digitatum* (Galun *et al.*, 1987), *Saccharomyces cerevisiae* (Volesky and May-Phillips, 1995), *Rhizopus nigricans* (Zhang *et al.*, 1998), *A. niger* (Junior *et al.*, 2003; Kapoor *et al.*, 1999). This could be explained by the increase in density of the negative charge on the cell surface, causing proton removal on the cell bonding sites, thereby increasing its biosorption capacity (Junior *et al.*, 2003).

The low bio-removal potency of the two tested fungal species for the heavy metals at low pH values, with some differences, was attributed to hydrogen ions that compete with metal ions on the sorption sites (Huang *et al.*, 1991; Tsezos and Volesky, 1981). In other words, at lower pH, due to protonation of the binding sites resulting from a high concentration of protons, negative charge intensity on the sites was reduced, resulting in the reduction or inhibition of the binding of metal ions (Kapoor *et al.*, 1999). Most microbial surfaces are negatively charged because of the ionization of functional groups, thus contributing to the metal binding (Huang *et al.*, 1988; Hughes and Poole, 1989). At low pH, some functional groups will be positively charged and may not interact with metal ions (Fourest *et al.*, 1994; Tobin *et al.*, 1984). In addition, Galli *et al.* (2003) show that the removal process was highly dependent on the pH of the metal solutions, which affects the surface charge of the biosorbents and the degree of ionization.

The optimum biomass dry weight production by *S. delica* was obtained in case of the median lethal dose of Pb at pH values of 6 and 8 followed by Zn whereas Cd treatments suppressed the biomass dry weight. It was noted that pH values which promoted the biomass dry weight production in the media were nearly the same which activated the bio-removal potency by *S. delica* and *T. viride* for the three tested heavy metals. The production of biomass dry weight by *T. viride* treated with Zn, Pb and Cd was attained at pH 6 for the three heavy metals and the maximum value was obtained at Zn supplement. Apart from the initial heavy metal concentration, the maximum yield of biomass dry weight by *T. viride* was found at all

pH values of Cd treatment, followed by Pb whereas Zn output was the lowest and acidic medium was inducible compared with the alkaline medium.

The optimum bio-removal ability of *S. delica* for the three tested heavy metals at their median lethal doses was found at the incubation temperature 20°C despite that the bioaccumulation potency was high at 25°C. The removal efficiency of *S. delica* for Zinc was highest than that for Pb at all incubation temperatures and Cd bio-removal by *S. delica* was the lowest of the three heavy metals. The removal efficiency of the three applied heavy metals by the terrestrial fungus *T. viride* was obtained at 25°C. Higher temperatures (30°C and 35°C) were more efficient in the removal of Pb and Cd than low temperatures (15°C and 20°C) and vice versa in case of Zn removal. In this regards, some authors (Chen and Yiacaumi, 1997; Gadd, 1990; Veglio and Beolchini, 1997) reported that the temperature was a variable affecting the biosorption process by microorganisms. Kapoor and Viraraghavan (1998) reported that *Aspergillus versicolor* pretreated with autoclave reduced the biosorption of cadmium, copper, and nickel. By the same way, Yan and Viraraghavan (2000) reported that *Mucor rouxii* biomass pretreated with autoclave reduced the biosorption of heavy metals. Also, Wu and Li (2002) indicated that the removal of Pb^{2+} from aqueous solution by the mycelial pellets of *Phanerochaete chrysosporium* affected by pH and temperature. Under the optimum biosorption temperature (27°C), the highest lead uptake was observed with mycelial pellets. In addition, Ozer and Ozer (2003) investigated the biosorption of Pb(II), Ni(II) and Cr(VI) ions onto inactive *S. cerevisiae* as a function of initial pH, and temperature. The Pb(II), Ni(II) and Cr(VI) biosorption depending on temperature and the maximum metal ions uptake obtained at optimum biosorption temperature of 25°C. The reduction of biosorption capacity in heat and autoclaved *A. versicolor* and autoclaved *Metarrhizium anisopliae* var. *anisopliae* biomasses may be attributed to the loss of intracellular uptake (Cabuk *et al.*, 2005). However, Li and Yuan (2006) studied the effects of temperature and initial pH of the solution on biosorption of Cd (2+) from water via biosorption using *Rhodotorula* sp. Caustic and heat treatments showed different influences on the biosorption capacity, and the highest metal uptake value was obtained by boiling yeast cells. In addition, Xinjiao (2006) found that the optimum conditions of Cu(II) biosorption by *Cladosporium* sp. were at pH 5.0 and temperature of 35°C.

The optimum incubation temperature for the production of the biomass dry mycelia by *S. delica* was 20°C at the three heavy metals at their median lethal doses. Also, the biomass production of dry weight was high at the incubation temperature 25°C but it was comparatively of lower amount than that found at 20°C. The biomass production of dry weight was decreased descindingly with rising the temperature. The biomass yield of the dry weight of *S. delica* treated with Zn was the highest at all incubated temperatures, except at 15°C, compared with that of Pb and Cd treatments. The low incubation temperature (15°C) was also inhibitory for the biomass dry weight production at the three heavy metals. The production of biomass dry weight production by *T. viride* treated with the individual heavy metals was obtained at 25°C incubation temperature. The maximum yield of biomass dry weight was attained in case of Zn treatment at the different incubation temperatures followed by Cd whereas Pb output was the lowest.

The results of this study showed that the tested fungi; *S. delica* and *T. viride* were successful as biosorbents for the removal of the heavy metals Zn, Pb and Cd from the aqueous media. The two tested fungal species (zoosporic fungus and the terrestrial fungus) can be easily obtained. In addition, the two tested fungi also grown in substantial amounts using cheap inexpensive growth media. Therefore, bio-removal carried out by these fungi could serve as an economical mean of treating effluents and the polluted water areas charged with toxic metallic ions. *T. viride* was more efficient in removing high quantities of the heavy metals compared with *S. delica* and this attributed to its affinity to tolerate high concentrations of the applied heavy metals. *S. delica* is a zoosporic fungal species strictly live as saprophyte in aquatic habitats and seemed adapted to remove heavy metals polluted water sources. In addition, only minor investigation to expire using zoosporic fungi as biosorbents for the heavy metals from aqueous media and it is the first an attempt to testify *S. delica* as a biosorbent. The bio-removal efficiency was optimum around neutral pH values for the two tested fungal species and at 20°C in case of *S. delica* and at 25°C for *T. viride*. These temperatures were expected because they are the optimum for the growth of the two fungal species at the normal conditions.

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