Polish Journal of Microbiology
2007, Vol. 56, No 4, 257–264

ORIGINAL PAPER

Comparative Study of the Effect of Stress by the Heavy Metals Cd^{+2}, Pb^{+2}, and Zn^{+2} on Morphological Characteristics of *Saprolegnia delica* Coker and *Dictyuchus carpophorus* Zopf.

ESAM H. ALI

Botany Department, Faculty of Science, Assiut University, Assiut, Egypt

Received 11 April 2007, 1st revison 20 June 2007, 2nd revison 23 October 2007, accepted 5 November 2007

Abstract

The effects of essential (Zn^{+2}) and non-essential (Cd^{+2} and Pb^{+2}) heavy metals on morphogenesis of two represantatives of informal group zoosporic fungi namely; *Saprolegnia delica* Coker and *Dictyuchus carpophorus* Zopf. were studied. These two species varied in their tolerance of each amended heavy metal. Lead had the most potent effect amongst the tested heavy metals in inhibiting the radial extension of the vegetative hyphae of the two tested species. The vegetative hyphae of *S. delica* and *D. carpophorus* assumed different morphological alterations compared with that at controls depending upon the applied heavy metal and the dose concentration. Both zoosporangial formation and discharges of the two tested fungi were greatly inhibited even at the low concentrations of Cd. Zoosporangia of *D. carpophorus* appeared curved at high concentrations of Cd. Zoosporangial formation and discharge of the two zoosporic fungi showed variable deformation when treated with Pb. The different applications of Zn nearly stimulated sporangial elongation in both zoosporic fungi. Sex organs varied in their numbers and morphogenesis at each treatment of the applied heavy metal. The gemmae of *S. delica* were greatly reduced or missed at the elevated toxic levels of Cd whereas they enhanced in numbers and size at most Pb treatments and little affected at Zn applications.

Key words: *Saprolegniaceae*, zoosporic fungi representatives, morphogenesis influenced by heavy metals

Introduction

The rate of the global deposition of the heavy metals Cd^{+2}, Pb^{+2}, and Zn^{+2} have dramatically increased over the past two centuries (Candelone *et al*., 1995). Industrial inputs and the agronomic application of fertilizers, pesticides, and metal-contaminated sewage continue to contribute to metal accumulation (Herland *et al*., 2000). Both essential and nonessential heavy metals can be toxic above a critical concentration which depends on the organism (Blaudez *et al*., 2000). Among heavy metals necessary for fungal growth is zinc. Nonessential metals commonly include cadmium and lead. Toxic heavy metals can inhibit the growth, cause morphological changes and affect the reproduction of organisms. However, some aquatic microorganisms (protista) can selectively take up various heavy metal ions from aqueous systems, and therefore are important for the regulation of environmental pollution and the recovery of useful metals from nature. Intensive studies have been done about the impact of heavy metals on fungal activity. The morphological changes induced by heavy metals are common among all groups of the fungi. Some reports are available on the effect of the heavy metals on morphogenesis of the vegetative hyphae (*e.g*. Darlington and Rauser, 1988; Gabriel *et al*., 1996a; Baldrain, 2003), sporulation (Abel and Baerlocher, 1984; Leyval *et al*., 1994; Duarte *et al*., 2004) and sexual and asexual reproduction (Perlman, 1948; Vega and Le Tourneau, 1974; Chiu *et al*., 1998) of some fungal groups. However, studies dealing with the morphogenesis of zoosporic fungi, especially *Oomycetes* responses to increased levels of heavy metals are less frequent (Hendrix *et al.*, 1969; Halsall, 1977; Lundy *et al*., 2001).

Therefore, the aim of our present studies is to explore the effects of some heavy metal ions on the growth, sporulation and sexual and asexual reproduction of two common zoosporic fungi (*Saprolegnia delica* and *Dictyuchus carpophorus*) belonging to family *Saprolegniaceae*, class *Oomycetes* collected from highly polluted waters drainages across Nile Delta (Lower Egypt).

Corresponding author: Esam H. Ali, Botany Department, Faculty of Science, Assiut University, Assiut, Egypt;
e-mail: ibraheem55@yahoo.com

Experimental

Materials and Methods

Organisms selection. Two species of zoosporic fungi namely; *Saprolegnia delica* Coker and *Dictyuchus carpophorus* Zopf. belonging to the family *Saprolegniaceae*, class *Oomycetes* were chosen for this study. They are frequent in highly polluted water drainages in Nile Delta of Lower Egypt (Ali, 2007).

Medium used for growing of zoosporic fungi. Water-sesame seeds cultures were used for growing the two selected zoosporic fungal species during this investigation. This medium was proved to be the best for growing of zoosporic fungi (Khallil, 1984). Six sterilized germinating sesame seeds were introduced into sterilized Petri-dishes (10 cm in diameter each) to which 20 ml of deionized, sterilized, distilled water were added.

Inoculation, incubation and microscopical examination. For fungal inoculation of Petri dishes containing the desired concentrations of each applied heavy metals as follows: for Cd^{+2}; 0.5, 1.0, 1.5, 2.0, 4.0 µg/ml, for Pb^{+2}; 4.0, 6.0, 8.0, 10.0, 15.0 µg/ml and for Zn^{+2}; 10.0, 20.0, 30.0, 40.0, 50.0 µg/ml. The heavy metals were used in the following chemical formulations: zinc sulfate for Zn^{+2} ($ZnSO_4 \times 7H_2O$), lead acetate $(CH_3COO)_2Pb$ for Pb^{+2} and cadmium chloride for Cd^{+2} ($CdCl_2$). The concentrations of the heavy metals were carefully chosen for the study after it has been established through preliminary experiments. Two ml of zoospores suspension of the tested fungal species were applied for each Petri dish. Zoospores suspension was prepared by growing the tested fungal species on water sesame seeds cultures for ten days at 20±2°C during which zoospores are matured and developed as inoculation propagules. The experiment was done in triplicates. Petri dishes were then incubated at 20°C. Microscopical examinations and observations on the developing colonies were carefully followed starting from the second day of incubation and were continued daily during the period of experiment (thirty days). Morphological abnormalities of the two tested zoosporic fungal species at the different treatments of the heavy metals were observed, described and were almost photographed at the same magnifications. Morphological features of the two species of zoosporic fungi as affected by the different applications of the heavy metals stress, which were followed included; the appearance of vegetative hyphae, zoosporangial formation and spores discharge, sexual reproductive structures (oogonia and antheridia) and gemmae formation (in case of *S. delica* because *D. carpophorus* formed no gemmae). The average numbers of each of zoosporangia, zoosporangial discharge, oogonia and antheridia per sesame seeds (representing one vegetative colony) of the two tested species of zoosporic fungi were counted and assessed. They were expressed as high, moderate, low and rare numbers as follows:

(A) For *S. delica*; high number (more than 25 per one colony), moderate number (25 to 12 per colony), low number (11 to 6 per colony) and rare number (less than 6 per colony).

(B) For *D. carpophorus*; high number (more than 20 per one colony), moderate number (20–10 per colony), low number (9–5 per colony) and rare number (less than 5 per colony).

Mycelial growth rate. Mycelial growth rate was also measured on sesame seeds. After incubation of the plates at 20±2°C for three weeks, the diameters of colonies were measured in centimeters.

Results

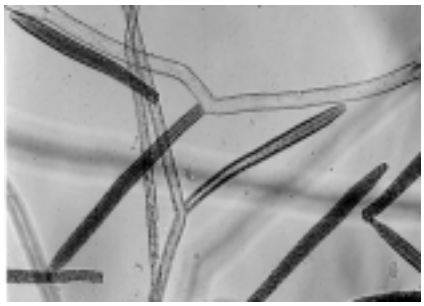
Effect of Cd^{+2}. The results presented in Table I indicate that *S. delica* can resist against Cd^{+2} concentrations until 4.0 µg/ml. The higher concentrations were lethal. In case of *D. carpophorus*, the maximum withstand of Cd^{+2} stress was 2.0 µg/ml concentration and higher concentrations were lethal. As shown in Table I the diameters of colonies of both *S. delica* and *D. carpophorus* descendingly decreased with increasing the toxicity level of Cd^{+2}.

The vegetative hyphae of *D. carpophorus* treated with the different concentrations of Cd^{+2} showed several morphological alterations compared with that at

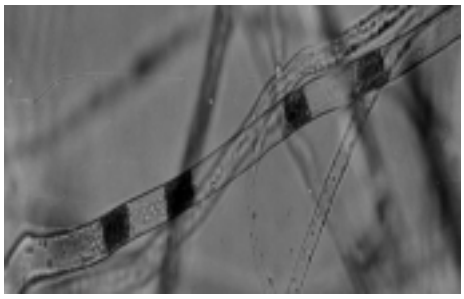
Table I
Effects of different tolerant rates of the the heavy metals; Cd^{+2}, Pb^{+2} and Zn^{+2} on the diameters of the vegetative colonies (cm) of *S. delica* and *D. carpophorus*

Heavy metals	Tolerant Concs. µg/ml	Diameters of vegetative colonies (cm)	
		Saprolegnia delica	*Dictyuchus carpophorus*
Cd^{+2}	Control	2.9	2.6
	0.5	2.1	2.2
	1.0	1.3	1.7
	1.5	0.8	1.4
	2.0	0.6	1.0
	4.0	0.3	NG
Pb^{+2}	Control	2.9	2.6
	4.0	1.4	1.9
	6.0	0.7	1.7
	8.0	0.6	0.6
	10.0	0.5	0.3
	15.0	0.2	NG
Zn^{+2}	Control	2.9	2.6
	10.0	3.1	2.8
	20.0	2.5	2.6
	30.0	1.8	2.0
	40.0	1.3	1.7
	50.0	1.1	1.5

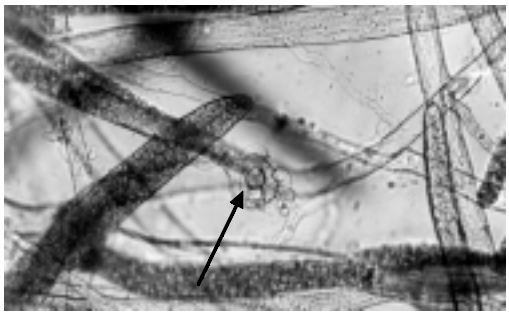
NG: Means no vegetative growth appeared at these concentrations.



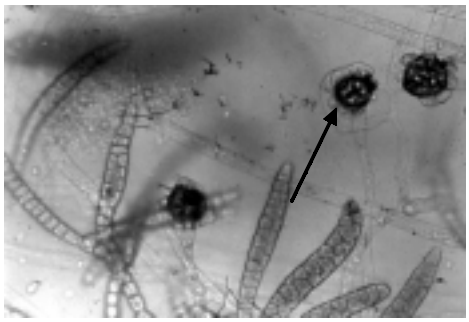
A.: Vegetative hyphae of *D. carpophorus* bearing dictyoid shaped sympodial sporangia at control.



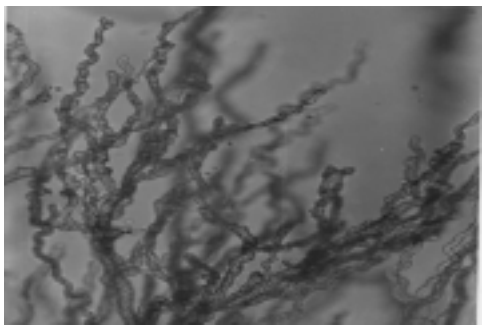
B.: Abnormal protoplasmic accumulation in *D. carpophorus* vegetative hyphae at 0.5 µg/ml of Cd.



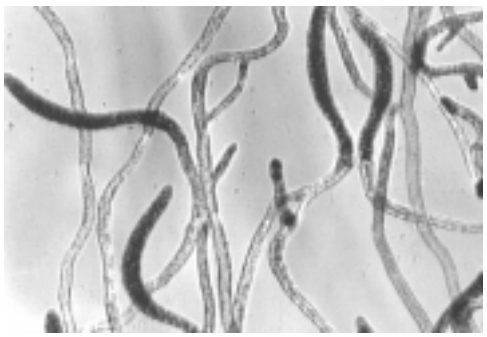
C.: Eccentric oogonia (arrow) characteristic for *D. carpophorus* at control.



D.: Immature oogonia (arrow) of *D. carpophorus* at 0.5 µg/ml of Cd.



E.: Coiled vegetative hyphae of *D. carpophorus* as observed at 1.5 µg/ml of Cd.



F.: Zoosporangia of *D. carpophorus* appeared curved at 1.5 µg/ml of Cd.

Fig. 1. Some morphological characteristics of *Dictyuchus carpophorus* at controls and as affected by different treatments of the heavy metal Cd.

controls (Fig. 1A). In this regards, unusual protoplasmic accumulations (Fig. 1B) were recorded inside the vegetative hyphae of *D. carpophorus* at the minimum inhibitory concentration (0.5 µg/ml). Sporangia often occurred at this concentration but sex organs appeared in low numbers and they were just appeared after 10 days of the incubation period. Normal eccentric oogonia were observed only at controls (Fig. 1C) whereas they appeared immature (Fig. 1D) at this treatment (0.5 µg/ml). Uprising Cd concentration to 1.0 µg/ml diminished the sporangial formation in *D. carpophorus* (Table II) where they were less frequent. At this treatment, only low numbers of undifferentiated oogonia were remarked after 12 days of incubation but male antheridia were completely missed. The vegetative hyphae of *D. carpophorus* were spiral (Fig. 1E) in their appearance at 1.5 µg/ml concentration of Cd and they were provided with crippled ends. Sporangia were observed in low rates at this supplement and they were severely bent being as forced for curvature (Fig. 1F) compared with that at control. Sexual reproductive organs totally disappeared at this concentration although prolongation of the incubation period. With increasing Cd supplements until 2.0 µg/ml (sublethal dose), the vegetative hyphae showed depleted protoplasmic material and seemed vacuolated and displayed abnormal branching at ends. The vegetative hyphae were also sterile bearing

Table II
Effects of various concentrations of the heavy metals; Cd^{+2}, Pb^{+2} and Zn^{+2} on some morphological aspects of *S. delica* and *D. carpophorus* which included sporangial formation (SF), sporangial discharge (SD), sexual reproduction organs (SR) and gemmae formation (GF) as were observed on cultures of water sesame seeds

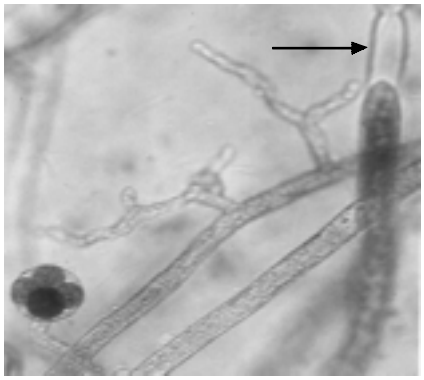
Heavy metals	Concs. µg/ml	Tested zoosporic fungi					
		Saprolegnia delica				*Dictyuchus carpophorus*	
		SF	SD	SR	GF	SF	SR
Cd^{+2}	Control	H	H	H	H	H	H
	0.5	H	H	H	H	H	L
	1.0	M	R	M	M	M	Ro
	1.5	L	R	M	R	L	–
	2.0	R	–	R	R	–	–
	4.0	–	–	–	–	NG	NG
Pb^{+2}	Control	H	H	H	H	H	H
	4.0	L	L	H	H	H	L
	6.0	L	–	R	H	H	R
	8.0	R	–	R	H	L	Ab
	10.0	R	–	–	M	R	–
	15.0	–	–	–	–	NG	NG
Zn^{+2}	Control	H	H	H	H	H	H
	10.0	H	H	H	M	H	M
	20.0	M	M	H	M	H	M
	30.0	M	L	R	L	H	Ab
	40.0	L	R	Ro	–	M	Ab
	50.0	–	–	–	–	R	–

Where: – Means that these fungal structures did not appear at this concentration
NG: No fungal growth (lethal concentration), Ro: only rare number of oogonia appeared, Ab: only antheridia appeared, H: High numbers, M: Moderate numbers, L: Low numbers, R: Rare numbers
For *S. delica*: H; >25 per one seed, M; 25–12 per one seed, L; 11–6 per one seed and R; <6 per one sesame seed.
For *D. carpophorus*: H; >20 per one seed, M; 20–10, L; 9–5 per one seed and R; <5 per one sesame seed.

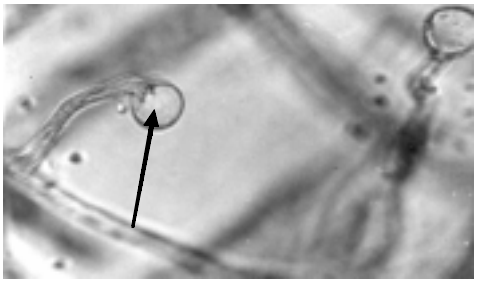
neither sporangia nor sexual reproductive organs whatever the period of incubation.

S. delica can resist concentrations of Cd until 4.0 µg/ml and these concentrations were the most effective amongst the heavy metals in reducing its radial mycelial growth compared with Pb and Zn supplements (Table I). The different tolerant rates of Cd affected the morphological features of *S. delica* as compared with normal shapes at control (Fig. 2A). The vegetative hyphae showed inner protoplasmic aggregations at different applications of Cd, which increased with rising the concentration. As indicated in Table I, the radial growth of the vegetative colonies of *S. delica* lowered with rising the stress concentrations of the heavy metal Cd. At the concentration of 0.5 µg/ml of Cd the ends of the vegetative hyphae appeared coiled compared with that at control. Also, at this minimal inhibitory concentration, zoosporangial formation, zoospores discharge, and gemmae formation of *S. delica* were little harmed and they were still in high numbers (Table II). However, this concentration promoted oogonial and antheridial formation. Oogonia characterized by its wide diameter and high density of oospheres content compared with the control. At 1.0 µg/ml treatments of Cd, zoosporangia were assessed in moderate numbers and they rarely showed spore release compared with the control. Oogonia and antheridia were observed in moderate numbers. Oogonia appeared with unusual coiled oogonial stalks and they did not show complete cytoplasmic cleavage. Also, gemmae enlarged in their size as compared with the control although they were depressed in their numbers (moderate number). Zoosporangia of *S. delica* were counted in low numbers at 1.5 µg/ml of Cd and they rarely showed zoospores discharge. At this supplement (1.5 µg/ml), oogonia and antheridia were also formed in moderate numbers and oogonia showed no ooplasm cleavage whatever the extension of the period of incubation. The ends of the vegetative hyphae of *S. delica* severely showed coiled ends at 2.0 µg/ml of Cd and zoosporangia (rare numbers) were small, deformed and un-discharged. At this concentration (2.0 µg/ml) of Cd, rare numbers of rudimental oogonia and non-functional antheridia were formed and gemmae were also sharply reduced (rare numbers). Only feeble sterile mycelial growth, without reproductive structures, appeared at 4.0 µg/ml of Cd and other higher concentrations were found lethal for fungal growth.

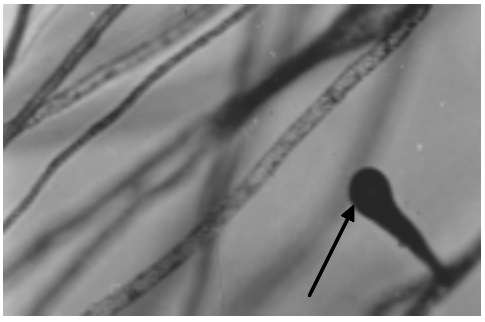
Effects of Pb^{+2}. With respect to *D. carpophorus*, the fungus can resist concentrations of Pb until



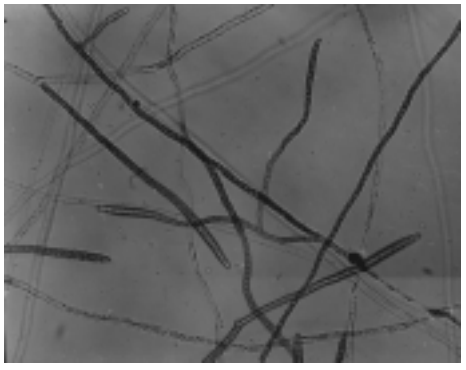
A.: *Saprolegnia delica* at control showing vegetative hyphae, sporangial proliferation (arrow) and sex organs (arrowheads).



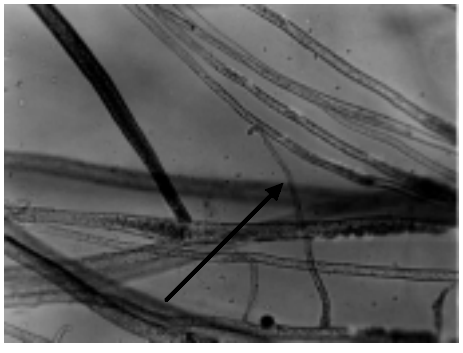
B.: Empty oogonia (arrow) of *D. carpophorus* as seemed at 6.0 µg/ml of Pb.



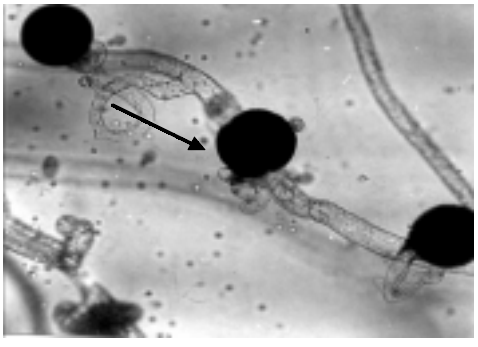
C.: Premature oogonia (arrow) of *S. delica* at 8.0 µg/ml of Pb.



D.: Vast sporangial elongation in *D. carpophorus* at 30.0 µg/ml of Zn.



E.: Non-functional antheridia (arrow) in *D. carpophorus* as appeared at 40.0 µg/ml of Zn.



F.: Premature oogonia antheridia (arrow) of *S. delica* as observed at 30.0 µg/ml of Zn.

Fig. 2. Morphological alterations in *Saprolegnia delica* and *Dictyuchus carpophorus* treated with various concentrations of the heavy metals Pb and Zn.

10.0 µg/ml and higher concentration were found lethal for spores germination. The different applications of Pb were the strongest in inhibiting the radial growth of the vegetative colonies compared with Cd and Zn treatments as presented in Table I. As the concentration of Pb raised the diameters of the vegetative colonies decreased. The vegetative hyphae at 4.0 µg/ml of Pb (the minimal inhibitory concentration) were thicker and stunted compared with that at control. Zoosporangia were highly occurred although they were shorter and thicker than that at control. Oogonia (undifferentiated) and antheridia were of low numbers and they delayed in their appearance (after 8 days of incubation) compared with the control. At 6.0 µg/ml applications of Pb zoosporangial formation little affected and still of high number. Rare numbers of vacuolated-oogonia (Fig. 2B) and antheridia appeared at this concentration of Pb after prolonged incubation period (18 days). Thick vegetative hyphae bearing low numbers of short zoosporangia were obviously

appeared at 8.0 µg/ml treatment of Pb. At this application (8.0 µg/ml), sexual reproductive organs represented only by sporadic short antheridia. A very restricted mycelia growth was formed at 10.0 µg/ml of Pb and the vegetative hyphae were tumoured showing apical protuberances. Only abortive sporangia were formed in rare numbers and no sexual structures appeared at this sublethal concentration.

S. delica tolerated concentrations of Pb^{+2} until 15.0 µg/ml and the higher concentrations were found lethal. In addition, the most remarkable phenomenon was the increase of *S. delica* gemmae numbers (high numbers) at the lowest three concentrations (4.0, 6.0 and 8.0 µg/ml) of Pb being as response of the fungus to face stress conditions of the heavy metal. The minimal inhibitory concentration of Pb for the fungal growth was 4.0 µg/ml at which the lateral vegetative hyphae showed abnormal branching. Both zoosporangial formation and discharge greatly reduced and they were appeared in low numbers (Table II). Oogonia and antheridia were differentiated and were of high numbers at this treatment. At 6.0 µg/ml treatments, the vegetative hyphae were slightly thicker and spiral compared with the control. Zoosporangial formation greatly deformed in their shapes compared with the control and they did not show zoospores release whatever the incubation period used despite zoospores cleavage. Oogonia and antheridia declined in their numbers (rarely formed) and the majority of oogonia did not show any cytoplasmic cleavage. Rare numbers of sporangia were observed at 8.0 µg/ml. These sporangia failed in their cleavage and so no spore liberation realized. Rudimental oogonia (Fig. 2C) and non-functional antheridia were observed in rare numbers at this treatment. At 10.0 µg/ml treatment of Pb, zoosporangia were also observed in rare numbers and they were very reduced in size showing no zoospores cleavage. At this concentration, gemmae enlarged in size and appeared in moderate numbers. No sexual reproductive organs appeared whatever the period of incubation. Only sterile stunted vegetative hyphae appeared at the sub-lethal concentration (15.0 µg/ml) and other distinguished morphological fungal structures disappeared completely whatever the period of incubation.

Effects of Zn^{+2}. In case of *D. carpophorus*, the fungal zoospores can germinate and grow until 50.0 µg/ml concentrations of Zn and thus they running out *S. delica* in their tolerance of the heavy metal as indicated in Table I. The low concentrations of Zn until 30.0 µg/ml were almost promotive for the radial growth of the vegetative hyphae and sporangial formation of *D. carpophorus*. The results presented in Table II show that zoosporangia were of high numbers at the low concentrations (10, 20 or 30 µg/ml) of Zn. At these treatment sporangia were pronouncedly elongated (Fig. 2D) compared with the control. Also, these treatments stimulated the germination of the encysted spores within their sporangia. Oogonia (eccentric) and antheridia moderately formed at 10.0 and 20.0 µg/ml of Zn (minimal inhibitory doses of sex organs) after 4 and 6 days of incubation. However, only twined, elongated antheridial branches were observed at 30.0 µg/ml. Zoosporangia were counted in moderate numbers at 40.0 µg/ml supplements of Zn and they still of unusual elongation. Only non-functional antheridia (Fig. 2E) appeared at this treatment and oogonia completely missed whatever the period of incubation used. Only sterile vegetative hyphae, which were spiral in their appearance, were observed at the sublethal concentration of Zn (50.0 µg/ml).

The low concentration of Zn (10.0 µg/ml) promoted hyphal elongation of *S. delica* as compared with the control (Table I). Both zoosporangial formation and discharge were highly activated and were of high numbers as shown in Table II. Oogonia and antheridia fully differentiated and they were of high numbers. The most distinct morphological feature at this concentration (10.0 µg/ml) is the stimulation of oogonial germination compared with the control. Ripening eggs (oospores) inside oogonium germinated via long germ tubes. Gemmae were also recorded in high numbers. Zoosporangia and sporangial release were observed in moderate numbers at 20.0 µg/ml of Zn (the minimal inhibitory concentration for sporangial formation) but sporangia were longer as compared with the control. Oogonia and antheridia encountered in high numbers at this treatment and most of the fertilized eggs within oogonia also germinated forming germlings. Gemmae slightly dropped at this application and they were found in moderate numbers. With increasing Zn supplements for 30.0 µg/ml, moderate numbers of elongated and multi-branched sporangia, which showed low rate of zoospores release, were encountered. Undifferentiated oogonia and antheridia (Fig. 2F) were remarked in rare numbers and enlarged gemmae were formed in low numbers. At 40.0 µg/ml concentration of Zn, low numbers of deformed sporangia appeared and these sporangia rarely discharged. At this treatment, rudimental rare numbers of oogonia were shown and no antheridia formed at all. Only un-fruitful spiral hyphal threads were observed at the sublethal concentration of Zn^{+2} (50.0 µg/ml) whatever the period of incubation used.

Discussion

The results showed that Cd was the strongest heavy metal in inhibiting the growth and morphogenesis of the two tested zoosporic fungi. In accordance with these results, Tham *et al*. (1999) reported that the toxicity of heavy metals in *Ganoderma lucidum* decreased in the order: Hg > Cd > Cu > U > Pb > Mn = Zn.

The maximum tolerance rates of *D. carpophorus* and *S. delica* for Cd were 2.0 and 4.0 µg/ml. The diameters of their vegetative colonies decreased with rising Cd toxicity. Morphological abnormalities of the vegetative hyphae varied according to the organism tested and the applied Cd concentration. The vegetative hyphae of the two tested fungal species were spiral and vacuolated compared with the control at most of the tolerant Cd concentrations. In this regard, Purkayastha *et al*. (1994) reported that Cd at 0.05 mM caused a strong growth reduction of *Pleurotus sajor-caju* and *Inonotus obliquus* (Baldrain and Gabriel, 1997), and the growth of *P. sanguineus* and *T. versicolor* was inhibited completely by the addition of 0.1 mM Cd to a stationary liquid culture (Pointing *et al*., 2000). In addition, Lundy *et al*. (2001) found that the heavy metals Cu, Co, Hg, Zn and Cd at concentrations between 0.05 and 3.0 mM decreased the mycelial area and radial extension of *Achlya bisexualis*. In the presence of 3 mM Hg the hyphae displayed spiral growth.

The morphological changes induced by the heavy metal Cd are also common among other groups of fungi (Darlington and Rauseer, 1988; Gabriel *et al*., 1996a; Baldrain, 2003; Jaeckel *et al*., 2005).

D. carpophorus and *S. delica* tolerated concentrations of the heavy metal Pb until 10.0 and 15.0 µg/ml, respectively. The heavy metal Pb was the most effective metal in inhibiting the radial growth of the two tested organisms. As a result, the vegetative hyphae of the two fungal species were thicker and stunted compared with the control. This finding is similar to that obtained by Ropek and Para (2002) who found that the growth of *Verticillium lecanii* was inhibited by Pb supplements.

D. carpophorus and *S. delica* tolerated parallel concentrations of Zn (50.0 µg/ml). The low concentrations of Zn until 30.0 µg/ml were promotive for the hyphal extension and elongation. In this regard, zinc has shown to be essential for the growth and nutrition of some species of *Phycomyces* (Odegard, 1952), *Fusarium* (Saraswathi-Devi, 1955), *Helminthosporium* (Peterson and Ketelson, 1956; White and Johnson, 1968), *Arthrobotrys* (Coscarelli and Pramer, 1962) and *Monoascus purpureus* (McHan and Johnson, 1970). In addition, Jaeckel *et al*. (2005) found that *Heliscus lugdunensis* and *Verticillium* cf. *alboatrum* showed a remarkable difference in their tolerance to Zn. The growth of *V.* cf. *alboatrum* was not inhibited at 1 mM Zn, whereas for *H. lugdunensis* no growth occurred above 0.3 mM Zn. On the other hand, Babich and Stotzky (1978) found that Zn at 10 mM concentration completely inhibited mycelial growth of *Rhizoctonia solani*.

Elevated concentrations of Cd diminished the number of formed sporangia in *D. carpophorus*, which appeared in curvature-fashion. The different Cd doses inhibited zoosporangial numbers in *S. delica* and the process of zoospores formation and release. It was found that heavy metals are harmful for asexual reproduction in different taxonomic groups of fungi. The reproductive stage of spore formation and conidium production are much more sensitive to heavy metals than mycelial growth in saprophytic and mycorrhizal soil fungi (Leyval *et al*., 1994) and also in aquatic hyphomycetes (Abel and Baerlocher, 1984).

Sporangial formation in *D. carpophorus* and *S. delica* sharply declined and sporangia became shorter with rising the heavy metal Pb concentration. In accordance, Ropek and Para (2002) found that the ions of Pb inhibited the growth and sporulation of *Verticillium lecanii*.

The low treatments of Zn until 30.0 µg/ml enhanced sporangial formation and elongation in *D. carpophorus* and also flourished the germination of encysted spores. However, higher concentrations (40.0 µg/ml) of Zn lowered sporangial numbers before they totally disappeared at 50.0 µg/ml. Elevated concentrations of Zn had an inhibitory action on sporangial formation and zoospores release in *S. delica* and sporangia assumed different alter shapes according to Zn concentration. In this respect, Halsall (1977) reported that low concentrations of zinc (10^{-7} to 10^{-6} M) increased the numbers of zoosporangia formed by *Phytophthora cinnamomi*. This probably reflects the requirement by *Phytophthora* species for traces of zinc (Hendrix *et al*., 1969). However, Halsall (1977) mentioned that the total inhibition of *P. cinnamomi* and *P. drechsleri* mycelial growth occurred between 1 and 5×10^{-5} M Cu^{+2} whereas total inhibition of sporangial formation occurred between 1 and 5×10^{-7} M Cu^{+2}. At copper concentrations between 10^{-5} M and 5×10^{-7}M, many *P. drechsleri* zoosporangia were abnormal in appearance and nonviable; in many cases the zoosporangial wall had ruptured, releasing the zoospores as a nonmotile cohesive mass.

These results are also comparative with that obtained by Duarte *et al.* (2004) who noted that zinc concentration and exposure time inhibited fungal production and affected fungal reproduction by either stimulating or inhibiting sporulation rates of aquatic hyphomycetous fungi colonizing alder leaf discs in microcosms.

Sexual reproductive organs in *D. carpophorus* seriously affected by the heavy metal Cd and they were observed only at 5.0 and 1.0 µg/ml after prolonged incubation period. Oogonia of *S. delica* enlarged in diameter and increased in their contents of eggs at the lowest concentration of Cd whereas the higher doses were inhibitory for sex organs formation. The sensitivity of formation of sex organs, in response to Cd stress, was also observed in other taxonomic groups of fungi (Gabriel *et al*., 1996b; Chiu *et al.*, 1998).

The supplements of the heavy metal Pb were generally promotive for sex organs formation and their related activities at the low concentrations while they were suppressive at high doses in the two tested zoosporic fungi.

Gemmae formation in *S. delica* treated with the heavy metal Cd was sensitive where they lowered in number and size with rising the concentration. Gemmae of *S. delica* increased in numbers and enlarged in size at most concentrations of Pb being to resist the stress action of the heavy metal. Higher concentrations of Zn were only the effective in delimiting the gemmae formation in *S. delica*. Similar results were obtained by Perlman (1948) who mentioned that concentrations higher than 1 mg/liter of Zn resulted in increased sclerotial formation in *Sclerotium delphinii*. Moreover, Vega and Le Tourneau (1974) reported that sclerotial production by *Whetzelinia sclerotiorum* occurred if Zn was added to cultures grown for periods of 1–22 days.

Further investigations on the effects of these heavy metals on the metabolisms and biosorption of zoosporic fungi would be necessary for precise evaluation. The results of these experiments could prove the applicability of zoosporic fungi as cheap and safe biosorbents in bioremediation to treat different wastes contaminated with heavy metals.

Literature

Abel T.H. and F. Baerlocher. 1984. Effects of cadmium on aquatic hyphomycetes. *Appl. Environ. Microbiol.* 48: 245–251.

Ali E.H. 2007. Biodiversity of zoosporic fungi in polluted water drainages across Niles' Delta region, Lower Egypt. *Acta Mycologica* 42: 99–111.

Babich H. and G. Stotzky. 1978. Toxicity of zinc to fungi, bacteria, and coliphages: Influence of Chloride ions. *Appl. Environ. Microbiol.* 36: 906–914.

Baldrain P. 2003. Interactions of heavy metals with white-rot fungi. *Enzyme Microb. Technol.* 32: 78–91.

Baldrain P. and J. Gabriel. 1997. Effect of heavy metals on the growth of selected wood-rotting basidiomycetes. *Folia Microbiol.* 42: 521–523.

Blaudez D., C. Jacob, K. Turnau, J.V. Colpaert, U. Ahonen-Jonnarth, R. Finlay, B. Botton and M. Chaloot. 2000. Differential responses of ectomycorrhizal fungi to heavy metals in vitro. *Mycol. Res.* 104: 1366–1371.

Candelone J.P., S. Hong, C. Pellone and C.F. Boutron. 1995. Post-industrial revolution changes in large-scale atmospheric pollution of the northern hemisphere by heavy metals as documented in central Greenland snow and ice. *J. Geophys. Res.* 100: 16605–16616.

Chiu S.W., Y.H. Chan, S.C. Law, K.T. Cheung and D. Moore. 1998. Cadmium and manganese in contrast to calcium reduce yield and nutritional values of the edible mushroom *Pleurotus pulmonarius*. *Mycol. Res.* 102: 449–457.

Coscarelli W. and D. Pramer. 1962. Nutrition and growth of *Arthrobotrys conoides*. *J. Bacteriol.* 84: 60–64.

Darlington A.B. and W.E. Rauser. 1988. Cadmium alters the growth of the mycorrhizal fungus *Paxillus involutus*: a new growth model accounts for changes in branching. *Can. J. Bot.* 66: 225–229.

Duarte S., C. Pascoal and F. Cassio. 2004. Effects of zinc on leaf decomposition by fungi in streams: studies in microcosms. *Microb. Ecol.* 93: 366–374.

Gabriel J., O. Kofronova, P. Rychlovsky and M. Krenzelok. 1996a. Accumulation and effect of cadmium in the wood-rotting basidiomycete *Daedalea quercina*. *Bull. Environ. Contam. Toxicol.* 57: 383–390.

Gabriel J., M. Capelari, P. Rychlovsky, M. Krenzelok and F. Zadrazil. 1996b. Influence of cadmium on the growth of *Agrocybe perfecta* and two *Pleurotus* spp. and translocation from polluted substrate and soil to fruit bodies. *Toxicol. Environ. Chem.* 56: 141–146.

Halsall D.M. 1977. Effects of certain cations on the formation and infectivity of *Phytophthora* zoospores. 2. Effects of copper, boron, cobalt, manganese, molybdenum, and zinc ions. *Can. J. Microbiol.* 23: 1002–1010.

Hendrix J.W., S.M. Guttman and D.L. Wightman. 1969. Cation and sterol effects on growth of *Phytophthora parasitica* var. *nicotianae*. *Phytopathology* 59: 1620–1624.

Herland B.J., D. Taylor and K. Wither. 2000. The distribution of mercury and other trace metals in the sediments of the Mersey Estuary over 25 years 1974–1988. *Sci. Total Environ.* 253: 45–62.

Jaeckel P., G. Krauss and G. Krauss. 2005. Cadmium and zinc response of the fungi *Heliscus lugdunensis* and *Verticillium* cf. *alboatrum* isolated from highly polluted water. *Sci. Total Environ*. 346: 274–279.

Khallil A.M. 1984. M. Sc. Thesis, (Studies on aquatic fungi in El-Ibrahimia canal, Egypt). Bot. Dept. Fac. Sc. Assiut University. Assiut. Egypt

Leyval C., I. Weissenhorn, A. Glashoff and J. Berthelin. 1994. Influence des metaux lourds sur la germination des spores de champignons endomycorhiziens a arbuscules dans les sols. *Acta Botanica Gallica* 141: 523–528.

Lundy S.D., R.P. Payne, K. Giles and A. Garrill. 2001. Heavy metals have different effects on mycelial morphology of *Achlya bisexualis* as determined by fractal geometry. *FEMS Microbiol. Lett.* 201: 259–263.

McHan F. and G.T. Johnson. 1970. Zinc and amino acids: Important components of a medium promoting growth of *Monoascus purpureus*. *Mycologia* 62: 1018–1031.

Odegard K. 1952. On the physiology of *Phycomyces blakesleeanus* Burgeff. I. Mineral requirements on a glucose-asparagine medium. *Physiol. Plantarum* 5: 583–609.

Perlman D. 1948. On the nutrition of *Sclerotium delphinii*. *Amer. J. Bot.* 35: 360–363.

Peterson E.A. and H. Ketelson. 1956. The effect of trace elements on growth of *Helminthosporium sativum* and several related species. *Can. J. Microbiol.* 2: 441–446.

Pointing S.B., V.V.C. Bucher and L.L.P. Vrimoed. 2000. Dye decolorization by subtropical basidiomycetous fungi and the effect of metals on decolorizing ability. *World. J. Microbiol. Biotechnol.* 16: 199–205.

Purkayastha R.P., A.K. Mitra and B. Bhattacharyya. 1994. Uptake and toxicological effects of some heavy metals on *Pleurotus sajor-caju* (Fr.) Singer. *Ecotoxicol. Environ. Saf.* 27: 7–13.

Ropek D. and A. Para. 2002. The effect of heavy metal ions and their complexions upon the growth, sporulation and pathogenecity of the entomopathogenic fungus *Verticillium lecanii*. *J. Inverteb. Pathology* 79: 123–125.

Saraswathi-Devi L. 1955. Heavy metals requirements of fungi. *Proc. Indian Acad. Sci.* 41B: 145–150.

Tham L.X., S. Matsuhashi and T. Kume. 1999. Response of *Ganoderma lucidum* to heavy metals. *Mycoscience* 40: 209–213.

Vega R.R. and D.L. Le Tourneau. 1974. The effect of zinc on growth and sclerotial formation in *Whetzelinia sclerotiorum*. *Mycologia* 66: 256–264.

White J.P. and G.T. Johnson. 1968. Growth and pigmentation of *Helminthosporium cynodontis*. *Bact. Proc.* 68: 40.